

Optimization of clear liquid fermentation condition for ethanol production from *Canna edulis Kerl*

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ABSTRACT

In this paper, clear liquid fermentation condition for ethanol production from *Canna edulis Kerl* with orthogonal design was studied. We have studied five factors that influenced clear liquid fermentation condition for ethanol production from *Canna edulis Kerl* by the single-factor test. The five factors were α -amylase amount, glucoamylase amount, pH, nitrogen and auxiliary materials amount. Selected the best amounts of α -amylase, glucoamylase, pH, nitrogen source and auxiliary materials by means of the single-factors test. And then, we used the Orthogonal test to optimize the fermentation process. The study showed that the best fermentation conditions were α -amylase amount 7 u/g *C.eduli*, glucoamylase amount 145u/g *C. edulis*, pH 4.4 and the amount of urea 0.08%, 1% of rice bran as auxiliary materials. The rate of alcohol which was produced by the best fermentation conditions is 21.5% that increased by 1.3% than the rate of alcohol by the single-factor test.

Keywords: Alcohol; *Canna edulis Kerl* ; Clear Liquid Fermentation; Orthogonal Design

1. INTRODUCTION

Canna edulis Kerl belongs to *Cannaceae* and *Canna L*, in chinese herbs it was called jiang-yu or jiao-ou. At the first of 20th century, it was planted widely at slope and hills in Sichuan, Guizhou and Yunan because of its excellent feature. The content of starch in rhizomes of *Canna edulis Kerl* is very abundant. The content of starch is about 40%–60% [8-13]. So *Canna edulis Kerl* has great development potential in supply of starch. The planting of *Canna edulis Kerl* can increase earning for montanic people. At the same time it has continuable-development significance in regulating agricultural configuration and developing rural economics. But the starch of *Canna edulis Kerl* is made into noodle

largely only now, so its value is low. The molecular structure and physical and chemical properties of the starch of *Canna edulis Kerl* were studied largely. Now, many scholars are investigating other purposes of the starch of *Canna edulis Kerl*. For example, the starch of *Canna edulis Kerl* has achieved a breakthrough in alcoholic fermentation. The solid-state fermentation, clear liquid fermentation of *Canna edulis Kerl* and the comprehensive utilization of fermentation residue have been researched largely in laboratory [1,7-11]. On this basis, we have got the best clear liquid fermentation process from *Canna edulis Kerl* with the single-factor test. Then we improve the utilization rate of *Canna edulis Kerl* raw after optimizing the technics of clear liquid fermentation condition for ethanol production from *Canna edulis Kerl* [8,12,15,16].

2. MATERIALS AND METHODS

2.1. Experimental Material

Canna edulis Kerl: Collected from Xingyi Guizhou, the content of Starch is 60.1%, the content of moisture is 14.8%;

Thermal Resistance α -amylase: wuxi xing-da Bio-Engineering Co., Ltd., enzyme activity: 20000u/mL;

New liquid glucoamylase: wuxi xing-da Bio-Engineering Co., Ltd., enzyme activity 100000u/ML.

2.2. Methods

2.2.1. The Preparation of Clear Liquid Culture Medium

Firstly, mixed the powder of *Canna edulis Kerl* with water in a certain proportion, joined in α -amylase, then heated them to 85–90°C rapidly, keep them together 30–60min. When the time was over, cooled them to 60°C, adjusted pH 4.2. A certain proportion moderate glucoamylase was joined into the mixture solution, a few minutes later, the Solid-state glycation Saccharification dregs and clear liquid can be gained by centrifugal technology, then put the clear liquid into the autoclave whose

temperature was 121°C, the clear liquid was taken out after twenty minutes. When the clear liquid temperature fell to 30°C, the yeast which was activated was inoculated into the clear liquid, the fermentation started in the incubator [2,3].

2.2.2. Determination of Alcohol Volume Fraction

The clear liquid, which was the outcome of *Canna edulis Kerl* fermented, was measured 100mL by measuring graduates (100mL), inoculated into a rockered flask (500ml) which contained 100mL distilled water. The distillation was collected by volumetric flask (100mL), determined alcohol level by alcohol meter [4-6].

2.2.3. Calculation of the Producing Ethanol Rate of Raw Materials and Starch Utilization [17]

The producing ethanol rate of raw materials =

$$\frac{95\% \text{ ethanol yield (kg)}}{\text{amount of raw materials (kg)}} \times 100\%$$

The starch utilization =

$$\frac{\text{the actual liquor rate of starch}}{\text{the theoretical liquor rate of starch}} \times 100\%$$

2.2.4. Determination of Reducing Sugar Concentration

The distilled water was inoculated into the volumetric flask which contained separated clear liquid 0.1mL till 25mL. The mixed solution was determined for reducing sugar concentration by DNS analytical method [5].

2.2.5. Determination of Mass Concentration of Total Sugar

The distilled water was inoculated into the volumetric flask which contained separated clear liquid 0.1mL till 25mL. The mixed solution was determined for total sugar concentration by H₂SO₄-Pheul analytical method [5].

3. RESULTS AND ANALYSIS

3.1. Single-Factor Test and Medium Optimization

Based on former experiments, we have found the best solid-state fermentation conditions of the ethanol production from *Canna edulis Kerl*, the best conditions were ratio of material to water 1:2.2, α -amylase amount 5u/g *C.edulis*, liquefaction time 45 min, glucoamylase amount 150u/g *C.edulis*, saccharification time 23.2 min, pH 5.5, urea 0.1%, fermentation temperature 27.9 °C, ADY 0.15%, fermentation time 48h and inert carrier 1%.

On this basis, the paper studied the clear liquid fermentation conditions of the ethanol production from

Canna edulis Kerl. By a large number of experiments, we found that α -amylase amount, glucoamylase amount, pH and urea amount have an important influence on the whole fermentation technology.

So we were proceeding to the in-depth study for these four factors, these studies as follows:

3.1.1. The Effect of α -amylase Amount on the Alcohol Fermentation

In order to confirm the appropriate amount of α -amylase on clear liquid fermentation from *Canna edulis Kerl*, different amount of α -amylase were tested on the ethanol rate of raw materials (*Canna edulis Kerl*) [7-11,14]. The results were showed in **Figure 1**.

The **Figure 1** showed that when the α -amylase amount was 6u/g *C.edulis*, the ethanol rate of raw materials (*Canna edulis Kerl*) was highest. So the conclusion was α -amylase amount 6u/g was the best condition on clear liquid fermentation for ethanol production from *Canna edulis Kerl*.

3.1.2. The Effect of Glucoamylase Amount on the Alcohol Fermentation

Glucoamylase played an important role in the clear liquid fermentation for ethanol production from *Canna edulis Kerl*, it can influence the cost of production. Different glucoamylase amount were tested on the ethanol rate of raw materials [7-11].

The different results were revealed in **Figure 2**.

The **Figure 2** described that when the glucoamylase amount exceeded 150u/g, the ethanol rate of raw materials (*Canna edulis Kerl*) was steady. In order to cut down the consumption of glucoamylase, the glucoamylase amount 150u/g was appropriate on clear liquid fermentation for ethanol production from *Canna edulis Kerl*.

3.1.3. The Effect of Different pH Conditions on Alcohol Fermentation

The pH of Clear Liquid fermented were adjusted to 3.6, 4.2, 4.5, 5.0 and 6.0 (the pH of *Canna edulis Kerl* Glycosylated liquid was original) [7-11]. The outcomes were dr-

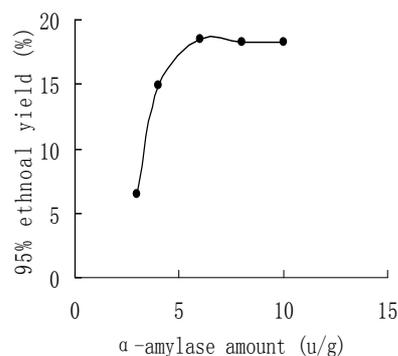


Figure 1. The effect of α -amylase amount on the alcohol fermentation.

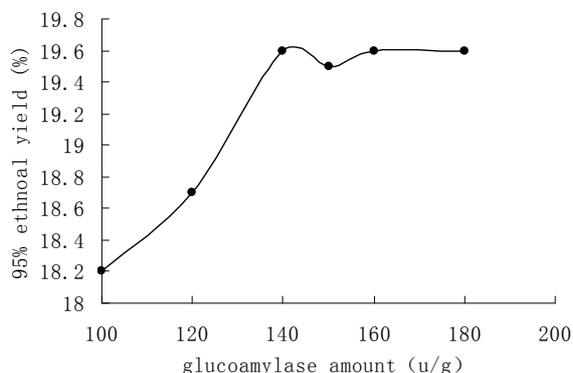


Figure 2. The effect of glucoamylase amount on the alcohol fermentation.

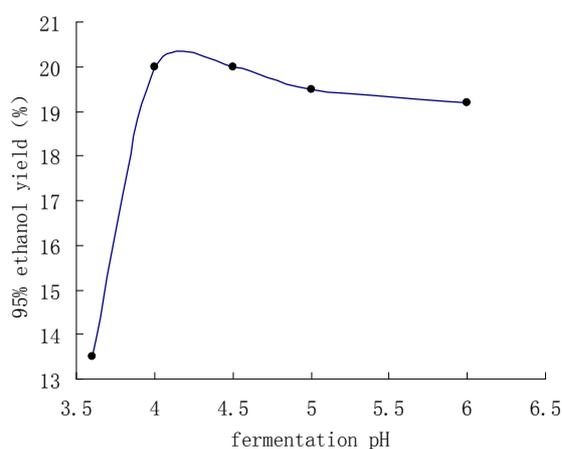


Figure 3. Effect of pH on ethanol fermentation.

ew in **Figure 3**.

The conclusion can be ascertained: the yield of alcohol was gradual increasing when pH was 3.5 to 4.2. When pH was 4.2 to 6.0, the yield of alcohol had a basically stable situation and the best effect was pH 4.2.

3.1.4. The Effect of Nitrogen on the Alcohol Fermentation

Under suitable environment and nutritional conditions, yeast can bear alcohol in high concentration, but the vigour of yeast was lower when nitrogen was scarce in culture medium. In this article, the yeast was restrained because the amount of nitrogen was deficient in glycosylated liquid. For accelerating growth of yeast and enhancing the ability of alcohol-resistant yeast, a certain amount of nitrogen was necessary to be added in the culture medium. With an eye to the results were various because of different nitrogen, therefore, urea, ammonium sulfate, ammonium bicarbonate, peptone and yeast extract were choiced as the element inspected in this experiment. The amount of nitrogen must be based on 0.1% nitrogen content of urea, the nitrogen content of urea, ammonium sulfate, ammonium bicarbonate, pep-

tone and yeast extract were 46.7%, 20%, 17.7%, 13.5%, 7% respectively. The outcomes were shown in **Figure 4**.

It was found that five kinds of nitrogen had great influence on alcohol fermentation from *Canna edulis Kerl* although urea showed the best consequence, maybe it can protect some enzymes, for example the alcohol dehydrogenase, promoted the activity of enzyme.

In order to confirmed the best amount of urea, at the next experiment, different dosage of urea which were 0.05%, 0.1%, 0.15%, 0.2% and 0.3% were studied on clear liquid fermentation condition for ethanol production from *Canna edulis Kerl* at the same condition. The results were revealed in **Figure 5**.

It was showed that the rate of alcohol reduced gradually from 0.15% to 0.3% dosage of urea, the rate of alcohol rised steadily from 0 to 0.1% dosage of urea. The best data appeared at 0.1% dosage of urea.

3.1.5. The Effect of Inert Carrier on Alcohol Fermentation

Fine-Grained material maybe reduce the content of CO₂ which could suppress yeast colony, different content of bran and corn cob were tested respectively on ethanol

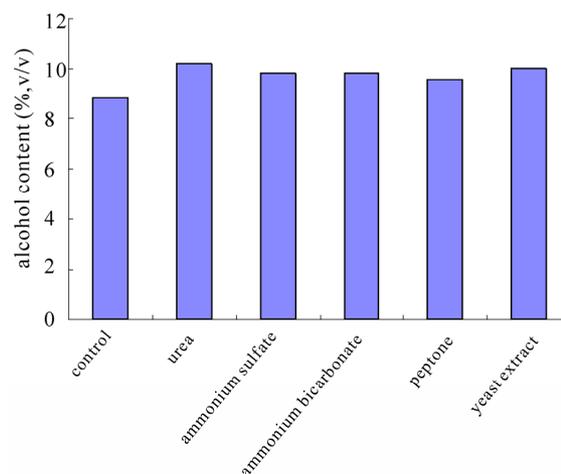


Figure 4. The effect of nitrogen on alcohol fermentation.

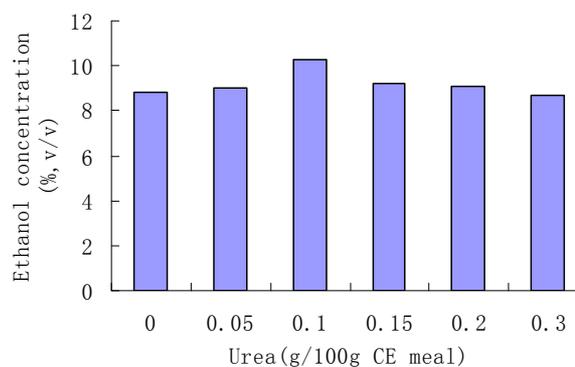


Figure 5. Effect of urea concentration on ethanol fermentation.

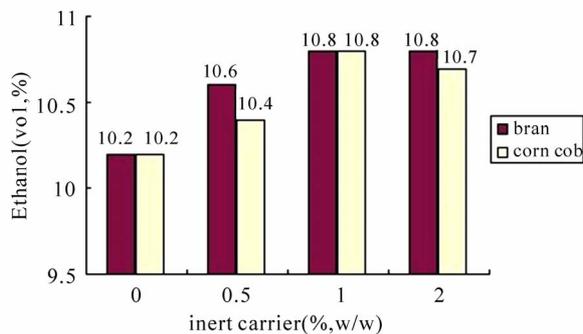


Figure 6. Effect of inert carrier on ethanol fermentation.

fermentation. The content of them were 0, 0.5%, 1%, 2% respectively. The results were showed in **Figure 6**.

The diagram indicated that bran and corn cob can promote alcohol fermentation of *Canna edulis Kerl*. 1% bran had the best effect. The same information had been reported in other documents.

3.2. Orthogonal Test

According to the results of the single-factor test, α -amylase amount, glucoamylase amount, pH and the amount of urea were optimized by orthogonal experiment [8,12,15].

The α -amylase amount 6u/g *C.edulis*, glucoamylase amount 150u/g *C.edulis*, pH 4.2 and the amount of urea 0.1% were used as intermediate level in the orthogonal tests. The experimental design and results of analysis were showed in **Table 1**, **Table 2** and **Figure 7** (In the **Table 1** and **Table 2**, A, B, C and D stand for the

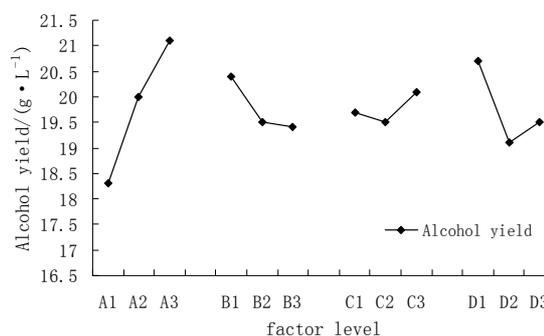


Figure 7. The trend of factors and indexes in orthogonal experiment.

α -amylase amount, glucoamylase amount, pH and urea amount respectively. In the **Tables 1** and **2** stand for three different amount levels of the four factors respectively.)

The **Table 2** showed that α -amylase amount had a great influence on clear liquid alcohol fermentation of *Canna edulis Kerl*. Based on the results of experiment, the influence of the other factors on the clear liquid alcohol fermentation of *Canna edulis Kerl* were ordered from larger to little as the amount of urea>glucoamylase amount>pH.

As can be seen from **Figure 7**, the best combination was A₃B₁C₃D₁, that is to say, the α -amylase amount 7u/g *C.edulis*, the glucoamylase amount 145u/g *C.edulis*, pH 4.4, and the amount of urea 0.08%.

At last, after three parallel experiments which have been carried out on ethanol fermentation by the optimal

Table 1. Factor and level of orthogonal experiment.

level	α -amylase amount (u/g)	glucoamylase amount (u/g)	pH	the amount of urea(%)
1	5	145	4.0	0.08
2	6	150	4.2	0.1
3	7	155	4.4	0.12

Table 2. Results and analysis of orthogonal experiment.

Test No.	factor				Alcohol yield(%)
	A	B	C	D	
1	1	1	1	1	19.8
2	1	2	2	2	17.1
3	1	3	3	3	18.0
4	2	1	2	3	20.1
5	2	2	3	1	2.0
6	2	3	1	2	18.8
7	3	1	3	2	21.3
8	3	2	1	3	20.5
9	3	3	2	1	21.4
mean 1	18.3	20.4	19.7	20.7	
mean 2	20.0	19.5	19.5	19.1	
mean 3	21.1	19.4	20.1	19.5	
pole difference	2.8	1.0	0.6	1.7	

technological conditions, we found the production rate of alcohol were 21.7%, 21.4%, 21.5% respectively, and the average was 21.5%.

4. CONCLUSIONS

1) *Canna edulis Kerl* is a non-alimentarn crop. The advantages of fuel ethanol produced by *Canna edulis Kerl* were raising the utilization rate of resources, reducing the pollution of the environment, lower manufacturing Cost and higher productivity. The fuel ethanol produced from *Canna edulis Kerl* can not only solve the problem of food security, but also promote the development of fuel alcohol industry in china.

2) We have known that α -amylase amount, glucoamylase amount, pH, nitrogen and auxiliary materials can influence clear liquid fermentation for ethanol production from *Canna edulis Kerl*. Then optimizing four factors by the orthogonal test .The best process conditions are α -amylase amount 7u/g *C.edulis*, glucoamylase amount 145u/g *C.edulis*, pH 4.4, the amount of urea 0.08% and 1% of rice bran. The production rate of alcohol was 21.5% which increased by 1.3% than the production rate of alcohol by the single-factor test.

3) We are researching the best conditions of clear liquid fermentation for ethanol production from *Canna edulis Kerl*. These investigations laid a good foundation for reducing the cost of alcohol production, comprehensive utilization of Resources and efficient, saving, unde-filed technics of alcohol production. So clear liquid fermentation for ethanol Production from *Canna edulis Kerl* was an epoch-making event in the realm of industrial alcohol.

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