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Microbial dynamics in ethanol fermentation from glycerol

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ABSTRACT

Bioethanol is a clean and renewable biofuel, which can be produced from low cost and renewable feedstocks via fermentation process. The microbes present in an upflow anaerobic sludge blanket (UASB) reactor for wastewater treatment were tested for application in batch fermentation of ethanol at varying glycerol concentrations, and pHs of culture medium. Ethanol was the main fermentative product in this work. Ethanol concentration between 3.9 and 11.1 g L^{-1}; and ethanol yields between 0.66 and 0.81 mol ethanol mol^{-1} glycerol were obtained. The ethanol concentration and yield were highly dependent on the initial glycerol concentration. The highest ethanol concentration of 11.1 g L^{-1} was obtained after 72 h of fermentation at the initial glycerol concentration of 45 g L^{-1}. Ethanol production and glycerol utilization were highly correlated. Denaturing gradient gel electrophoresis (DGGE) pattern revealed there were microbial selection and adaption during the fermentation. 16S rRNA gene sequences showed that the seed culture comprised largely of uncultured bacteria with various *Proteobacteria*, *Bacteroidetes*, and *Firmicutes*. Enrichment of *Proteobacteria*, particularly Enterobacter and Klebsiella bacteria occurred within 24 h of the fermentation and the ethanol producing microcosm at 72 h was dominated >95% by these 2 strains. The major ethanol producers were identified as Enterobacter and Klebsiella strains. This work demonstrated the potential use of biodiesel derived glycerol as a feedstock for the ethanol production, and revealed the major roles of Enterobacter and Klebsiella in the ethanol production from glycerol.

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Introduction

Swift growth and extensive expansion of biodiesel production in recent years has generated a tremendous amount of glycerol by-product from transesterification of triglycerides in vegetable oils or animal fats. In general, approximately 10% (w w^{-1}) of total biodiesel generated is released as glycerol by-product [1]. The dramatically increase of the glycerol over demand of consumption greatly caused its price drop [2]. Low price of commercial glycerol tends to limit the economic viability of crude glycerol purification. Consequently, crude glycerol will soon become waste, requiring disposal or treatment with costs. Development of processes to convert crude glycerol into higher value compounds is a sustainable way to deal with the crude glycerol. Previous studies demonstrated that glycerol can be fermented and converted to more valuable chemicals such as 1,3-propandiol [3–5], dihydroxyacetone [6,7], succinic acid [8], citric acid [9,10], and ethanol [11–14]. For ethanol, its cost of production is mainly governed by feedstock and operational costs. Use of crude glycerol as a feedstock instead of traditional feedstock (e.g. sugar, molasses, and lignocellulosic biomass) could reduce the production cost approximately 40% [2]. Furthermore, working under non-sterile conditions using mixed cultures could lower the operational costs of ethanol production. Due to being constituted higher microbial diversity, the mixed cultures are more advantageous than pure cultures when dealing with the crude glycerol with variable impurities [15]. The aim of this study is to investigate the feasibility of using a mixed consortium as an inoculum seed for ethanol production from glycerol. Kinetic data of the fermentation process, dynamics of microbial diversity, and microbial species at the optimal ethanol production are determined. The work thus provides a potential approach for efficient utilization of glycerol and would improve the profit for the biodiesel industry.

Materials and methods

Inoculum

Anaerobic granule collected from a full scale upflow anaerobic sludge blanket (UASB) system (Malee Sampran Public Co., Ltd, Nakorn Prathom Province, Thailand) was used as a microbial seed throughout the study. The microbial seed was washed with distilled water twice to remove wastewater and debris coming with the seed before using in the experiments.

Culture medium

A cultural medium was prepared in the distilled water with the following constituents (per liter); 7.0 g of K_2HPO_4, 5.5 g of KH_2PO_4, 1.0 g of $(NH_4)_2SO_4$, 0.25 g of $MgSO_4.7H_2O$, 0.021 g of $CaCl_2.2H_2O$, 0.12 g of $Na_2MoO_4.2H_2O$, 2.0 mg of nicotinic acid, 0.172 mg of Na_2SeO_3, 0.02 mg of $NiCl_2$ and 10 mL of trace element solution containing 0.5 g of $MnCl_2.4H_2O$, 0.1 g of H_3BO_4, 0.01 g of $AlK(SO_4)_2.H_2O$, 0.001 g of $CuCl_2.2H_2O$ and 0.5 g of Na_2EDTA [11]. The laboratory grade glycerol (QRec, Auckland, New Zealand) was used as carbon source and energy sources. The medium was autoclaved at 121 °C, 15 psi for 15 min before use.

Cultivation of microbial consortium

All experiments were setup in 100-mL serum bottles with 50-mL working volume. Mixed culture was dispensed into the serum bottle containing sterile cultural medium. The final concentration of biomass in the serum bottle was 0.13 g dry cell weight L^{-1}. Experiments were setup under various conditions, initial glycerol concentrations (0, 10, 25, 45, 65, 128 g L^{-1}), and pHs (4, 5, 6, 6.8, 7), at 30 °C. The experiment was conducted in duplicates. After all components were placed in the bottle, the rubber plug and aluminum cap were closed and nitrogen gas was purged into the bottle content through the 0.45 μm syringe filter prior to incubation at 37 °C with rotary shaking at 150 rpm. Liquid samples were collected periodically (0, 24, 40, 56, 72, 168, and 240 h) for analysis of glycerol, ethanol and fermentative products. Microbial cells were collected by centrifugation for molecular phylogenetic analysis.

Analysis of fermentative products

Glycerol concentration in the fermentation broth was determined on a high performance liquid chromatograph (Agilent LC1200 Series, USA) equipped with a UV/RI detector, and an Aminex HPX-87H column of 300 × 7.8 mm (Bio-Rad, USA). Sample of 20 μL was injected into the HPLC system and eluted with 5 mM H_2SO_4 flow rate 0.6 mL min^{-1} using isocratic mode. Ethanol and volatile fatty acids (VFAs) were determined with Gas Chromatography (Shimadzu GC-7A, Japan) equipped with a flame ionization detector (FID) and fitted with a Stabilwax DA capillary column (30 m × 0.32 mm, i.d., 0.25 μm film thickness). The temperature of the injection port, detector, and column were 240, 230, and 80 °C [16].

Denaturing gradient gel electrophoresis of 16S rRNA gene fragments

Total genomic DNA was extracted from the microbial cell pellet using the benzyl chloride method [17]. The purified DNA

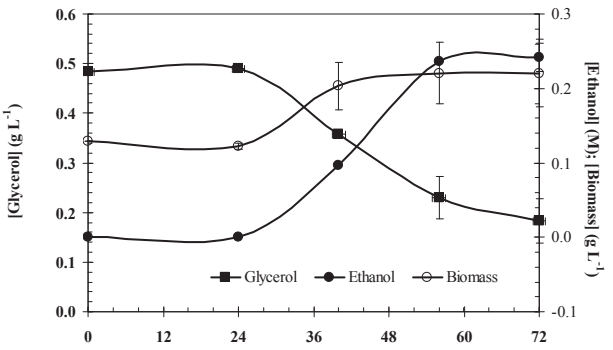

Fig. 1 – Batch fermentation using initial glycerol concentration of 45 g L^{-1}, pH 7 and 30° C. Symbols represent mean values of duplicate measurements.