Glycerol waste to value added products and its potential applications

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Abstract
The rapid industrial and economic development runs on fossil fuel and other energy sources. Limited oil reserves, environmental issues, and high transportation costs lead towards carbon unbiased renewable and sustainable fuel. Compared to other carbon-based fuels, biodiesel is attracted worldwide as a biofuel for the reduction of global dependence on fossil fuels and the greenhouse effect. During biodiesel production, approximately 10% of glycerol is formed in the transesterification process in a biodiesel plant. The ditching of crude glycerol is important as it contains salt, free fatty acids, and methanol that cause contamination of soil and creates environmental challenges for researchers. However, the excessive cost of crude glycerol refining and market capacity encourage the biodiesel industries for developing a new idea for utilising and produced extra sources of income and treat biodiesel waste. This review focuses on the significance of crude glycerol in the value-added utilisation and conversion to bioethanol by a fermentation process and describes the opportunities of glycerol in various applications.

Graphic abstract

Keywords Crude glycerol · Bioethanol · Biofuel · Valuable products

Introduction
As fossil fuels worldwide are becoming increasingly concerned, the need for renewable energy sources has become more urgent than ever due to the increase in energy demand, lack of global oil supply, and changes in the climate [1]. Energy sources are rationed worldwide. There are no energy shortages in developed countries whereas developing countries like Malaysia, China, India, Thailand, etc. need more
energy to boost development programs. However, most nations either developed or developing countries are trying to promote the use of renewables to meet their rising energy demand and to decrease import fuel dependence and partly to seek agreement to minimise greenhouse gas emissions (GHGs) [2]. According to the International energy outlook (IEO), at present, the most used source of energy is liquid fuels which are approximately 205 quadrillions Btu whereas renewables are 120 quadrillions Btu and are expected to use renewables more than petroleum worldwide till 2050 as shown in Fig. 1 [3].

The EIA (energy information administration) issued an annual energy outlook of 2021 and forecasted the biofuel usage will steadily increase by 2050. Although the COVID-19 pandemic affected the market for all liquid fuels last year, the EIA states that biofuel consumption has not fallen as much as petrol-based fuels. EIA plans to raise and steadily expand the percentage of biofuel mixed with the US transportation fuel pool by 2050. The production of biodiesel in the reference case is expected to increase slightly, maintaining a steady supply level by 2050. The output of renewable diesel is expected to rise at a higher pace. In the later years of the prediction era, ethanol consumption is projected to return to pre-COVID levels, which will continue to increase until 2050 because the higher ethanol blends are being introduced into the road transport fuel EIA. Biodiesel production is bound to grow by 0.5% by 2050, reaching 130,000 barrels a day.

Many researchers focused on the expansion of biodiesel due to eco-friendly fuel. The use of biodiesel as an alternative fuel to Petro diesel is seen as a significant transitional strategy in the quest for new sources of fuel [4]. Biodiesel is commonly used as a fuel substitute, is made from a range of renewable feedstocks, including plant oils, animal fats, and reused oil [5]. Particularly the biodiesel produced from plant oil from babassu, canola, castor bean, crambe, jatropha, lupine, palm oil, seed radish, peanut, sunflowers, soybeans, peanut, and macauba. It is also formed from cooking oil waste and photosynthetic algae, respectively [6]. Animal fats and plant oils containing fatty acids are transformed into fatty acid methyl or ethyl esters in a transesterification reaction during the biodiesel production process.

The primary byproduct in biodiesel processing is larger quantities of crude glycerol, representing approximately 10% of total biodiesel [7]. About 10 lb of biodiesel produces 1.10 lb of glycerol [8]. The rise in the price of biodiesel is increasing from 0.015£/liter and the price decrease by 0.079£/Lb. in the sale of glycerol. With the growth of biodiesel plants, increased quantities of rough glycerol are produced, which has affected the market price of glycerol and the economy of biodiesel production [9]. To mitigate this effect, a range of approaches of crude glycerol like direct burning as heating oil, purification for commercial glycerol sale, hydrogen reforming, and microbial conversion into valuable products [10]. The first three solutions that use further energy are less profitable, require off-gas processing for direct combustion, and control toxic gas emissions [11].

Further purification procedures to eliminate impurities such as water, methanol, soap, and fat are necessary to increase glycerol concentration by over 80% before marketing with low-value crude glycerol, ranging from 35 to 50%. Not only has this surplus crude glycerol significantly affected consumer prices, but it also has caused environmental problems because treatment is needed before discharge into the atmosphere [12].

Crude glycerol upgrading to valuable products impairs a significant impact on the biodiesel economy [13]. It also falls under the fourth-generation biofuel approach to produce minimal waste [14]. Clearing raw glycerol is a tedious approach, and thus the use of raw glycerol is intact as a source for any industrial product [15]. The study of new applications for crude glycerol is necessary to reduce the projected surplus supply. Therefore, various outlets were tested for the disposal and use of crude glycerol. Major biodiesel producers have upgraded crude glycerol to pure glycerol through expensive purification processes [16]. However, alternatives to use crude glycerol as the main feedstock to generate value for money products employing chemical conversion and thermochemical/biological conversion are promising for affordable output [17]. Biological methods

Fig. 1 Most used energy source by 2050
for converting crude glycerol into various valuable chemicals and fuels like 1, 3-propanediol, hydrogen, citric acid, dihydroxyacetone, and ethanol were considerably developed [18]. This review focuses on the industrial byproduct of crude glycerol waste from the biodiesel industry. The crude glycerol bioconversion into ethanol is described and valuable products from glycerol with various applications are reviewed.

**Glycerol production from the biodiesel industry**

In conjunction with the efforts to ensure a sustainable expansion of the biodiesel industry, a necessary policy for renewable diesel could be built to cover the mandates, tax incentives, and subsidiaries in the future. This campaign widely opened biodiesel’s prospects to globally replace fossil fuels as the world’s leading energy source.

Biodiesel is produced from the following methods like blending, micro emulsification, pyrolysis, and transesterification process as shown in Fig. 2. In the blending process, the ratio of 10–40% (w/w) preheated vegetable/animal oils were blended with petro-diesel. The resulting oil-diesel mixture is used in the diesel engine. The advantage of this process does not require chemicals and no requirement for engine modifications. Micro emulsification is a pollution-free and simple process in which the vegetable or animal oil is solubilised in solvent (alcohol) and surfactant until required viscosity was obtained [19].

In the pyrolysis process, vegetable/animal oils were preheated and broken down at high temperatures above 350 °C in the presence or absence of the catalyst. Various products, including gas and liquid, were analysed to determine the exact product according to their boiling temperature range. The process is a practical, waste-free, and simple process with no washing, drying, and filtering [4]. The commonly used method is transesterification and as shown in Fig. 3. Transesterification is one of the most economic processes with high biodiesel yield and therefore the most adaptive method for commercial production of biodiesel. The vegetable/animal oils and fats were reacted with alcohol and alkali or acid catalyst, then the mixture of methyl/ethyl esters (biodiesel) and glycerol by-product undergo separation and purification steps before further usage. The biodiesel production approaches with pros and cons are summarised in Table 1 [13].

Biodiesel is essentially derived from the triglyceride transesterification process in which 90 wt percent of methyl ester (biodiesel) and 10wt percent of glycerol are produced [20]. The selection of catalysts depends on the fatty acid content of oil. Alkali catalysts improve the process to achieve a higher biodiesel yield [21]. Alkaline catalysed transesterification has several advantages like a fast reaction rate, which requires less time, and an easy setup than acid catalysts, [22].

Glycerol is traditionally obtained from four different processes, i.e. soap production, fatty acids production, fatty esters production, and microbial fermentation. For over a century, the reactions of vegetable oils and animal fats directly transformed into glycerol and methyl esters have been identified. Transesterification of triglycerides like rape-seed, palm, soybean, and sunflower oils has become important for manufacturing biodiesel fuel of high quality. The key focus of development is currently on glycerol byproducts from the biodiesel industry. Typically, three mol of methanol reacts to the formation of methyl esters and glycerol in three steps with the presence of a catalyst. In the first step, methanol reacts with triglycerides to form diglycerides and methyl esters. Later, methanol reacts again with diglycerides to monoglycerides and methyl ester. These monoglycerides react with methanol again and eventually form glycerol and methyl esters [23, 24]. According to Alexandre et al., the schematic summary of the generation of glycerol and its alternative route is shown in Fig. 4 [17]. Glycerol is refined from unrefined glycerol is an expensive process is used in the chemical, textile, pharmaceutical, and food industries. An alternative application of unrefined glycerol for fuel additives, development of fuel cells, to produce hydrogen, methanol, and ethanol, co-digestion and co-gasification, and for the waste treatment.
In literature, the expressions of glycerol, glycerine, glycerin, and 1,2,3-propanetriol are preferred to use. The word glycerol usually refers to the pure substance. Though glycerine refers to commercial products containing more than 95% glycerol solutions in water. These products vary in glycerol content, colour, odour, and impurity traces. Crude-glycerol contains catalysts, dissolved salts, fatty acids, water, and 70–80% glycerol in the biodiesel production process [25]. Hydrolysis, saponification, and the byproduct of transesterification reaction of animal fats and vegetable oils are the most popular routes for glycerol synthesis [26]. At the same time, other methods such as the fermentation process and carbohydrate hydrogenolysis are non-industrial. The alkyl esters were obtained from the transesterification of animal fats and vegetable oils with alcohol. Primarily the simple catalysts are the key route in the biodiesel industry as a byproduct single glycerol molecule is produced for each of three methyl or ethyl ester molecules that form the basis of biodiesel [27].

According to SDA’s glycerine and oleochemical Division, over 1500 glycerol applications include hundreds of food and foam categories, as well as cosmetics and pharmaceutical [28]. The vast array of applications reflects the broad range of chemical reactions of glycerol for synthetic precursors to obtain more valuable products with insertion choice in various fields is described in Fig. 5. which illustrates the main chemical reactions of glycerol transformation. Crude glycerol found from biodiesel synthesis contains alcohol (particularly methanol), catalysts, free fatty acids, mono, di- and triacylglycerols, salts, and water contents which differ
with the usage of raw material, catalytic process, and the stages of biodiesel preparation and purification [29].

A significant characteristic feature of the glycerol chain link with the chain of the biodiesel industry. The increase in biodiesel manufacture resulted in substantial surpluses of glycerol, which have a negative impact on its marketplace. The market value for crude glycerol from 0.065£ and 0.072£/Lb. However, the price of pure glycerol is between 0.22 and 0.37£/Lb. Approximate crude glycerol production will reach 50 billion liters in 2021 [30].

The biodiesel industry produces energy and sells the commodity that lacks the treatment with crude glycerol. Crude glycerol by combustion and uncontrolled burning leads to severe risks to human health due to the formation of unsaturated aldehyde [31].

Pure glycerol found on the market is refined and includes less than 5% impurities. This is not the case with crude glycerol produced from the production of biodiesel. Crude glycerol is either produced by oil saponification (16% impurities), hydrolysis (10–12% impurities), and transesterification (25–30% impurities) [32]. The market for glycerol previously had a strong connection with demand patterns in the medicinal, food products, cosmetics, polymer, and other chemical industries. The applications of glycerol in drugs and pharmaceuticals for the treatment of wounds, skin burns, and skin grafts, also acts as holding agents for tablets and in cough syrups, glycerol is utilised in the blood bank to maintain blood cells from freezing, personal care products like moisturiser cream, cosmetics for the skin, mouth wash, and soap. Glycerol is used to preserve food, sweetener and acts as thickening agents in distilled spirits. The various application of glycerol is shown in Fig. 6 [6].

Pinheiro categorised the emergence of two consumer markets for glycerol. First, is the existing market demanded a higher purity product by market standard requirements primarily as additives for food, pharmaceuticals, and cosmetics. Second the use of biodiesel crude glycerol for the transformation of products or chemical intermediates. This market is characterised by higher product volume demand, lower quality standards, and lesser costs [6].

Biodiesel industries concentrate on the technical challenges of biodiesel manufacture, storage, and treatment of crude glycerol waste. This implies enterprise strategies creating innovative claims to enable the extra value and the exploration of new markets. This fact does not support technical advancement concerning the byproduct of crude glycerol by biodiesel production firms. However, there is a trend for non-biodiesel companies to seek technical solutions in the utilisation of crude glycerol for other valuable products [33].

Glycerin is available for various applications depends on chemical and physical characteristics like less volatile nature, hygroscopic, elastic, softness and flexibility, solvent strength, solubility, high miscibility, materials compatibility, stability, high viscosity, antifreeze property, no toxicity, and emollient quality. The application of crude glycerol is restricted due to its purity that affects physical, chemical, and biological properties lowering the metabolite concentration [34, 35]. The crude glycerol has impurities in the form of inorganic salts. The higher levels of Na and K in
the diet result in electrolyte imbalance in animals. As the unregulated glycerol product from biodiesel affects animal metabolism. Methanol is one of the reactants in biodiesel production. The higher levels above 150 ppm are unsafe for animal feed causing blindness by destruction of optical nerve [36].

**Metabolic pathway of glycerol**

Since crude glycerol is polluted with many biodiesel chemicals, purification is not economically advantageous. Auto-thermal reform of crude glycerol produces hydrogen, but greenhouse gas emissions are a matter of concern. Microbial fermentation is thus defined as an option that can benefit the metabolic pathways leading to the desired products (biohydrogen and bio alcohol), thus minimising the formation of other side products by using ideal conditions and proper micro-organisms [37]. Several microbes can aerobically metabolise glycerol and few microbes can anaerobically metabolise. Crude glycerol can be converted into value-added products by *Escherichia coli*, Klebsiella, Enterobacter, Glucanobacter, Clostridium, Candida, Aspergillus is shown in Table 2.

Glycerol has a higher rate of reduction than sugars and is thus cheaper and more readily available. The almost exclusive synthesis of reduced products during glycerol fermentation represents a very reducible glycerol level compared to glucose fermentation. Conversion of glycerol to phosphoenolpyruvate or pyruvate is higher than glucose. The fermentation of glycerol produced ethanol and formic acid (or ethanol and hydrogen), for example, generated twice as much in total as glucose fermentation as half of the glucose was lost as carbon dioxide in glucose conversion [38]. Furthermore, the use of crude glycerol often alleviates the glucose effect of carbon catabolic repression in the case of glucose use. For carbon catabolite repression, a fast-metabolising source of carbon like glucose curbs the gene expression that encodes protein needed to use other sources of carbon like glycerol and lactose.

Glycerol is a small, uncharged symmetrical molecule. Development of a small number of metabolic routes for channelling by using glycerol as a terminal acceptor of electrons anaerobically to the central carbon for biomass and energy production. The first step in all pathways is the transportation of glycerol into the cell. Depending on the micro-organism, different modes of transport are used. Bacteria primarily use facilitated diffusion in the cell to glycerol network. The facilitator proteins are primarily simple permeases. For eukaryotes including yeasts *S. cerevisiae* and *Y. lipolytica*, glycerol is mainly taken up with Glycerol/H+ antiporters via an active transport method.

Microbes utilise two key metabolic paths in which glycerol is converted to the necessary glycolytic mediates for cell
development [39] is shown in Fig. 7. In the aerobic route with the presence of oxygen, glycerol is converted to G3P (glycerol-3-phosphate) with a glycerol kinase. In the next process, DHAP (dihydroxyacetone phosphate) undergoes oxidation to create one mole of NADH through a NAD+ based G3P dehydrogenase [40].

Glycerol oxidation to dihydroxyacetone (DHA) producing a single mole of NADH is the first stage of microaerophilic

Table 2: Crude glycerol conversion by a micro-organism into valuable products

| Micro-organism | Type of strain | Product formed | Yield or concentration | Productivity (g/L/h) | References |
|---------------|----------------|----------------|------------------------|----------------------|------------|
| Citrobacter   | C. freundii FMCC-B294 1,3 propanediol 0.48 mol/mol 0.79 g/L/h | [41] |
|               | C. werkmonii DSM 17,579 0.62 mol/mol 2.84 g/L/h | [50] |
|               | C. freundii H3 Hydrogen 0.94 mol/mol – | [51] |
| Clostridium   | C. pasteurianum (immobilised) n-butanol 0.43 mol/mol 0.074 g/L/h | [52] |
|               | Clostridium CT7 11.8 g/L – | [53] |
|               | C. butyricum AKR102a 1,3 propanediol 0.63 mol/mol 3.3 g/L/h | [15] |
|               | C. butyricum VPI 3266 0.65 mol/mol 10.3 g/L/h | [54] |
|               | Engineered C. acetobutylicum 0.66 mol/mol 3 g/L/h | [55] |
|               | C. butyricum (DL07) 94.2 g/L 3.04 g/(L h) | [56] |
| Enterobacter  | Enterobacter aerogenes TISTR 1468 Ethanol 0.59 mol/mol – | [57] |
|               | Enterobacter MT491125 1,3 propanediol & 2,3-butanediol 0.70 g/ml & 0.88 g/ml – | [58] |
|               | Enterobacter sp. LU1 Succinic acid 0.38 ± 0.21 mol/mol (Micro-aerobic) 0.58 ± 0.11 mol/mol (Anaerobic) | [59] |
|               | E. coli Engineered E. coli Sy03 Ethanol 1 mol/mol 0.051 g/L/h | [60] |
|               | E. coli AC521 Lactic acid 0.9 mol/mol 0.97 g/L/h | [61] |
|               | Engineered E. coli Succinate 0.8 mol/mol – | [62] |
|               | Engineered E. coli Succinate 0.93 mol/mol – | [63] |
|               | E. coli YY-GS011 Succinate 0.92 mol/mol – | [64] |
|               | Engineered Escherichia coli butanol 6.9 g/L 0.18 g/L/h | [65] |
| Klebsiella    | Engineered K. pneumonia Ethanol 0.89 mol/mol 1.2 g/L/h | [66] |
|               | K. pneumonia encapsulated 1,3 propanediol 0.65 mol/mol batch 4.46 g/L/h | [67] |
|               | K. oxytoca lactate deficient 0.41–0.53 mol/mol 0.63–0.83 g/L/h | [68, 69] |
|               | K. pneumonia inactivated ADH 0.70 mol/mol 1.07 g/L/h | [70] |
| Lactobacillus | L. acidipropionici Propionic acid 0.88 mol/mol 0.085 g/L/h | [73] |
| Mixed culture | R. palustris CGA009 Hydrogen 6 mol/mol – | [74] |
|               | P. acerans Hydrogen 0.801 mol/mol | [75] |
| Fungi         | L. edodes Single cell oil Oxalic acid 0.1 g/g | [76] |
|               | A. niger Single cell oil 0.411 g/g – | [77] |
|               | Thamnidium elegans Single cell oil (SCO) – | – | [78] |
| Yeast         | Engineered S. cerevisiae Ethanol – | – | [79] |
|               | Y. lipolytica wrastilavua AWG7 Citric acid 0.33 mol/mol 1.16 g/L/h | [80] |
|               | Yarrowia lipolytica Succinic acid 0.45 mol/mol 1.45 g/L/h | [81] |
|               | Cryptococcus curvatus Single cell oil 52% lipid – | [82] |
|               | Rhodotorula glutinis 36.5% lipid – | [83] |
| Microalgae    | S. limacinum SR21 DHA – 0.52 g/L/h | [84] |
or anaerobic conditions, tailed from dihydroxyacetone to dihydroxyacetone phosphate using dihydroxyacetone kinase.

Glycerol oxidation is paired with a reductive pathway under low oxygen conditions via glycerol as a concluding electron receiver. These paths are coded in a single regulon called DHA regulon (dihydroxyacetone regulon). In particular, glycerol converted to 1,3-propanediol, the electrons from oxidation then transferred to reduction as NADH [41]. The route of reduction includes an enzymatic activity. The initial process is glycerol dehydration with a glycerol dehydratase to form hydroxy propionaldehyde (3-HPA). The next step is to reduce 3-HPA to 1,3-PDO with 1,3 propanediol oxidoreductase. Dehydratase glycerol is a complicated enzyme divided into two subcategories depending on the enzyme reactivation [42].

Adenosyl radical is the fundamental catalytic principle in both subcategories. The most important thing to prevent an unwanted reaction in which the radical is lost in the enzyme activity. Both subclasses vary with the radical adenosyl created. The initial category of glycerol dehydrates employs reactivation; vitamin B12 relies on the process. The enzyme is arranged like a heterotrimer dimer and genes for the complex reactivation with dehydrated glycerol in the DHA operon [43].

The three GldA, GldB, and GldC genes encrypt the individual subunits of the enzyme, and two additional GrdB and GrdA genes encode the factor for reactivation. This subclass is tolerant to oxygen. The enzyme inactivated by an excess of glycerol includes in the community of species *Lactobacillus, Clostridium, Klebsiella*, and *Citrobacter* [44].

The second subgroup of glycerol dehydrates utilises an independent re/activation mechanism of vitamin B12. They consist of two subunits that create a homo-dimer. Both proteins are encoded again in the DHA operon with genes (DhaB1 and DhaB2). This group of glycerol dehydrates oxygen-sensitive and glycerol-inactivated suicidally [45]. Until now, only a single strain was shown to have glycerol dehydrates. However, genome analyses for another microorganism that ferments glycerol showed that independent glycerol dehydratase contains vitamin B12 like *C. difficile, C. botulinum*, and other *Clostridium* species [44].
The sequence review of knowledgeable glycerol dehydratases based on vitamin B12 showed that both groups of glycerols were not homogeneous and formed from separate predecessors. Interestingly, some species also comprise all subclasses of glycerol dehydratases [46].

Another study of 2000 prokaryotic genomes has shown a very unusual distribution of DHA regulon. Around 111 of the prokaryotic genomes have a minor portion of the DHA regulon and various taxonomic phyla. For instance, actinobacteria, firmicutes (Clostridia, Lactobacillies), or Proteobacteria). Only the whole DHA regulon, such as Klebsiella spp was present in a few bacterial genomes [47]. The genomic study also indicates that gene distribution appears to be unequal for the oxidative and reductive branches. The reduction pathway genes are present in a more significant number of microbes, while the oxidation pathway gene distribution has been more restricted [48].

Yarrowia lipolytica is a micro-organism that channels glycerol into central carbon metabolism by an aerobic route through glycerol’s phosphorylation, followed by oxidation into dihydroxyacetone phosphate shown in Fig. 3. Many yeasts exhibit an aerobic pathway to catabolise glycerol. Other carbon and electron flows are affected by eco-friendly considerations (nutrient restriction, pH, temperature, dissolved oxygen) but also by strain sources and their sources of isolation [49].

Glycerol into value-added products

In the last decades, many researchers and industrialists found the valuable conversion of crude glycerol through chemical routes shown by publications and a patent filed in many countries. Industrialists create a supply chain by the purification process of the glycerol, originally from a residue of the biodiesel. However, many manufacturers converted into valuable products from these raw materials and gaining high value in different sectors. The crude glycerol is converted into value-added products like propionic acid, single cell oil, citric acid, ethanol, polyunsaturated fatty acid (DHA, EPA), biopolymers like PHA, and PHB with applications are described below [85].

Ethanol

Ethanol is one of the most significant organic substances used in consumer and industrial products. The primary industrial uses of this aliphatic alcohol are intermediate in other chemicals and solvent production [86]. In medicines, plastics, lacquers, polishes, plasticisers, and cosmetics, ethanol is used [87]. Ethanol is a topical anti-infective in medicine and an antidote for ethylene glycol or overdose of methanol. Ethanol-containing commercial products include drinks, fragrances, aftershaves and colognes, medicinal products, mouth washing, liniments, and some rubbing alcohols. It is the prominent alcohol formed in the fermentation process. It is used as a solvent, aerosols, paints, detergents, and thermometers [88].

Ethanol plays a vital role in fuel application due to its clean-burning features and lowers greenhouse gas emissions, primarily carbon dioxide [89]. Also, the crude agro-industrial residues are used as raw materials for alcoholic fermentation and an alternative substrate to solve the disposal issue [90]. However, ethanol with high solubility with water, biodegradability, and low toxicity. Ethanol as fuel is used as a blend with gasoline with 5–85%. E85 is the most standard blend with 85% bioethanol and 15% gasoline. The other blends like E20 possess 20% bioethanol and 80% gasoline, E10 contains 10% bioethanol and 90% gasoline commonly known as gasohol in the USA [91]. Whereas in Brazil, gasohol is the mixture with 24% of bioethanol and 76% gasoline. The higher concentrations of bioethanol are used in flexible fuel vehicles (FFV) that can function with the mixtures up to 85% bioethanol [92]. Bioethanol is converted into ethyl tertiary butyl ether that can be used as a 15% blend with gasoline [93]. The potential bioethanol production is an important bioprocess in the industrial sector.

Ethanol blends are influenced by various factors like climate, geographic location, and government policies. In general, about 10% anhydrous ethanol applicable in conventional combustion engines, while blends to 100% utilised in Flexi-fuel engines. Apart from using the spark-ignition engine, ethanol is also used in diesel engines as a transportation fuel because of its enhanced efficiency with near-zero particulate emission. The ED95 fuel comprises 95% v/v ethanol used in diesel engines in buses and trucks with 43% efficiency. Ethanol utilised in vehicles with fuel cells to evade pollution from combustion and eradicate the risk accompanied by hydrogen storage by lowering greenhouse gas emissions [94]. Ethanol is a well-known solvent in the industry. It is a colourless and flammable oxygenated hydrocarbon. Synthesis of ethanol from glycerol through chemical method is available, but the biological method by fermentation is the standard process.

Bioethanol is one of the fermentation products generated by anaerobic fermentation from glycerol [95]. The productivity of ethanol in the soil of a bacterium tested as a member of the Bacillus genus with 7.0–9.6 g/L ethanol production from the enriched glycerol-algal mixture. Jervis et al. found that Klebsiella planticola derived from the rumen was the main product of glycerol fermentation [96]. Due to its quality and the potential of reducing particulate emissions, bioethanol is considered an alternative to biofuels. At present, the bulk of bioethanol production originates from crops such as maize, sugarcane, wheat, and soy [97]. The unwanted effects on food production, including food

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price rises, a lack of feed, and growing land competition. Therefore, the use of biomass or glycerol waste to produce bioethanol has a substantial potential to ease these unwanted effects on food production [98].

Glycerol is used as the route for bioethanol by transforming into ethanol and hydrogen, and other chemical products. The conversion of ethanol depends on the glycerol concentration. *Enterobacter aerogenes* HU-101, Klebsiella sp. HE1 and *Escherichia coli* obtained a maximum yield of 0.6–1 mol ethanol/mol glycerol at a 10 g/L glycerol concentration [99]. The ethanol production from crude glycerol using microorganisms is shown in Table 3.

The *Kluyvera cryocrescens* S26 converts the crude glycerol to 27 g/L bioethanol (80% yield) with 0.61 g/L/h productivity under microaerobic conditions [100]. A staged batch process achieved 28.1 g/L by *Pachysolen tannophilus* (CBS4044) using crude glycerol as substrate [101]. Ethanol production improved using *K. pneumoniae* (GEM167) with a maximum output level of 21.5 g/L and productivity of 0.93 g/L/h [102]. Glycerol of 34.5 g/L and organic nitrogen at 6.42 g/L produced 1.00 mol/mol of ethanol using *E. coli* SS1. Most of the confirmed ethanol fermentation with glycerol as a substrate carried out using a serum bottle and 500 mL bottles at the laboratory level was described by Adnan et al. [103]. There is a window of opportunity for ethanol fermentation by Very high gravity (VHG) that saves energy for ethanol distillation. Because this technology produces high ethanol at lower cost and low waste, it could be more efficient in the industry. Still, ethanol production for Industrial-scale is challenging for the researcher that makes it possible from laboratory to industrial scale with low cost and high yield. [104].

1,3 propanediol: This three-diol carbon is a colourless viscous fluid used to manufacture polymers including polytrimethylene terephthalate (PTT). Aliphatic polyester, co-polyesters, adhesives, composites, layers, mouldings, laminates, wood lacquers, and anti-freeze products are commonly used. This compound has many significant uses for medicines, polymers, cosmetics, foodstuffs, adhesives, lubricants, solvents, and other products [14]. *Enterobacter* sp. MU-01 produced 0.70 g/L of 1,3-propanediol and 0.88 g/L of 2,3-butanediol at 10 g/L crude glycerol [58]. The co-fermentation of glycerol and glucose using *C. beijerinckii* CCIC 22,954 produced 23.3 g/L of 1,3-propanediol [105]. *K. pneumoniae* strain DSMZ 2026 successfully metabolised pure glycerol and biosynthesized 1.3-PD with 0.42 g/g yield and 1.57 g/L/h productivity after 12 h of cultivation. Unfiltered crude glycerol fraction had an important impact on the production of 1,3-PD (0.21 g/g yield, 0.81 g/L/h productivity) [71].

### Hydrogen

Hydrogen, the only byproduct fuel that produces water, is considered a potential environmentally friendly fuel. Crude glycerol is used as the substrate in microbial fermentation to produce hydrogen. The wide range of usable substrates in fermenting hydrogen allows the energy use of biomass for hydrogen to be combined with waste materials simultaneously. Microbial fermentation produced hydrogen is an appropriate alternative since hydrogen fuel only produces water as a byproduct that dramatically reduces CO₂, NOₓ, particulate matter, and other pollutants, typically followed by fossil fuels [112]. Increased concentrations of crude glycerol co-digested with sanitary sewage by anaerobic consortium bacteria in anaerobic batch reactors at 30 °C and initial pH 7.0 produced biohydrogen. The higher H₂ generation of 35.82 mmol L⁻¹ was observed by 63.9%

**Table 3** Ethanol production from glycerol/crude glycerol from micro-organisms

| Sno | Micro-organism                     | Yield or productivity | Substrate     | References |
|-----|------------------------------------|-----------------------|---------------|------------|
| 1   | *K. cryocrescens* strain           | 0.40 g/g and 0.61 g/L/h | Crude glycerol | [100]      |
| 2   | *P. tannophilus* CBS4044           | 0.06 g/L/h            | CG            | [101]      |
| 3   | *K. oxytoca* FMCC-197              | 25.2 g/L              | CG            | [106]      |
| 4   | *Enterobacter aerogenes* Hu-101    | 0.83 g/L/h            | CG            | [102]      |
| 5   | *Pachysolen tannophilus* CBS4044   | 0.06 g/L/h            | CG            | [107]      |
| 6   | *Enterobacter aerogenes* Hu-101    | 0.5 g/L/h             | Glycerol      | [38]       |
| 7   | *K. pneumoniae* GEM167             | 21.5 g/L of EtOH in fed-batch bioreactor | CG | [102] |
| 8   | *Klebsiella oxytoca* M5al          | 19.5 g/L and productivity 0.56 g/L/h | CG | [108] |
| 9   | *Enterobacter aerogenes* ATCC 29,007 | 5.38 g/L            | CG            | [109]      |
| 10  | *E. coli* ATCC 11,505 immobilised  | 96.7 g/L             | CG            | [110]      |
| 11  | *Escherichia coli* EH05            | 20.7 g/L of ethanol   | CG            | [108]      |
| 12  | *E. aerogenes*                     | 204 mM of ethanol     | CG            | [111]      |
consumption of crude glycerol [113]. Microbial immobilisation has enhanced cumulative hydrogen production (CHP) and hydrogen yield (HY). PVA-alginate is used to immobilise microbes. In the case of immobilisation microorganisms, the highest CHP and HY were 64 mL/100 mL and glycerol 0.52 mol H₂/mol glycerol than suspended microorganisms with glycerol 9 mL/100 mL and glycerol 0.29 mol H₂/mol glycerol [114].

**Propanoic acid**

It is a universal preservative originating directly from a metabolic pathway analogous to the succinic acid pathway. Propanoic acid is increasingly used to develop a biotechnological production method for solvents, pesticides, artificial flavors, heat plastics, and pharmaceuticals through its various industrial applications. The main strains employed to convert glycerol to propionate are *C. acnes*, *C. propionicum* and *P. acidipropionicici* [115]. Propionic acid is produced from crude glycerol in an anaerobic fluidized bed reactor. The reactor is operated with hydraulic retention times varying from 8 to 0.5 h under mesophilic conditions. The maximum yield of 0.48 ± 0.06 g propionic acid g COD⁻¹ with 4.09 ± 1.29 g/L/h productivity was noticed [116]. The propionic acid production’s metabolic reaction is a cyclic reaction dependent upon pyruvate and NADH equivalents. The exogenous CO₂ supply varied from 1.56 to 2.94 g/L/day propionic acid production. Enhanced metabolism of glycerol and increased volume productivity were found when CO₂ was delivered to the dissimilation glycerol process [117]. Using *Propionibacterium freudenreichii* subsp. *shermani*, over-expressing native propionyl-CoA: succinate CoA Transferase (CoAT) has been examined on glucose, glycerin, and mixtures as a carbon source on propionic acid fermentation. The propionic acid production with 10% yield and 46% productivity is shown with the mutant. Metabolic flux analysis has shown that CoAT overexpression has diverted more carbon fluxes to propionic acid, resulting in higher propionic acid with purity and preference for glycerol over glucose [118]. Propionic acid production using waste substrates like glycerol is promising and cost-effective than other sugars [119].

**Single-cell oil**

In industrial applications, single-cell oil or SCO has been identified in microbial lipids that can replace triacylglycerol plants. Fed-batch systems seem to allow lipid and cell density to be increased. *S. limacinum* SR21, a marine microalga found to grow faster in crude glycerol and accumulate high lipid levels. In the case of batch cultivation using crude glycerol, cell growth was substantially inhibited at crude glycerol levels of up to 35 g/L due to the inhibition of substrate and the presence of methanol. The optimal substrate concentration of the processed crude glycerol was increased to 35 g/L with high lipid content of 73.3% (w/w) [120]. Fed-batch operations generally have obtained greater lipid than batch operations because the substrate inhibition is effectively alleviated [80, 121]. Further improvement was made by using a two-stage fed-batch operation. A study of 12 days *Cryptococcus curvatus* with crude glycerol derived from yellow grease. The two-stage fed-batch strategy achieved a greater cell density of 32.9 g/L and lipid content of 52% w/w compared to the one-stage fed-batch operation of 31.2 g/L cell density and 44.2% lipid content w/w [80].

**Butanol**

The development of biobutanol is particularly important because it has better physical characteristics as an alternative fuel. It is applied in plastics, polymers, lubricants, and brake fluid. It can be used as a fuel source. As an eco-friendly and effective solvent for product cleaning and polishing. N-butanol is present in many drinks and foods in the USA and is used as a food artificial aroma. Used in the cosmetics sector, shampoos, shaving products, and soaps. As a chemical intermediate, other essential compounds like glycol ether, acrylate esters, amino resins, acetates, and amines will be generated. The promising n-butanol producer *Clostridium acetobutylicum* KF158795 was stated to use glycerol for substrate and to produce 13.57 g/L of butanol in 96 h under optimised conditions [122]. Butanol production of 16.6 g/L was achieved with a yield of 0.43 g/g by glycerol using *Clostridium* sp. strain CT7. The same species used directly to convert crude glycerol to 11.8 g/L butanol without primary treatment [53]. Butanol production from glycerol was studied in polyvinyl alcohol particles entrapped by *Clostridium pasteurianum*. The glycerol of different impurities from biodiesel production achieved butanol productivity of 2.90 g/L/h and 1.76 g/L/h [123]. *Clostridium* sp. strain CT 7 produced 41.9 g/L of butanol and 0.4 g/L of ethanol from 103.3 g/L of glycerol with pervaporation [124].

**Glyceric acid**

Glyceric acid is also known as hydroxyacetic acid. Glyceric acid is used in the garment industry for dyeing, and tanning agents in food manufacturing, a flavoring agent, a preservative, and an agent for skincare in pharmaceutical manufacturing. It is also used in plastic and adhesives. In emulsion polymers, solvents, and additives, glyceric acid is often used to enhance flow properties and provide gloss. *Gluconobacter* sp. NBRC3259 was used to produce glyceric acid from crude glycerol using activated charcoal pre-treatment. Glyceric acid of 49.5 g/L and dihydroxyacetone of 28.2 g/L was produced from 174 g/L of glycerol [125].
Citric acid

Citric acid is used as an emulsifier in ice creams, as a purifier in the pharmaceutical sector, in cosmetics, and so forth. Citric acid is an acidulant, buffering agent, emulsifier, flavorant, preservative, and sequestant commonly used in many industries in food, beverages, pharma, nutrient, and cosmetic products. The first notable new use as a joint producer with zeolites, primarily in concentrated fluid detergents, is household detergents and dishwashing cleaners. The citric acid serves as a builder, chelating the hardness of Ca\(^{2+}\) and Mg\(^{2+}\) ions, but does not contribute to eutrophication of aquatic systems, as opposed to phosphate builders. The glycerol sources from three biodiesel industry ROTHSAI, BIOLIQ, and BIOCARDEL was used to produce citric acid of 18.70 g/L, 12.0 g/L, and 8.30 g/L respectively at 96 h using *Yarrowia lipolytica* SKY7 [126]. *Y. lipolytica* strain Gut1 and Gut2 using crude glycerol as substrate produced 42.5 ± 2.4 g/L isocitric acids [127].

Polyunsaturated fatty acids

DHA (docosahexaenoic acid), as well as EPA (eicosapentaenoic acid) both, are significant omega-3 PUFA because of their critical function in cancer therapy, cardiovascular disease, and Alzheimer’s. Most of the PUFA are fish are fish that are less favored because they accumulate undesirable odors and harmful contaminants [128]. EPA and DHA are known for their engagement in exercise performance to enhance fatigue recovery and stamina as well as preserve immune function. Also, exhaustive, or unusual exercise induces muscle tiredness and retarded onset of muscle soreness (DOMS). Oxidative stress and inflammatory reactions occur simultaneously [129].

DHA foods are helpful because DHA is important for brain functioning. The use of abundant DHA in the diet enhances brain growth and learning skills. It is good for the eyes and helps to heal from such vision problems. DHA was reported for the prevention of and treatment of senile dementia, hypertension, asthma, depression, diabetes mellitus, myocardial infarction, thrombosis, cardiac disease, and certain types of cancer DHA has a positive impact [130]. Production of DHA with 17.25 ± 0.33 g/dm\(^3\) by *Schizochytrium* sp. grown on waste glycerol as organic carbon source on glycerol waste [131]. The mixed substrate of glucose and glycerol by fed-batch fermentation with *Thraustochytriidae* sp. PKU\#Mn16 using produced DHA yield of 8.65% and productivity of 100.7 ± 2.9 mg/L/h [132].

EPA was derived from fungus *Pythium* irregular waste glycerol with a final concentration of 90 mg/L and productivity of 14.9 mg/L/day [133]. The algal species *Schizochytrium limacinum* SR21 developed with a DHA productivity of 0.51 g/L/day with waste glycerol [134].

Polymer compounds: Polymer compounds like acrolein, polyhydroxyalkanoates, polyhydroxy butyrates exhibit their significance. Acrolein is a significant chemical source for acrylic acid industries, that mostly used in paints, plastics, and adhesives. It is widely used for the manufacture of superabsorbent polymers and n-butyl acrylate. Acrolein can be derived as an oxidation result of glycerol dehydration [135]. Crude glycerol is converted to acrolein with 56% and 81.1% yield by supercritical process (380 °C & 27.6 MPa) and subcritical water process (335 °C & 20 MPa) using sulphuric acid as catalyst [136]. Nonthermal Plasma Induced Fabrication of Solid Acid Catalysts like HSiW-Al and HSiW-Si have shown 98.9 ± 1.8 and 93.5 ± 1.8 conversion (mol %) of glycerol dehydration to acrolein [137].

The glycerol oxidation reaction offers the ability to turn glycerol into value-added goods, boost the economy of biodiesel production and provide new alternative chemical sources for the industry. The produced acrylic acid and its esters exhibit characteristic features with clarity, simple adherence, and plasticity [138]. Sequential dehydration and oxidation of crude glycerol convert to 86% of acrylic acid [139].

The biopolymers were noticed with crude glycerol as a biodiesel byproduct of the small volume of nitrogen and phosphate. Many scientists documented the development of bio-polymers via crude glycerol as a substrate of carbon source through microbial fermentation [140].

Polyhydroxyalkanoates (PHAs) have characteristic features very similar to synthetic plastics. The special features that make them attractive biomedical materials are their biocompatibility biodegradability, and non-toxicity. PHAs suitable for many medical applications have been found for bio-control agents, drug carriers, biodegradable implants, tissue engineering, memory enhancers, and anticancer drugs [141]. The current PHA industry is affected by the high price of the carbon substrate. The use of low-value crude glycerol from the biodiesel industry could reduce the production cost and thus makes PHA more marketable. The crude glycerol has been proven as the potential low-cost feedstocks for PHA production. Polyhydroxyalkanoates (PHAs) reflect natural biopolymers formed by the fermentation of many microbial strains using glycerol via intracellular fermentation. The intracellular accumulation of 56% PHA in *Halomonas* sp. SA8, the soil bacteria of Finnish soils and sediments identified by fermentation with crude glycerol by a mineral medium [142]. *Bacillus thuringiensis* EGU45 produces 1.5–3.5 g/L PHA using crude glycerol and nutrient broth [143]. The combination of crude glycerol and activated sludge with 3 hydroxybutyrate and hydroxy valerate produced 80 Wt% of PHA [144]. The waste glycerol from the biodiesel industry produced 5.63 g/L with 64% PHA by *Burkholderia cepacia* BPT1213 [145]. PHA content of 48% CDW in 48 h with a maximum PHA productivity
of 13.16 mg/L/h produced by *Pseudomonas mosselii* TO7 [146].

Polyhydroxy butyrate (PHB) is biodegradable and biocompatible and, in turn, defines its ecotoxicity and human toxicity in terms of the environment. Consequently, PHB has found useful applications for tissue engineering and related biomedicines such as surgical sutures, thermogels as a controlled release medication supply vehicle, surgical mesh, wound dressing and absorbable nerve guides, bone tissue, and nerve regeneration tissue scaffolding, cardiovascular and cartilage support. PHB is a powerful biomedical and packaging substitute. However, due to secondary crystallisation and slow nucleation speeds, numerous physical disadvantages, such as high production costs, heat instability, and poor mechanical properties restricted its competitiveness with conventional plastics in industrial and biomedical applications [147]. Transformation of glycerol up to 60% polyhydroxybutyrate with *Bacillus megaterium* was performed [148]. PHA production yield 0.44 g/g with 59% PHA content (CDW) using mixed microbial cultures and crude glycerol as feedstock [149] (see Fig. 7).

Water treatment: Various natural source wastes from chicken feathers, sheep wool, human hair, etc. are used for water processing [150, 151]. Wastes from biodiesel industry i.e. unrefined glycerol used in a variety of other ways. Recently, Bodk et al. explored the use of biodiesel waste as a source of organic carbon for a municipal wastewater treatment plant’s denitrification. The experiments were first devised in the laboratory and, after obtaining promising results, they were scaled up. The biodiesel waste is used in the denitrification phase as a result of the findings [152]. Fountakis et al. found that anaerobic digesters successful for the treatment of sewage sludge with the aid of unrefined glycerol to improve the biogas production if the concentration does not exceed 1% (v/v) in the sludge [153].

Co-digestion and cogasification: Glycerol has also served as a biogas source in digesters that work with the aid of heat and a carbon source to be codigested raw material for anaerobic decomposition. The addition of glycerol to a biogas degrading swine manure results in an increase in methane production according to Odorica et al. [154]. The biogas production crude glycerin, a by-extracted biodiesel ingredient, is an appropriate carbon source when added to cattle manure with 5% to 10% has helped increase rural biodigester output and improve the quality. Glycerol can also act as a substrate to aid in the production of hydrogen and methane from industrial and domestic wastewater. The possibility of using unrefined glycerol and hardwood chips up to 20% as feedstock in a fixed-bed downdraft gasifier to manufacture syngas, which is made up of CO, CO₂, CH₄, N₂, and H₂ [33]. Biodiesel production revalorisation does possess several advantages including low nutrient requirements, enhanced efficiency, and the generation of methane, such as those mentioned by Silva [155].

**Animal feed**

Crude glycerol from the biodiesel industry for many years used as animal feed. As animal nutrition demands are anticipated to rise, a greater amount of glycerol is expected to be consumed by the animal. There has been considerable analysis of the possibility of using glycerol instead of corn grain in lactating cow diets. Biodiesel unrefined glycerol contains impurities. However, about 10% used as animal feed exhibited beneficial growth performance in pigs [156]. Similarly, the same level of crude glycerol as a diet supplement to catfish has no negative impact on weight, feed intake, feed conversion, and lipid utilisation.

**Other applications**

Glycerol is called an outstanding additive for improving concrete by cement performance with compression strength, facilitating stronger grinding and handling properties [157].
Additives may be used between 150 and 500 g per ton of cement. A more thorough investigation into glycerol application on cement clinkers revealed that glycerol can interfere with both the chemical and physical. The studies conducted have concluded that glycerol during the course of grinding alters the particles’ surface tension [158].

Future prospective

The massive global production of glycerol by biodiesel industries challenges the researchers to develop new technology which can be commercially viable. It also assists in the valorisation of glycerol with the purpose of glycerol surplus that does not affect the biodiesel industry’s sustainability, i.e., contributes to the biodiesel industry’s environmental economics. This industry can also be profitable and contribute to the biorefinery. Microbial cell factories proposed the micro-organism by engineering depends on glycerol for developing several potential products. The market scenario of biofuels will change because of the creation of new technologies involving the production of ethanol. Rather than seeing crude glycerol as waste, biodiesel industries become aware as a potential material for other potential industries by the fermentation process, which plays a significant role in the current industries and leads to a new pathway for the biofuels sector. Researchers reported pre-treatment is a better way to utilise crude glycerol for ethanol production. Therefore, further research is required, as collective removal methods for various kinds of impurities will lead to an industrial process that will be useful to produce ethanol soon. Apart from that Some researchers also carried out ethanol from crude glycerol without pre-treatment for saving cost. The use of crude glycerol in the formulation of value-added material shows that crude glycerol can be a critical part of the bio-refining industry. The government needs to develop a new regulation and protocol to deal with biodiesel industries for the utility of byproduct glycerol waste that leads to protect the environment.

Besides, genetically engineered microbial strains need to develop and boost the crude glycerol’s ethanol output ability. However, many of the current technologies available but still require more advancement to make the integration into biorefineries cost-efficient and operationally feasible.

Conclusion

Biodiesel is the prominent fuel for the future as considering the environmental issue and reduce carbon footprint. The increase in biodiesel production has contributed to an increase in glycerol byproducts as waste. It is important to find alternative, safe technologies for the use of crude glycerol. The flexibility of glycerol allows being used in many industrial segments as a raw material for the manufacture of chemical intermediates or goods. The value-added chemicals formed from the crude glycerol utilised in cosmetics, pharmaceuticals, adhesives, detergents, paints, paper, and feed products. The bioconversion approach appears to be a feasible source for the development of the economy and environmental maintenance.

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