Journal of Industrial and Engineering Chemistry xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of Industrial and Engineering Chemistry

journal homepage: www.elsevier.com/locate/jiec

Process strategy for 2,3-butanediol production in fed-batch culture by acetate addition

Q1 Sang Jun Lee[a], Han Suk Choi[a], Chan Kyum Kim[a], Laxmi Prasad Thapa[a], Chulhwan Park[b,*], Seung Wook Kim[a,*]

[a] Department of Chemical and Biological Engineering, Korea University, 145 Anam-Ro, Seongbuk-Gu, Seoul 02841, Republic of Korea
[b] Department of Chemical Engineering, Kwangwoon University, 20 Kwangwoon-Ro, Nowon-Gu, Seoul 01897, Republic of Korea

ARTICLE INFO

Article history:
Received 23 March 2017
Received in revised form 3 July 2017
Accepted 9 July 2017
Available online xxx

Keywords:
2,3-Butanediol
Enterobacter aerogenes
Acetate supplementation
Lactate dehydrogenase
Fermentation

ABSTRACT

Various strategies were studied to enhance 2,3-butanediol production using *Enterobacter aerogenes* SUMI014. The synergistic effect of acetate improved yield and productivity, and resulted in 32.3% increase in 2,3-butanediol production, compared to the wild strain. Optimizing the fermentation conditions successfully increased the 2,3-butanediol production. In batch fermentation, 93.75 g/L of 2,3-butanediol was obtained within 54 h, along with 0.49 g/g of yield and 1.74 g/L/h of productivity. The highest 2,3-butanediol production achieved was in fed-batch fermentation with acetate addition strategy, with production, yield and productivity of 126.10 g/L, 0.38 g/g and 2.10 g/L/h, respectively.

Introduction

The biorefinery concept integrates the processes and technologies for the production of biofuels and biochemicals from biomass [1]. The biorefinery has recently attracted significant attention as an alternative, that can compensate for the defects of oil refineries such as depletion of resources and environmental pollution [2]. Many platform chemicals from oil refineries can be produced biologically from renewable raw materials as well. One such chemical is 2,3-butanediol, which can be used as a precursor for the production of synthetic rubber, plastics, flavor enhancer and fuel additive [3,4]. Biological production of 2,3-butanediol appears to be inexpensive, compared to the chemical method owing to its less energy intensive processes with microorganisms. Furthermore, much higher production could be allowed than other bio-products because of the lesser toxicity of 2,3-butanediol to microorganisms in biological production [5,6]. Thus, 2,3-butanediol is considered as a promising product in the biochemical industry.

Microbial 2,3-butanediol production is usually performed with bacteria, owing to their specific ability for 2,3-butanediol formation. Until now, *Klebsiella* species have been well-studied and are regarded as good strains for high 2,3-butanediol titer [7]. However, these strains are pathogenic bacteria, which can cause opportunistic infections, leading to safety problems in industrial-scale fermentation [8]. In contrast, nonpathogenic bacteria, such as *Bacillus licheniformis* and *Bacillus subtilis*, show much lower efficiency in 2,3-butanediol production than do pathogenic bacteria [9,10]. Under these circumstances, *Enterobacter aerogenes* can be a promising 2,3-butanediol producer. Even though *E. aerogenes* is classified as pathogenic bacteria, some of its species are biosafety level 1 strains, whereas *Klebsiella* species are biosafety level 2. In addition, *E. aerogenes* have a broad spectrum of substrate (including glucose, xylose, mannitol and various hydrolysates of biomass) and can grow rapidly under both aerobic and anaerobic conditions [11,12].

Several strategies have been employed to improve 2,3-butanediol production such as screening new microorganisms, developing recombinant strains and optimizing fermentation conditions. In terms of genetic modification, heterologous genes, related to the 2,3-butanediol formation, are introduced to the host strains or genes, in competition with 2,3-butanediol synthesis, are eliminated [13–17]. These genetic manipulations efficiently improved 2,3-butanediol production. Moreover, acetic acid supplementation is known to increase 2,3-butanediol production [12,18,19]. In this regard, we have previously demonstrated that acetate stimulates a transcriptional level of genes cording for key enzymes related to the 2,3-butanediol synthesis in *E. aerogenes* [20]. Even though the significance of the acetate effect was verified, the application of acetate in 2,3-butanediol production has not

* Corresponding authors. Fax: +82 2 926 6102.
E-mail addresses: chpark@kw.ac.kr (C. Park), kimsw@korea.ac.kr (S.W. Kim).

http://dx.doi.org/10.1016/j.jiec.2017.07.008
1226-086X/

been sufficiently studied. Therefore, methodological experiments regarding the effects of acetate in the fermentation strategies on 2,3-butanediol production are necessary.

We previously constructed a genetically engineered *E. aerogense* SUMI014 strain by deleting *ldhA* gene coding for lactate dehydrogenase, resulting in improved ethanol production concomitant with a decrease in the lactate production from glycerol media [21]. Therefore, *E. aerogense* SUMI014 was used for 2,3-butanediol production with glucose as a carbon source in this study. The synergistic effect of the mutant strain and acetate on the 2,3-butanediol production was evaluated and the optimal fermentation parameters (induction time of acetate, pH and aeration) were determined as well. Furthermore, the batch and fed-batch fermentations were performed based on the optimal conditions to assess its industrial importance in the production of 2,3-butanediol.

Materials and methods

Strain and medium condition

In this study, *E. aerogenes* ATCC 29007 and *E. aerogenes* SUMI014 were used for the 2,3-butanediol production. *E. aerogenes* ATCC 29007 was purchased from the American Type Culture Collection, while *E. aerogenes* SUMI014, *ldhA* deleted mutant of *E. aerogenes* ATCC 29007, was obtained from our previous study [21]. The cells were maintained on Nutrient agar plates at 4 °C and sub-cultured every month.

Seed medium for inoculating to production medium was Nutrient broth (2 g/L yeast extract, 1 g/L meat extract, 5 g/L peptone, 5 g/L NaCl). The production medium for 2,3-butanediol consisted of 3 g/L KH_2PO_4, 6.8 g/L Na_2HPO_4, 5 g/L yeast extract, 5.35 g/L $(NH_4)_2SO_4$, 10 g/L casamino acid, 0.75 g/L KCl, 0.28 g/L Na_2SO_4, 0.42 g/L citric acid, 0.26 g/L $MgSO_4$ and 0.3 mL of solution of trace elements, containing 34.2 g/L $ZnCl_2$, 2.7 g/L $FeCl_3$, 10 g/L $MnCl_2$ 0.85 g/L $CuCl_2$ and 0.31 g/L H_3BO_3. Glucose (50% (w/v)) was separately sterilized and then added to the production medium.

Culture condition

Seed cultivation was performed aerobically with 50 mL of seed medium in a 250 mL Erlenmeyer flask at 37 °C and 180 rpm in a rotary shaking incubator for overnight, and seed culture was then inoculated into the production medium. All the batch fermentations for 2,3-butanediol productions were carried out in a 5 L bioreactor (Hanil Science Inc., Korea) with a 2 L working volume. Comparative experiments (wild strain, mutant strain, with acetate and without acetate) were performed at 37 °C, pH 6.8, 200 rpm and 1 vvm in the bioreactor with 100 g/L of the initial glucose concentration and potassium acetate was initially added to the production medium; therefore, the potassium acetate concentration reached 0.1 M. Fed-batch fermentation was also performed with a 2 L working volume and 100 g/L of initial glucose concentration. After 12 h of cultivation, 100 mL of glucose solution (stock solution was 800 g/L glucose) and 20 mL of potassium acetate solution (50%) was added into the bioreactor every 6 h. Moreover, antifoam B emulsion (Sigma, USA) was added whenever needed. Other comparative experiments for optimizing fermentation conditions are described in the following "Operational parameters" section.

Operational parameters

Several parameters, including pH, acetate induction and aeration rate (vvm), were optimized for efficient 2,3-butanediol production through the batch fermentation in the bioreactor at 37 °C and 200 rpm with 100 g/L of glucose. The effect of pH on 2,3-butanediol production was investigated under various pHs (pH 5.5, 6, 6.5 and 7). The pH was maintained by auto-titration with 10 N KOH and 5 N HCl. In order to investigate the optimal induction time of acetate, potassium acetate was added into production media at 0, 1, 2, 3, 4 and 5 h during fermentation. Moreover, the effect of aeration on the cell growth and 2,3-butanediol production was subsequently investigated at 0, 0.5, 1, 1.5, 2 and 2.5 vvm.

Analytical method

Cell growth was monitored by measuring the optical density of samples at 600 nm using a UV–vis spectrophotometer (Uvmini-1240, Shimadzu, Japan). Dry cell weight for the biomass concentration was calculated using the standard curve relating the optical density to the dry cell weight. The concentrations of glucose, 2,3-butanediol, succinate, acetate and ethanol were detected by high-performance liquid chromatography (HPLC) using an Aminex HPX-87H column (300 × 7.8 mm, Bio-Rad, USA) and a refractive index detector (RID-10A, Shimadzu, Japan). The temperature of the column and detector was maintained at 50 °C. Mobile phase was 0.005 N H_2SO_4 and applied at a flow rate of 0.6 mL/min.

Results and discussion

Synergistic effect of ldhA *mutant and acetate on 2,3-butanediol production*

In a mixed acid fermentation process, various end-products such as succinate, formate, lactate, acetate and ethanol are produced from pyruvate; and these end-products compete with 2,3-butanediol in utilizing pyruvate. Jung et al. demonstrated that 2,3-butanediol is a dominant product, followed by lactate, ethanol, succinate and acetate in the glucose media and 2,3-butanediol production is increased by deletion of lactate dehydrogenase in *E. aerogenes* KCTC 2190 [22]. Based on these results, we used our engineered strain *E. aerogenes* SUMI014 for 2,3-butanediol production and also evaluated the synergistic effect of acetate and mutant strain on the 2,3-butanediol production.

In order to compare the effects of mutant and acetate on 2,3-butanediol production, batch fermentations were performed using *E. aerogenes* ATCC 29007 and *E. aerogenes* SUMI014 with acetate or without acetate in 100 g/L of glucose media (Table 1). In wild-type strain, 100 g/L of glucose was completely consumed within 24 h, resulting in 27.26 g/L of 2,3-butanediol without acetate and 32.73 g/L of 2,3-butanediol with acetate, respectively. Whereas the concentrations of biomass, succinate and ethanol were similar in both with and without acetate, lactate production was decreased from 12.6 to 8 g/L in the acetate added media. Comparing the fermentation results by the wild and mutant strains, 2,3-butanediol production in the mutant strain increased by 16% without acetate and by 32.3% with acetate compared to the wild strain without acetate. Moreover, the concentrations of cell biomass, succinate and ethanol were slightly increased, while lactate production was not observed in the mutant strain. These results indicate that 2,3-butanediol and lactate are high contenders for pyruvate utilization, and acetate is favorable for pyruvate to convert into 2,3-butanediol rather than lactate. Lactate is one of the by-products derived from pyruvate; therefore, a decrease in the lactate production by deletion of the lactate forming gene (*ldhA*) leads to re-direction for pyruvate utilization, and increase of 2,3-butanediol and other metabolites. Moreover, the availability of NADH, a coenzyme for the synthesis of microbial products, increases by deletion of the *ldhA* [22].

Please cite this article in press as: S.J. Lee, et al., Process strategy for 2,3-butanediol production in fed-batch culture by acetate addition, J. Ind. Eng. Chem. (2017), http://dx.doi.org/10.1016/j.jiec.2017.07.008