DOI: https://doi.org/10.54966/jreen.v1i1.1253

Journal of Renewable Energies

Revue des Energies Renouvelables

journal home page: https://revue.cder.dz/index.php/rer

Conference paper

Conversion of glycerol into value-added products

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ARTICLE INFO

Article history:

Received July 31, 2024

Accepted September 4, 2024

Keywords:

Glycerol,

Fermentation,

Ethanol,

Microbial consortium.

ABSTRACT

Biomass residues and industrial waste are of great interest for their potential to produce a wide range of bioenergy and value-added products. Crude glycerol can be valorized into value added products by several microorganisms through microbial fermentation. Thus, Significant efforts have been made to develop biological methods to convert crude glycerol into various valuable chemicals and fuels, including 1, 3-propanediol, hydrogen and ethanol.

In this context, our work have use glycerol medium as source of carbon for investigate bioethanol and 1, 3-propandiol production, using a microbial consortium. All experiments was carried out in sealed bottles, the reaction medium is placed in the shaker incubator at 37°C with stirring at 130 rpm. . Nitrogen gas was injected to create anaerobic conditions. The highest concentration of ethanol and 1,3 – propanediol obtained were 3.47 (g/l) and 4.8 mg/l respectively . These results highlight promising avenues for the valorization of biomass residues and industrial wastes in sustainable bioenergy production.

1. INTRODUCTION

Biodiesel is a renewable energy source that can significantly contribute to a coun-try's long-term energy needs. It is widely used for this purpose (Chilakamarry, et al. 2022). The market for biodiesel is growing rapidly affecting the cost and availability of glycerol (Monteiro, et al. 2018).

During biodiesel production processes, approximately 10% of crude glycerol is re-leased, with the majority being discarded as waste into the environment (Dobson, Gray et Rumbold 2012). Glycerol is also generated by bioethanol process (Choi, et al. 2011) .The strategy of valorizing this by-product into value-added products appears to be very promising (Varrone, Giussani, et al. 2012). Glycerol can be converted using physicochemical or biological methods, however due to high probability to

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ISSN: 1112-2242 / EISSN: 2716-8247

contamination of crude glycerol and high purification costs, physicochemical method was discarded (Meyer, et al. 2008). Therefore, converting glycerol through anaerobic fermentation into higher value products appears to be a promising pathway to enhance the economic sustainability of the biofuels industry (López, et al. 2009). Several researchers have investigated the conversion of waste into value-added products, such as bioethanol, 1, 3 propandiol, lactic acid, and propionate (Papanikolaou, Frick et Aggelis 2004). The metabolic pathways involved in the biosynthesis of most of these products under aerobic and anaerobic fermentation conditions are shown in are shown in Figure 1. Due to the size and structure of their molecules, microorganisms are able to bio-convert glycerol into metabolites with yields similar to those obtained from sugar metabolism (Yazdani, et al .Gonzalez. 2007). In order to improve the efficiency and stability of the process, the fermentation with a suitable microorganisms is necessary (Varrone, Giussani, et al. 2012) . Several wild-type bacteria including *Clostridium, Enterobacter, Bacillus, Citrobacter Klebsiella, Anaerobiospirillum, Propionibacterium,* and *Lactobacillus* species can be ferment glycerol to produce value-added products (Chanthoom, Tanikkul, et al. 2016) . Moreover, various researchers noticed that microbial consortium were highly efficient than pure strain for the conversion of a complex substrate like glycerol (Zhou, et al. 2017) (Jiang, et al. 2017). In this study, anaerobic fermentation of glycerol were performed using microbial consortium collected in biogas reactor in acidogenesis phases with cow dung as organic source . The current research focuses on bioethanol and 1, 3 propandiol production.

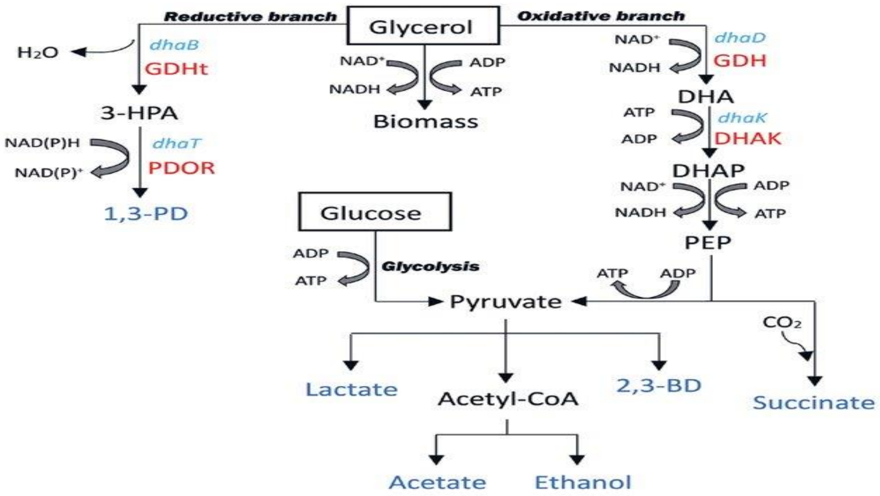

Fig. 1. Metabolic pathway of glycerol conversion (Yang 2018).

2. MATERIAL AND METHODS

2.1 Microorganisms and medium composition

2.1.1 Microorganisms

Microbial consortium was obtained from anaerobic biogas reactor in acidogenesis phases with fresh cattle manure as organic source.

2.1.2 Medium

The preculture medium contains (per liter of water) glycerol 30 g, NH4Cl 1 g, KH2PO4 3 g, Na_2HPO_4 6 g, 14.7 $CaCl_2$ mg, NaCl 58 mg, $MgSO_4$ 24 mg. The suspension is left for 24 h at 37° C under stirring.

2.1.3 Fermentation

The fermentation are carried out in 100 ml sealed bottles containing 50 ml of the synthetic medium with 10% of inoculum. The ethanol fermentations were conducted with and without glucose. The ratio of glucose to glycerol is 1: 20. All experiments was carried out in sealed bottles, the reaction medium is placed in the shaker incubator at 37°C with stirring at 130 rpm. Nitrogen gas was injected to create anaerobic conditions.

2.2. Analytical methods

Glycerol , ethanol and 1,3 propandiol were quantified by a high performance liquid chromatograph Jasco LC Net II/ADC equipped with Eurokat H column (10 µm, 300 × 8 mm) and RI detector (JASCO RI 4030). Elution was performed using sulfuric acid 5 mM as the mobile phase, at a flow rate of 0.6 ml/min. The column and the refractive index detector were maintained at 60 °C. The injection volume was 20 µl.

3. RESULTS AND DISCUSSIONS

Glycerol waste can inhibit microbial growth and then ethanol production was affected (Vikromvarasiri, et al. 2014) , in this case we used pure glycerol in our study in order to evaluate glycerol fermentation from microbial consortium. According to the results summarized in Table 1, we can noted that the use of glucose as co –substrate provided the highest concentration of bioethanol (3.47 g/l) ,while the lowest concentration (1.53 g/l) is obtained in fermentation with glycerol as sole carbone substrate. Although the fermentation of glycerol with the addition of glucose can increase the efficiency of ethanol production, glucose has a negative effect on the production of 1,3-propanediol, in fact the highest production (0.02 g/l) was obtained in fermentation without the addition of glucose, whereas 0.005 g/l was produced in the fermentation with glucose as co-substrate. These data strongly suggest that oxidative pathways are more favorable than reductive ones.

Our results are in agreement with Boonyawanich et al (2021), who have shown that it is possible to improve ethanol production by adding glucose as a co-substrate. In fact, in their study, ethanol production was achieved 232.8mM with a glycerol/glucose ratio of 100:1, compared to fermentation without glucose, when they achieved 251.1mM (Boonyawanich, Haosagul et & Pisutpaisal 2021).

Acetic acid can also be used as a co-substrate, effectively Sawasdee et al (2023) showed that the production of bioethanol was improved after an addition of low concentration of acetic acid. They obtained 272.64±3.30 mM with 100:1 ratio (gly/ HAc), while 142.73mM was produced at ratio 10:1 (Sawasdee, Vikromvarasiri et & Pisutpaisal 2023).

Table 1. Results of glycerol fermentation.

Substrate (g/l)	Residual glycerol (g/l)	Ethanol (g/l)	1,3PDO (g/l)	$Y_{Ethanol}$ g/g
ratio glycerol/glucose (1: 20)	14.7	3.47	0.005	0.24
Glycerol 30g/l	14.85	1.53	0.02	0.1

In addition, we noted that the consumption of glycerol is not complete, in fact approximatively 49% of the initial concentration of glycerol was fermented by the consortium, in the twice fermentations, this result are similar to Ito et al. 2005, who reported that the consumption of glycerol is not complete when the initial concentration of glycerol is up than 25 g/l (Ito, et al. 2005). Furthermore, Gonzalez (2013) reported ethanol yields ranging from 0.2 to 0.47 (g/g), which is almost the same as our ethanol yield of 0.24 (g/g) (Clomburg 2013). In the previous studies, Temudo et al (2008) (Temudo, et al. 2008) carried out glycerol fermentation using mixed cultures derived from distillery effluent and a sludge solution from an acidifying tank used in potato starch processing, to enhance the production of hydrogen and 1, 3-PDO, Selembo et al 2009 (Selembo, et al. 2009) used mixed cultures from two different soils.

Microbial consortium involves multiple interacting species, the performance and stability of the microbial consortium during fermentation deserves attention (Chanthoom, Tanikkul, et al. 2016) , thus type of bacteria strains have an important impact to glycerol fermentation. On the other hand several microorganisms have demonstrated their ability to convert glycerol using pure strains, such *Clostridium*, *Enterobacter*, *Bacillus*, *Citrobacter Klebsiella*, *Anaerobiospirillum*, *Propionibacterium*, and *Lactobacillus species* can be ferment glycerol to produce value-added products (Chanthoom, Tanikkul, et al. 2016). Genetically modified yeasts like Saccharomyces cerevisiae, *Pachysolen tannophilus* can also be used to produce bioethanol from glycerol (Sawasdee, Vikromvarasiri et & Pisutpaisal 2023). The efficiently fermentation of glycerol in *S. cerevisiae* was obtained by an engineering of glycerol pathways and rewriting of the NADH pathway (Khattab et & Watanabe 2021). Chilakamarry et al (2022) (Chilakamarry, et al. 2022) found that the concentration of glycerol had a significant effect on ethanol formation. High substrate saturation causes low conversion. Various studies have shown that glycerol conversion is influenced by a number of parameters and that a suitable microorganism is required to achieve maximum yield (Adnan, et al. 2014).

4. CONCLUSION AND PERSPECTIVES

In this study, feasibility conversion glycerol into ethanol and 1, 3 PDO was demonstrated ,utilizing microbial consortium. Bioethanol production was boosted by using glucose as a co-carbon source fermentation. However parameters study is necessary to improve the glycerol degradation into bioethanol and 1, 3 PDO formation.

The fermentation of crude glycerol can also be investigated further. The expected results of this study will add potential approaches for the efficient use of glycerol waste to improve value for the biodiesel and refined glycerol industries, leading to a waste-free process.

In addition, some micro-organisms may be difficult to use on an industrial scale, thus isolate an indigenous appropriate micro-organism would be preferable to improve conversion efficiency.

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