Biochemical Engineering Journal 47 (2009) 127–131

Contents lists available at ScienceDirect

Biochemical Engineering Journal

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

Adsorptive bioconversion of ethylene glycol to glycolic acid by *Gluconobacter oxydans* DSM 2003

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ARTICLE INFO

Article history:
Received 23 December 2008
Received in revised form 6 June 2009
Accepted 25 July 2009

Keywords:
Gluconobacter oxydans
Glycolic acid
Ethylene glycol
Product inhibition
Integrated bioprocess
In situ product removal

ABSTRACT

An integrated bioprocess for the production of glycolic acid from ethylene glycol with *Gluconobacter oxydans* DSM 2003 and in situ product removal were investigated. A slight substrate inhibition was observed as substrate concentration was above 20 g/l and the product inhibition was much stronger. Bioconversion of glycolic acid is an end-product-inhibited reaction. In order to increase the productivity of glycolic acid and reduce the end-product inhibition of bioconversion, an adsorptive bioconversion for glycolic acid production from ethylene glycol catalyzed by resting cells of *G. oxydans* DSM 2003, was developed by using anion exchange resin D315 as the adsorbent for selective removal of glycolic acid from the reaction mixture. This approach allowed the yield of glycolic acid to be increased to 93.2 g/l, compared to 74.5 g/l obtained from a conventional fed-batch mode.

1. Introduction

Gluconobacter oxydans DSM 2003 strain is Gram-negative, aerobic, and rod-shaped acidophilic bacteria. Its unique capacity to incompletely oxidize polyol substrates has led to numerous production processes for the synthesis of compounds such as vitamin C, gluconic acids, dihydroxyacetone and vinegar. Nowadays, these processes are being further optimized whilst new processes for the synthesis of compounds such as L-ribulose, D-tagatose, miglitol, and chiral aldehydes and acids are being developed [1–4].

Glycolic acid is one of the most important fine chemicals, extensively used in adhesives, metal cleaning, textiles, leather processing [5], biodegradable polymers [6], and as a component in personal care product [7]. Ethylene glycol is one of the cheap starting materials for the production of glycolic acid through an oxidation reaction. Microbial conversion of ethylene glycol to glycolic acid was expected to be an attractive alternative method for the value-added production of glycolic acid with no by-production [8].

Although the production of glycolic acid by microbial means was very attractive, the inhibition of glycolic acid was a key limitation for industrial application. The end-product inhibition by glycolic acid resulted in several problems, in which the most important one was low concentration. Therefore, an integrated approach with separation of product in situ gained a lot of interest in bioconversion process. One possible approach was adsorptive bioconversion which involves bioconversion with in situ separation of glycolic acid. This in situ product removal (ISPR) could eliminate end-product inhibition and increase product yield, final product concentration and reactor productivity by keeping the product concentration below the inhibitory value [9,10].

The use of adsorbent resin system offered a simple way to remove products from an aqueous phase into a second solid phase [11,12]. Bioconversion of glycolic acid with in situ separation by anion ion-exchange resin involved considerably less operational and maintenance cost. Therefore, the present studies employed the anion exchange resin in the glycolic acid bioconversion.

The extreme pH was a problem in an adsorptive bioconversion process via anion exchange resin, because it was harmful to cells. To overcome this problem, the hollow-fiber membrane-based extraction which could provide nondispersive phase contact was used before the anion exchange resin extraction. With the membrane protection, cells could be protected from direct contact with extreme pH solution, and the toxicity could be avoided.

In this work, the bioconversion of ethylene glycol to glycolic acid using resting cells of *G. oxydans* DSM 2003 was evaluated in a membrane bioreactor. Anion exchange resins (D301, D315 and D345) were evaluated for glycolic acid extraction from aqueous solution. Suitable macroporous anion exchange resin was chosen and its application in the fed-batch bioconversion of ethylene glycol was studied, which led to an enhancement of glycolic acid yield.

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doi:10.1016/j.bej.2009.07.016

Table 1
Physical property of macroporous anion exchange resins.

Resins	Moisture (%)	Bulk density (g/ml)	Specific density (g/ml)	Particle size (mm)
D301	50–60	0.65–0.75	1.03–1.07	0.315–1.25
D315	47–57	0.68–0.80	1.07–1.12	0.315–1.25
D345	45–55	0.65–0.80	1.15–1.25	0.315–1.20

2. Materials and methods

2.1. Chemicals

Glycolic acid was purchased from Sigma–Aldrich. Ethylene glycol was purchased from Shanghai Chemical Reaction Co. Ltd. The macroporous anion exchange resins used were purchased from shanghai Huazhen Science and Technology Co. Ltd. (Shanghai, China). Detailed properties of these resins were shown in Table 1. The hollow-fiber membrane module (0.2 μm) was purchased from Tianjin MOTIMO Membrane Technology Ltd. (Tianjin, China). All other chemicals were of analytical grade and were commercially available.

2.2. Microorganisms and culture conditions

The strain *G. oxydans* DSM 2003 used for the bioconversion of ethylene glycol was grown in a liquid medium containing: 73.0 g/l sorbitol, 18.4 g/l yeast extract, 1.50 g/l $(NH_4)_2SO_4$, 1.52 g/l KH_2PO_4 and 0.47 g/l $MgSO_4{\cdot}7H_2O$. All media were sterilized by autoclaving at 115 °C for 20 min. Precultures were inoculated with a single cell colony from a Sorbitol Broth Agar plate and incubated in an orbital shaker (250 rpm, 28 °C) until late exponential growth phase (~22 h). The seed culture was repeated twice in order to adapt the culture to the fermentation environment. A 10% (v/v) inoculum of these cells was added to the fermentor and grown at 28 °C for 22 h.

2.3. Assay for the activity of the resting cells

The activity assay system contained 0.1 M buffer solution of potassium phosphate (pH 6.0), 20 g/l cells, and 20 g/l ethylene glycol. The reaction was carried out at 28 °C for 1 h. The amount of glycolic acid produced in the reaction mixture was assayed by high-performance liquid chromatography (HPLC). One unit of the activity of the resting cells was defined as the amount of enzyme, which converts 1 μmol of ethylene glycol to glycolic acid per minute under the assay conditions.

2.4. Substrate and product inhibition

Effect of initial substrate concentration on the activity of the resting cells was studied. Cells were harvested at the end of the exponential growth phase by centrifugation for 15 min at 7000 rpm. Cells were washed twice with physiological saline and resuspended in 0.1 M buffer solution of potassium phosphate (pH 6.0), then incubated in 28 °C after injection of ethylene glycol. The concentrations of ethylene glycol in reaction mixture were 10, 20, 40, 60, 80 and 100 g/l, respectively. After 1 h reaction, the reaction mixture was centrifuged at 12,000 × *g* for 10 min and the resulting supernatant was analyzed by HPLC.

Effect of initial glycolic acid concentration on the activity of the resting cells was also investigated. The glycolic acid was added in the beginning of the bioconversion. The initial concentrations of glycolic acid in reaction mixture were 0, 10, 20, 30, 40, 50, 60, 70 and 80 g/l, respectively. The initial concentration of ethylene glycol in reaction mixture was 20 g/l. After 1 h reaction, the reaction mixture was analyzed by HPLC.

2.5. Resins and its preparation

To evaluate the performance of adsorption system, three anion exchange resins which have high exchange capacity including D301, D315 and D345 were studied. Before use, the resins were soaked in 50–60 °C hot water several times to remove impurities. The following steps were involved in saturation. Firstly, the resin columns were treated with five times the volume of 1.0 M NaOH solution and then washed with deionized water to remove the alkali. Secondly, the resin columns were treated with five times the volume of 1.0 M HCl solution and then washed with deionized water to remove the acid. Finally, a repeat operation of the first step was done.

Exchange capacity of ion-exchange resins for glycolic acid was measured as follows: 10 ml 85 g/l glycolic acid solution was transferred into 50 ml flask. The pH value of the solution was adjusted to 5.0. The resin was added into the flask and shaken at 250 rpm for 1 h at 30 °C. The capacity of D315 at different pH value was also investigated. Five groups of experiments were performed, in which the pH value of glycolic acid solution was 2.0, 3.0, 4.0, 5.0 and 6.0, respectively. Adsorption was carried out at 250 rpm for 30 min at 30 °C. The effect of temperature on adsorbing glycolic acid by D315 resin was also studied. Concentration of glycolic acid in the aqueous phase was analyzed by HPLC.

2.6. Selection of washing and elution conditions

100 ml glycolic acid (80 mg/ml, pH 2.0) was loaded on a column (size 2.6 cm × 20 cm) packed with 60 g of wet resin. The loaded resin was taken out from the column and filtered. The amount of loaded glycolic acid on resin was 133.33 mg/g bases on material balance calculation. One gram of wet D315 resin was placed in each flask. Ten milliliters of different eluants with 1.0 M concentration of HCl, H_2SO_4, NaCl, $NH_3{\cdot}H_2O$ and NaOH were added into these test flasks, respectively. These flasks were shaken at 250 rpm in a shaking incubator for 1 h and concentration of glycolic acid in eluate was determined by HPLC.

2.7. Fed-batch bioconversion

The fed-batch bioconversion of ethylene glycol was carried out in a 3.7-l bioreactor (Bioengineering AG) with 2-l working volume. The pH was controlled at 5.4 by automatic addition of 4 M NaOH. The impeller speed was adjusted at 700 rpm and the aeration rate was controlled at 300 l/h. The fermentor pressure was controlled at 0.5 bar. The experiment was carried out in a fed-batch mode, and kept the concentration of ethylene glycol at approximately 20 g/l during the reaction. The original feeding rate was 3 g/(l h). The initial activity of the resting cells was 713.3 U/l in the reaction system. Samples were taken and analyzed at time intervals of 2–10 h.

2.8. Adsorptive bioconversion

The adsorptive bioconversion of glycolic acid was carried out in a 3.7-l bioreactor (Bioengineering AG) with 2-l working volume. The hollow-fiber membrane bioreactor was connected to a column (size 5.5 cm × 50 cm) packed with the anion exchange resin D315 for the glycolic acid separation. The description of bioreactor system was shown in Fig. 1. The impeller speed was adjusted at 700 rpm. The experiment was carried out in a fed-batch mode, by consecutively adding ethylene glycol to a concentration of approximately 20 g/l. The original feeding rate was 3.1 g/(l h). The initial activity of the resting cells was 730.5 U/l in the reaction system. The effluent of hollow-fiber module (flow rate 10 ml/min) was recycled back to the reactor. The flow rate through the resin column was 5 ml/min. The operations of the hollow-fiber module and the resin column were