Lactic Acid Production from 1,2-Propanediol by Jar Culture and Resting Cells of *Arthrobacter oxydans*

Osami YAGI and Yasuji MINODA*

The National Institute for Environmental Studies, Tsukuba-gun, Ibaraki

**Department of Agricultural Chemistry, Faculty of*

Agriculture, The University of Tokyo, Tokyo

Received September 6, 1978

Arthrobacter oxydans (PG21-1) which was isolated from soil preferably assimilated 1,2-propanediol and produced remarkable amount of lactic acid. Cultural conditions for lactic acid production from 1,2-propanediol were studied using a 15 liter jar fermentor. Optimum pH and urea concentration for the production of lactic acid were 8.0 and 0.3%, respectively. Under these conditions, the yield of lactic acid was 17.8 g/liter in 7 day cultivation. About 70% of the utilized 1,2-propanediol was converted to lactic acid. The production of lactic acid by resting cells was also investigated. Resting cells of *Arthrobacter oxydans* showed a high activity to produce lactic acid at alkaline pH and the amount of the acid reached to 10.2 g/liter in 12 hr incubation. The accumulation of lactic acid at alkaline side was considered to due to the strong suppression of the degradation of lactic acid.

In the previous paper,¹⁾ it was reported that *Arthrobacter oxydans*, which was isolated from soil, produced lactic acid from 1,2-propanediol and that pH and urea were the effective factors to promote the accumulation of lactic acid.

Lactic acid is used in various fields, such as food, drink, leather and textile industries. Moreover, lactic acid is thought to be useful as a monomer of synthetic substances because of having two functional groups in the molecule. At present, lactic acid is made by chemical synthesis or bacterial fermentation. However, the price is fairly high. 1,2-Propanediol is one of the petrochemicals and a relatively cheap and safe material. Therefore, lactic acid production from 1,2-propanediol by microorganisms is of great advantage.

In this paper, optimal conditions of lactic acid production from 1,2-propanediol using a jar fermentor and the accumulation of lactic acid by resting cells were investigated.

MATERIALS AND METHODS

Microorganism. *Arthrobacter oxydans* PG21-1, which was isolated from soil by screening procedures as described in the previous paper¹⁾ was used throughout this investigation.

Medium. The composition of basal medium was as follows: 1,2-propanediol 30.0 g, urea 3.0 g, K_2HPO_4 1.0 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, KCl 0.5 g, $FeSO_4 \cdot 7H_2O$ 0.01 g, yeast extract 0.5 g and tap water to make 1 liter.

Cultivation. Inoculum was prepared as follows: A loopful of cells grown on 1,2-propanediol agar slant for 2 days was inoculated to 50 ml of basal medium in a 500-ml reciprocal flask and incubated for 36 hr at 30°C. Main cultivation was carried out in a 15-liter jar fermentor containing 8 liters of liquid medium with an agitation speed of 500 rpm and aeration of 8 liters per min at 30°C. pH was continuously controlled with 5 N NaOH and 4 N HCl.

Preparation of resting cells. Cells grown at 30°C for 36 hr in basal medium were harvested by centrifugation at $10,000 \times g$ for 10 min and washed two times with 1/15 M potassium-sodium phosphate buffer (pH 7.0). The cells thus obtained were suspended in various kinds of buffer and used as resting cell suspension.

Lactic acid production by resting cells. The activity of resting cells for lactic acid production was assayed by measuring the amount of the acid produced in the reaction mixture containing 5 ml of resting cell suspension, 40 ml of 0.5 M buffer and 5 ml of 1,2-propanediol after incubation for definite time at 30°C on a shaker.

Oxygen uptake experiment. Warburg flask containing 0.1 ml of cell suspension (2 mg dry cells), 0.3 ml of substrate solution, 1.6 ml 1/15 M various buffer and 0.3 ml of 20% KOH solution (in center well) in a total volume 2.3 ml were incubated at 30°C with shaking

and oxygen uptake of resting cells was measured manometrically.

Determination of cell growth, 1,2-propanediol and lactic acid. Cell growth was determined by the optical density at 660 nm. 1,2-Propanediol was measured by gas chromatography using a Hitachi Gaschromatograph K-53 with flame ionization detector. Conditions of gas chromatography was as follows: Stainless steel column (1 m long, 3 mm i.d.) was packed with Chromosorb 101. Column and injection temperature were 170°C and 210°C respectively. Carrier gas was N₂ and flame gas was a mixture of H₂ and air. Lactic acid was determined with the same way as mentioned in the previous paper.¹⁾

1,2-Propanediol. 1,2-Propanediol was obtained from Junsei Chemical Co., Ltd. and used without further purification.

RESULTS

Effect of pH on lactic acid production

In the previous paper,¹⁾ it was found that initial pH was one of the most important factors on lactic acid production by a reciprocal flask. This time, effect of pH was studied using a jar fermentor with pH control apparatus. pH of the culture medium was kept at various levels by adding appropriate amount of 5 N NaOH and 4 N HCl. In order to clarify the cultural characteristics, accumulation of lactic acid, cell growth and consumption of 1,2-propanediol were investigated throughout the cultivation.

Figure 1 shows the time course of lactic acid production using a 15-liter jar fermentor. pH was not controlled. Cells grew exponentially for 60 hr after a short lag and the maximum cell concentration reached to about 4.5 g/liter. The accumulation of lactic acid increased in proportion to the cell growth and continued after the cell growth stopped. The maximum amount of lactic acid was about 5.1 g/liter. pH of the culture broth rose to 9.0 initially but pH was lowering after 30 hr. These results were almost the same as those of the cultivation using a 500 ml reciprocal flask reported in the previous paper.¹⁾

Figure 2 shows the time course of lactic acid production when pH was adjusted to 7.0. The

FIG. 1. Time Course of Lactic Acid Production. ●—●, lactic acid; ○—○, growth; △—△, 1,2-PD.

FIG. 2. Lactic Acid Production by Jar Fermentor. Cultivation was carried out at pH 7.0. ●—●, lactic acid; ○—○, growth; △—△, 1,2-PD.

maximum cell concentration was obtained after 45 hr cultivation. The growth rate and final cell concentration were higher than those of the cultivation of which pH was not controlled. But the accumulation of lactic acid was almost the same. Most of 1,2-propanediol was consumed after 85 hr cultivation, then 240 g of 1,2-propanediol was added at that time (shown by arrow) and the cultivation was continued. As shown in Fig. 2, the addition of 1,2-propanediol caused the increase in lactic acid accumulation but the cells were scarcely increased.

Figure 3 shows the time course of lactic acid production at pH 8.0. The cell growth became maximum in 70 hr but lactic acid production increased linearly until 1,2-propanediol

FIG. 3. Lactic Acid Production by Jar Fermentor. Cultivation was carried out at pH 8.0. ●—●, lactic acid; ○—○, growth; △—△, 1,2-PD.

was consumed completely.

Effect of concentration of urea on lactic acid production

In general, accumulation of organic acid is closely related to concentration of nitrogen source.²⁾ In this fermentation, urea as nitrogen source was suitable for lactic acid production and cell growth. Therefore, effect of concentration of urea on lactic acid production using a 15 liter jar fermentor was studied. pH of culture broth was controlled at 8.0 and the concentration of 1,2-propanediol was about 3% in all cases.

Figure 4 shows effect of urea concentration on lactic acid production. Under the condition of 0.03% urea, the cell growth and the

FIG. 4. Effect of Urea Concentration on Lactic Acid Production.

Cultivation was carried out for 6 days using a 15 liter jar fermentor. pH of culture medium was kept at 8.0. ●—●, lactic acid; ○—○, growth.

accumulation of lactic acid were both depressed. However, in the case of 0.05% urea, the cell growth was abundant but the production of lactic acid was low. The best concentration of urea was 0.3%.

Lactic acid production by resting cells

Effect of pH on lactic acid production by resting cells grown on 1,2-propanediol medium is shown in Fig. 5. Lactic acid was most abundantly accumulated at pH 8.0 in 12 hr incubation and the amount of lactic acid reached to 10.2 g/liter. However, in 48 hr culture the accumulation of lactic acid was maximum at initial pH of 9.5 and at that time the pH of culture was decreased to 8.0. In the alkaline side, the accumulation of lactic acid markedly increased but in the acidic side, the accumulation of lactic acid remarkably decreased.

FIG. 5. Lactic Acid Production by Resting Cells.

Concentration of resting cells in the reaction mixture was 9.0 g/liter. pH 5.0, 6.0, 7.0, phosphate buffer; pH 8.0, 8.5, 9.0, 9.5, Tris buffer. Incubation time: ■, 12 hr; □, 48 hr.

Table I shows the lactic acid production by resting cells cultivated with various carbon sources. 1,2-Propanediol was the best carbon source for the preparation of resting cells. Resting cells grown on bouillon medium had a relatively high activity to produce lactic acid.

O₂ Uptake for 1,2-propanediol and lactic acid by resting cells

In order to elucidate effect of pH on lactic acid production, O₂ uptake for 1,2-propanediol and lactic acid by resting cells was studied.

TABLE I. LACTIC ACID PRODUCTION BY RESTING CELLS GROWN ON VARIOUS CARBON SOURCES

Resting cells grown in basal medium containing 1% of each carbon source instead of 1,2-propanediol were used. Incubation was carried out using 0.5 M phosphate buffer (pH 8.0). Resting cell concentration of the reaction mixture was 4.8 g/liter. 1,2-Propanediol concentration was 3%.

Carbon sources	Lactic acid (g/liter)	
	incubation time 6 hr	24 hr
Meat extract	0.25	1.68
Glucose	0.47	0.93
Mannose	0.23	0.36
Sucrose	0.10	0.03
Sorbitol	0.29	0.50
1,2-Propanediol	1.20	3.50

Figure 6 shows effects of pH on O_2 uptake for 1,2-propanediol. The maximum O_2 uptake was found at pH 7.0 in 2.25% of 1,2-propanediol. When 0.75% of 1,2-propanediol was used, O_2 uptake was almost the same in the range of pH 6.0 to 9.0, and O_2 uptake in 2.25% of 1,2-propanediol was higher than in 0.75%.

FIG. 6. Effect of pH on Oxygen Uptake for 1,2-Propanediol. pH 5.0, 6.0, 7.0, phosphate buffer; pH 8.0, 8.5, 9.0, Tris buffer; pH 9.5, borate buffer. Concentration of 1,2-PD: ●—●, 2.25%; ○—○, 0.75%.

Figure 7 shows effect of pH on O_2 uptake for lactic acid. O_2 uptake was much affected by the concentration of lactic acid and pH. In the high concentration of lactic acid, the optimum pH for O_2 uptake was 7.0 but in the low concentration, the optimum pH for O_2 uptake was 5.0.

FIG. 7. Effect of pH on Oxygen Uptake for Lactic Acid.

Concentration of Na-lactate: ○—○, 2.25%; ●—●, 0.75%; △—△, 0.13%.

DISCUSSION

Arthrobacter oxydans which was isolated from soil preferably assimilated 1,2-propanediol and produced remarkable amount of lactic acid. As shown in Figs. 2 and 3, high growth was observed but lactic acid was not much accumulated at neutral pH while at alkaline side the rate of cell growth became slow but lactic acid was accumulated abundantly. The most favourable pH for lactic acid production was 8.0. It is interesting that an organic acid is produced in a large quantity in alkaline condition.

Urea was the most favourable nitrogen source for lactic acid production. The optimum urea concentration was 0.3%. Usually, low concentration of nitrogen source is favorable for the production of organic acid. But in this cultivation, much more amount of urea than that required for the sufficient cell growth was necessary for lactic acid production. The reason was not clarified.

Under the most favorable conditions for lactic acid production using a 15 liter jar fermentor, the amount of lactic acid reached to 17.8 g/liter in 7 day cultivation and the consumed 1,2-propanediol was 26.0 g/liter. Therefore the yield of lactic acid from 1,2-propanediol was about 70%.

Lactic acid was produced associating with

the cell growth in early phase and the acid production continued after the stopping of cell growth. If the cultivation is carried out under the favorable conditions of the cell growth in early phase and then under the favorable conditions of lactic acid production in later phase, the production rate of lactic acid is expected to be extremely high.

As shown in Fig. 5, resting cells of *Arthrobacter oxydans* possessed a high ability of lactic acid production and the production by resting cells was much affected by pH similarly to that by growing cells. Though buffer action of 0.5 M buffer was not sufficient to keep the pH of the reaction mixture constant, the optimum pH for lactic acid production was 8.0 and the amount of lactic acid was 10.2 g/liter in 12 hr incubation. The production rate was 0.095 g/hr/g dry cell. This rate was about 2 times higher than that of growing cells. However, the decomposition of lactic acid was observed in a long period of incubation. Since the activity of lactic acid production by resting cells is stable for a long time and the degradation of

lactic acid is inhibited completely, the use of resting cells is very advantageous.³⁾

To clarify the reason of lactic acid accumulation at the alkaline side, O₂ uptake for 1,2-propanediol and lactic acid was studied. O₂ uptake for 1,2-propanediol was not much affected by pH and the concentration. However, O₂ uptake for lactic acid extremely decreased at higher pH than 8 in any concentrations. The accumulation of lactic acid at alkaline side was considered to be owing to the strong suppression of the degradation of lactic acid. The optimum pH for O₂ uptake on lactic acid changed with the concentration of lactic acid. The reason is not obvious.

REFERENCES

- 1) O. Yagi and K. Yamada, *Agric. Biol. Chem.*, **33**, 1587 (1969).
 - 2) T. Kodama, U. Kotera and K. Yamada, *J. Ferment. Technol.*, **49**, 93 (1971).
 - 3) R. Uchio and I. Shiio, *Agric. Biol. Chem.*, **36**, 1169 (1972).
-