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A Review on Current Research Activities: Biological Conversion of Crude Glycerol from Biodiesel Industry into Value-Added Products

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Abstract: Glycerol is a by-product obtained from biodiesel industry. Recently, the biodiesel production is increasing exponentially, the crude glycerol produced from the transesterification reaction of vegetables oils has also been produced in a large quantity. The pure glycerol plays an important role in food, pharmaceutical, cosmetics, and many other industries, it is too costly to refine the crude glycerol to a high purity, especially for medium and small biodiesel producers. Several research projects and studies have been conducted and innovative consumptions of the crude glycerol are under investigations. It will be beneficial to the research people as well as biodiesel industry in understanding the progress of glycerol for value-added applications. In the present paper, we have been clearly summarized the current available studies and possible ways on the utilizations of crude glycerol generated from biodiesel industry using biological conversion process.
Keywords : Biodiesel, Crude glycerol, Biological Conversion Process, value-added products.

Introduction

The 20th century was the first era to be dominated by fossil fuels. The advances of human civilization have led to an unprecedented rise in energy consumption, and a 16-fold increase in the use of fossil fuels since 1900. The limited supply of fossil fuels and the potential increase in costs of extraction from fossil fuel reserves have triggered global debates on the need for energy conservation and renewable energy. "Reserves are small, well-explored shares of the total mineral resources in the earth's crust that can be extracted with the available techniques at an acceptable cost. Advances in exploration and extraction constantly transfer fossil fuels from the broader and only poorly known resource category to the reserve pool. Resource exhaustion is thus not a matter of actual physical depletion but rather one of eventually unacceptable costs"[1]. The relatively rapid atmospheric warming due to the combustion of fossil fuels has emerged as the foremost global environment concern. Revelle and Suess have stated in their 1957 study that "Within a few centuries, we (humans) are returning to the atmosphere and the oceans, the concentrated organic carbon stored in the sedimentary rocks for over hundreds of millions of years"[2]. The combustion of fossil fuels is the largest source of anthropogenic emissions of SOx and NOx, the oxidation of which produces sulphates and nitrates that are responsible for acid depositions. Due to these concerns of human development, energy security and climate change, it has become an urgent necessity to implement energy conservation measures and replace fossil fuels with renewable sources of energy wherever possible. Renewable energy is a general term which includes solar energy, wind energy, hydropower, biomass energy and energy from wastes[1].

Biodiesel is a promising alternative to fossil fuels and its production is increasing worldwide. This rise in production of biodiesel is slow due to the high cost of production[3]. Glycerol is the principal by-product of

the biodiesel industry, accounting for nearly 10% (w/w) of the total product output [3,4]. The increasing amounts of glycerol produced from the biodiesel industry have led to a significant decrease in the price of glycerol to almost half its actual cost, and it is now considered a waste. However, the price of biodiesel is high due to the high cost of raw materials required for production, and biodiesel is currently in use as a blend with petroleum-based diesel. A decrease in the cost of biodiesel would urge consumers to opt for this renewable and less toxic alternative to fossil fuels. This lowering of the cost can be brought about by producing commercially-relevant chemicals from the by-product glycerol [3]. Many chemicals can be obtained from glycerol through either biological or chemical conversions. Some examples are 1,3-propanediol, 3-hydroxypropionaldehyde, polyhydroxyalkanoates, hydrogen, ethanol, citric acid, polyunsaturated fatty acids, lipids, carotenoids, succinic acid, biosurfactants, acrolein, syngas, and butanol [3,5].

Glycerol

Glycerol is a colourless and odourless organic liquid which has applications in many products such as cosmetics, pharmaceuticals, food, and so on. It is a by-product of the transesterification process of triacylglycerols (TAGs) to obtain biodiesel. The glycerol backbone of TAGs is substituted with an alcohol in the presence of a catalyst at elevated temperatures, giving Fatty Acid Alkyl Esters (FAAEs) and glycerol as major products [6]. The crude glycerol obtained after the transesterification process contains some impurities and varies in composition depending on the manufacturer and source of TAGs. These impurities may affect the conversion of crude glycerol into other products.

Differences between types of glycerol from biodiesel industry [7].

Parameter	Crude glycerol	Purified glycerol	Refined/commercial glycerol
Glycerol content (%)	60–80	99.1–99.8	99.20–99.98
Moisture content (%)	1.5–6.5	0.11–0.8	0.14–0.29
Ash (%)	1.5–2.5	0.054	<0.002
Soap (%)	3.0–5.0	0.1–0.16	0.04–0.07
Acidity	0.7–1.3	0.10–0.16	0.04–0.07
Chloride (ppm)	ND	1	0.6–9.5
Colour (APHA)	Dark	34–45	1.8–10.3

ND = Not Determined

1,3-Propanediol

1,3-propanediol is an important intermediate in the manufacture of polyethers, polyurethanes, polyesters, biocidesand heterocyclic compounds, and is also widely applied in the food, cosmetics and pharmaceutical industries. It is also useful as a coolant or antifreeze, or to improve the properties of solvents, lubricants, laminates and adhesives. It also plays a role in the synthesis of the polymer polytrimethylene terephthalate (PTT) which can be used to make carpets, special textile fibres, monofilaments, films, and non-woven fabrics. PTT is also used in the engineering thermoplastics area. Currently, 1,3-propanediol is synthesized via one of two chemical routes. The Degussa/DuPont route involves the conversion of acrolein to 1,3-propanediol via 3-hydroxypropionaldehyde. The Shell process involves the conversion of ethylene oxide to 1,3-propanediol via 3-hydroxypropionaldehyde. These production routes produce toxic intermediates, involve high equipment costs [8] and rely on fossil fuel derivatives as feedstock. The biological conversion of glycerol to 1,3-propanediol occurs through anaerobic fermentation by certain bacterial strains, such as some species of the genus *Lactobacillus*, thermophilic organisms and species from the families Enterobacteriaceae and Clostridiaceae.The fermentation of glycerol occurs through two parallel pathways, an oxidative pathway and a reductive pathway. 1,3-propanediol is a product of the reductive pathway via 3-hydroxypropionaldehyde. Pyruvate formed by the oxidative pathway competes with 3-hydroxypropionaldehyde for the NADH-linked oxidoreductase enzyme to form ethanol, citric acid, acetic acid, butanol, etc., thus decreasing the yield of 1,3-propanediol.The purity and concentration of the crude glycerol, and the fermentation conditions influence substrate consumption and 1,3-propanediol yield. In the family Clostridiaceae, the main producers of 1,3-propanediol from glycerol were found to be *Clostridium butyricum, C. acetobutylicum, C. pasteurianum, C. beijerinckii* and *C. diolis*. *Clostridium* sp. were found to be able to catabolize glycerol only under anaerobic

conditions. Ke-Ke Cheng*et al* [9], found that an aerotolerant strain of*Klebsiella pneumoniae* isolated from the micro-alkaline soil of Xinjiang in China produced a high concentration of 1,3-propanediol (12.2 g/L) after 8 hours during batch fermentation at 40°C, with pH at 8.0, glycerol concentration 20 g/L and ammonium sulphate as nitrogen source. They found that higher glycerol concentrations and low pH and temperature inhibited the formation of 1,3-propanediol by *K. pneumoniae*.During fed-batch fermentations under the same conditions, 1,3-propanediol concentration reached 38.1 g/L after consumption of 66.4 g of glycerol. In 2014, de Souza and co-workers studied the effectiveness of immobilization of *K. pneumoniae* on calcium alginate beads on 1,3-propanediol production. They found that immobilized cells were able to produce more 1,3-propanediol in a shorter time (22.22 g/L after 12 hours) when compared to free suspended cells (19.51 g/L after 16 hours) and the immobilized cells could be reused several times with or without a resting period. The diameter of the bead and cell concentration in the alginate solution were important parameters which affected the production of 1,3-propanediol. Immobilization may decrease the pathogenic opportunity of *K. pneumoniae*, creates a stable anaerobic environment in which the organism can grow and makes separation of cells from fermentation medium easy, thus reducing the cost of the downstream processing and making the cells available for reuse [10].

Polyhydroxyalkanoates

Widespread use of petroleum-based plastics has caused long-term problems due to depletion of fossil fuel reserves, accumulation of plastic waste in the environment, and rising oil prices. These reasons have led scientists to conduct research into alternative plastics that are environmentally-friendly, biodegradable under appropriate conditions and obtained from cheap source [11]. The class of biodegradable polymers or bioplastic, polyhydroxyalkanoates (PHAs), were first observed as lipid-like intracellular storage granules in *Bacillus megaterium* [11,12], and are synthesized by over 30% of soil-inhabiting bacteria [13]. PHAs are a versatile class of linear-structured polymers with more than 100 different monomer constituents [12,13].The most commonly found type of PHA produced by microorganisms is poly-3-hydroxybutyrate (PHB). These polymers are produced by microorganisms under nutrient stress conditions using acetyl-CoA as an initial material. The stress conditions under which PHAs get accumulated are an excess of carbon source and the absence or limited supply of an essential nutrient such as oxygen, phosphorous, nitrogen or sulphur [11,14]. Their mechanical properties such as brittleness, stiffness, molecular weight and glass transition temperature are comparable to some of the conventional petrochemical-derived plastics[15]. They do not degrade under normal conditions of storage, and are stable in air [12]. These polymers are useful in areas such as pharmaceuticals, biomedical products, packaging and agriculture, and are also immunologically compatible with human tissue[16]. Some bacterial genera such as *Methylobacterium*, *Ralstonia*, *Pseudomonas* and *Bacillus*[14]are able to accumulate PHAs using glycerol as a substrate. In studies on *Paracoccus denitrificans* and *Cupriavidus necator* JMP134 it was shown that NaCl present in crude glycerol hindered the growth and PHA production of these organisms [14,17,18]. It was suggested that the harmful effects of NaCl could be decreased by mixing crude glycerol from different manufacturers, thus diluting the NaCl [17]. *Zobellella denitrificans* MW1 was able to produce PHB to high concentrations using crude glycerol as carbon source, especially in the presence of NaCl [19]. *Pseudomonas putida* was found to be able to accumulate medium chain length PHAs (mcl-PHAs, PHAs with monomeric repeat units of C6 to C14 carbons in length) using crude glycerol as the sole carbon source. The strain *P. putida* KT2440, grown on minimal medium (M9) supplemented with 30 g/L crude glycerol as the sole carbon source, was able to accumulate PHA to 34.5% of its cell dry weight (1.46 g/L) in batch culture. Other *P. putida* strains were not able to accumulate as much of the polymer as KT2440 (10% to 27% CDW)[20].Another Pseudomonad, *P. mediterranea* 9.1, was able to accumulate 1.63 g/L (60% CDW) PHAs on 2.5% (w/v) crude glycerol after 48 hours of cultivation, with 2.5 mM diammonium hydrogen phosphate and 0.1% meat or yeast extract as nitrogen source, at a pH of 6.9 and a temperature of 27°C[21]. Rosa Palmeri and her colleagues compared the PHA yields of *P. mediterranea* 9.1 with *P. corrugata* 388 and A1, and found that *P. mediterranea* 9.1 produced a maximum of 2.93 g PHA/L in 2% crude glycerol, while *P. corrugata* 388 and A1 produced their maxima at 1.28 g/L in 2% crude glycerol and 2 g/L in 5% crude glycerol, respectively, after 72 hours of cultivation[22]. Burkholderia *cepacia* is a common soil and water bacterium used at the College of Environmental Science and Forestry at the State University of New York (SUNY ESF) to produce PHAs from different substrates. The highest yields of PHAs were seen when *B. cepacia* was cultivated in 2.5% crude glycerol. At higher crude glycerol concentrations, growth was inhibited[23].

Hydrogen and Ethanol

Hydrogen has a very high energy content. Combustion of hydrogen produces only water as a by-product. Therefore, it can be considered as a promising clean fuel. Glycerol can be used as a substrate for hydrogen production by microorganisms. Crude glycerol has a higher energy content than purified glycerol, due to the presence of impurities like methanol and traces of biodiesel and fatty acids. Theoretically, 8mol of H_2 can be obtained per mol of glycerol. Soap and methanol were found to be the major inhibitors of microbial growth and hydrogen production[24].Methanol can be removed from crude glycerol by autoclaving 72, distillation or rotary evaporation, since its boiling point is 65℃ 7, Denver 2008. Soap can be removed by precipitation by adjusting the pH 7 and then centrifuging[25]. This process is advantageous as the pH used for precipitating soap out is conducive for hydrogen production by a wide range of microorganisms. Sodium can be removed by crystallization/precipitation of hydroxyapatite through the co-addition of lime and phosphoric acid 74. Alternatively, halotolerant organisms can be cultivated in glycerol containing NaCl, avoiding the need for removal of the salt.

Conversion of glycerol to hydrogen by microbial fermentation is accompanied by the production of other metabolites such as acetic acid, butyric acid, ethanol and butanol [24]. Co-culture with acetogonic bacteria can help to convert these end products to hydrogen 89. It was observed by Varrone and co-workers[26] that hydrogen and ethanol yields decreased with an increase in production of 1,3-propanediol. This is probably due to the fact that the pathway for 1,3-propanediol production is an alternative one to the ethanol-hydrogen production pathway.

Enterobacter

Enterobacter aerogenes is the most studied organism for hydrogen production from crude glycerol [27].Higher concentrations of glycerol and presence of NaCl in the glycerol decreased production of hydrogen and ethanol. The presence of methanol in crude glycerol did not inhibit growth and glycerol consumption rates of *E. aerogenes*. Added nitrogen and nutrient sources such as tryptone, yeast extract and other minerals helped to increase the growth rate and production of hydrogen and ethanol. Higher yields of ethanol and hydrogen were achieved at low glycerol concentrations of 1.7 g/L and 5 g/L. Increase in concentration of crude glycerol in the medium decreased hydrogen and ethanol production, as well as the production of the by-products acetate and formate. However, the production of other by-products like lactate and 1,3-propanediol increased with the glycerol concentration[28].Sarma and colleagues [24] found that soap and methanol present in crude glycerol had negative effects on the hydrogen productivity of *E. aerogenes*, while NaCl had a positive effect.

Clostridium

A community of bacteria dominated by species from the genus *Clostridium* was able to produce 1.1mol H2/mol glycerol or 15.2% of the total gases accumulated at optimal conditions of 40 ℃, 1 g/L substrate concentration and pH 6.5 after 48 hours. At the same conditions, only 2.4 mM of ethanol was obtained. However, 4.7 mM ethanol was produced at pH 6.5, 40 ℃ and 5 g/L substrate concentration after 48 hours [29].

Rhodopseudomonas

Rhodopseudomonas palustris is capable of producing hydrogen when grown on glycerol, but the presence of contaminants like saponifiable fatty acids (SFAs) was found to be inhibitory to growth and H2 production. Various methods for removal of these SFAs were studied, and it was found that treatment with activated carbon, pH reduction, calcium precipitation and solvent extraction combined with pH adjustment were effective.

Mixed Microbial Consortium

A microbial consortium enriched from activated sludge was able to utilize crude glycerol at an initial concentration of 15 g/L as the sole substrate for hydrogen production. This consortium was dominated by Enterobacteriaceae (90%), with the genera *Klebsiella* and *Escherichia*/*Shigella*, followed by Burkholderiaceae (10%), with the genus *Cupriavidus*, and was able to produce 2690 mL H_2/L/day, with a yield close to the stoichiometric yield. The average hydrogen content in the biogas at the end of fermentation reached 54.05% [26].

The hydrogen production rates can be increased by improving the retention of microorganisms in the fermentation vessel. The use of immobilized cells have many advantages like reusability, process stability, high tolerance to environmental interference and high cell retention[30].

Lipids and Polyunsaturated Fatty Acids

Microbial lipids are secondary metabolites produced under conditions of excess carbon source and a limited nutrient source such as nitrogen. Lipids are produced by all microorganisms for use in membranes and other cellular structures. Organisms which are capable of accumulating intracellular lipid bodies in quantities higher than 20% of their dry cell weight are known as oleaginous microorganisms [31].It was found that not all organisms are able to accumulate lipids to more than 20% of their dry cell weight. Yeast, fungi and a few bacteria were found to have the ability to accumulate extractable lipids, which have similar composition as plant oils [32]. Generally, bacteria are not lipid-accumulating organisms. They usually produce complex lipoids such as polyhydroxyalkanoic acids (PHAs) as a means of energy storage [33].

Microbial lipids containing high proportion of polyunsaturated fatty acids (PUFAs) have nutritional and pharmaceutical importance. These are called single cell oils (SCOs) and are produced by some prokaryotic and eukaryotic organisms. They are similar in composition to that of edible and non-edible oils from plant and animal sources [32]. Demand for PUFAs is constantly increasing while the sources producing them are not increasing at the same pace [32,34]. Current sources of essential fatty acids are some higher plants, animal entrails and oily fish. These sources have some disadvantages. Plants are unable to synthesize fatty acids with more than 18 carbons due to a lack of specialized enzymes [34]. Therefore, essential fatty acids like arachidonic acid cannot be obtained from plants. Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) which are omega-3 essential fatty acids are obtained from marine fish oils. Marine fish oils have objectionable tastes and odours and have high cholesterol content and toxic impurities which are difficult to remove. The production of PUFAs from plant and animal sources are generally low in content and vary with season, climate and geographical location. Therefore, the quantity and quality of PUFAs from conventional sources may not meet the growing demand [35,36,37].This led to a screening of microorganisms for lipids. Fungi belonging to the orders Mucorales and Mortierellales have been found to be promising for the production of gamma-Linolenic Acid (GLA), which has important pharmaceutical and nutritional properties for mammals[38,39] as it has selective anti-cancer properties and alleviates many diseases [32,40,41].

Microbial lipids also present an alternative feedstock for the production of biodiesel [42]. Bacteria, fungi and algae have been considered as alternate sources of Single Cell Oils (SCOs) for oleochemicals including biodiesel[43]. The use of microbial triacylglycerides (TAGs) for biodiesel production has many advantages over the use of plant oils, such as low soil consumption, less labour and lower land resources, easy scale up, short life cycle and low affection by venue, season and climate, leading to a more sustainable biodiesel production process [5,44]. The production process is currently not economically feasible due to the high cost of substrates. Therefore, the re-use of waste glycerol from the biodiesel industry as a substrate to produce microbial TAGs can reduce the manufacturing costs of biodiesel significantly[42]. From previous studies, it can be seen that oleaginous microorganisms are mainly bacteria, fungi, yeasts and microalgae, with lipid content in yeasts, fungi and microalgae being higher (70% to 90%) than in bacteria (20% to 50%) [6,45].

Physical Parameters

pH

In yeasts, it was found that the PUFAs content increased with increase in pH [32]. Lindberg and Molin [46]showed that the oleaginous mould *Mortierella alpina* achieved rapid growth and high lipid content at pH 5.5 and 6.5. *M. ramanniana var. ramanniana* showed a high GLA production at pH 5.0 in dextrose yeast extract broth [47]. In *Mucor* spp RRL001, maximum lipid production occurred at pH 6.5, while maximum GLA production occurred at pH 5.0[48].Li et al [49] reported that in *Mucor recurvus*, total lipids, PUFAs and GLA were highest at pH 6.0.

Temperature

Low fermentation temperatures positively influenced production of unsaturated fatty acids, as part of the adaptive response to cold environments [50,51,52]. In a study conducted by Hansson and Dostalek [53], it

was found that moulds of the genus *Mortierella* were able to produce fatty acids with a higher degree of unsaturation at lower temperatures (25 °C). It was also found that GLA content increased with decrease in temperature.Shimizu,*et al*[54] found that in *M. alpina*, the eicosapentaenoic acid (EPA) content decreased significantly above 12 °C, and production of EPA stopped at 20 °C, while the optimum temperature for production of arachidonic acid (AA) was found to be 28 °C. This study concluded that EPA production increased with decreasing temperature while AA content decreased with decreasing temperature. Mamatha [32] conducted a study in which *Mucor rouxii* was able to produce GLA optimally at a pH of 5.5 and a temperature of 28 °C for biomass build-up and 15 °C for GLA accumulation.

Nutrient Sources

Lipids are produced by oleaginous microorganisms as a secondary metabolite under conditions of nutrient stress, that is, excess carbon source and limited source of a nutrient such as nitrogen [31]. Commercial microbial oil production requires the use of nutrients such as yeast extract which are expensive and add to the high cost of production. Kiran and his colleagues replaced yeast extract with hydrolysates of whole rapeseed meal as nitrogen-rich nutrient, mixed with crude glycerol as carbon sourcein fed-batch fermentations by *R. toruloides*. *Aspergillus oryzae* was used to break down proteins in the rapeseed meal into peptides and amino acids in order to make the nitrogen source more accessible to the oleaginous *R. toruloides*. Kiran and his co-workers reported that with medium containing yeast extract and 50 g/L crude glycerol in batch fermentations, *R. toruloides* gave 6.1 g lipid/L in 72 hours, while with the rapeseed hydrolysate as nitrogen source, 9.5 g lipid/L was obtained in the same time. In fed-batch fermentation with glycerol concentration at 50 g/L of medium, yeast extract as nitrogen source gave 9.4 g lipid/L while medium with rapeseed hydrolysate gave 13 g lipid/L after 120 hours of fermentation. The FAMEs produced in this study had a high degree of unsaturation, confirming that the fatty acids produced by this medium had the potential to be used in biodiesel production[55]. A similar study employing the hydrolysate of whole sunflower meal (SFM) and fractionated sunflower meal (pre-treated sunflower meal, PSFM) as nutrient source with crude glycerol for microbial oil production by *R. toruloides*, *L. starkeyi* and *C. curvatus*was performed by Leiva-Candia and colleagues[31]. The hydrolysates were prepared using *A. oryzae* in Solid Substrate Fermentation (SSF). PSFM was produced by fractionation into lignocellulosic fraction, protein-rich fraction and intermediate liquid fraction. The lignocellulosic fraction was used as a substrate in SSF and the protein fraction was treated to isolate the proteins. The residue from both these treatments was mixed with the intermediate liquid fraction to give PSFM. *R. toruloides* produced highest microbial oil yield of 9.4 g/L with PSFM hydrolysate and 100 g/L initial glycerol concentration in the fermentation medium.*C. curvatus* gave its highest yield of 9.5 g/L microbial oil with SFM hydrolysate and 100 g/L initial glycerol concentration. *L. starkeyi* produced its highest yield at 5.1 g/L of microbial oil with SFM hydrolysate and 100 g/L initial glycerol concentration.

Organisms

Fungi and Yeasts

Lipids from yeasts such as *Yarrowia lipolytica*, *Rhodosporidium toruloides*, *Cryptococcus curvatus*, *Lipomyces strarkeyi* and *Rhodotorula glutinis* have been used in biodiesel production at laboratory scale. The production has been performed in a three-step process involving drying of the biomass, extraction of lipids and transesterification to FAAEs. A few researchers have performed direct transesterifications without extracting the lipids which may help in reducing the cost of the process by eliminating the drying and extraction steps [8,56].

Liu and Zhao [8] optimized the direct transesterification of the oleaginous yeast *Lipomyces starkeyi* using acid catalysts under different conditions. At a temperature of 70 °C and a biomass to methanol ratio of 1:20, they found that with 0.4 mol/L of H_2SO_4 as catalyst, a yield of 96% FAME was achieved. However, black precipitates were observed at or above this concentration. With 0.6 mol/L of HCl, a yield of 90% was obtained, but the excess acid created harsh conditions due to which side reactions such as acid-promoted polymerization of unsaturated fatty acids occurred. When studying the time course of direct methanolysis, they found that both 0.2 mol/L H_2SO_4 and 0.4 mol/L HCl gave similar yields of around 90% at around 24 hours of reaction time at 70 °C. During temperature optimization and methanol loading studies, they found that 0.2 mol/L H_2SO_4 gave a yield of 96.8% at 70 °C with the optimal biomass to methanol ratio being 1:20. They further applied the optimized conditions of transesterification reaction on *Rhodosporidium toruloides* (yeast) and *Mortierella*

isabellina (filamentous fungus), and found that the yields of FAME from both were around 90%, indicating that direct transesterification is a robust procedure which can be applied to many microorganisms.

Thliveros and his co-workers [57] investigated the yield of FAMEs with base-catalysed direct transesterification reaction of *Rhodosporidium toruloides* Y4. They found that the optimum reaction conditions were 50 °C temperature, with 4 g/L NaOH as catalyst at a biomass to methanol ratio of 1:20 (w/v) for 10 hours, giving a yield of 97.7%. On comparing with the optimized acid-catalysed reaction, they found that the base-catalysed reaction used approximately 10 times less catalyst (4 g/L NaOH instead of 39.2 g/L H_2SO_4), at a temperature 20 °C lower than the acid-catalysed reaction (50 °C instead of 70 °C) in a 20 hour less reaction time (10 hours instead of 30 hours), and gave a slightly higher yield (97.7% instead of 95.0%).

Cheirsilp and Louhasakul [56] studied the effects of three different transesterification procedures on the yield of biodiesel from*Yarrowia lipolytica*. The first procedure involving extraction of lipids and transesterification produced highest yield of 72% within 3 hours of reaction time with a molar ratio of methanol to oil of 84:1. The second procedure involving direct transesterification of dried biomass without extraction of lipids yielded 65 – 69% FAME within 1 hour reaction time with a methanol molar ration of 167:1 and 209:1. The third procedure which involved direct transesterification of the wet biomass gave the highest yield of 72% in the shortest reaction time of 1 hour with a methanol molar ratio of 209:1 and 73% in 6 hours with a methanol to molar ratio of 167:1. The direct transesterification methods reduced the number of steps and reaction times, but required a higher volume of methanol. The advantage is that the removal of the extraction step eliminated the use of solvents like chloroform and the evaporation step for removal of the solvents from the extracted lipid. It is not that expensive to use a larger volume of methanol as the unreacted methanol can be recovered and reused. Therefore, the use of higher amount of methanol would be preferable economically than the use of the conventional three-step process which uses more solvents and takes a longer time.

Emission studies performed by Wahlen and his colleagues [58] on the biodiesel obtained from the yeast *Cryptococcus curvatus*, which contained mainly C16 and C18 monounsaturated fatty acids, was found to give the least CO emissions (0.048% ± 0.004 of total gas emissions) as compared to the other sources of biodiesel used in the study. Further, the high amount of oleic acid in the oil of yeast should be advantageous for oxidative stability without greatly affecting the cold flow properties.

Algae

Some strains of microalgae are capable of accumulating TAGs amounting to 20 to 50% of their total dry weight [59]. Recent research has shown that crude glycerol can be fermented by the alga *Schizochytrium limacinum* to produce docosahexaenoic acid (DHA) [25], which is an important omega-3 PUFA that has been shown to have many beneficial health effects. The major commercial source of omega-3 PUFAs is fish oil, which faces challenges such as bad odour and taste, heavy metal contamination and limited supply (Barclay, Meager, & Abril, 1994). There has been an increase in the price of fish oil due to low supply and high demand. Therefore, developing DHA-containing microalgae is an excellent opportunity to provide alternative omega-3 sources [60].In an emission study on biodiesel from various sources conducted by Wahlen and his co-workers [58], it was reported that the biodiesel obtained from the microalga*Chaetoceros gracilis* contained a substantial amount of palmitoleic methyl ester (C16:1) and myristic methyl ester (C14:0), which are rarely found in plant oils. The presence of palmitoleic acid in biodiesel fuels has been shown to be beneficial by improving oxidative stability without sacrificing cold flow performance, and short chain fatty acids like myristic acid improve the NOx emissions of the biodiesel. The microalgal biodiesel produced 24% lower NOx emissions than the petroleum diesel used as a standard in the study, which was a surprising result since biodiesel is usually associated with higher NOx emissions as compared to petroleum diesel. Lipid production by different cultivation processes using glycerol and glucose as substrates individually or mixed was studied in *Chlorella vulgaris* [61]. They found that batch cultures with 10 g/L glycerol as sole carbon sourceyielded a higher amount of lipids and had a higher lipid content (55.31 ± 3.03 mg/g biomass and 26.90 ± 0.21%, respectively) than cultures with 10 g/L glucose as sole carbon source (32.48 ± 2.90 mg/g biomass and 18.88 ± 0.36%, respectively). In two-stage batch culture when 10 g/L glucose was followed by addition of 10 g/L glycerol in the medium, the yield of lipids and lipid content were 38.69 ± 1.47 mg/g of biomass and 24.37 ± 0.81%, respectively. In the two-stage fed-batch culture A, 10 g/L glucose in the medium as batch culture was followed by a fed-batch culture with addition of 1 g/L of either glycerol every 10 hours. The lipid yield was 36.98 ± 2.43 mg/g of biomass and the lipid content was 30.56 ± 0.67%.In the two-stage fed-batch culture B, a batch culture

with 10 g/L glucose was followed by the fed-batch stage with additions of 1 g/L glycerol every 4 hours. This method gave a better yield of 59.14 ± 1.11 mg/g of biomass than the type A culture method, and gave a lipid content of 30.01 ± 0.20%. When crude glycerol was used instead of pure glycerol in the fed-batch experiments, the yield and lipid content were found to be higher than their corresponding experiments with pure glycerol, at 44.15 ± 3.11 mg/g of biomass and 31.04 ± 0.34%, respectively in the type A method and 84.14 ± 2.03 mg/g of biomass and 36.39 ± 0.17%, respectively in the type B method.

Bacteria

Bacteria belonging to the actinomycetes group, such as *Streptomyces*, *Nocardia*, *Rhodococcus*, *Mycobacterium*, *Dietzia* or *Gordonia* are capable of accumulating lipids and TAGs under nitrogen-limited conditions [62,63].*Bacillus subtilis* and *Pseudomonas* spp have also been shown to be able to accumulate microbial lipids (Patnayak & Sree, 2005).*Rhodococcus opacus* strain PD630 grown on sugarcane molasses medium was able to accumulate intracellular lipids to 93% of their cell dry weight. Of these lipid bodies, TAGs made up 50% (Gouda, Omar, & Aouad, 2008). In a study conducted by Sriwongchai *et a.l,* [63] maximum growth (3.17 g/L) of *Rhodococcus erythropolis* was observed when the glycerol concentration in the medium was 30 g/L, with 1 g/L ammonium nitrate as nitrogen source. At this concentration, 1.51 g/L, which made up 47.72% of the total dry biomass, was produced. Increase in glycerol concentration led to a decrease in biomass and lipid content. It was suggested that increased glycerol concentrations led to an increase in osmotic stress on the cells, thus decreasing the growth rate. Using urea as a nitrogen source led to a higher substrate consumption and increased production of biomass (3.77 g/L). The highest amount of cellular lipid (1.87 g/L, lipid content 46.97% of total dry biomass) was accumulated when urea was added to the medium at a concentration of 0.75 g/L. The optimum pH and incubation time were 7.4 and 96 hours, respectively. The predominant fatty acids seemed to be C16:0, C18:1, C16:1 and C14:1.

Conclusion

From the foregoing the following conclusions are made:

1. Even though glycerol can be produced through routes (saponification, hydrolysis, esterification and the transesterification), but its large production is currently achieved through the transesterification process.
2. The waste by-product derived from transesterification process can be converted into biodiesel using biological conversion process.
3. It was reported that the present glycerol market is low due its abundance and presence of impurities in the crude glycerol.
4. To make it of commercial grade, it should be treated and refined through filtration, chemical additions, and fractional vacuum distillation.
5. Finally, the achievement of high-grade glycerol products will help in raising the standards for biodiesel activities.

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