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Enantioselective oxidation of racemic 1,2-propanediol to D-(−)-lactic acid by *Gluconobacter oxydans*

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Abstract—*Gluconobacter oxydans* DSM 2003 was firstly used in the production of (*R*)-2-hydroxy-propionic acid through microbial oxidation of racemic 1,2-propanediol. The biotransformation was processed with high enantiomeric excess (>99%) and near theoretical yield (48% of racemic 1,2-propanediol) when the substrate concentration was lower than 20 g/L. When the substrate concentration was increased, maintaining the pH at 6.0 helped to improve the enantioselectivity.

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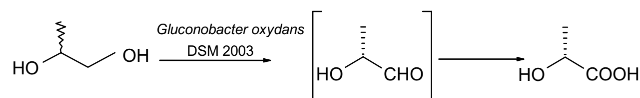
1. Introduction

Biotransformations have many useful advantages over chemical synthesis, such as chemo-, regio- and enantioselectivity under mild conditions. Whole microbial cells are often used when the reaction needs co-factors and the system of co-factor regeneration. The oxidation of sugars, alcohols and acids sometimes employs *Gluconobacter oxydans*, which is used industrially in the production of vitamin C.[1] The organism has several dehydrogenases while the co-factor recycle system involved in the metabolism, can be characterized by incomplete oxidation in which partially oxidized organic compounds are accumulated as end-products.[1–3] Moreover, they can be employed for the enantioselective oxidation of different racemic primary alcohols for the production of enantiomerically pure carboxylic acids.[4,5] The mechanisms of the enantioselectivity brought about by the quinoehaemoprotein dehydrogenases have recently been proposed.[6] The dehydrogenation of 2-methylbutanol, 2-phenylpropanol and other racemic primary alcohols with *G. oxydans* has recently been studied and the reaction conditions optimized.[4,7,8]

1,2-Propanediol is a cheap commercial product with great potential for microbial processes, since it is water-soluble and nontoxic. Herein, we report the production of (*R*)-2-hydroxy propionic acid [D-(−)-lactic acid] with *G. oxydans* from racemic 1,2-propanediol as the raw material. D-(−)-Lactic acid is a valuable chiral building block for the asymmetric synthesis, for example D-amino acids. It is also involved in the synthesis of poly-lactic acid, which is the material of biodegradable plastics.[8] The industrialized production of lactic acid is from the fermentation of lactic acid bacteria with the products mainly being L-(+)-lactic acid: So far there has been no report about the production of D-(−)-lactic acid from 1,2-propanediol employed acetic acid bacteria.



2. Results

G. oxydans DSM 2003 were selected from the screening of several acetic acid bacteria. The selection was based on the oxidation activity of 1,2-propanediol. Sorbitol was chosen as the optimal carbon source. The highest concentration of biomass was achieved after 24 h (Fig. 1).

Biotransformations were then carried out in phosphate buffers with the addition of 1,2-propanediol and resting cells. D-Lactic acid was produced by the oxidation of (*R*)-1,2-propanediol with the highest yield being up to

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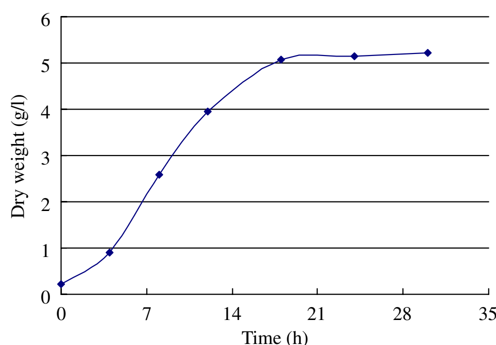

Figure 1. Growth of *G. oxydans* DSM 2003.

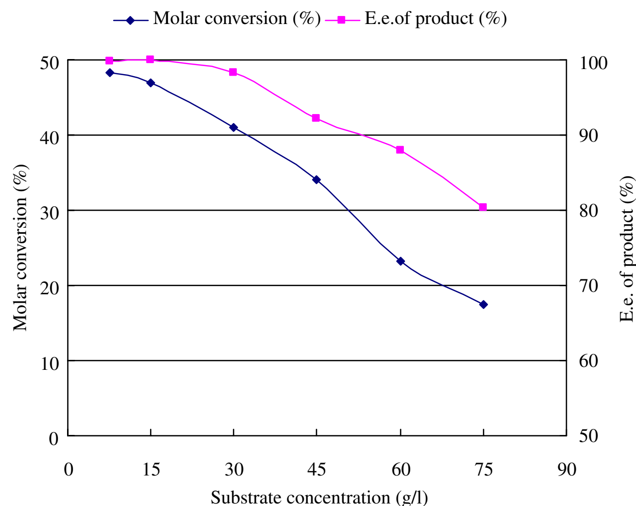

Figure 2. The different molar conversion and enantioselectivity of biotransformation at different 1,2-propanediol concentrations with *G. oxydans* DSM 2003.

48% (based on racemic 1,2-propanediol) in 20 h. After that, there was no racemization or further consumption of D-lactic acid in the reaction system.

The accumulation of D-lactic acid was not due to the consumption of L-lactic acid. This can be proven by the observation that when enantiomerically pure L-lactic acid was added to the reaction system, it did not decrease even after 24 h.

In previous reports of dehydrogenation with alcohol dehydrogenases from acetic acid bacteria along with reviews of the *Gluconobacter* strains, the optimal reaction conditions were ranged with pH's 6.0–6.5 and at temperatures 28–30 °C with few exceptions; changes within this narrow range did not significantly influence the reaction.[4,7–9] Therefore, initial conditions for the oxidation were chosen at pH 6.0 at 28 °C. We studied the influence of substrate concentration on the molar conversion and enantio-selectivity under these conditions (Fig. 2).

From the results it can be seen that the molar conversion and enantiomeric excess (ee) of D-lactic acid were at their best when the concentration of 1,2-propanediol was lower than 20 g/L, while they were reduced when the substrate concentration increased. Some papers have attributed the drop of enantioselectivity to the simultaneous actions of different dehydrogenases, which display different enantioselectivities.[10,11] The most notable impact on the reaction of high substrate concentration was a drop in pH. The reaction times with or without pH adjustment (Fig. 3) showed that maintaining the pH at 6.0 improved the enantioselectivity. This is possibly because the lower pH influences the dehydrogenases on the selectivity of different configurations. However the yield of D-lactic acid did not markedly improve on pH adjustment, which needs further approaches such as semi-continuous addition of the substrate or the immobilization of the cells.[12]

3. Conclusion

The synthetic monomer D-(−)-lactic acid was obtained via the enantioselective oxidation of racemic 1,2-pro-

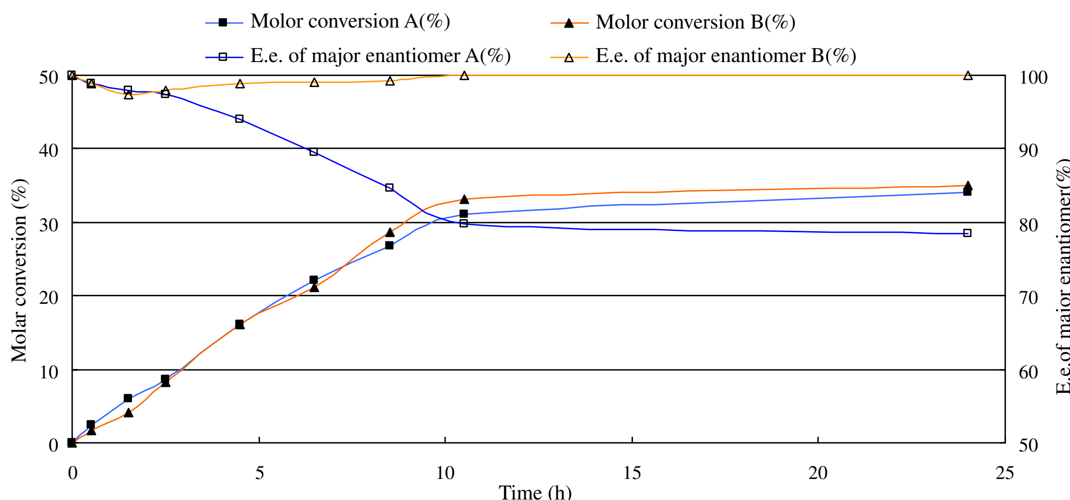

Figure 3. Biotransformation of 1,2-propanediol at the concentration 35 g/L. The reactions were either performed without pH adjustment (A) or with the pH being maintained at 6.0 (B).

panediol employed *G. oxydans* DSM 2003 under mild conditions. The biotransformation occurred with high molar conversion (98% of D-(−)-1,2-propanediol) and high enantioselectivity (ee 100%) when the substrate concentration was lower than 20 g/L. When the substrate concentration was higher, a decrease in pH in the oxidation reaction was shown to influence the enantioselectivity. The maintenance of pH improved the selectivity (ee from 80% to 100%). As a result, we have shown an approach for D-(−)-lactic acid production that is technically and economically interesting.

4. Experimental

4.1. Microorganism, growth and biotransformation conditions

The *G. oxydans* DSM 2003 strains were from our own collection. For the highest growth of organism, several media were tested with the best being chosen: Y-S medium (pH 6.0), consisted of 8 g of sorbitol, 2.4 g of yeast extract and other factors. The organisms were incubated at 28 °C with shaking at 250 rpm for 24 h. The cultivated cells were collected by centrifugation at 8000 rpm for 8 min. The cells were washed twice with distilled water and then dried at 110 °C for 24 h for determining the dry weight. Biotransformations were directly carried out with growing cells or with cells centrifuged and suspended in phosphate buffers (0.1 M) at various pH values. Different concentrations of substrates were added directly to the suspensions and flasks shaken at 250 rpm and incubated at 28 °C, which were the optimal conditions based on a previous report of biotransformations using *Gluconobacter* strains.

4.2. Analytical methods

The production of lactic acid was routinely determined by HPLC analysis using a ZORBAX SB-AQ column (Agilent Technologies, USA) and an aqueous acidic solution (H_3PO_4, 1%) as the eluent in 1 mL/min. Samples were taken at intervals, brought to pH 1.0 by the addition of HCl (1 M) and extracted with an equal volume of $CHCl_3$. The enantiomeric composition was also determined by HPLC analysis using an SUMICHIRAL OA-5000 column (Sumika Chemical Analysis, Japan) and the eluent composed with 2 mM copper sulfate in water/2-propanol (95/5, v/v). The retention times of the enantiomers were 7.151 min (L-lactic acid) and 9.105 min (D-lactic acid). The absolute configuration of the obtained acid was determined by the comparison of the specific rotation of authentic samples of the enantiomerically pure compounds. The specific rotational value of the product is $[\alpha]_D^{20} = -1.8$ (*c* 8, H_2O). The stereochemical outcomes of the biotransformations were expressed as enantiomeric excesses (ee's) of the major enantiomer.

Acknowledgements

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