Bioresource Technology xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Bioresource Technology

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

Production of sodium gluconate from delignified corn cob residue by on-site produced cellulase and co-immobilized glucose oxidase and catalase

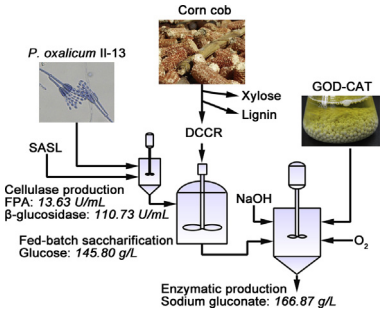
Xiaolong Han [a], Guodong Liu [a], Wenxia Song [b], Yinbo Qu [a,*]

[a] State Key Laboratory of Microbial Technology, Shandong University, Jinan 250100, China
[b] School of Life Science, Qufu Normal University, Qufu 273165, China

HIGHLIGHTS

- On-site production of cellulase was performed for lignocellulose saccharification.
- 145.80 g/L glucose was obtained by fed-batch saccharification of DCCR.
- 166.87 g/L SG was produced by co-immobilized GOD-CAT under optimal conditions.
- The co-immobilized GOD-CAT could being reused at least 6 times.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 26 April 2017
Received in revised form 13 June 2017
Accepted 14 June 2017
Available online xxxx

Keywords:
Cellulase
Sodium gluconate
Immobilized enzyme
Lignocellulose

ABSTRACT

The production of sodium gluconate by enzymatic catalysis of delignified corn cob residue (DCCR) hydrolysate was studied. *Penicillium oxalicum* I1-13 was used for the production of cellulase with high β-glucosidase activity. A fed-batch saccharification process was developed to obtain high yields of glucose. At the end of hydrolysis, the concentration of glucose reached 145.80 g/L. Glucose oxidase and catalase were co-immobilized to catalyze DCCR hydrolysate to produce sodium gluconate. Under the optimum conditions, 166.87 g/L sodium gluconate was obtained after 56 h of reaction, with a yield of 98.24%. The immobilized enzymes could still maintain more than 60% of the activity after repeated use for 6 times. This study provides a potential route for the production of valuable chemicals by enzymatic conversion of lignocellulosic materials.

1. Introduction

Lignocellulosic materials as inexpensive feedstocks can be converted into a variety of products, such as ethanol, butanol, 2,3-butanediol, organic acids, etc., through enzymatic hydrolysis and microbial fermentation (Menon and Rao, 2012; Sindhu et al., 2016). As a substitute for starch or glucose used as raw materials in fermentation industry, lignocellulose hydrolysate often contains substances that inhibit microbial growth (Nichols et al., 2008; Palmqvist and Hahn-Hagerdal, 2000). Therefore, the fermentation period is often longer than those using starch or glucose, which greatly limits the application of lignocellulose in fermentation industry. Bio-ethanol is currently one of the few cases getting close to industrialization of lignocellulose bioconversion (Qu, 2007; Santos et al., 2016).

Sodium gluconate (SG) has a wide range of industrial applications. It can be used as a chelating agent, surface cleaning agent of steel or glass, high-efficiency retarder and superplasticizer in

* Corresponding author.
E-mail address: quyinbo@sdu.edu.cn (Y. Qu).

http://dx.doi.org/10.1016/j.biortech.2017.06.109
0960-8524/

the concrete industry, etc. (Ma et al., 2015; Ramachandran et al., 2006). The only difference in the structure of gluconic acid from that of glucose is the carboxyl group instead of aldehyde group at C-1 position. Gluconic acid can be produced by the fungus *Aspergillus niger* with glucose as raw material (Ramachandran et al., 2006). The glucose oxidase system in *A. niger* is capable of specific oxidation of glucose to gluconic acid, and does not produce other miscellaneous acids (Ramachandran et al., 2006; Wong et al., 2008). *A. niger* has been used to produce gluconic acid from dry dilute acid pretreated corn stover hydrolysate, and the high efficiency of the crude product as cement additive was evidenced (Zhang et al., 2016b). In addition, the bacterium *Gluconobacter oxydans* was also used for fermentative production of gluconic and xylonic acids from corn stover hydrolysate (Zhang et al., 2016a, 2017).

Despite the wide use of microbial fermentation for gluconic acid production, two enzyme preparations, glucose oxidase (GOD) and catalase (CAT), are sufficient to effectively catalyze the production of gluconic acid from glucose *in vitro* (Wong et al., 2008). GOD catalyzes the oxidation of glucose to gluconic acid and produces hydrogen peroxide. Hydrogen peroxide has a negative effect on the activity of glucose oxidase. Therefore, the addition of CAT which catalyzes the decomposition of hydrogen peroxide to water and oxygen can eliminate its inhibitory effect on and provide oxygen for glucose oxidase. In the reaction process, sodium hydroxide solution is slowly added to the reaction system in order to maintain the suitable pH environment, protect enzyme activity, and to finally ensure the formation of SG.

In this study, enzymatic conversion of delignified corn cob residue (DCCR) to SG was explored. Crude cellulase produced by *Penicillium oxalicum* was used for high-solids saccharification of DCCR. The hydrolysate was then converted to SG by co-immobilized GOD and CAT. Compared with fermentation method, this enzymatic route for SG production does not require the detoxification of substrate and the culture of fermenting microorganisms, thus resulting an improvement in productivity.

2. Materials and methods

2.1. Cellulase production

2.1.1. Strain

Cellulase was produced by *P. oxalicum* I1-13 (Yao et al., 2016). I1-13 is a cellulase high-producing strain that uses *bgl2* promoter to drive BGL1 overexpression in strain RE-10 (*gpdA(p)-clrB-ptra*; Δ*bgl2*::*hph*; Δ*creA*::*bar*). Compared to RE-10, I1-13 has significantly improved production of β-glucosidase, which makes a great contribution to DCCR hydrolysis.

2.1.2. Cellulase fermentation

Cellulase fermentation was performed using the stirring fermentor BioFlo 310 (New Brunswick, USA). The *P. oxalicum* I1-13 spores kept in 30% (v/v) glycerinum vials were inoculated on slant and cultured for 5 days at 30 °C (Han et al., 2017), followed by another 5 day-culture at 30 °C on a new wheat bran extract slant. Spores on the slant were collected by 30 mL normal saline (0.85% NaCl, 1‰ Triton X-100, w/v) and inoculated into 100 mL seed medium in 500 mL Erlenmeyer flasks at 10^6 spores/mL. The seeds were cultured for 48 h at 200 rpm and 30 °C.

The BioFlo 310 fermentor (New Brunswick, USA) with a volume of 7.5 L was filled with 4.5 L fermentation medium and sterilized for 30 min at 121 °C. The fermentor was inoculated with adding 10% (v/v) of the seed culture, with agitation speed and temperature set to 300 rpm and 30 °C, respectively. Aeration rate varied in different stages, with 0.4 VVM in the first 12 h, 0.6 VVM from 12 to 24 h and 0.8 VVM after 24 h. Within the first 10 h of fermentation, spent ammonium sulphite liquor (SASL) from wheat straw pulping process (Tranlin Group, Shandong, China) was added at a continuous flow rate of 0.1 mL/L/min. The flow rate doubled every 10 h and was kept at 3.2 mL/L/min from 50 h to 100 h. Samples were collected every 12 h during the fermentation process. After finishing the fermentation at 168 h, the fermentation broth was centrifuged for 20 min at 9000 rpm and 4 °C, and its pH was adjusted to 4.8 by adding 1 M pH 4.8 citric acid-sodium citrate buffer solution. The resulting crude cellulase preparation was kept in refrigerator at 4 °C.

The seed medium contained 10 g/L DCCR, 10 g/L wheat bran, 10 g/L peptone, 10 g/L glucose, 2 g/L $(NH_4)_2SO_4$, 3 g/L KH_2PO_4, and 0.5 g/L $MgSO_4{\cdot}7H_2O$.

The fermentation medium contained 20 g/L DCCR, 6 g/L microcrystalline cellulose, 46.5 g/L wheat bran, 10 g/L soybean cake powder, 2 g/L $(NH_4)_2SO_4$, 2.79 g/L $NaNO_3$, 1 g/L urea, 3 g/L KH_2PO_4, and 0.5 g/L $MgSO_4{\cdot}7H_2O$.

2.2. Enzymatic saccharification of DCCR

The substrate DCCR was provided by Shandong Longlive Biotechnology Co., Ltd. (Shandong, China). DCCR is the residue from xylose production with corncob, and most lignin was removed by alkaline extraction (Zhao et al., 2008). DCCR was crushed into uniform particles (<200 meshes) for saccharification, and the composition was analyzed as previously described (Tan et al., 2013). The saccharification was first carried out in 300 mL Erlenmeyer flasks with a reaction volume of 100 mL, while the substrate concentration and cellulase loading varied as indicated in the text. Sodium citrate buffer solution (pH 4.8) was added at 0.05 M and 1‰ (w/v) NaN_3 was used as the bacteriostatic agent. The temperature for saccharification was 50 °C and the rotating speed was 200 rpm. The scale-up of saccharification was accomplished in the BioFlo 310 fermentor at 50 °C with an agitation speed of 100 rpm, and the fermentor with a volume of 7.5 L was filled with a reaction volume of 5.0 L. Instead of adding NaN_3, steam sterilization of substrate was performed before the addition of enzyme. pH was maintained at 4.8 by feedback control using 0.1 M HCl and 0.1 M NaOH.

2.3. Preparation of co-immobilized GOD and CAT

Commercial GOD (NS28166) and CAT (NS28196) were provided by Novozymes (China). Polyvinyl alcohol (PVA) and sodium alginate (SA) were used for co-immobilizing GOD and CAT (Idris et al., 2008). PVA with a polymerization degree of 1750 ± 50 and SA with a viscosity of 0.02 Pa.s in 10 g/L aqueous solution at 20 °C were purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). PVA and SA solutions of different concentrations were prepared with distilled water by simultaneous heating at 85 °C and stirring. The solutions were cooled to 30 °C, added with different amounts of GOD and CAT solutions (enzyme activity ratio = 1:100), thoroughly mixed with a magnetic stirrer, and then let stand for 1 h. Then, the solution was injected into saturated boric acid solution which contained 1.5% (w/v) $CaCl_2$ by using a 50 mL injector. After the cross-linking reaction in refrigerator at 4 °C for 4 h, the immobilized enzyme particles were rinsed several times with distilled water. Finally, the particles were dehydrated by absorbent paper and stored in refrigerator at 4 °C.

2.4. Enzyme assays

2.4.1. Cellulase activities

Samples were taken every 12 h and centrifuged at 4 °C for 10 min, and the supernatants were collected and suitably diluted.

Please cite this article in press as: Han, X., et al. Production of sodium gluconate from delignified corn cob residue by on-site produced cellulase and co-immobilized glucose oxidase and catalase. Bioresour. Technol. (2017), http://dx.doi.org/10.1016/j.biortech.2017.06.109