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Bacterial isolation for efficient 2,3 butanediol production from glycerol waste discharged from bio-diesel processing

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Abstract

Glycerol waste generated from biodiesel production is a major by-product in the biodiesel process. This study aims to demonstrate the effective use of bacterial isolation isolated from natural sources as a biocatalyst to convert 2,3-butanediol (2,3-BD) using crude glycerol as a sole carbon source. We report the microorganisms converting glycerol to 2,3-BD using F1, F2, and F3 isolates, and all three isolates were tested to produce 2,3-BD from glycerol waste and MA medium. The 2,3-BD was produced in a batch experiment with an initial glycerol concentration of 15 g/L, an incubator temperature of 37 °c, and shaking at 150 rpm for 24 hours. The results showed that the isolate F3 was capable of 2,3-butanediol production of 0.35 g/L and achieved that highest OD_{600} reading of 0.851 after 24 hours.

Keywords: Glycerol waste, 2,3-butanediol, Bacterial Isolation

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1. Introduction

Nowadays, Climate change, the end of oil, overpopulation, and land use are existential problems for the human race, with innovative approaches such as using microbes to produce better biofuels. Implementing principles of green chemistry also indicates increased awareness of environmental safety and protection. Considering the technology growth, it is a transformation from regular pathways of production processes towards economically improved, cost-effective processes, and 2,3-Butanediol (also called diol or 2,3-butylene glycol) (2,3-BD) is an essential raw material for basic organic chemicals and fine chemicals. One of the significant factors for showing interest in bio-butanediol by companies is to reduce the use of petroleum-based feedstock in light of global oil prices. 2,3-BD is a chemical and liquid fuel that has great potential in the manufacturing process and is used in Chemicals, food, fuels, aeronautical, and other fields. Microbial production of 2,3-BD from renewable resources is a promising to conventional chemical process. 2,3-BD is a value-added biobased chemical in diverse applications. 2,3-BD is known to be produced by a range of sugar or citrate fermenting microbes. The organisms are *Enterobacter aerogenes*, *Klebsiella pneumoniae*, and *Serratia marcescens*. However, bioconversion of biodiesel-derived glycerol as a substrate into 2,3-BD promotes an eco-friendly environment because, during the biodiesel production process, glycerol is a primary by-product for every 9 kg of biodiesel produced, about 1 kg of a product, crude glycerol is formed. Moreover, glycerol is an efficient and low-cost feedstock for the fermentation industry. In this work, we were isolated from natural sources as a biocatalyst to convert 2,3-butanediol (2,3-BD) using crude glycerol as a sole carbon source to demonstrate the effective use of bacterial isolation.

2. Methods

2.1 Parameter of glycerol waste

Crude glycerol from the biodiesel pilot plant Prince of Songkla University, Pattani Campus, Pattani Province, Thailand. The chemical compositions of the glycerol waste were 77.56% (w/v) total solid (TS), 72.77% (w/v) volatile solid (VS), 4.79% (w/v) ash, 563.04 g/L chemical oxygen demand (COD) and 375 g/L glycerol. Natural sources (utilize palm oil mill effluent-POME)

2.2 Bacteria isolation for 2,3-BD-producing bacteria

A strain of bacteria was isolated from natural sources (utilize palm oil mill effluent-POME) from the mill processing; Palm Pattana Southern Burder Co., Ltd Pattani, Thailand, was used as the inoculum source. 5 g of POME was added to the 250 mL flask containing the sterilized enrichment medium (working volume 120 mL). The enrichment medium contains 15 g/L of glycerol as a substrate with trace elements (Ripoll et al., 2012). The trace element solution (all in g/L): 34.2 $ZnCl_2$, 2.7 $FeCl_3.6H_2O$, 10 $MnCl_2.H_2O$, 0.85 $CuCl_2.2H_2O$, 0.3 H_3BO_3, 23.8 $CoCl_2$, 0.75 KCl, 1.38 $NaH_2PO_4.2H_2O$, 5.35 $(NH_4)_2SO_4$, 0.28 Na_2SO_4, 0.26 $MgSO_4.7H_2O$, 0.42 HNO_3 and 2 yeast extract. The enrichment medium was adjusted to a pH of 7. Samples were incubated in an orbital shaker at 150 rpm and an incubation temperature of 37 ◦C. Every 7 days, the culture broth (10% v/v) was transferred to the flask containing 30 mL of MA medium supplemented with 15 g/L glycerol. This step recurred until a soil-free enrichment was observed. The soil-free enrichment cultures obtained from facultative anaerobe cultivation were serially diluted (10^{-1}–10^{-6}), and broth culture was spread on an MA agar plate, then

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incubated at 37 ◦C in an incubator for 2 days. Single colonies from each dilution series were picked up and transferred to the sterilized medium for testing their 2,3-BD production performance. During fermentation, the broth was collected and analyzed for 2,3-BD by gas-chromatography and glycerol concentrations by high-performance liquid chromatography (HPLC). The isolated strain that produces the highest 2,3- BD was bringing to identify the strain by 16s rDNA sequencing.

2.3 The fermentation process and sample preparation for 2,3-BD production

The batch fermentation experiment contains the MA medium used for the bioconversion of glycerol to 2,3-BD in anaerobic fermentation. Three milliliters of inoculum were transferred to the serum bottle containing 27 mL of sterilized growth medium (MA), incubated at 37 ◦C for 72 h in an incubator shaker at 150 rpm. After 24 h of incubation, 1 mL of the broth fermentation was collected in a 1.5 mL microcentrifuge tube, centrifuged at 10,000$\times g$ for 5 min, and the supernatant was collected. This study collected samples from 0, 4, 8, 12, 16, 20, 24, 48, 60 and 72 h incubation. And 1 μL was injected into GC-FID for production.

2.4 Analytical methods

Bacterial growth was measured spectrophotometrically from the absorbance at 600 nm (OD600) using Shimadzu UV–-visible spectrophotometer UV-1603. The pH values of the culture broth medium were determined using an AB15 pH meter. The products from fermentation broth 2,3-BD concentrations production were identified using gas chromatography with a flame ionization detector (HP 6890 Series, HP Hewlett Packard) using helium as the carrier gas.

3. Result and discussion

3.1 Isolation and identification of bacteria

Bacterial strains were isolated from natural sources (utilize palm oil mill effluent-POME), and the three isolates F1, F2, and F3 were tested to produce 2,3-BD. Among these, the isolate F3 showed the production of 2,3-BD (0.35 g/L). Based on this result, The morphologies of the three isolates by Gram staining. Isolate 1 was white colored with a circular shape and smooth surface, isolate 2 was white colored with a circular shape and rough surface, and isolate F3 could be used as the inoculum in the next step. However, the morphology of isolate F3 is white-colored with a rhizoid shape and wrinkled colonies. Therefore, strain F3 was selected as the inoculum throughout this study. (Fig. 1) and The experiment was performed in broth medium in a 50 mL flask, incubated at 37 °C for 1 day, and then the fermentation broth was analyzed for 2,3-BD production using GC-FID. The results are shown in Table 2.

a b c

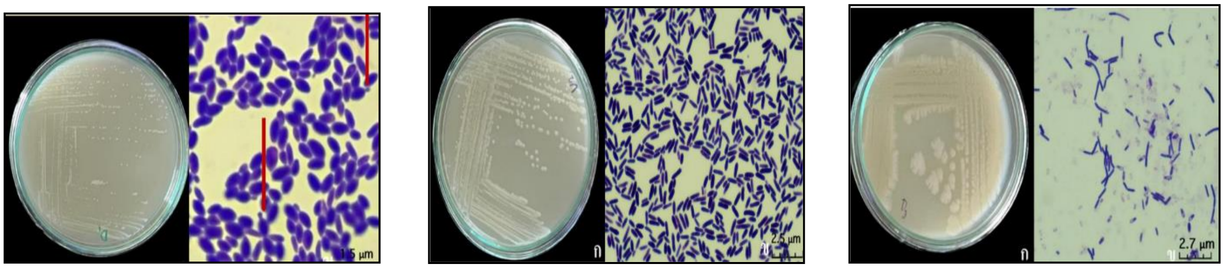

Fig. 1 a Colony morphology of F1 **b** Colony morphology of F2 and **c** Colony morphology of F3

Table 1 Colony characteristics, conditions, and concentrations of 2,3-BD in agar medium containing 15 g/l glycerol concentration of each isolate.

Isolate	Colony morphology	2,3-BD production (g/L)
F1	white colored with a circular shape and smooth surface	ND
F2	white colored with a circular shape and rough surface	ND
F3	white colored with a rhizoid shape and wrinkled colonies	2.46

- ND is Not detected.

3.2 The fermentation process and sample preparation for 2,3-BD production

The production of 2,3-butanediol was studied under batch fermentation incubated at 37◦C for 72 h in an incubator shaker at 150 rpm. During the fermentation process, samples were collected for analysis every 4 hours. The results from the study of OD_{600} from fermentation were collected at the following times: 0, 4, 12, 16, 20, 24, 32, 40, 48, 60, and 72 hours. The results showed that isolate F3 could produce a 2,3-butanediol concentration of 0.35 g/L. from the growth curve experiment. The maximum production of 2,3-BD concentration was 0.82 g/L and achieved the highest OD_{600} reading of 0.950 after 12 hours. (Fig. 2)

a **b**

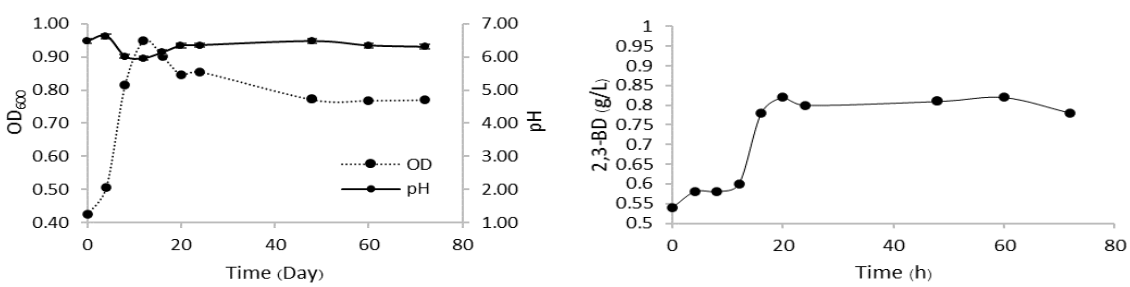

Fig. 2 An OD and pH value of F3 **b** 2,3-BD production of F3

4. Conclusion

In this study, We have to demonstrate The microorganisms converting glycerol to 2,3-BD using the new isolates F1, F2, and F3. The isolate F3 was capable of producing a 2,3-butanediol concentration of 0.35 g/L, and The isolate F3 achieved the highest OD_{600} reading of 0.851 after 4-12 hours. Therefore, the isolated F3 could be a better microbial for anaerobic conversion of glycerol to 2,3-BD production.

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