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Kinetics of Bioethanol Production from Glycerol by *Enterobacter aerogenes*

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Abstract

Kinetics of ethanol production from glycerol as sole carbon source using *Enterobacter aerogenes* were evaluated in batch fermentation with initial glycerol range from 10 to 120 g L^{-1} under 240 hr incubation at 30°C and pH 7. *E. aerogenes* was able to grow and produce the maximum ethanol concentration of 136.7 mM at the initial glycerol concentration of 20 g L^{-1}. At this glycerol concentration, glycerol was completely utilized after 40 hr fermentation and ethanol yield was 0.7 mol mol^{-1}. Microbial growth appeared to decrease when the initial glycerol concentrations were increased. In the initial glycerol concentration range of 10-45 g L^{-1}, glycerol utilization was approximately 90% after 40 hr fermentation. Minimal amount volatile fatty acids and 1, 3-propanediol were detected.

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Keywords: ethanol; biofuel; fermentation; glycerol; *Enterobacter aerogenes*

1. Introduction

Biodiesel is widely used as a substitute of diesel, fossil fuel, to be less dependent on the increased price fossil fuel and to mitigate greenhouse gas emissions [1]. Biodiesel is produced by transesterification of vegetable oils or animal fats, with ethanol or methanol (alcoholysis). With every 100 lbs of biodiesel produced, 10 lbs of crude glycerol is generated [2]. Crude glycerol derived from biodiesel production is a

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low value product because of impurities such as water, methanol, soap and fat [3]. Glycerol as the main by-product from production of biodiesel was purified to over 80% before marketing. Rapid industrial biodiesel production generates high amount of glycerol by-product. This contributed to the decrease of glycerol price leading to unprofitable in production of pure glycerol. The glycerol by-product is considered environment problem without proper treatment, therefore it needs optimal treatment before discharge into water sources [4]. Previous research have shown that the waste or raw glycerol can be converted to various chemical products by biological method for various industries such as food, pharmaceutical, cosmetics, pulp and paper [5]. Generally, glycerol can be utilized as carbon source for several microorganisms and can be biologically converted to other products such as 1, 3- propanediol [2], hydrogen [6], succinic acid [7], ethanol [6], and dihydroxyacetone [8]. The current study evaluated the impact of glycerol concentration on the ethanol production yield, microbial growth and glycerol utilization in batch fermentation.

2. Materials and methods

2.1 Microbial Inoculum

E. aerogenes was purchased from the Institute of Science Research and Technology of Thailand (TISTR). The culture was maintained at 70% (v/v) glycerol stock and kept at -20°C. Volume of stock solution of *E. aerogenes* was recultivated in 250 mL flask containing 50 mL nutrient broth (NB) (per 1 L contained peptone 5 g, beef extract 3 g) and cultivated for 24 hr (30°C with agitation 150 rpm). The recultivated culture (10 % v/v) was subsequently transferred to 100 mL NB medium (24 hr incubation), third transferred into 500 mL NB medium (18 hr incubation) prior to harvesting the cell. The cells were washed by simple medium (SM) [6], and determined cell dry weight (CDW) before using as seed.

2.2 Batch fermentation

Glycerol was prepared in pH 7 SM medium of 47.5 mL by various initial glycerol concentrations (10 to 120 g L^{-1}). The medium was added into 100 mL serum bottle, before autoclaved at 121°C, 15 psi for 15 min. The serum bottle was sealed with rubber stoppers and aluminium cap. The concentrated cell (2.5 mL) was inoculated into the serum bottle to achieve the initial cell concentration of 1.5 g CDW L^{-1}. The reactor content was purged with nitrogen gas for 2 min to achieve anaerobic condition. Fermentation condition was set up at 30°C with agitation 150 rpm. Liquid and gas samples were periodically collected for analyses.

2.3 Analysis of fermentative products

The cell concentration was measured by CDW. Liquid sample of 1.5 mL was periodically collected and centrifuged (8000 rpm, 4°C 15 min) to collect the supernatant for analyses of volatile fatty acids and ethanol. After that the supernatant was filled with cellulose acetate membrane 0.45 μm. The concentrations of Ethanol and volatile fatty acids were determined using gas chromatography (GC-7A, Japan) [9]. The concentrations of CO_2 and H_2 were determined by gas chromatography (GC- 2014) [10]. The glycerol concentration were determined using an high performance liquid chromatography device column (300x78 mm; Aminex HPX-87H; Bio-Rad) equipped with a refractive index detector, The mobile phase was 5 mM H2SO4 and run at flow rate 0.6 mL min-1, the column temperature at 60°C.

3. Results and discussion

Cell dry weight of *E. aerogenes* increased after inoculation the cell until 56 hr and remained constantly for the rest of the process for the initial glycerol rang 10-60 g L^{-1}. However, at the initial glycerol concentration of 120 g L^{-1}, no CDW was increased. Maximum and minimum CDW was found at the glycerol concentration of 20 and 120 g L^{-1}, respectively. Glycerol was mainly utilized during first 24 hr fermentation and slightly dropped after 24 hr fermentation for all cases. The CDW increased corresponding to the decrease of glycerol concentration. Maximum glycerol utilization (18.4 g L^{-1}) was observed when the initial glycerol 20 g L^{-1} was fermented for 40 hrs (Fig. 1). The increase of glycerol concentration resulted in the decrease of glycerol utilization.

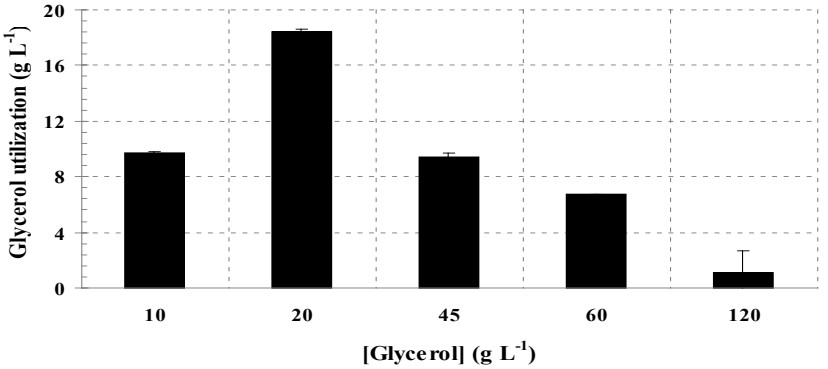

Fig. 1. Glycerol utilization after 40 hr fermentation. Histograms represent mean values of triplicate measurements, error bars represent one standard deviation.

Maximum ethanol concentration (6.3 g L^{-1}) and yield (0.7 mol mol^{-1}) was also observed at the medium glycerol concentration of 20 g L^{-1}. The ethanol concentration decreased as initial glycerol concentration increased (Fig. 2) whereas minimal amount of volatile fatty acids such as acetic and butyric acids, and 1, 3- propanediol were detected. The previous study [10] obtained ethanol concentration and yield of 3.4 g L^{-1} and 0.35 mol mol^{-1} when the fermentation was setup in the same conditions but only mixed culture used as an inoculum. The results suggested that diversified microbes in the mixed culture may be not be always advantageous compared to a pure strain since some species convert glycerol to other products and caused a low ethanol yield.

4. Conclusion

The ethanol production by biological fermentation using microorganisms and glycerol as a carbon source is cost effective due to the fact that glycerol is obtained as byproduct of biodiesel production. The current work demonstrated that *Enterobacter aerogenes* is able to convert glycerol as carbon source to the ethanol at the maximum concentration of 136.7 mM (6.3 g L^{-1}) at the glycerol concentration of 20 g L^{-1}. The results can be used as a role model for experiment on a larger scale.

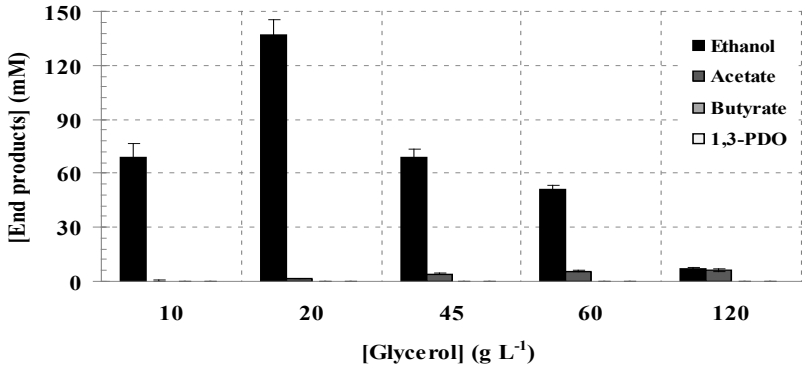

Fig. 2. Concentrations of ethanol and other fermentative products after 40 hr fermentation. Histograms represent mean values of triplicate measurements, error bars represent one standard deviation.

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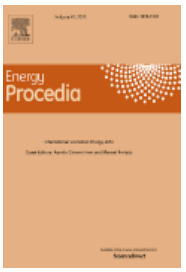

Biography

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