Study on the Variation of Microbial Diversity during the Fermentation of Potato Liquor Based on Biolog ECO Technology

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Abstract. There are many kinds of microorganisms in the fermentation process of potato liquor. Using the technology of Biolog Eco, the changes of microbial diversity in different fermentation periods were studied. The results showed that the AWCD value of microorganism in the fermentation process of potato liquor was increasing, and the AWCD value of the sample on the sixth day of fermentation was higher than that of other fermentation time samples. The results showed that the values of dominance index and diversity index of the samples on the sixth day of fermentation were higher than those of the samples in other fermentation periods, but the overall difference was not significant; the value of richness index of the samples before loading was the highest, and the value of richness index decreased after the start of fermentation, and the difference in the later stage of fermentation was small. The utilization of various carbon sources by microorganisms showed that in the process of potato liquor fermentation, microorganisms mainly used carbohydrate, polymer and amino acid carbon sources, but less used carboxylic acid, amine and phenolic acid carbon sources. The results of principal component analysis of microbial community showed that the highest carbon source was D-xylose / pentanose, and the lowest carbon source was D, l-α-Glycerin phosphate.

1. Introduction
Potato is a kind of potato plant, its tuber contains a lot of starch, and has a series of unique properties. There is a long history of using potato to brew liquor in foreign countries. Using potato brewed liquor, in its fermentation process involves many physical and chemical changes, these changes directly affect the product quality and safety, accurate understanding of the changes in microbial fermentation process is particularly important for the formation of good quality and good flavor products \cite{1}, the core and the potato liquor fermentation is the role of microorganisms in the fermentation process, therefore in the process of potato liquor fermentation the change rule of microbial diversity research, is of great significance for optimizing the potato liquor fermentation process \cite{2}. With the continuous development of microbiology and neighborhood, at present a lot of methods to explore microbial diversity, because of Biolog ECO technology simple operation, good sensitivity, wide detection range,
according to microbial utilization of different carbon source, the changes of microbial community metabolism and function display features [3], has become an important tool to study the microbial functional diversity, and is widely applied in microbiology, agriculture, food science, medicine and other fields.

Therefore, this paper takes the microorganisms in the process of potato liquor fermentation as the research object, studies the microbial flora dynamics in the process of potato liquor fermentation, objectively and accurately grasps the change law of microorganisms in the process of potato liquor fermentation, and lays a theoretical basis for further analysis of the change law of microbial diversity in the process of potato liquor fermentation.

2. Materials and Methods

2.1. Sample collection
According to the standard of DB 34 / T 2264-2014, the fermented grains in the process of potato liquor fermentation are sampled with sterile sampling bags at regular intervals every day. After sampling, the samples are stored in a refrigerator at -20℃ for standby.

2.2. Main instruments and equipment
EnSpire automatic enzyme labeling instrument (Perkin Elmer Enterprise Management Co., Ltd.), WE-2 dual function water bath thermostatic oscillator (Beijing Haitian Youcheng Technology Co., Ltd.), LRH-250F biochemical incubator (Wuxi marrett Technology Co., Ltd.), YXQ-LS-18SI portable pressure steam sterilization pot (medical equipment factory of Shanghai bosun Industry Co., Ltd.), BIOLOG ECO board (American Biolog company).

2.3. Determination method

2.3.1. Weigh 10.0g fermented grains accurately, pour them into conical flask, add 90ml (0.85% NaCl, w/V) of sterile physiological saline, and seal them with sterile cotton plug.

2.3.2. Put the conical flask with fermented grains into the water bath constant temperature oscillator, shake and mix for 30 minutes, take out and stand for 15 minutes, use the pipette gun to transfer 10ml of supernatant on the sterile operation platform to another empty conical flask, and then add 90ml of sterile physiological saline.

2.3.3. Dilute the fermented grains to 10-3g/ml according to the operation method of 2.3.2.

2.3.4. Suck 150μl of 10-3g/ml fermented grains suspension on the sterile operation platform with a pipette gun, and inoculate it into each micropore of the Biolog ECO plate in turn.

2.3.5. Cover the inoculated Biolog ECO plate and put it into a biochemical incubator at 25℃ for 6 days.

2.3.6. The absorbance value of each micropore was measured at 590nm and 750nm every 24h with the full-automatic enzyme labeling instrument, and the data was recorded.

2.4. Data processing
The difference between the OD value of 590nm and that of 750nm represents the metabolic activity of microorganisms. When the difference is less than 0.06, the correction result is 0 [4]. The average activity of microorganisms was evaluated by AWCD of Biolog ECO plate [5], and the calculation formula was as follows:

\[
AWCD = \frac{\Sigma(Ci-R)}{n}
\]
In the formula, $C_i$ and $R$ are the difference between the reaction pores and the control pores $OD_{590}$ and $OD_{750}$ of the eco plate respectively. If the $C_i-R$ value is less than zero, then zero treatment is used for calculation; $n$ is the type of carbon source [6].

The richness index Shannon (H), dominance index Simpson (D), diversity index McIntosh (U) are used to analyze the functional diversity of microbial community [7], and the calculation is based on the data of 96h culture [8], and the formula is shown in Table 1.

| Diversity index | purpose                                      | formula                                      | Remarks                                                                 |
|-----------------|----------------------------------------------|----------------------------------------------|-------------------------------------------------------------------------|
| Shannon index   | Assess richness and average                  | $H = -\Sigma pi \ln pi$                     | $pi$ is the ratio of the relative absorptivity (C-R) of i holes to the relative absorption value of the whole plate |
| Simpson index   | Evaluate the dominance index of some of the most common species | $D = \Sigma \left(\frac{n_i(n_i-1)}{N(N-1)}\right)$ | $n_i$ is the relative absorption value (C-R) of the i hole; $N$ is the sum of the relative absorption values; Simpson index is expressed by $1/D$ value |
| Mcintosh index  | Evaluation of diversity index based on eucidian distance in multi-dimensional space of community species | $U = \sqrt{\Sigma n_i^2}$                   | $n_i$ is the relative absorption value (C-R) of the i pore               |

SPSS statistics 23 software was used for data statistical analysis.

3. Results and Analysis

3.1. Analysis of microbial metabolic activity in potato liquor fermentation

The change of AWCD value of microorganisms in fermented grains at different fermentation time is shown in Figure 1. It can be seen from Figure 1 that the AWCD value of each sample is on the whole increasing trend, and the AWCD value of each sample increases the fastest in the 48h-96h range; the AWCD value of the sample on the sixth day of fermentation is significantly higher than that of other fermentation time. It shows that during the culture period of samples, all kinds of microorganisms use microplate for rapid growth and propagation, and the activity of microorganisms is the most frequent in 48h-96h, and the overall metabolic activity of microorganisms in the sixth day of fermentation is higher than that in other fermentation times [9].
Figure 1. AWCD value curve of different samples.

It can be seen from Figure 1 that the total difference of AWCD value among different samples cultured for 96h is the largest, so we use the data of cultured for 96h to explore the overall characteristics [10], the results are shown in Figure 2, the AWCD value of samples fermented for 0-2 days shows a downward trend, the AWCD value starts to increase after the second day, the AWCD value reaches the maximum value on the sixth day, and the AWCD value starts to slowly decline after the sixth day. In the process of potato liquor fermentation, within 2 days after the start of fermentation, the microorganisms that do not adapt to the fermentation environment begin to die or the environment has inhibited the growth of microorganisms [11]. After 2 days, the dominant population begins to grow and propagate rapidly and carry out various life activities. On the sixth day, the microorganisms are most active. After 6 days of fermentation, the increase of acidity and alcohol concentration in the fermentation tank inhibited the growth and reproduction of microorganisms, and the overall metabolic activity of microorganisms began to decline [12].

Figure 2. AWCD curve of each sample cultured for 96h.
3.2. Utilization of various carbon sources by microorganisms in potato liquor fermentation

There are 31 different carbon sources in the Biolog ECO panel, which can be divided into 6 categories: amino acids, polymers, sugars, phenolic acids, carboxylic acids and amines. The utilization of carbon sources by different microorganisms will be different. The AWCD value of each type of carbon source will be calculated in the experiment, and the experimental data will be drawn as shown in Figure 3 below. It can be seen from Figure 3 that during the fermentation process of potato liquor, microorganisms are relatively beneficial the largest utilization rate is carbohydrate carbon source, followed by polymer carbon source; the relative utilization rate of carboxylic acid carbon source increases gradually, amino acid carbon source decreases gradually, and the relative utilization rate of amine and phenolic acid carbon source is less, and the daily relative utilization rate change is not significant [13].

![Figure 3. Distribution of utilization ratio of samples to different carbon sources.](image)

According to the specific situation of microorganism using different kinds of carbon sources, carry out comparative analysis, select the best three kinds of carbon sources used in each fermentation stage, and the results are shown in Table 2.

| sample               | carbon source                                                                 |
|----------------------|-------------------------------------------------------------------------------|
| Before the altar     | Glycogen, D-xylose / pentanose, L-arginine                                   |
| Fermentation day 1   | D-xylose / pentanose, Glycogen, D-glucosamine                                |
| Fermentation day 2   | Glycogen, β-methyl-D-glucoside, D-xylose / pentanose                         |
| Fermentation day 3   | D-xylose / pentanose, L-phenylalanine, putrescine                            |
| Fermentation day 4   | D-xylose / pentanose, Glycyl-L-glutamate, Glycogen                           |
| Fermentation day 5   | D-xylose / pentanose, L-asparagine, Tween 80                                 |
| Fermentation day 6   | N-acetyl-D-glucosamine, D-xylose / pentanose, 2-hydroxybenzoic acid          |
| Fermentation day 7   | D-xylose / pentanose, Itaconic acid, N-acetyl-D-glucosamine                  |
| Fermentation day 8   | D-xylose / pentanose, N-acetyl-D-glucosamine, Methyl pyruvate                 |
| Fermentation day 9   | D-xylose / pentanose, L-asparagine, Glycogen                                |
| Fermentation day 10  | D-xylose / pentanose, D-galacturonic acid, α-butanone acid                    |
It can be seen from table 2 that D-xylose / pentanose of sugar is the most widely used carbon source in the whole fermentation process of potato liquor, followed by liver sugar of polymer, and N-acetyl-D-glucosamine of sugar.

3.3. Analysis of microbial diversity in potato liquor fermentation

According to the calculation method of Yang Yonghua et al. [14], calculate the dominance index Simpson (D), richness index Shannon (H), diversity index McIntosh (U) of each sample, and the calculation results are shown in Table 3.

| Sample                  | Simpson (D) | Shannon (H) | McIntosh (U) |
|-------------------------|-------------|-------------|--------------|
| Before the altar        | 0.9332      | 3.2325      | 0.9636       |
| Fermentation day 1      | 0.9235      | 3.0144      | 0.9742       |
| Fermentation day 2      | 0.9225      | 2.8586      | 0.9635       |
| Fermentation day 3      | 0.9375      | 2.8385      | 0.9785       |
| Fermentation day 4      | 0.9459      | 2.8432      | 0.9743       |
| Fermentation day 5      | 0.9632      | 2.8687      | 0.9815       |
| Fermentation day 6      | 0.9875      | 2.9623      | 0.9963       |
| Fermentation day 7      | 0.9844      | 2.8897      | 0.9887       |
| Fermentation day 8      | 0.9765      | 2.8541      | 0.9756       |
| Fermentation day 9      | 0.9631      | 2.8456      | 0.9653       |
| Fermentation day 10     | 0.9653      | 2.8494      | 0.9765       |

It can be seen from table 3 that the difference between the dominance index value and diversity index value of each sample is small, and the dominance index value and diversity index value of the sample on the sixth day of fermentation are the largest compared with other fermentation time samples; the comparison between the sample before loading and other sample data shows that the richness index value is the highest, followed by the richness index of the sample on the first day of fermentation, and the richness index of other samples the difference is not obvious.

3.4. Principal component analysis of functional diversity of microbial community in potato liquor fermentation

Based on the data of 96h culture, standardized transformation was carried out, and the utilization of different carbon sources by microorganisms in the fermentation process of potato liquor was further described by principal component analysis. It can be seen from Figure 4 that the characteristics of microbial community metabolism can be directly reflected after dimensionality reduction treatment. The contribution rate of principal component 1 (PCA1) is 69.119%, and that of principal component 2 (PCA2) is 20.945%. According to the distribution characteristics in the figure, six types of carbon sources can be divided into three parts: the first part is amino acids, sugars, carboxylic acids, amines, the second part is polymers, and the third part is phenolic acids Thus, we can know the overall distribution of carbon sources used in the fermentation process of potato liquor.
Figure 4. The first and the second main composition and dispersion point diagram of six types of carbon sources.

In order to further analyze the utilization of 31 kinds of carbon sources in the whole process of potato liquor fermentation, the characteristic chart of microbial community utilization of carbon sources was drawn. After standardizing the sample data of 96h culture, principal component analysis was carried out the contribution rate of principal component 1 (PCA1) is 46.623%, the contribution rate of principal component 2 (PCA2) is 21.248%, and the contribution rate of principal component 3 (PCA3) is 11.243%. There are differences in the spatial distribution of different carbon sources. Through statistical analysis of the contribution rate and correlation of the three principal components, the relative ranking of the utilization rate of carbon sources in the whole process of potato liquor fermentation is:

D-xylose / pentanose > N-acetyl-D-glucosamine > Glycogen > L-asparagine > Tween 80 > D-galacturonic acid > D-glucosamine acid > Tween 40 > Methyl pyruvate > 4-hydroxybenzoic acid > Glycyl-L-glutamate > D-mannitol > i-erythritol > γ-hydroxybutyric acid > 1-glucose phosphate > α-cyclodextrin > Putrescine > L-serine > 2-hydroxybenzoic acid > α-butanone acid > L-Phenylalanine > β-methyl-D-glucoside > D-malic acid > L-threonine > Phenylethylamine > L-arginine > D-cellobiose > α-D-lactose > D-galactoselγ-lactone > Itaconic acid > D, L-α-glycerin phosphate.

Figure 5. Principal component analysis of utilization ratio of 31 carbon sources by microbial community.
4. Conclusion
In this study, Biolog ECO technology was used to analyze the diversity of microorganisms in the fermentation process of potato liquor. The average absorbance value (AWCD) can reflect the overall metabolic activity and carbon source utilization capacity of microorganisms, and the diversity index can reflect the changes of microorganisms in the fermentation process of potato liquor [15]. According to the analysis results, the AWCD value of each fermentation sample showed an overall increasing trend, indicating that during the sample culture period, microorganisms were making more and more use of carbon sources in the ECO board. The AWCD value of the samples on the sixth day of fermentation was the largest compared with other samples, indicating that the metabolic activity of microorganisms on the sixth day was the strongest. The results of Simpson (D) and McIntosh (U) also indicated that the microbial growth and reproduction capacity of the fermentation samples on the sixth day was the strongest. The richness index Shannon (H) and the AWCD curve of 96h of sample culture showed the overall trend of the microbial quantity in the fermentation process of potato liquor. After the fermentation of fermented grains, the microbial quantity in the fermented grains first began to decrease, then began to increase after the second day of fermentation, and reached the highest level on the sixth day. Potato liquor fermentation microorganisms during the process of utilization of carbon sources, sugar carbon source is the microbial use most of the carbon source [16], based on the whole potato liquor fermentation process of carbon source utilization rate of relative principal component analysis data show that microbial communities throughout the fermentation process of D-xylose / pentanose relative efficiency is the highest, further suggests that D-xylose / pentanose is the best carbon source in the potato liquor fermentation process.

The experimental results laid a foundation for more effectively grasping the rule of microbial changes in the process of potato liquor fermentation, provided theoretical reference for improving the yield of potato liquor and other optimization processes, and further demonstrated the reliability and simplicity of Biolog ECO technology in the field of food.

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