Study on the Effect of cellulolytic strain MYB3 for Corn Stover Fermentation

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Abstract. The effects of corn stover fermentation with the *Bacillus megaterium* MYB3 was studied in this paper. The results showed that the decomposition rates of cellulose and hemicellulose were 49.6%, 43.46% after 20 days respectively, after fermentation, pH was changed to 5.68, and adjusted to corn stover initial pH 3 to achieve the purpose of sterilization. The decomposition rate was significantly increased by adding corn flour. After adjusting fermentation composes with the ratio of the corn stove to corn flour was 15 : 1, the decomposition rate of cellulose would be 52.37% for 10 days.

1. Introduction

The total annual output of straw in China ranks the first in the world. Although the quantity of straw is huge, but the utilization rate of straw is low. Crop straw accounts for 12 % - 19 % of total organic fertilizer resources in China and it contain extremely abundant nutrient elements such as N, P, K and so on [1-2]. With the gradual prohibition of burning straw in Jilin Province, the processing mode of crop straw was promoted. Crop straw consists of cellulose, hemicellulose and lignin [3]. Straw resources are mainly the use cellulose and hemicellulose. And achieving rapid decomposition of straw by microbial inoculum, which has the ability of cellulose decomposing, and converting the straw cellulose, hemicellulose and other substances, which are difficult to decompose into sugars and so on to provide microbial nutrients or to produce fermented straw feed, which could realize the recycling of energy effectively and is one of the main methods of straw recycling [4]. Straw is also a research hotspot in the world as an energy resource [5].

Silage fermentation agent in the market is mainly composed of cellulase, pectinase, *Saccharomyces*, *Bacillus subtilis*, lactic acid bacteria, etc. These starters have certain fermentation effects, and the main purpose is to preserve straw, but quite few microorganisms are directly used for cellulose and hemicellulose decomposition, and the main purpose was preserving straw. For example, the principal and theory of making silage mainly use lactic acid bacteria fermentation to produce organic acid so that the pH of straw was reduced to 3 - 5, which could prevent the breeding of spoilage bacteria and improve the palatability of the animal. At present, according to the research reports on screening or domestication of cellulose decomposition bacteria [6-9], the microorganisms which had were fermented straw feed except *Bacillus subtilis* and some mould. *Bacillus megaterium*, used as biological fertilizer, has phosphate solubilizing effect. Some *Bacillus megaterium* is also added in diet to improve the utilization of nutrients and the effect of intestinal flora, as a bacteria which has huge potential ability [10-13].

In this paper, fermentation of corn stover with different treatments was studied by *Bacillus megaterium* MYB3 isolated from our laboratory.

2. Materials and Methods

2.1. Materials

The strain MYB3 was a kind of cellulose decomposing microorganism with the ability of acid and alkali resistance (pH 3 - 11) and was isolated our laboratory [14]. Corn stover was collected from farmland and the content of moisture is 7.32%, content of cellulose is 29.17% and hemicellulose is 28.8%.

2.2. Analysis of cellulose and hemicellulose content

The content of cellulose in corn stover was determined by sulfuric acid potassium dichromate oxidation method of Wang Jinzhu [15]. Hemicellulose content was analyzed by hydrochloric acid hydrolysis method.

2.3. Fermentation experiment and changed pH

Corn stover was chopped to 2 ~ 5 cm length and adjusting the moisture content to 70%, adjusting pH of straw to 3 by 80% lactic acid and then sealing in a room-temperature for 1 - 2 day. After that the germicidal efficacy was detected by CFU plate count method. The advanced cultured bacteria MYB3 was evenly sprayed in the sterilized corn stover and sealing, and then fermenting at 30℃.

2.4. Effects of corn flour addition on fermentation

In order to study the effect of adding different amounts of corn flour on the decomposition of cellulose and hemicellulose in the corn stover, the proportion of different corn flour was divided into groups (table 1) for fermentation. The control group was inoculated strain MYB3 and the treatment group was inoculated with 3.5% MYB3 cultivation, which were fermented at 30℃. Fermentation of corn stover was taken after inoculation 5d, 10d, 15d, and the decomposition rate of cellulose and hemicellulose was analyzed.

Table 1 Fermentation for the different ratio of corn stover and corn flour

Treatment	Combination
Control	corn stover
Treatment 1	corn stover + MYB3
Treatment 2	corn stover + corn flour（15:1）+ MYB3
Treatment 3	corn stover + corn flour（10:1）+ MYB3
Treatment 4	corn stover + corn flour（ 5:1）+ MYB3

3. Results and Discussions

3.1. Cellulose, hemicellulose and pH changes during the fermentation of the corn stover

First of all, lactic acid was used to sterilize. Then, straw was fermented by inoculation of strain MYB3. After 20 day's fermentation, the cellulose content decreased from 23.89% (3 d) to 14.7% and the decomposition rate was 49.6% (Fig.1). The content of hemicellulose decreased from 25.91% (3 d) to 16.30%, and the decomposition rate was 43.46% (Fig.2). The results showed that after adjusting the pH of the corn stover to 3 with lactic acid, the purpose of killing spoilage bacteria was achieved, and the preservation of straw and the palatability of animals were also improved. The cellulose decomposition rate of 20 d was close to 50%, and the hemicellulose content was more than 40% after fermentation, fermented corn stover could improve the nutritive value of straw feed, increase the digestibility of straw feed, and meet the reuse of a straw.

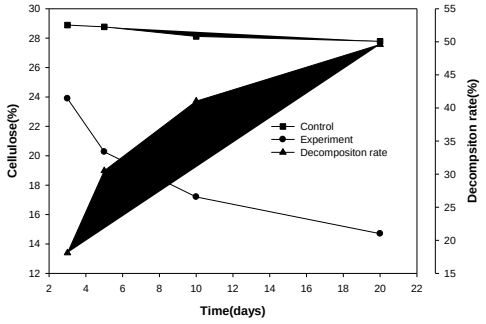

Fig. 1 Analysis for cellulolytic decomposition rate of corn stover

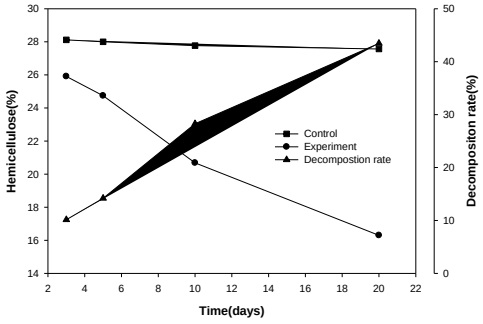

Fig. 2 Analysis for hemicellulolytic decomposition rate of corn stover

When the corn stover was fermented with lactic acid after adjust pH, the pH increased, and the pH in 20 d was 5.68, and the pH of fermented matter was not changed at 20th day (Fig.3). The organic acids such as lactic acid and more and more in silage were important quality indexes [16]. When pH was more than 5, other miscellaneous bacteria were easy to breed and thus this experiment used 80% lactic acid to pretreat. In the fermentation process, when reducing pH to below 5, the purpose of inhibiting the reproduction of undesirable microorganisms was reached and feed value of straw was improved. For corn silage, some experimental results showed that the daily consumption of dry matter increased by 1.5 kg when pH was 5.5, and the silage consumed was the largest [17] at 5.7. When the straw as feed, the optimum pH was 5.6, because the animal has a complex set of an acid-base regulating system which maintains pH of rumen generally at 5.5 ~ 7. The too high or low rumen pH the animal feed-intake during a meal. In this experiment, when the 20th day of corn stover fermentation conforms to the best pH in the rumen of animals the cellulose and hemicellulose in corn stover decreased by about 50% at this time as they turned into sugars, organic acids, protein, improved the nutritional value of straw feed and to the feed conversion rate of animal.

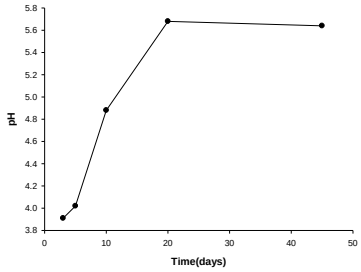

Fig. 3 Changes of pH in fermentation of corn stover

3.2. Effects of corn flour addition on fermentation in corn stover

Treatment 1 without corn flour, cellulose decomposition rate was 31.79% at 5 d; the cellulose decomposition rate of treatment 2 (15 : 1) was 43.14%; Treatment 3 (10 : 1) was 61.74%; the decomposition rate of treatment 4 (5 : 1) reached 70.56%. At the 10 days, cellulose decomposition rates of treatment 1, 2, 3 and 4 were 41.09%, 52.37%, 64.87%, 75.21% respectively. At 15 d, it were 43.18%, 62.05%, 67.96%, 77.26%. However, cellulose decomposition rates in the control group were 1.76%, 2.77%, 2.19% at 5 d, 10 d, and 15 d, as shown in Fig. 4. Treatment 1 without corn flour, hemicellulose decomposition rate was 13.96% at 5 d; the hemicellulose decomposition rate of treatment 2 (15 : 1) was 16.08%; Treatment 3 (10 : 1) was 17.97%; the decomposition rate of treatment 4 (5 : 1) reached 19.15%. At the 10 days, hemicellulose decomposition rates of treatment 1, 2, 3 and 4 were 24.85%, 26.46%, 27.87%, 30.61% respectively. At 15 d, were 27.9%, 31.5%, 31.98%, 32.59%. However, hemicellulose decomposition rates in the control group were 1.53%, 1.79%, 1.98% at 5 d, 10 d, and 15 d, as shown in Fig. 5.

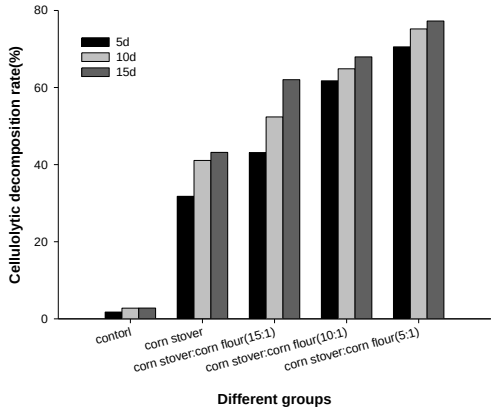

Fig. 4 Effect of different groups on cellulolytic decomposition rate of corn stover

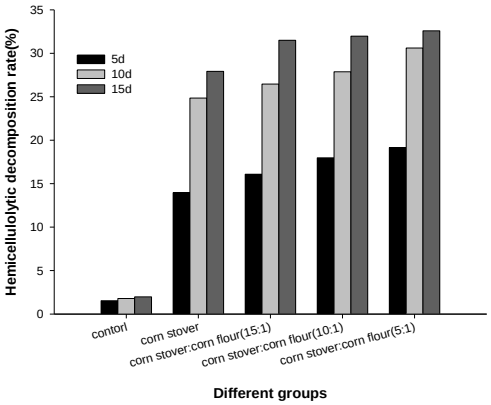

Fig. 5 Effects of different groups on hemicellulolytic decomposition rate of corn stover

Generally, the additive could be regulated the physical and chemical properties in the fermentation process of straw. According to the study of Li Guoxue [18], the effect of additives on the straw, the straw additive was divided into additive regulator, microbial inoculants, specific modulators or osteoporosis agent etc. Additive can not only help to improve the fermentation environment, accelerate the process of fermentation, but also it can reduce the loss of nitrogen and other nutrients, which was a good for maintaining nutrient contents of straw and adjusting the content of various nutrient elements at the same time in the fermentation and finally improving the quality of fermentation [19]. The content of water-soluble carbohydrates and other substances in corn flour were relatively high, so adding corn flour could supplement the shortage of water-soluble carbohydrates in straw fermentation [20]. The study results of Yu Zhu [21] showed that, the addition of corn flour in straw fermentation could be obviously optimize the fermentation quality of straw fermentation, and improve the nutritional value of straw fermented feed. In this experiment, when compared to the treatment 1 without corn flour, the cellulose decomposition rate of treatment 4 (corn stover : corn flour = 5 : 1) reached 77.26% at the 15 d and it was increased 44.1%. Dai Fang [22] added the additives to the study, the degradation rates of hemicellulose and cellulose were increased 5.7% and 10.7% respectively. Li Sa [23] added cellulase to the test, the cellulose decomposition rate was increased 10%. The results of this experiment were consistent with the Dai and Li study's results, and corn flour had a greater impact on cellulose decomposition in straw fermentation.

With the increase of corn flour, the decomposition rate of cellulose was increased. However, the cost of making straw fermented feed was increased gradually, and did not achieve the purpose of saving resources. When the treatment 4 (corn stover : corn flour = 5 : 1) was fermented for 15 d, the cellulose decomposition rate reached the best effect of 77.26%. However, the cost of production was also the highest. Therefore, in accordance with the cellulose decomposition rate of more than 50% as the purpose of this experiment, the treatment 2 (corn stover : corn flour = 15 : 1) for fermentation at 10 d was selected and cellulose decomposition rate reached 52.37%, which could be reduced the cost and meet the purpose of cellulose decomposition.

4. Conclusions

In the experiment of corn stover fermentation, after 20 days, the decomposition rates of cellulose and hemicellulose were 49.6%, 43.6%, pH 5.68 after adjusting pH to 3 by lactic acid. This method could prevent the breeding of spoilage bacteria, and improved the preservation of corn straw. Corn flour as additive could be significantly increased the cellulose and hemicellulose decomposition rate of corn stover. The 10 d and corn stover : corn flour = 15 : 1, which was the best method for making corn stover fermented feed.

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