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Biological carbon monoxide conversion to acetate production by mixed culture

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HIGHLIGHTS

- Undefined mixed cultures from wastewater treatment plant were used as inocula for CO conversion.
- Fermentation conditions for HAc production were optimized using RSM with BBD.
- Higher HAc production was achieved at 20.81% CO, 41.38% CO_2 and 7.18 pH.
- The 23.6 g/L of high HAc production was achieved at continuous gas condition.

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ABSTRACT

To utilize waste CO for mixed culture gas fermentation, carbon sources (CO, CO_2) and pH were optimized in the batch system to find out the center point and boundary of response surface method (RSM) for higher acetate (HAc) production (center points: 25% CO, 40% CO_2, and pH 8). The concentrations of CO and CO_2, and pH had significant effects on acetate production, but the pH was the most significant on the HAc production. The optimum condition for HAc production in the gas fermentation was 20.81% CO, 41.38% CO_2, 37.81% N_2, and pH 7.18. The continuous gas fermentation under the optimum condition obtained 1.66 g/L of cell DW, 23.6 g/L HAc, 3.11 g/L propionate, and 3.42 g/L ethanol.

1. Introduction

Carbon monoxide (CO) is a toxic waste gas produced in large quantity from petrochemical industries, electric power plants, and iron smelters processes. The utilization of CO gas as a feedstock for organic chemicals production through gas conversion reaction could create a potential profit of the industry from reducing waste disposal cost (Sim et al., 2008; Vega et al., 1990).

CO can be converted to various organic chemicals such as acetate (HAc), acetone, and ethanol by a microbiological CO conversion process, called gas fermentation (Mohammadi et al., 2011). The process has high specificity, mild operating conditions, ability of utilizing various types of gases, and resistance to toxic gases (Sim et al., 2008). Earlier gas fermentation studies were based on pure-culture fermentation systems (Guo et al., 2010; Kundiyana et al., 2010a,b; Younesi et al., 2005), however, the pure culture system requires a high operation cost to sustain sterilization conditions (Younesi et al., 2005). On the other hand, the mixed culture does not require the sterilization condition, and has high adaptation capacity to various substrates including toxic components. Therefore, the mixed culture can be more suitable for applying to large industrial systems (Kleerebezem and van Loosdrecht, 2007).

In the gas fermentation of CO gas, HAc can be produced as the major component in the large amount without any additional energy source. HAc is utilized as an end-product by itself as well as an intermediate for further chemical processes to produce value-added compounds (Batlle-Vilanova et al., 2016). Thus, enhancing HAc productivity in the gas fermentation results in maximization of carbon recovery efficiency and reduction of CO_2 emission from the fermentation process (Sim et al., 2008).

In the gas fermentation system, the type of carbon sources (e.g., CO and CO_2) and pH could significantly affect microbial growth and various metabolite production. As the energy and carbon source in the gas fermentation, the partial pressure of CO would influence the cell growth and formation of various products (Hurst and

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Lewis, 2010). Although CO_2 is not an energy source in the system, it can act as a starting material in most carbon-fixation processes because the formation of acetyl coenzyme A needs CO_2 (Heiskanen et al., 2007). Thus the partial pressure of CO_2 can also affect the cell growth rate and final products. Variation of pH conditions in the system causes the final product yield, and the composition of metabolites (i.e., organic acids). At low pH (4–5.5), butyrate and HAc were the main products in the gas fermentation; however, at high pH (10–12), HAc was selectively formed with over 70% of the total organic acid in the system (Jankowska et al., 2015; Temudo et al., 2007).

In this study, the concentration of CO ([CO]), [CO_2], and pH were optimized to maximize HAc production in a mixed culture gas fermentation system using response surface methodology (RSM). The statistical approach using RSM was also implemented to quantify the relationships among three measurable responses with the one vital factors. Finally, the optimized condition was applied to a continuous gas fermentation system to design a high-efficiency CO conversion process model using the mixed culture system.

2. Materials and methods

2.1. Preparation of gas fermentation inoculums

Raw sludge was obtained from a domestic wastewater treatment plant (Daegu, South Korea). To select organic-acid-producing microorganism, the sludge was heated at 90 °C for 30 min to kill methanogens. Inocula for gas fermentation were acclimatized in a 5 L fermenter (Biotron Inc., South Korea) under anaerobic conditions, then 3 L of the heat-treated inocula and 2 L of anaerobic modified M9 medium ($CaCl_2$ 0.015 g/L, Na_2HPO_4 6 g/L, KH_2PO_4 3 g/L, NaCl 0.5 g/L, NH_4Cl 1 g/L, $MgSO_4$ 0.5 g/L, and yeast extract (BD, USA) 0.5 g/L) were supplied to the fermenter. In the medium, the nitrogen source (e.g., yeast extract and NH_4Cl) constitutes 12.5% of total nutrient composition because microorganisms use it to create energy to grown and reproduce (Sim et al., 2008).

The pH was controlled between 6.0 and 6.5 by an automatic pH controller (Model KB-250, K&B, South Korea) using 5 M NaOH (Samchun, South Korea) and 1 M HCl (Deajung, South Korea) solutions. Temperature was maintained at 37 °C and stirring rate was maintained at 200 rpm. After acclimation, 12 mL/min of mixed gas (25% CO, 25% CO_2, 50% N_2) was supplied to the fermenter by a gas mixer (Automatic gas mixing system SHGM-4000, Sehwa Corp., South Korea).

2.2. Batch experimental preparation

Batch experiments were performed in 280-mL serum bottles with 100-mL working volume. The modified M9 medium which was purged with nitrogen gas for 10 min to maintain anaerobic condition was used. Experiments were conducted with 0.22 g/L of the pretreated inocula. The initial pH of the medium was adjusted using potassium phosphate buffer. The batch experiment was conducted in three steps optimization of [CO], [CO_2], and pH in serial order. First, volumetric percentage of [CO] was regulated at 0%, 25%, 50%, 75% or 100% with [N_2]. Based on the best [CO], volumetric percentage of [CO_2] was then adjusted at 0%, 25%, 50% or 75% with [N_2]. Then pH values of 4, 6, 8 or 10 were applied at the best combination of [CO], [CO_2] and [N_2] (Heiskanen et al., 2007). All batch experiment was conducted in duplicate. The serum bottles sealed with butyl rubber stoppers and aluminum seal caps were purged by the mixed gas for 6 min. The bottles were put into operation in a shaking incubator at 200 rpm and 37 °C. Liquid (2 mL) and gas (100 μL) samples were collected every 12 h for further analysis.

2.3. Analytical methods

The pH in the reactor was continuously monitored using a pH meter (405-DPAS-SC-K85, METTLER TOLLEDO, Switzerland). Organic acids were quantified using a high performance liquid chromatograph (HPLC, Agilent Technology 1100 series, Agilent Inc., USA) equipped with an organic acid and alcohol analysis column (Aminex HPX-87H, BIORAD Inc., USA), a refractive index detector, and a diode array detector. [CO], [N_2], and [CO_2] were analyzed using a gas chromatograph (GC, Model 6890N, Agilent INC, USA) equipped with a capillary column (Supelco Carboxen™-1010 PLOT capillary column, 30 m × 0.32 mm, Sigma–Aldrich, USA) and a pulsed discharged detector. The carrier gas was He with flow rate of 1.9 mL/min; the temperatures of oven, inlet and detector were 120, 150, and 240 °C, respectively.

CO consumption [mg/L] and CO consumption rate [mg/L/day] were calculated as follows:

$$\text{CO consumption} = \frac{[\text{CO}]_i - [\text{CO}]_a}{\text{reactor working volume}} \tag{1}$$

$$\text{CO consumption rate} = \frac{\text{CO consumption}}{t} \tag{2}$$

where $[\text{CO}]_i$ is the initial volume of the [CO], $[\text{CO}]_a$ is the actual volume of the [CO] at sampling time, and t is the time until all CO was consumed.

HAc production yield Y [g/g CO] and HAc production rate [g/L/h] was calculated as follows:

$$\text{HAc production yield } Y = \frac{[\text{HAc}]_a - [\text{HAc}]_c}{A} \tag{3}$$

$$\text{HAc production rate} = \frac{[\text{HAc}]_a}{t} \tag{4}$$

where $[\text{HAc}]_a$ [mg/L] is the HAc concentration, $[\text{HAc}]_c$ is HAc concentration at [CO] = 0% (control condition), A [mg/L] is inlet CO volume, and t is the time until achieve the highest HAc concentration.

The cell dry weight (cell DW) was estimated by filtering a cell suspension sample through a pre-dried and pre-weighed 0.45-μm nitrocellulose filter (Millipore, USA). The sample was dried at 110 °C and then weighed by the difference.

Cell DW yield [g/g CO] was calculated as follows:

$$\text{Cell DW yield} = \frac{\text{cell DW}_a - \text{cell DW}_i}{A} \tag{5}$$

where cell DW_a [g/L] is the actual cell DW, cell DW_i is the initial cell DW, and A [g/L] is inlet CO volume.

2.4. Response surface methodology (RSM)

A 3^K factorial Box–Behnken model was used as an experimental design to optimize the process parameters (pH, [CO], [CO_2]) to maximize HAc yield. In the three-factor condition, the Box–Behnken design (BBD) has the advantage of requiring fewer runs than other RSM protocols (Jo et al., 2008; Sim et al., 2008). The BBD is efficient to estimate second degree quadratic polynomial and to combine values optimizing the response within the region of the three-dimensional observation space (Annadurai et al., 1999). To develop the regression equation, the relation between the coded- and actual values are shown as follows:

$$x_i = \frac{(X_i - X_i^*)}{\Delta X_i} \tag{6}$$

where x_i is the coded value of the ith independent variable, X_i is the uncoded value of the ith independent variable, X_i^* is the uncoded