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Short communication

Improving the performance of cell biocatalysis and the productivity of xylonic acid using a compressed oxygen supply

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ABSTRACT

Xylonic acid is useful and producing it in bacteria cost-effectively would be good because of potential applications and high yield. Production in bacteria like *Gluconobacter oxydans* is hampered by low xylose utilization and poor bacterial tolerance to contaminants. Here we exploited the oxygen-dependence of NAD^+ regeneration and the lack of gas release during xylose metabolism in *G. oxydans* to develop a high-oxygen tension bioreactor with increased productivity. In this design we maintained gas outlets closed, which eliminated all bubbling and media foaming, and added a compressed pure oxygen inlet, which increased oxygen tension. Biocatalysis of xylose in this bioreactor yielded 3 times higher (586.3 g/L xylonic acid) than the best previous output. Moreover, we directly produced 143.9 g/L of xylonic acid from the diluted sulfuric acid pre-hydrolysates of corn stover without a detoxification process and at 1.0 g/L/h volumetric productivity. The central features of this bioreactor design are scalable and thus would enable cost-competitive bacterial xylonic acid production.

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1. Introduction

Thus far, the efficient utilization of xylose remains a critical bottleneck for the commercialization of lignocellulosic biorefineries products [1]. As a result, new technologies and products must be developed in order to achieve economic feasibility. D-Xylonic acid (XA) has been identified as a valuable platform chemical. This chemical is ranked in the top 30 high-value chemicals by NREL and PNNL and has the potential to be used in a wide variety of applications including as a chelator, as a dispersant of concrete, a precursor of 1,2,4-butanetriol, a retardant for oil wells and so on [2]. XA and xylonate can be produced in laboratories by enzymatic [3], electrochemical or chemical oxidation. However, these methods are inaccessible for industrial production because of their pollution and cost. Fortunately, the bioconversion of xylose to XA has a promising future because of its high efficiency [4,5].

In this study, the performance of whole-cell biocatalysis was tested and compared in Erlenmeyer shaken flasks and in an aerated stirred tank reactor (ASTR) (Fig. 1). In nature, the metabolism of xylose in *Gluconobacter oxydans* is a close-coupling biooxidation reaction of the dehydrogenation and the cellular respiration chain that depends heavily on the oxygen supply. D-Xylose is first oxidized to release and form xylono-δ-lactone, an equilibrium form of xylonate, by the catalysis of xylose dehydrogenase which is bound to cytoplasmic membrane. The electrons stored in the coenzyme (NADH/NADPH) were transferred forwards to the oxygen which was the terminal electron acceptor by a series of transporter complexes that constitute the respiration chain [6–8]. Specifically, oxygen is a final driving force that runs the serial oxidation reactions that maintain a continuous bioconversion process for catalyzing xylose to XA. Therefore, a new method of compressed oxygen supply in a sealed aerated stirred tank reactor (COS–SSTR) (Fig. 1) was presented and investigated. Additionally, the foaming issue due to strong aeration could be alleviated.

2. Materials and methods

2.1. Microorganism

G. oxydans NL71 from the strain ATCC 621 and obtained from Nanjing Forestry University of China, was maintained in colonies on sorbitol agar (sorbitol 50 g/L, yeast extract 5 g/L, agar 15 g/L) at 4 °C.

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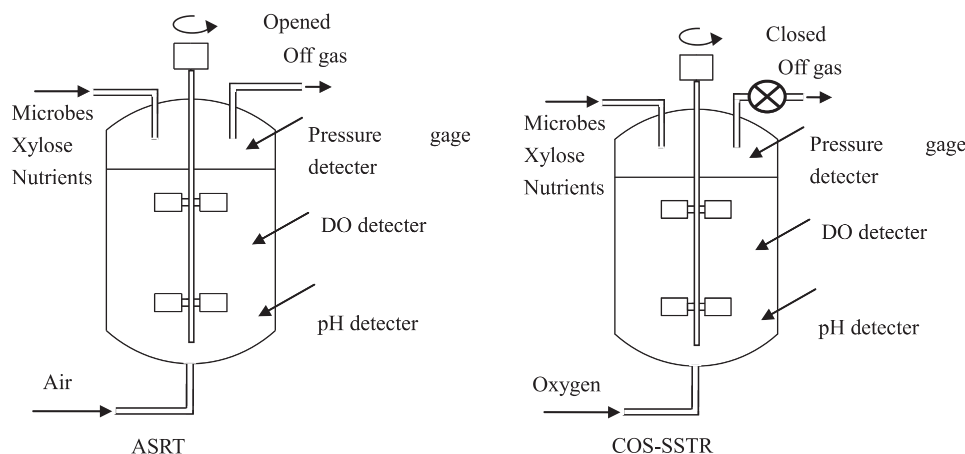

Fig. 1. Diagram of the ASRT and COS–SSRT designs.

2.2. Media and culture conditions

The inocula of *G. oxydans* NL71 were prepared in 250-mL Erlenmeyer shake flasks containing 50 mL media (sorbitol 100 g/L, yeast extract 10 g/L) and cultured for 24–36 h at 220 rpm and 30 °C. For large-scale proliferation, the inocula were proliferated in a 3.0-L ASTR (New Brunswick Gelligen 115) containing 1.0 L of broth at 30 °C and 500 rpm and with airflow of 3 vvm. The pH was stabilized at 5.5–6.5 by the automatic addition of 10% NaOH. The foaming was automatically controlled by adding the defoamer polyether–ether–ketone. The cell pellet was harvested at a density of approximately 2.0 g/L by centrifugation at 5000 rpm for 10 min.

The cell biocatalysis medium: 5.0 g/L yeast extract, 0.5 g/L $MgSO_4$, 1.0 g/L KH_2PO_4, 2.0 g/L K_2HPO_4 and 5.0 g/L $(NH_4)_2SO_4$. In total, 5 g glucose per 100 g xylose was used as the xylose-cofactor in pure xylose [9]. The cell biocatalysis was performed identically to proliferation conditions in Erlenmeyer shaken flasks and in a 3.0-L ASTR. The sodium hydroxide solution was substituted with calcium carbonate powder to control the pH. In addition, pure oxygen was also used to supply oxygen in a COS–SSTR, which was connected to an oxygen cylinder (purity ≥ 99.9%) at a gas inlet pressure of 0.02–0.05 MPa. Because of the neutralization reaction between $CaCO_3$ and newly generated Xylonic acid in COS–SSTR, we adjusted the oxygen inlet valve and opened the reaction system every 6–12 h.

Lignocellulosic pre-hydrolysates were prepared from corn stover that was pretreated with 1% (w/v) sulfuric acid at 150 °C for 30 min. Before whole cell biocatalysis, the pre-hydrolysates were concentrated by vacuum evaporation and neutralized to pH 5.5–6.5 using $CaCO_3$ powder. Then, the solid precipitate was discarded by centrifugation at 6000 rpm for 10 min.

2.3. Analytical methods

Sugar (arabinose, galactose, glucose, xylose and mannose) and sugar acids (xylonic acid (XA); gluconic acid (GluA); arabonic acid (AraA); galactonic acid (GalA); and mannonic acid (ManA)) were simultaneously determined using high performance anion-exchange chromatography coupled with pulsed amperometric detection (Dionex ICS-3000) with a CarboPac™ PA10 column [10]. Inhibitors, including acetic acid, formic acid, levulinic acid, furfural and 5-hydroxymethyl-furfural, were determined using high performance liquid chromatography (Agilent 1260) equipped with an Aminex Bio-Rad HPX-87H column, with 5 mM H_2SO_4 at 0.6 mL/min as the mobile phase.

The yield of XA was calculated as follows: the product of XA, divided by the total xylose loading, multiplied by the constant of 0.934. The volumetric productivity of XA was calculated from the concentration of XA divided by the reaction time.

3. Results and discussion

3.1. Cell biocatalysis performance in shaken flasks

With batch operations, a certain amount of xylose can be completely catalyzed to XA without further decomposition by the cell biocatalysis of *G. oxydans* in the Erlenmeyer shaken flasks [5,8]. However, the yield and the volumetric productivity significantly decreased when the initial xylose was over 350 g/L (Fig. 2). In contrast, the whole cell biocatalysis performance was improved greatly, by over two-fold, even at the total substrate loading of 450 g/L xylose when we used fed-batch operation. The results show that the cell catalytic capacity was suppressed in the high xylose loading medium. It is likely that oxygen transport was limited in the Erlenmeyer shaken flasks or that the high concentration of xylose would impose inhibitory effects on oxygen dissolving and on the cell biocatalysis of *G. oxydans*. When xylose is used as a raw material, the whole cell biocatalysis performance of XA yield

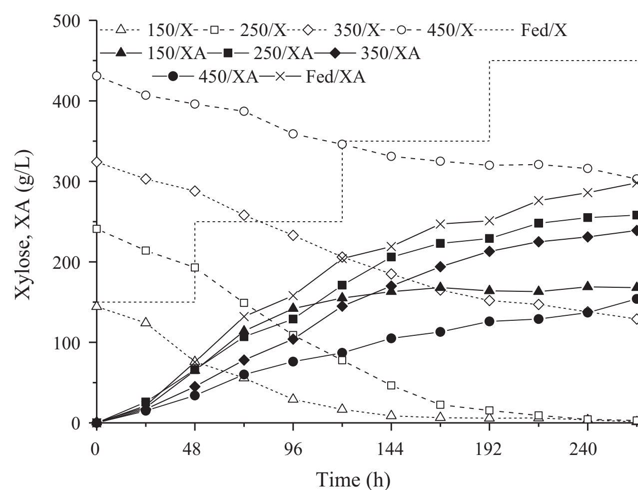

Fig. 2. Whole cell biocatalysis kinetics in Erlenmeyer shaken flasks. In total, 50 mL medium was added to 250-mL Erlenmeyer shaken flasks. X, xylose; XA, xylonic acid. Fed, fed-batch operation, with three batch additions of 100 g/L xylose and 20 g/L $CaCO_3$, at 24, 96, 168 h.

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