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Co-production of 3-hydroxypropionic acid and 1,3-propanediol by *Klebseilla pneumoniae* expressing *aldH* under microaerobic conditions

Yanna Huang [a], Zhimin Li [a,*], Kazuyuki Shimizu [b], Qin Ye [a,*]

[a] State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai 200237, China
[b] Department of Bioscience and Bioinformatics, Graduate School of Computer Science and Systems Engineering, Kyushu Institute of Technology, Iizuka, Fukuoka 820-8502, Japan

HIGHLIGHTS

- *Klebseilla pneumoniae* expressing *E. coli aldH* was cultured under microaerobic conditions.
- Aeration enhanced 3-hydroxypropionate production but lowered 1,3-propanediol formation.
- The highest 3-hydroxypropionate was 48.9 g/L in 28 h at the aeration rate of 1.5 vvm.
- The specific formation rates of metabolites were compared.
- Carbon and redox balances were performed.

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ABSTRACT

Fed-batch cultures of *Klebsiella pneumoniae* expressing *Escherichia coli aldH* were performed under microaerobic conditions to investigate the effects on metabolites production. Increasing the aeration rate enhanced cell growth and 3-hydroxypropionic acid (3-HP) production, but reduced 1,3-propanediol (1,3-PDO) formation. The recombinant strain *K. pneumoniae*/pUC18*kan-aldHec* produced 48.9 g/L of 3-HP and 25.3 g/L of 1,3-PDO with an overall yield of 0.66 mol/mol in 28 h at an aeration rate of 1.5 vvm; however, under fully aerobic condition, no 3-HP and 1,3-PDO were produced due to the repression of *dha* operon. The flux through the reaction catalyzed by glycerol dehydratase and the split ratio of 1,3-PDO were negatively correlated with the aeration rate, even though the 3-HP level showed a positive trend. This study demonstrated that the relative amounts of 3-HP and 1,3-PDO can be controlled by the aeration rate.

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1. Introduction

1,3-Propanediol (1,3-PDO) is a bulk chemical widely used in cosmetics, foods, lubricants, medicines, and the polymer polytrimethylene terephthalate (PTT) (Deckwer, 1995). Another platform chemical, 3-hydroxypropionic acid (3-HP), also has a wide range of industrial applications for synthesis of such specialty chemicals as acrylic acid, methyl acrylate, propiolactone, malonic acid, acrylamide and so on. Both 1,3-PDO and 3-HP are classified in the third group of top value added chemicals that can be derived from biomass (Werpy and Petersen, 2004). Since the biodiesel industry produces a surplus of glycerol (Andrea et al., 2010; Forrest et al., 2010), conversion of this chemical to higher-value products is essential for economic reasons.

Klebsiella pneumoniae is a facultative anaerobic organism that produces 1,3-PDO from glycerol under anaerobic and microaerobic conditions (Ruch et al., 1974; Ruch and Lin, 1975). In the oxidative route, glycerol is dissimilated to pyruvate via dihydroxyacetone phosphate (DHAP) and NADH is formed. Pyruvate is further converted to acetate, lactate, ethanol and other compounds. In the reductive route, glycerol dehydratase (GDHt), a coenzyme B_{12}-dependent enzyme, converts glycerol to 3-hydroxypropionaldehyde (3-HPA), which is subsequently reduced to 1,3-PDO by 1,3-propanediol oxidoreductase (PDOR) concomitant with oxidation of NADH to regenerate NAD^+ (Zeng et al., 1996). When an NAD^+-dependent aldehyde dehydrogenase is over-expressed, 3-HPA is converted to 3-HP and the formed NADH can be used for 1,3-PDO production (Zhu et al., 2009), and the amount of byproducts from the oxidative route can be reduced.

GDHt is a critical enzyme for 1,3-PDO and 3-HP production. It is expressed under anaerobic conditions and undergoes inactivation by oxygen in the absence of substrate (Wanger et al., 1966). Although GDHt is sensitive to oxygen, some studies indicated that 1,3-PDO production was improved in microaerobic cultures (Chen et al., 2003). It was also reported that transcription of the *dha*

* Corresponding authors.
E-mail addresses: lizm@ecust.edu.cn (Z. Li), qye@ecust.edu.cn (Q. Ye).

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operon was not repressed but promoted under microaerobic conditions since the specific activity of GDHt at an aeration rate of 0.04 vvm was 59% higher than that in the absence of air supply (Wang et al., 2011).

The efforts aiming at improving 3-HP production based on the aldehyde dehydrogenase activity manipulation were also made. For example, a higher level of 3-HP (2.8 g/L) was achieved in a process composed of an aerobic phase (6 h) and a microaerobic phase (18 h) using *K. pneumoniae* over-expressing *aldA* from *Escherichia coli* in a 3-L bioreactor. The AldA activity was the highest in aerobic culture, followed by microaerobic culture, and the lowest in anaerobic culture (Zhu et al., 2009). Ashok et al. (2011) constructed a recombinant *K. pneumoniae* Δ*dhaT* (*puuC*) strain that produced 16.0 g/L of 3-HP and 16.8 g/L of 1,3-PDO with an overall yield of 3-HP plus 1,3-PDO of 0.51 mol/mol glycerol at an aeration rate of 1.0 vvm. Luo et al. (2011) reported that 6.8 g/L 3-HP and 22.7 g/L 1,3-PDO were produced in 48 h at an aeration rate of 0.5 vvm using a strain *K. pneumoniae* AK expressing an indigenous *aldH* gene encoding NAD^+-dependent aldehyde dehydrogenase. These examples suggest that microaerobic condition is favorable to 3-HP production.

We constructed a strain *K. pneumoniae*/pUC18*kan-aldHec* expressing *aldH* from *E. coli*, and the levels of 3-HP and 1,3-PDO reached 24.4 and 49.3 g/L, respectively, with an overall yield of 3-HP plus 1,3-PDO of 0.61 mol/mol based on consumed glycerol in a 5-L reactor under anaerobic condition (Huang et al., 2012). In the present study, fed-batch cultures were conducted at different aeration rates to investigate the effects on 3-HP and 1,3-PDO production.

2. Methods

2.1. Bacterial strain and media

K. pneumoniae/pUC18*kan-aldHec* expressing *aldH* encoding the γ-glutamyl-γ-aminobutyraldehyde dehydrogenase from *E. coli* K12 (Huang et al., 2012) was used and stored in 25% (w/w) glycerol at -20 °C.

The preculture medium contained (per liter): glycerol 20 g, yeast extract 3 g, citric acid 0.42 g, KH_2PO_4 2 g, K_2HPO_4 1.6 g, NH_4Cl 5.4 g, $MgSO_4{\cdot}7H_2O$ 0.2 g, and 1 mL of a trace elements solution ($FeCl_3$ $6H_2O$ 5 g, $MnCl_2{\cdot}4H_2O$ 2 g, $ZnCl_2$ 0.684 g, $CoCl_2{\cdot}6H_2O$ 0.476 g, $CuCl_2{\cdot}2H_2O$ 0.17 g, H_3BO_3 0.062 g, $Na_2MoO_4{\cdot}2H_2O$ 0.005 g, and 10 mL of concentrated HCl per liter). Fermentation was carried out in the same medium, but the glycerol and yeast extract concentrations were 40 and 2 g/L, respectively.

2.2. Fed-batch cultivation of K. pneumoniae/pUC18kan-aldHec

The inoculum was prepared in two 250-mL flasks each containing 100 mL of the preculture medium supplemented with 20 μg/mL of kanamycin. Each flask was inoculated with 1 mL of the stock culture and was incubated aerobically at 37 °C and 220 rpm for 12 h. The precultures were combined to inoculate 2.8 L of the fermentation medium in a 5-L bioreactor (BG-5, Baoxing Biotech Co., Shanghai, China). The fed-batch experiments were carried out at 37 °C, and the pH was controlled automatically at 6.8–7.0 with 5 M NaOH. For the fed-batch culture under microaerobic condition, sterile air was sparged at 0.1, 0.4, or 0.6 vvm with an agitation speed of 250 rpm, or at 1.0 or 1.5 vvm with agitation at 400 rpm. Fed-batch culture under fully aerobic condition was performed at an aeration rate of 1.5 vvm to keep the dissolved oxygen (DO) above 5% of air saturation by changing the agitation speed. As a control, anaerobic fermentation was performed by sparging nitrogen at 0.1 vvm with agitation at 400 rpm. A feed of glycerol solution (1.26×10^3 g/L) was added through a peristaltic pump to maintain the concentration between 10 and 20 g/L. When the fermentation was close to termination, feeding of glycerol was stopped to exhaust the residual glycerol. Samples were withdrawn periodically to determine cell mass and metabolites concentrations.

2.3. AldH expression and gel electrophoresis

SDS–PAGE was conducted on a 15% polyacrylamide gel using the method of Sambrook and Russell (2001) with a WH-300-LCD Protein Electrophoresis System (Yirui, Shanghai, China). Samples were removed at 10 h from fed-batch cultivations and centrifuged at 10,000*g* and 4 °C for 2 min for SDS–PAGE.

2.4. Analytical methods

Biomass was determined by measuring the optical density of appropriately diluted samples at 650 nm (OD_{650}) with a UV–visible spectroscopy system (Xinmao, Shanghai, China). The value of the optical density was converted to dry cell weight (DCW) by using a calibration equation (one unit of OD_{650} was equivalent to 0.284 g DCW/L).

The substrate and metabolites including glycerol, 3-HP, 1,3-PDO, ethanol, succinate, lactate, acetate and formate were determined by HPLC (LC-10AT, Shimadzu, Japan) equipped with an Aminex HPX-87H column (300 × 7.8 mm, Bio-Rad, USA) as well as refractive index (SPD-20A) and UV/vis (SPD-20AV) detectors. Culture supernatant obtained by centrifugation at 10,000g and 4 °C for 10 min and filtration through a 0.22 μm membrane filter was injected into and eluted from the column at 50 °C. The mobile phase was 2.5 mM H_2SO_4 at a flow rate of 0.5 mL/min. The residual glycerol concentration was monitored every 2 h by titration using $NaIO_4$ (for control of glycerol) and by HPLC (together with other metabolites). The 3-HP standard was purchased from Tokyo Chemical Industry Co., Japan, and other standard chemicals were obtained from Sigma.

Carbon balance was based on the carbon of total glycerol consumed and that contained in the end products. The fraction for cell growth was calculated assuming the elemental composition of $C_4H_7O_2N$ (Zeng et al., 1993), with an ash content of 3.6% (Ashok et al., 2011).

3. Results and discussion

As it was shown that 3-HP and 1,3-PDO can be produced simultaneously from glycerol using the recombinant *K. pneumoniae*/pUC18*kan-aldHec* under anaerobic condition (Huang et al., 2012), fed-batch cultivations were conducted at different aeration rates to examine the effect on the production of both metabolites.

3.1. Anaerobic fed-batch cultivation

The profiles of cell density, residual glycerol and metabolites concentrations in the anaerobic fed-batch culture are shown in Fig. 1. The average specific growth rate was 0.17 h^{-1} during the initial 4 h. The maximum cell density of 2.43 g/L was achieved at 15 h, and 16.6 g/L 3-HP and 38.4 g/L 1,3-PDO were produced with the yields of 0.14 and 0.39 mol/mol, respectively, at the end of the cultivation. The overall yield of 3-HP plus 1,3-PDO was 0.53 mol/mol, 15.1% lower than that obtained previously at a nitrogen rate of 0.4 vvm (Huang et al., 2012). This could be attributed to the oxygen introduced by the glycerol feed and the alkali solution for pH control and low K_La of the reactor at 0.1 vvm.

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