Bioresource Technology 102 (2011) 7177–7182

Contents lists available at ScienceDirect

Bioresource Technology

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

Use of glycerol for producing 1,3-dihydroxyacetone by *Gluconobacter oxydans* in an airlift bioreactor

Zhong-Ce Hu, Yu-Guo Zheng *, Yin-Chu Shen

Institute of Bioengineering, Zhejiang University of Technology, Hangzhou 310032, People's Republic of China

ARTICLE INFO

Article history:
Received 14 December 2010
Received in revised form 23 April 2011
Accepted 23 April 2011
Available online 29 April 2011

Keywords:
1,3-Dihydroxyacetone
Gluconobacter oxydans
Biodiesel
Glycerol
Airlift bioreactor

ABSTRACT

1,3-Dihydroxyacetone can be produced by biotransformation of glycerol with glycerol dehydrogenase from *Gluconobacter oxydans* cells. Firstly, improvement the activity of glycerol dehydrogenase was carried out by medium optimization. The optimal medium for cell cultivation was composed of 5.6 g/l yeast extract, 4.7 g/l glycerol, 42.1 g/l mannitol, 0.5 g/l K_2HPO_4, 0.5 g/l KH_2PO_4, 0.1 g/l $MgSO_4{\cdot}7H_2O$, and 2.0 g/l $CaCO_3$ with the initial pH of 4.9. Secondly, an internal loop airlift bioreactor was applied for DHA production from glycerol by resting cells of *G. oxydans* ZJB09113. Furthermore, the effects of pH, aeration rate and cell content on DHA production and glycerol feeding strategy were investigated. 156.3 ± 7.8 g/l of maximal DHA concentration with 89.8 ± 2.4% of conversion rate of glycerol to DHA was achieved after 72 h of biotransformation using 10 g/l resting cells at 30 °C, pH 5.0 and 1.5 vvm of aeration rate.

1. Introduction

Biodiesel, a viable fuel and a fossil diesel additive, has attracted much concern in recent years. In China, the research and development of biodiesel have an early start, and large-scale production of biodiesel started in early 21st century. The production capacity of biodiesel increased very quickly in recent years (Zhong et al., 2010). Glycerol is the byproduct in manufacturing biodiesel fuel by transesterification of vegetable oils or animal fats with methanol (Ma and Hanna, 1999). With the fast increasing of biodiesel production, the supply of glycerol will be in excess of demand, which leads to a glycerol price decline, and many glycerol plants will be closed. It is necessary to find new application and market of glycerol, some value-added chemicals produced from glycerol were developed, such as epichlorohydrin, 1,3-dihydroxyacetone (abbreviated as DHA), 1,3-propanediol, propionic acid, polyhydroxyalcanoate (da Silva et al., 2009; Pagliaro et al., 2007), erythritol (Rymowicz et al., 2009), citric acid (Rywinska et al., 2009), hydrogen (Sabourin-Provost and Hallenbeck, 2009), mannitol (Khan et al., 2009), lipid (Liang et al., 2010), mixed acid (Forrest et al., 2010), and eicosapentaenoic acid (Athalye et al., 2009).

In this work, we reported use of glycerol for producing DHA, which is a value-added product and a kind of active ingredient in sunless tanning skincare preparation, and used extensively in the cosmetic industry. Meanwhile, DHA is an important precursor for the synthesis of various fine chemicals and precursors of pharmaceuticals, and serves as a versatile building block for the organic synthesis of a variety of fine chemicals (Enders et al., 2005; Hekmat et al., 2003).

Many researches on DHA production from glycerol by *Gluconobacter oxydans* have been reported in recent years Hekmat et al., 2003, 2007; Hu et al., 2010a; Mishra et al., 2008). DHA can be manufactured by traditional fermentation over *G. oxydans* and by whole cell biotransformation of glycerol with *G. oxydans* (Hu and Zheng, 2009; Hu et al., 2010b; Mishra et al., 2008). The disadvantage of DHA fermentation is that the composition of fermentation broth is complex, which results in difficulty of DHA purification and crystallization, and increases the production cost. Furthermore, the production cost for DHA extraction, purification and crystallization is the main part of total cost. Thus, to limit the impurities in broth is essential for reduction of DHA production cost. On the other hand, the reaction mixture from biotransformation of glycerol to DHA using resting cells of *G. oxydans* has little impurities. Thus, it is much easier to extract and purify DHA from the reaction mixture relative to fermentation broth, and the production cost of DHA for biotransformation of glycerol to DHA will decrease dramatically. During the bioprocess of biotransformation of glycerol to DHA in *G. oxydans*, oxygen is required for the glycerol dehydrogenation. Since oxygen is employed as the final acceptor of electrons formed during the oxidation of glycerol (Claret et al., 1994; Wei et al., 2007a). In order to achieve high DHA production, it is necessary to prepare whole cells of *G. oxydans* with high

* Corresponding author. Fax: +86 571 88320630.
E-mail address: zhengyg@zjut.edu.cn (Y.-G. Zheng).

0960-8524/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2011.04.078

activity of glycerol dehydrogenase for biotransformation of glycerol to DHA (Wei et al., 2007b).

Airlift bioreactor, characterized by its simple design and the complete absence of mechanically moving internals, is a class of bioreactors where a region of gassed liquid is connected to a region of ungassed liquid, the difference in hydrostatic pressure between the two regions resulting in circulation of the liquid phase. It provides several advantages during operation and maintenance, such as high fluid circulation, mass and heat transfer, low shear stress, low energy consumption and low operating and maintenance costs. It has been widely applied in fermentation, biochemical reaction, and biological wastewater treatment bioprocesses (Kang et al., 2000; Klein et al., 2002; Sikula et al., 2007; Zheng et al., 2005, 2001). However, few literatures reported about DHA production by biotransformation in airlift bioreactors (Hu et al., 2010a). Due to oxygen requirement for biotransformation of glycerol to DHA and the characteristics of internal loop airlift bioreactor, the applicability and performance of the internal loop airlift bioreactor in DHA production from glycerol were investigated in this work.

The objective of this work is to produce DHA from glycerol by biotransformation in an internal loop airlift bioreactor. The bioprocess can be separated into two stages: firstly, cultivation conditions for *G. oxydans* cells growth were optimized by response surface methodology (RSM) for obtaining resting cells with high activity of glycerol dehydrogenase. Secondly, the bioprocess conditions of glycerol biotransformation to DHA were investigated in an internal loop airlift bioreactor.

2. Methods

2.1. Microorganism and medium

Gluconobacter oxydans ZJB09113, a mutant strain from *G. oxydans* ZJB-605 (CCTCC No. M208069) by ion beam implantation, was employed in this work, stored at 4 °C on GY agar slant, and transferred monthly.

The medium GY was composed of 25 g glucose, 5 g yeast extract, 20 g agar per liter.

The medium *M* was composed of 25 g mannitol, 5 g yeast extract, 3 g peptone, 0.5 g K_2HPO_4, 0.5 g KH_2PO_4, 0.1 g $MgSO_4{\cdot}7H_2O$, and 2 g $CaCO_3$ per liter.

The medium BM was composed of 0.5 g K_2HPO_4, 0.5 g KH_2PO_4, 0.1 g $MgSO_4{\cdot}7H_2O$, 2 g $CaCO_3$ per liter.

All media were autoclaved for 20 min at 121 °C.

2.2. Shaking flasks cultivation

Liquid seed was prepared by transferring cells from 24-h-old slant cultures into 500-ml flasks containing 30 ml of liquid medium *M*. Then, the flasks were incubated on a rotary shaker (XWW 25/450, the orbital diameter of 25 mm, Leshan Changzheng Pharmaceutical Machine Co., Ltd.) with 150 rpm for 24 h at 30 °C.

1.0 ml of seed culture was inoculated into 500-ml flasks containing sterile 30 ml liquid medium with different composition according to the RSM design. The flasks were incubated at 30 °C for 24 h on a rotary shaker with 150 rpm.

2.3. Experimental design for medium optimization

In order to optimize the cultivation conditions for obtaining resting cells with high glycerol dehydrogenase activity, response surface methodology (RSM) using a Box–Behnken factorial design with three factors and three replicates of central point was performed (Liu et al., 2008). Initial pH, yeast extract, carbon sources (glycerol and mannitol in a 1:9 mixture) were chosen as independent variables. The range and levels of experimental variables are presented in Table 1. And the other components of medium for RSM experiment were the same as that in BM medium.

The test factors were coded according to the following equation (Eq. (1)):

$$x_i = \frac{X_i - X_0}{\Delta X} \tag{1}$$

where x_i is the coded value of the independent variable, X_i is the natural value of independent variable, X_0 is the value of independent variable at the central point, and ΔX is the step change value.

The response variable (enzyme activity of glycerol dehydrogenase) was fitted by a quadratic model (Eq. (2))

$$y = b_0 + \sum b_i x_i + \sum b_{ii} x_i^2 + \sum b_{ij} x_i x_j \tag{2}$$

where y is the response variable, b is the regression coefficient, and x is the coded level of the independent variable.

The DPS software (Version 11.5, Refine Information Tech. Co., Ltd. Hangzhou, China) was used for regression and statistical analysis. Bonferroni *t*-test was used to detect the significant differences.

2.4. Resting cell preparation for biotransformation

Cell cultivation experiments were carried out in a 15 l fermentor with 10 l of the working volume (Biostat C. B. Braun Biotech International). Temperature, pH, DO concentration, airflow rate and agitation speed were measured on-line. The operation temperature and pH were controlled automatically at 30 °C and 4.9, respectively. The airflow rate was measured in units of vvm (volumes of air/effective volume of reactor/minute). The foam was controlled automatically by the addition of polyethylene glycol as antifoam agent. Agitation speed and airflow rate were controlled at 400 rpm and 0.8 vvm, respectively. The cells were harvested by centrifugation (10,000*g*, 10 min; Avanti J-E, Beckmen, USA), and then suspended by 0.02 M phosphate buffer for next step experiment. Liquid seed was prepared as mentioned above, and an inoculum ratio of 5% (v/v) was used in the bioprocess.

2.5. Bioreactor and bioprocess

Schematic diagram of the 2 l home-made airlift bioreactor with an internal loop is showed in Fig. 1. The temperature was controlled at 30 °C by water circulation in the outer jacket on the bioreactor. pH of the culture was controlled manually by adding 1.0 mol/l NaOH/HCl. The foam was controlled by the addition of polyethylene glycol as antifoam agent. The airflow rate was measured in units of vvm (volumes of air/effective volume of reactor/min), and controlled manually in the range of 0.5–2.5 vvm using a flow-meter with a needle valve. To compensate for the volume reduction due to evaporation, appropriate volume of sterile water was added to the bioreactor during the experiments.

Samples were withdrawn from airlift bioreactor for testing pH, DHA, and glycerol. Conversion rate of glycerol to DHA and DHA productivity were defined as follows, respectively.

Table 1
Experimental range and levels of the independent variables.

Variables	Coded symbols	Range and levels		
		−1	0	+1
Initial pH	x_1	4.00	5.00	6.00
Yeast extract concentration (g/l)	x_2	4.00	5.00	6.00
Carbon sources concentration (g/l)	x_3	20.00	40.00	60.00