Efficient production of L-lactic acid from corn straw hydrolysate

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Abstract. In this study, L-lactic acid was produced from corn straw hydrolysate by the strain Lactobacillus sp. L47, the fermentation conditions such as nitrogen sources, mineral salts, metal ions, incubation periods, pH values and temperatures were investigated, and the optimal incubation conditions were obtained: corn straw hydrolysate of 243.1 ml/l, (NH4)2SO4 10 g/l, yeast extract 10 g/l, KH2PO4 0.5 g/l, ZnSO4•7H2O 0.3 g/l, MgSO4•7H2O 0.1 g/l, MnSO4•H2O 0.01 g/l, CaCO3 60 g/l, initial medium pH 6.0, controlled fermentation pH 6.0, 20 ml of seed, incubation temperature 46 ℃. Under the optimal fermentation conditions, the highest L-lactic acid production was 99.8 g/l in 40 h.

1. Introduction
L-lactic acid (2-hydroxypropionic acid, CH3CHOHCOOH) is a kind of organic acid which is most widely used in food, food related applications, pharmaceuticals and cosmetics [1, 2]. Lactic acid is also found to be an important commodity chemical, an intermediate for alkyl lactates, propylene glycol, propylene oxide, acrylic acid, and poly (lactic acid) production [3]. Furthermore, lactic acid-based biopolymers are also of high interest, and the demand is continuously increasing in these years. Lactic acid is usually produced by fermentation from carbohydrates such as sugars or starch, which is produced from food materials. However, as for China, food materials are relatively short due to the huge population, and the fermentable alternatives should be found for the chemical's production.

China is a leading agricultural nation, cellulosic resources including corn straw, corn cob, wheat straw, wheat bran, rice straw are very rich, and these feedstocks could be used for bio-chemicals or bio-energy production after pretreatment [4-6]. The biomass is difficult for the microorganisms directly utilization and pretreatment processes are important for lactic fermentation. Li et al. reported that wheat straw hydrolysate was used for lactic acid production by Rhizopus oryzae, and the yield could reach 92.5 g/l after optimization [7], but the production still very lower for lactic acid production in fermentation industry.

Corn straw is almost the most agricultural waste in China (more than 2 hundred million tons) [8], which should be used for high value products production. However, many fermentation inhibitors (eg.
5-hydroxymethylfurfural, formic acid, acetic acid, vanillic aldehyde and etc.) were found in the hydrolysate prepared from biomass, and these toxic substances play a key role in the bioconversion of lignocellulose. In this study, L-lactic acid was produced from the carbon source corn straw hydrolysate, and the culture medium and fermentation conditions were optimized, and the high yield of L-lactic acid was reached.

2. Materials and methods

2.1. Microorganism
*Lactobacillus* sp. L47, stored at 4°C on a MRS medium (peptone 10 g/l, meat extract 8 g/l, yeast extract 4 g/l, glucose 20 g/l, sodium acetate • 3H₂O 5 g/l, Tween 80 1 g/l, dipotassium hydrogen phosphate 2 g/l, triammonium citrate 2 g/l, magnesium sulfate • 7H₂O 0.2 g/l, manganese sulfate • 4H₂O 0.05 g/l, agar 15 g/l, pH 6.2.), slant, and sub cultured every three months.

2.2. Corn straw hydrolysate
The corn straw hydrolysate was prepared from corn straw with cellulase in the laboratory, and the steps were described as follows: 1) Pretreatment: The corn straw was ground, then the powder was sieved and the particle sizes \( \leq 1 \) mm were collected; 2) HCl treatment: The pretreated materials were treated with 6% HCl at 90°C for 1 h, then washed by water and adjusted to pH 4.8-5.0; 3) Cellulase hydrolysation: The powder of corn straw was hydrolyzed with complex cellulase (20-30 FPU/g dry mass, solid-liquid ratio was 1 : 20) at 50°C for 48 h then the liquid was collected and concentrated by a rotary evaporator, and the corn straw hydrolysate was obtained. The main sugars such as glucose, xylose, and arabinose in the hydrolysate were determined by a high-performance liquid chromatography (Agilent 1100 system; Agilent Technologies, USA; Aminex HPX-87P analytical column, 300×7.8 mm; BioRad, USA). The final concentrations of the hydrolysate used in this study were adjusted (concentrated by a rotary evaporator or diluted by distilled water) for the experiments utilization in this study.

2.3. Culture
*Lactobacillus* sp. L47 strain on the MRS medium slant was transferred to the 250 ml Erlenmeyer flask contains 50 ml of seed culture medium (containing of peptone 10 g/l, meat extract 8 g/l, yeast extract 4 g/l, glucose 20 g/l, sodium acetate • 3H₂O 5 g/l, Tween 80 1 g/l, dipotassium hydrogen phosphate 2 g/l, triammonium citrate 2 g/l, magnesium sulfate • 7H₂O 0.2 g/l, manganese sulfate • 4H₂O 0.05 g/l, pH 6.2.), the strain incubated at 42°C for 24 h in a biochemical incubator (SPX-250B, Shanghai Boxun Industry & Commerce Co. Ltd. Medical Equipments Factory, China), and the seed was obtained. And then 20 ml of seed was added to 1000 ml basal fermentation medium (containing of corn straw hydrolysate 243.1 ml (150 g sugars), (NH₄)₂SO₄ 3 g/l, ZnSO₄ • 7H₂O 0.2 g/l, NaH₂PO₄ 0.2 g/l, MgSO₄ • 7H₂O 0.08 g/l, CaCO₃ 60 g/l, pH 5.0) and incubated at 42°C for 48 h in the biochemical incubator.

2.4. Effect of the medium components and incubation conditions on the L-lactic acid production
10 g/l inorganic nitrogen sources such as (NH₄)₂SO₄, NH₃HCO₃, (NH₄)₂CO₃, NH₄NO₃, NH₄Cl, 10 g/l organic nitrogen sources including soybean meal, castor bean meal, soybean protein powder, cotton seed meal, peptone, groundnut meal, corn steep, yeast extract were evaluated for L-lactic acid by the strain L47. 0.4 g/l mineral salts such as K₂HPO₄, Na₂HPO₄, KNO₃, KCl and NaCl, metal ions such as Mg²⁺ (0.01 g/l, 0.05 g/l, 0.1 g/l), Mn²⁺ (0.01 g/l, 0.03 g/l, 0.05 g/l), Zn²⁺ (0.1 g/l, 0.3 g/l, 0.5 g/l), Cu²⁺ (0.01 g/l, 0.03 g/l, 0.05 g/l), Ba²⁺ (0.01 g/l, 0.03 g/l, 0.05 g/l), K²⁺ (0.01 g/l, 0.03 g/l, 0.05 g/l) and Co²⁺ (0.01 g/l, 0.03 g/l, 0.05 g/l) were also estimated for L-lactic acid production. Different initial pH values (4.0, 5.0, 6.0, 7.0, 8.0), controlled pH values (5.0, 5.5, 6.0, 6.5, 7.0), fermentation periods (10 h, 20 h, 30 h, 40 h, 50 h), incubation temperatures (20°C, 30°C, 40°C, 50°C and 60°C) were investigated in the L-lactic acid production by the strain L47. In these experiments, the total sugar of the corn straw hydrolysate was 150 g/l.
2.5. Analytical methods
Sugar (glucose, xylose and arabinose) concentration was determined with a high performance liquid chromatography system (Agilent 1100, Agilent Technologies, USA), and the L-lactic acid was measured by a SBA-40E Biosensor (Biology Institute of Shandong Academy of Sciences, China).

3. Results and discussion

3.1. Sugars in the corn straw hydrolysate

Table 1. Sugars in the corn straw hydrolysate.

| Glucose (g/l) | Xylose (g/l) | Arabinose (g/l) | Total (g/l) |
|--------------|-------------|----------------|-------------|
| 424          | 172         | 21             | 617         |

The glucose, xylose, arabinose and total sugars in the corn straw hydrolysate were listed in Table 1. Glucose was the main sugar in the corn straw hydrolysate, which was 68.7% of the total sugar, and xylose was 27.9% of the total sugar. The amount of glucose and xylose were the main fermentable sugars in the corn straw hydrolysate. In order to produce L-lactic acid, the straw hydrolysate was concentrated and the total sugars was 61.7%.

3.2. Effect of inorganic nitrogen sources on the L-lactic acid production

The effect of inorganic nitrogen sources (NH$_4$)$_2$SO$_4$, NH$_3$HCO$_3$, (NH$_4$)$_2$CO$_3$, NH$_4$NO$_3$, NH$_4$Cl on the L-lactic acid production by the strain L47 was shown in Figure 1. It could be found that the strain L47 could grow on these inorganic nitrogen sources, and the highest L-lactic acid production was of when (NH$_4$)$_2$SO$_4$ was used as inorganic nitrogen sources, and the L-lactic acid yield was 72.4 g/l, which was much higher than the basal medium (the L-lactic acid production was 65.2 g/l).

![Figure 1. Effects of the inorganic nitrogen sources on the L-lactic acid production](image-url)
3.3. Effect of organic nitrogen sources on the L-lactic acid production

The effect of soybean meal, castor bean meal, soybean protein powder, cotton seed meal, peptone, groundnut meal, corn steep, yeast extract on the L-lactic acid production by the strain L47 were estimated in the experiments and the results were summarized in Figure 2. And it could be concluded that peptone, corn steep liquid and yeast extract were better than the others in this study for the L-lactic acid by the strain L47, and yeast extract was the best organic nitrogen source because there were much more amino acids, minor metals or vitamins in it, which could apply more nutrients to the strain L47 in the fermentation processes, and the L-lactic acid yield could reach 76.7 g/l.

3.4. Effect of mineral salts on the L-lactic acid production

In Figure 3, five kinds of mineral salts were investigated for L-lactic acid production, and it was concluded that $K_2HPO_4$ and NaCl were suitable for L-lactic acid fermentation, and the highest yield was 78.4 g/l.
3.5. Effect of metal ions on the L-lactic acid production

6 kinds of metal ions and different concentrations were investigated in L-lactic acid fermentation with 150 g/l total sugars, and the results were shown in Table 2. Mg$^{2+}$, Mn$^{2+}$ and Zn$^{2+}$ could promote L-lactic acid production, and the concentrations were 0.1 g/l, 0.01 g/l and 0.3 g/l, respectively. While Ba$^{2+}$ and Co$^{2+}$ did not show significantly effect on L-lactic acid fermentation, and Cu$^{2+}$ showed negative effect on L-lactic acid fermentation.

Table 2. Effect of mineral salts on the L-lactic acid production.

| Mineral salts | Concentration (g/l) | L-lactic acid production (g/l) |
|---------------|---------------------|-------------------------------|
| Mg$^{2+}$     | 0.01                | 70.6                          |
|               | 0.05                | 71.2                          |
|               | 0.10                | 73.9                          |
|               | 0.01                | 75.9                          |
| Mn$^{2+}$     | 0.03                | 72.3                          |
|               | 0.05                | 70.1                          |
|               | 0.1                 | 76.4                          |
| Zn$^{2+}$     | 0.3                 | 80.7                          |
|               | 0.5                 | 74.3                          |
|               | 0.01                | 65.4                          |
| Cu$^{2+}$     | 0.03                | 61.3                          |
|               | 0.05                | 58.3                          |
|               | 0.01                | 71.4                          |
| Ba$^{2+}$     | 0.03                | 72.8                          |
|               | 0.05                | 72.1                          |
|               | 0.01                | 72.6                          |
| Co$^{2+}$     | 0.03                | 72.4                          |
|               | 0.05                | 71.9                          |

3.6. Effect of pH values on the L-lactic acid production

When the initial medium pH values ranged from 4.0 to 8.0, significant effects on the L-lactic acid production was observed in the experiments, and when the pH was 6.0, the highest L-lactic production was obtained (Figure 4). The controlled pH values (ranged from 5.0-7.0) during the fermentation were also investigated and shown in Figure 4, and the result was similar to the initial pH of medium, and the highest L-lactic yield was also appeared when pH=6.0, so pH 6.0 was selected for further experiments.
3.7. Effect of different fermentation periods on the L-lactic acid production

The fermentation periods of 10 h, 20 h, 30 h, 40 h and 50 h were estimated for L-lactic acid production, and the highest yield was observed in 40 h of fermentation period (Figure 5).

3.8. Effect of different incubation temperatures on the L-lactic acid production

Figure 6. Effect of the temperature on the L-lactic acid production
The effect of temperature on the L-lactic acid production was evaluated and the results were shown in Figure 6. When the temperature ranged from 38 °C to 48 °C, the highest L-lactic acid production reached 76.3 g/l when the temperature was 46 °C.

3.9. Time course of the L-lactic acid fermentation under optimal conditions

From the former experiments, the optimal condition for L-lactic acid fermentation with the strain \textit{Lactobacillus} sp. L47 was obtained: corn straw hydrolysate 243.1 ml/l, \((\text{NH}_4)_2\text{SO}_4\) 10 g/l, yeast extract 10 g/l, \(\text{KH}_2\text{PO}_4\) 0.5 g/l, \(\text{ZnSO}_4\) • 7\(\text{H}_2\text{O}\) 0.3 g/l, \(\text{MgSO}_4\) • 7\(\text{H}_2\text{O}\) 0.1 g/l, \(\text{MnSO}_4\) • \(\text{H}_2\text{O}\) 0.01 g/l, \(\text{CaCO}_3\) 60 g/l, initial medium pH 6.0, controlled fermentation pH 6.0, 20 ml of seed, incubation temperature 46 °C for 40 h in the biochemical incubator, and the L-lactic acid production reached 99.8 g/l (Figure 7).

![Figure 7. Time course of L-lactic acid fermentation under the optimal condition](image)

4. Conclusion

In this paper, L-lactic acid was produced from corn straw hydrolysate by the strain \textit{Lactobacillus} sp. L47, the fermentation conditions such as nitrogen sources, mineral salts, metal ions, incubation periods, pH values and temperatures were investigated, and the optimal incubation conditions were obtained (total volume 1000 ml): corn straw hydrolysate of 243.1 ml, \((\text{NH}_4)_2\text{SO}_4\) 10 g, yeast extract 10 g, \(\text{KH}_2\text{PO}_4\) 0.5 g, \(\text{ZnSO}_4\) • 7\(\text{H}_2\text{O}\) 0.3 g, \(\text{MgSO}_4\) • 7\(\text{H}_2\text{O}\) 0.1 g, \(\text{MnSO}_4\) • \(\text{H}_2\text{O}\) 0.01 g, \(\text{CaCO}_3\) 60 g, initial medium pH 6.0, controlled fermentation pH 6.0, 20 ml of seed, incubation temperature 46 °C. Under the optimal fermentation conditions, the highest L-lactic acid production was 99.8 g/l in 40 h. Glucose in the corn straw hydrolysate was the main carbon source for L-lactic acid production and the conversion substrate; however, there were also arabinose and xylose in the hydrolysate, which were not utilized by the strain L47. So novel strains should be developed for taking full advantage of the hydrolysate.

Acknowledgments

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