Chinese Chemical Letters Vol. 13, No. 12, pp 1213 – 1216, 2002
http://www.imm.ac.cn/journal/ccl.html

Biocatalytic Synthesis of Pyruvate from *DL*-lactate with Enzymes in *Pseudomonas sp.*

Jing Song GU[1, 2]*, Ping XU[1], Yin Bo QU[1]

[1]State Key Laboratory of Microbial Technology, Shandong University, Jinan 250100
[2]The school of Chemistry and Environmental Engineering, Jinan University, Jinan 250002

Abstract: A novel method of preparing pyruvate from *DL*-lactate catalyzed by enzymes from a bacterial strain of *Pseudomonas sp.* SM-6 was proposed. Catalytic processes of cell-free extract enzymes and immobilized enzymes were evaluated. The kinetic data were studied, too.

Keywords: Pyruvate, *DL*-lactate, biocatalyst, enzyme, *Pseudomonas*.

Pyruvate has received increasing attention in recent years as a potential precursor for the synthesis of L-amino acids[1]. Compared with other small non-chiral building blocks, pyruvate is relatively expensive. Conventional processes for preparing pyruvate include a process comprising reacting sodium cyanide and acetyl chloride to synthesize acetyl cyanide and hydrolyzing the acetyl cyanide, and a process comprising reacting tartaric acid and potassium hydrogen sulfate. These processes not only start with expensive raw materials but attain low yields[2]. Pyruvate formation by fermentation does not lead to high volumeric yield, either. Also the yield and the concentrations of pyruvate in fermentation are often not very high. In addition, being a quite active intermediate, pyruvate is difficult to accumulate in cells[3]. But pyruvate can be formed by extracellular biocatalytic processes. Both lactate dehydrogenase (LDH) and lactate oxidase (LOD) could transform lactate into pyruvate[4]. What should be emphasized is that racemic as well as stereochemically pure forms of lactate are much cheaper than pyruvate. In this study, enzymes from a bacterial strain of *Pseudomonas sp.* SM-6 was employed as catalyst for pyruvate production.

Exprimental

Pseudomonas sp. SM-6 was inoculated into lactate medium (50 mL in 300-mL conical flasks, containing 1% sodium *DL*-lactate, inoculating volume ratio was 1%). Cultivation was carried out at 30°C with shaking at 150 rpm. After 24 h to 48 h, the cells were harvested by centrifugation (10000 r/min, 15 min, 4°C), then washed twice with 20 mL, 67 mmol/L phosphate buffer (pH 7.4) or 10 mmol/L imidazole/HCl buffer

* E-mail: gujingsong@life.sdu.edu.cn

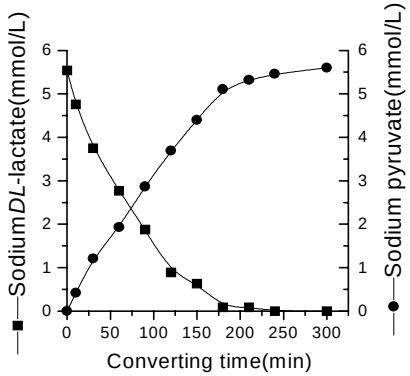
(pH 7.0) followed by resuspension in the same buffer. The next step involved disruption of the cells using ultrasonic oscillator (Sonic & Material Inc., Danbury CT. USA) for 5 min at 0°C. After disruption, the cell extracts were further centrifuged (12000 r/min, 15 min, 4°C), the supernatant was collected and used as an enzyme source. Crude enzymes protein was assayed by method of Bradford test[5]. The lactate concentrations were measured by SBA-40C lactate analyzer (The Academy of Science in Shandong Province, China). Pyruvate estimations were carried out spectrophotometrically as the 2,4-dinitrophenylhydrazone derivative[6]. Crude enzymes were immobilized with 2% calcium alginate.

Results and Discussion

Conversion curve of the biocatalyst

As shown in **Figure 1**, crude enzymes from *Pseudomonas sp.* SM-6 is a potential biocatalyst for pyruvate preparation. After the liquid crude enzymes were mixed with sodium *DL*-lactate and phosphate buffer, *DL*-lactate was rapidly transferred into pyruvate. The conversion reaction was carried out in total volume of 10 mL, in pH 7.4, at 37°C. Within 3 h, 70 mg/L of crude enzymes protein (final concentration), can convert 5.54 mmol/L (final concentration) of *DL*-lactate to pyruvate with yield of 92.0%, which corresponds to a space time yield of 40.8 mmol/L·d or about 4.49 g/L·d (sodium pyruvate). After 5 h, it can transform >99% *DL*-lactate (5.54 mmol/L) into pyruvate. Converting rate was almost constant in the first 2 h.

Figure 1 Converting curve of crude enzymes of SM-6



Reaction was carried out in a volume of 10 mL containing 70 mg/L of crude enzymes protein and 33 mmol/L phosphate in pH 7.4, at 37°C

The mechanism of lactate dehydrogenase (LDH) and lactate oxidase (LOD) to convert lactate to pyruvate are different[4] as shown in Eq.1 and in Eq.2.

$$\text{Lactate} + NAD^+ \leftrightarrow \text{Pyruvate} + NADH + H^+ \quad \text{(Eq.1)}$$

$$\text{Lactate} + O_2 \leftrightarrow \text{pyruvate} + H_2O_2 \quad \text{(Eq.2)}$$

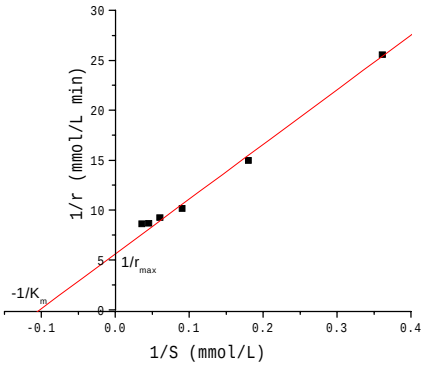
Expensive pyridine nucleotides (NAD^+) are required for the LDH reaction. LOD, as a flavoprotein, directly catalyzes the formation of pyruvate from lactate without requiring NAD^+ as a cofactor. Thus lactate oxidase would be very useful for the formation of pyruvate on a preparative scale. To determine what kind of enzyme mainly acts in the biocatalysis, we dialyzed crude enzymes against phosphate buffer to remove small molecules including NAD^+. According to the dialysis results (**Table 1**), a majority of LOD activity still remained after dialysis. So it was confirmed that the formation of pyruvate from lactate was catalyzed mainly by lactate oxidase rather than lactate dehydrogenase.

Table 1 Variation of catalytic rate after dialysis

	The amount of pyruvate formed by each mg of crude enzymes protein in the first 10 min (mg/mg)
Before dialysis	1.479
After dialysis	1.078

Kinetic Study of the biocatalyst

Figure 2 Linear relationship between 1/r and 1/S



Crude enzymes protein concentration: 70 mg/L, *DL*-lactate concentration varied from 2.8 mmol/L to 28 mmol/L

Initial catalytic rate increased when we enhanced the substrate concentration (*DL*-lactate). And we also found that, when initial substrate concentration varied from 2.8 mmol/L to 11 mmol/L, there was linear relationship between reciprocal of catalyzed rate (1/r) and reciprocal of substrate concentration (1/S) (**Figure 2**). The reaction kinetic equation was as follows:

$$1/r = 55.3 \cdot 1/S + 5.8$$

It was coincident with Linweaver and Burk equation.

$$1/r = K_m/ r_{max} \cdot 1/S + 1/ r_{max}$$

r: catalyzed rate (mmol/L·min) r_{max}: maximum catalyzed rate (mmol/L·min)

S: substrate concentration (mmol/L) K_m: Michaelis constant (mmol/L)

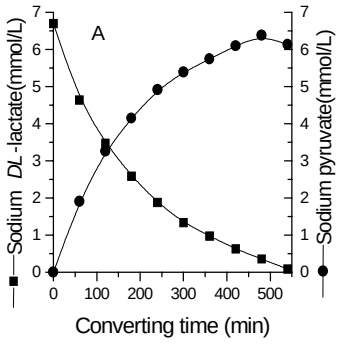
K_m and r_{max} was 9.53 mmol/L and 2.46 μmol/L·min·mg crude enzymes protein (0.17 mmol/L·min·70mg crude enzymes protein), respectively.

Higher concentrations than 16.6 mmol/L (1/S = 0.06 L/mmol) of *DL*-lactate caused the substrate inhibition of the enzyme activity.

Conversion of DL-lactate to pyruvate with immobilized biocatalyst

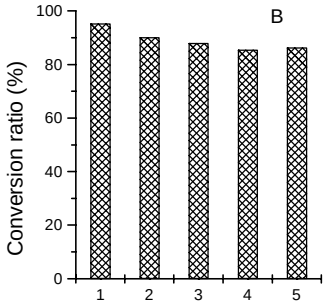
Immobilized crude enzymes of SM-6 with alginate still had relatively strong ability on substrate conversion. 150 mg /L of crude enzymes protein (concentration in total volume) could transform 95.3% of 6.7 mmol/L *DL*-lactate into pyruvate within 8 h (**Figure 3A**). And the immobilized crude enzyme with this method also indicated fine stability. Conversion ratio still could reach 85% after reused four times (**Figure 3B**).

Figure 3A Converting curve of immobilized SM-6 crude enzymes



Catalytic reactions were carried out with 150 mg/L of immobilized crude enzymes in pH 7.4 phosphate buffer at 37°C

Figure 3B Reusing of immobilized SM-6 crude enzymes with 6.7 mmol/L initial substrate concentration



Each converting time: 8 h

Acknowledgment

This work was supported by the National Natural Science Foundation of China (No. 39970020).

References

1. T. Tsujino, S. Ohigashi, H. Hayashi, *J. Molec. Catal.* , **1992**, *71*, 25pp.
2. M. Imanari, Pat. US Tokyo JP, 5,225,593 6 Jul. **1993,** 1pp.
3. H. Yanase, N. Mori, M. Masuda, *J. Ferment. Bioeng.* , **1992**, *73*, 287pp.
4. M. Dixon, E. C. Webb, *Enzymes*, 3rd Edition, Longman Group Ltd, London, **1979**, 220pp, 243pp.
5. M.M.Bradford, *Anal. Biochem.* , **1976**, *72*, 248pp.
6. C.T. Walsh, A.Schonbrunn, R.H.Abeles, *J. Biol. Chem.* , **1971**, *246*, 6855pp.

Received 13 March, 2002