Conversion of Waste Glycerol to Bioethanol via Fermentation: A Review

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***Abstract*— The exponential growth of population and industrialization lead to excessive consumption of fossil fuels. Dwindling fossil fuel stockpiles and elevated environmental dilemmas drive researchers to discover renewable energy sources. Bioethanol is captured worldwide attention as a biofuel due to its clean performance and renewable nature. However, the usage of traditional food crop feedstock for bioethanol production has caused a negative impact on food production and land competition. Thus, replacing the conventional feedstock with an alternative is pivotal. Approximately 10% of glycerol is formed as the primary co-product in biodiesel production during the process of transesterification. Crude glycerol consists of impurities that are harmful to human health. Purification of glycerol is convenient to convert it to a value-added commodity. The high cost associated with the purification process made the procedure infeasible for small and medium-sized producers. Within the circular economy, crude glycerol becomes an alternative to produce bioethanol through microbial fermentation. Aerobic and anaerobic are the major metabolic pathways associated with the fermentation procedure. Many microorganisms readily convert glycerol to bioethanol with various productivities under different physiological conditions. Optimization of fermentation parameters observes a high yield of bioethanol. Even the glycerol acts as a potential substrate, some undesirable drawbacks are still affiliated with bioethanol industrialization regarding favourable yield. This review mainly explores the conversion of low-cost glycerol waste to bioethanol via fermentation and fermentation kinetics of bioethanol. The value addition, characterization, and purification of glycerol are also highlighted along with future prospective.**

Keywords-bioethanol, glycerol, kinetics, microbial fermentation

I. INTRODUCTION

The increasing demand driven by a complex lifestyle and growing population has intensified resource utilization, predominantly from non-renewable sources unable to meet current needs, thereby escalating costs. This trend has spurred research into technologies that utilize industrial or domestic waste and byproducts to generate valuable resources [1–3]. Among these resources, ethanol has emerged as a focal point for global production advancements. Ethanol is primarily produced through two main methods: biomass fermentation and ethylene hydration. Pre-treatment, fermentation, distillation and dehydration are the sequential stages [1,4]. Ethylene hydration, a chemical process catalysed by phosphoric acid, converts ethylene and water into ethanol [5]. The United States, Brazil, Canada, China, and the European Union dominate the global ethanol production landscape. The world's biggest producer of ethanol is the United States which primarily utilizes corn [6].

The traditional way of sugar and starch crops utilization as feedstock for bioethanol production has considerably elevated a negative impact on food production, increment of food prices, a shortage of fodder, and growth of competition for land [7]. Considering the above undesirable impact on food production, the usage of alternative substrates for bioethanol production became important. Since worldwide crude glycerol prices are decreasing and availability has continued to increase; a surplus of crude glycerol has been created. With that, ethanol fermentation using glycerol as an alternative substrate has become a promising substitution for manufacturing bioethanol and eliminating harmful effects on the environment [1,8].

The terms glycerin, glycerol, and glycerine are often used interchangeably, though there are slight differences in their definitions and usage. Glycerol refers to pure 1,2,3-propanetriol. Glycerine is the common commercial name for products primarily composed of glycerol. Glycerin refers to purified commercial products containing 95% or more glycerol [9]. Refining pure glycerine from the waste by product mixture (crude glycerol) is challenging due to various complications. The high costs associated with purification methods make this process particularly expensive for small and medium-scale plants. Additionally, the historically low market price of crude glycerol and the negative environmental impacts of improper glycerol disposal drive researchers to develop methods for converting it into value-added products [10]. Their primary focus is on transforming low-cost glycerol into valuable products. One promising method is producing bioethanol from glycerol through fermentation. This technology is an attractive and viable option for utilizing waste glycerol, as it is feasible and economical for both small and large-scale plants, with minimal environmental impact. Moreover, it significantly contributes to the production of bioethanol, a widely used alternative biofuel [11]. The review aims to discuss a practical and cost-effective method of using a significant glycerol excess to produce bioethanol, a substitute energy source, through inexpensive fermentation, thereby contributing to meeting the rise in energy demand. It is also noted that there is still a knowledge gap about the fermentation kinetics of ethanol generation from glycerol.

Under such background, the review mainly focused on the conversion of crude glycerol to bioethanol via fermentation and fermentation kinetics of bioethanol. This also provides a review of glycerol purification techniques and qualitative and quantitative analysis of glycerol and glycerol-based value-added products.

II. Origination of Raw Glycerol from Biodiesel Production

Glycerol is obtained as the primary co-product of biodiesel production from vegetable oil through transesterification, which is considered the most economically viable method for biodiesel production. During the transesterification reaction, a 3:1 molar ratio of alcohol to triglyceride is used, producing three moles of esters and one mole of glycerol. Generally, crude glycerol is the main by-product of biodiesel production, with significant quantities (8% to 10%) produced during the transesterification process [8]. After a successful transesterification reaction, methyl ester and glycerol separate into distinct layers due to their polarity differences, allowing for easy separation. Methyl ester is nonpolar, while glycerol is polar [9,12].

III. Raw Glycerol Purification Techniques

In the literature on glycerol purification techniques, the most commonly applied methods are distillation, ion exchange resin, membrane separation technology, and acidification, followed by neutralization and solvent extraction [9]. Acidification is a common strategy to neutralize impurities like catalysts into inorganic salt [12]. Since the acidification process doesn't eliminate all impurities, it needs further purification to remove other contaminations like methanol, oil, water, and ester. However, the distillation process has some restrictions over others as it requires high energy input for vaporization and causes thermal decay [13]. Additionally, a high vacuum is also demanded [14]. Moreover, this operation involves high capital investment and maintenance expense, followed by considerable losses of glycerol [15]. In comparison, the ion exchange process is gathering wide acceptance due to its simplicity of operation, low power consumption, and energy demand, as well as the fact it has also proven efficient in taking off traces of impurities, colour, and odour [16]. In a resin-supported column of glass tube the ion exchange resins are used. They mainly absorb the free cations and anions which contaminate the glycerol [17]. Membrane filtration is an emerging technology and is much fetching because of its simple operability, low energy necessity and thus low cost, good purification performance of up to 90% weight glycerol output, and environmental friendliness [18]. A combination of any of these techniques in single or multi-stages has been utilized for glycerol purification, and according to research, this produces high-purity glycerol of more than 99.2% purity [13].

IV. Raw Glycerol Conversion to Value-added Products

An increment in the exploration of crude glycerol directly for the production of value-added products has been observed in recent times. Different technologies are available for glycerol conversion to value-added products. These are biological conversion, chemical conversion, thermochemical conversion and catalytic conversion [19,20].

A. Biological Conversion

Biological conversion utilizes microorganisms and enzymes for the conversion of glycerol either by aerobic or anaerobic metabolism [9,21]. These biological reactions are carried out in a large bioreactor under aerobic, anaerobic, or micro-aerobic conditions as per the demand of different microorganisms [22]. 1,3- propanediol, ethanol, citric acid, n-butanol are some value-added products obtained via biological conversion of glycerol using Klebsiella oxytoca, Enterobacter aerogenes, Yarrowia lipolytica and Clostridium pasteurianum respectively [18]. For a successful conversion it is important to use microorganisms tolerant to impurities in crude glycerol [19].

B. Chemical Conversion

Different value-added products are obtained such as polyglycerols, ethers, ketals, solketa, polyglycerol esters, 1,3-propanediol, malleated glycerides from the chemical processes in various industrial applications via different pathways such as polymerization, etherification, ketalisation, acetalization, esterification, dihydroxylation, and mallenization of glycerol respectively [18,19].

C. Thermochemical Conversion

Thermochemical conversion of glycerol via steam reforming, gasification, pyrolysis, and partial oxidation are acceptable methods for converting glycerol into useful forms. Carbon monoxide, methane, hydrogen, syngas, and carbon dioxide are major products produced by thermochemical conversion [9].

D. Catalytic Conversion

The conversion of glycerol to value-added products like tartronic acid and 1,2-propanediol with the use of catalysts either basic or acidic, homogeneously or heterogeneously is termed catalytic conversion. Mostly, due to the high selectivity heterogeneous catalysts are preferred commercially. When considering each conversion method, each of them has its pros and cons from both environmental and economic points of view. Biological conversion is more economical and less polluted than chemical, thermochemical and catalytic conversions. Biological conversion needs less capital investment and lower maintenance and labour costs than other methods [19].

V. Conversion of Glycerol to Bioethanol via Fermentation

Bioconversion of crude glycerol to ethanol is carried out via aerobic and anaerobic fermentation processes from microbes [23]. Ethanol produced in these ways is affected by the glycerol concentration [24]. The first step in the passage is the movement of glycerol into the cell by simple permeases, or the cell's facilitator proteins via diffusion or active transport mechanism. The aerobic and anaerobic metabolic routes are the two main methods by which microbes transform glycerol into essential glycolytic mediates [25]. Glycerol kinase aids in transforming glycerol into G3P (glycerol-3-phosphate) during the aerobic pathway when oxygen is present. Next, through a NAD+-based G3P dehydrogenase, DHAP (dihydroxyacetone phosphate) is subjected to oxidation and offers one mole of NADH (Nicotinamide adenine dinucleotide hydrogen) The oxidation of glycerol to dihydroxyacetone (DHA), which yields a single mole of NADH, is the initial step in both aerobic and anaerobic passage [26]. Dihydroxyacetone kinase then converts DHA to dihydroxyacetone phosphate. Then dihydroxyacetone phosphate is followed by pyruvate. Then pyruvate to acetyl CoA, then acetaldehyde, and finally to ethanol [8,26]. Research shows many microbes efficiently convert glycerol to ethanol via fermentation under different conditions as mentioned below. Saccharomyces cerevisiae and some other microorganisms can be successfully genetically modified for widespread usage with the

contribution of metabolic engineering [1]. The regulatory factors of bioethanol production are; temperature, pH, transportation of metabolites within and outside the cell, carbon source, etc. Even though glycerol utilization provides a high yield, undesirable drawbacks are still associated with industrialization such as slow consumption rates of glycerol and unfavourable productivity. With that industrial-scale bioethanol production with low cost and high yield is still challenging [8].

VI. Analysis of Ethanol and Glycerol and Product Characterization of Glycerol

Detection of ethanol and glycerol concentration during microbial fermentation is important. Gas chromatography (GC) paired with a flame ionization detector and HPLC; high-performance liquid chromatography with a Bio-Rad Aminex HPX-87H column are the most common methods applied in many research methodologies to detect ethanol and glycerol concentration [7,27]. Other than this, ethanol assay kits-Megazyme, K-ETOH and glycerol assay kits such as Megazyme, and K-GCROL have also been employed [28]. Another rapid, spectrophotometric method has been developed for glycerol concentration analysis. Which is the periodate-chromotropic acid method [29].

Characterization of crude glycerol is more important to its value-added conversion in many industries. The water content can be measured according to the standard method ISO 2098-1972 by using the Karl- Fisher titrator V20. The ash content can be analysed with the standard method ISO 2098-1972 by burning glycerol in a muffle furnace at 750 0C for 3 hours or by oven calcinations at 520 0C until white ash is produced. Regarding AOCS recommended practice Cc 17-95 and ASTM D 4662-08, the soap content of crude glycerol can be determined. The alkalinity of crude glycerol is found according to ASTM D 4662-08 and used to adjust the soap content. Elemental analysis of crude glycerol is done by ICP-MS (Inductively coupled plasma mass spectrometry) and Fourier transform infrared spectra (FT-IR), 1H NMR, and 13C NMR (nuclear magnetic resonance) spectra are also employed in research studies [30].

VII. Conclusion

Dependence on fossil fuels is increasingly untenable due to rising global energy demands, necessitating the development of alternative and renewable fuels for both economic and environmental sustainability. Bioethanol is in high demand as an alternative fuel, highlighting the importance of a continuous and systematic feedstock supply for ethanol production. Biodiesel production, which yields about 10% crude glycerol as a byproduct, faces the challenge of managing this surplus to maintain economic sustainability and meet environmental protocols. Researchers are exploring cost-effective methods to valorize crude glycerol, such as its use in bioethanol production through microbial fermentation, which can also contribute to waste utilization and renewable energy production. While some advancements have been made, including the use of genetically engineered microbial strains to increase ethanol yield, there remains a knowledge gap in the fermentation kinetics of ethanol production from glycerol, essential for industrial-scale efficiency. Thus, further research is needed to improve these technologies. Given the growing population and vehicle numbers, bioethanol alone cannot meet the energy demand, necessitating government regulations to ensure a sufficient bioethanol supply. Promoting bioethanol over petrol and diesel is also crucial. Biodiesel, derived from the transesterification of vegetable oil, cooking oil, or animal fat, produces glycerol as a byproduct, and its surplus has led to a low market value. Direct disposal of crude glycerol is hazardous due to impurities, making it preferable to convert to value-added products through biological and chemical methods. This glycerol can be used as a substrate for bioethanol production via microbial fermentation, feasible for small-scale industrialization. Understanding fermentation kinetics and optimizing reaction conditions are key to maximizing ethanol yield from various microorganisms, with techniques like HPLC and GC used for analysis. Bioethanol, a renewable and eco-friendly fuel, blends with gasoline and is effective in reducing greenhouse gas emissions while addressing fossil fuel scarcity. Additionally, ethanol has potential applications in various commercial industries.

Table 1. Microorganisms Used for Ethanol Production by Fermentation Process from Glycerol Waste

Microorganisms	Ethanol yield or productivity	Microorganisms	Ethanol yield or productivity	Microorganisms	Ethanol yield or productivity
K. cryocrescens S26	27 g/L, 0.61 g L^{-1} h^{-1}	*P. tannophilus Y−475*	0.63 ± 0.02 g L^{-1} h^{-1}	*Klebsiella variicola TB-83D*	14.6 g L^{-1}
Enterobacter aerogenes ATCC 13048	12.8 g L^{-1}	*Enterobacter sp.*	50.77 mmol L^{-1}	*Mixed cultures of E. coli CECT432, Enterobacter cloacae MCM2/1 E. coli CECT434,*	1.21 mol mol^{-1}
		Klebsiella sp.	51.09 mmol L^{-1}		
Enterobacter aeroegenes TISTR 1468	12.33 g L^{-1}	*Klebsiella aeroegenes ATCC 29007*	15.89 gL^{-1}	*Enterobacter aeroegenes KKU-S1*	116.2 mmol L^{-1}
Recombined strains Ogataea polymorpha	10.7 g L^{-1} 3.55 g L^{-1}	*K. pneumoniae Kp17*	17.3 gL^{-1}, 0.59 g $L^{-1}h^{-1}$	*Saccharomyces cerevisiae*	2.4 g L^{-1}
		Pachysolent annophilus CBS404	28.1 g/L, 0.11 g L^{-1} h^{-1}	*Escherichia coli K12*	3.6 g L^{-1}

(Source: [31], [32], [33], [34], [35], [36], [36], [37], [1], [38], [39], [40], [41], [42], [28])

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