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Optimization for Fermentation of Cattle Manure to Produce Bio-Fertilizer by Inoculating Complex Microbial Agents

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Abstract: In this study, in order to determine the optimization technology of bio-fertilizer, the aerobic fermentation test was conducted by using fresh manure and rice husk powder as materials. The inoculation, moisture content, C/N ratio and turning frequency analysed by single-factor test. Following this, the optimal conditions in the first fermentation were obtained through orthogonal experiments. After the first fermentation, the second fermentation was carried out by adding complex microbes with the ability to release potassium, dissolve phosphate and fix nitrogen. The termination time of second fermentation was determined by testing the growth condition of beneficial bacteria in the fermentation process. The results show, that the optimal parameter in the first fermentation contained moisture content of 70%, C/N ratio of 20:1, inoculation of 3‰, and turning frequency of once every three days. After a period of fifteen days of fermentation, the germination index reached 91.3%. Following this, second fermentation was conducted at the sixth day and the germination index was increased to 98.8%. Under this optimum condition, the material could heat up fast and the top temperature could be high with a long duration.

Keywords: Bio-organic fertilizer, cattle manure, complex microbial agents, fermentation.

1. INTRODUCTION

With the establishment of the large amount of intensive pasture, more manure has been produced [1, 2]. The manure has become a major source of ecological environmental pollution [3], which is not only harmful for the environment and human health, but also influences the sustainable development of livestock industry. Moreover, manure contains a large amount of organic matter and other nutrients composition such as N, P, S, K, *etc*. After processing, it can become a good agricultural fertilizer [4-6]. Aerobic fermentation is the most effective way of treating manure harmlessly and resourcefully [7, 8]. In the fermentation process, a large number of nitrogen, phosphorus and potassium compounds useful for plant growth are produced by microbial degradation. Moreover, the synthesis of new organic polymer - humus could be an important active substance for soil fertility [9]. However, due to limited indigenous microorganisms and fermentation technology, the traditional fermentation process has not been widely used before. Currently, some reports have indicated that the fermentation efficiency could be improved by inoculating microbial agents [10-14]. However, there is little research on the optimization of process parameters. In this study, the main parameter of first fermentation was optimized by single factor test and orthogonal experiments. In the second fermentation, the additional phosphate-dissolving bacteria released soluble phosphorus to plants by dissolving insoluble tricalcium phosphate. The silicate bacteria degraded silicate minerals and released the potassium for plant growth. Meanwhile, the nitrogen fixation bacteria fixed the nitrogen and supplied it to the plant. Therefore, these beneficial microbes can be added in the soil to provide crop nutrients or produce hormones to stimulate plants.

In this study, aiming at producing biological organic fertilizer, secondary fermentation process was conducted and different composite microbial agents were added at different stages of fermentation. The main process parameter was optimized and the optimal fermentation conditions were explored to improve the fermentation efficiency.

2. MATERIAL AND METHODS

2.1. Fermentation Materials

Fresh cattle manure and rice husk powder were used as fermentation materials. The main physical and chemical properties are shown in Table **1**.

2.2. Preparation of Complex Microbial Agents in the First Fermentation

Trichoderma viride, Aspergillus oryzae, Bacillus subtilis and *Pseudomonas* sp., which were isolated and saved in the laboratory, were respectively increased for expanding culture step by step to prepare the solid microbial agents. *Following this,Trichoderma viride, Aspergillus oryzae, Bacillus subtilis* and *Pseudomonas* sp., were mixed in the proportion of 2:1:1:2 to produce mixed microbial agents.

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Table 1. Main physical and chemical characteristics of compost material.

Material	Moisture Content (%)	pH Value	Organic Matter (%)	C / N Ratio	Total Carbon (%)	Total Nitrogen (%)
cattle manure	90	8.0	66.2	20.6	39.8	1.93
rice husk powder	10	7.0	71.6	70.6	45.6	6.46

2.4. Preparation of Complex Microbial Agents in the Second Fermentation

Azotobacter choococcum which could fix nitrogen, *Bacillus megaterium* which could dissolve phosphorus and *Bacillus mucilaginosus* which could dissolve potassium, were respectively cultured in liquid under the condition of 180rpm, 30°C. When the cell concentration reached 10^9CFU/mL, they were respectively mixed with sterilized bran in the proportion of 1:1 to prepare solid agents. The solid agents of *Azotobacter choococcum, Bacillus megaterium* and *Bacillus mucilaginosus* were mixed together with the same ratio to produce mixed microbial agents.

2.5. The First Fermentation Design

Static composting experiment was carried out by piling up the material to 2 meters long, 1 meters wide, and 0.8 meters high. Inoculation, moisture content, C/N ratio and turning frequency were analyzed by single factor test. In this study, temperature and germination indexes were considered as evaluation index to identify the optimal range of four factors. After testing four factors by orthogonal test, the optimum fermentation parameters were determined With the ambient temperature being about 30 °C.

2.6. The Second Fermentation Design

When the first fermentation temperature dropped to 45 °C, adding *Azotobacter choococcum*, *Bacillus megaterium* and *Bacillus mucilaginosus* released potassium, dissolved phosphate and fixed nitrogen to conduct a second fermentation,with the turning frequency being once a day and keeping the temperature suitable for bacterial growth and reproduction. Viable count of bacteria was tested to determine the terminal time of the secondary fermentation process.

2.7. Determination of the Indicators

The temperature was detected by a glass thermometer. The moisture content and C/N ratio were tested according to the organic research method [15] and potassium dichromate - sulfuric acid digestion method [16]. The seed germination index was tested as follows: 30 rape seeds were put into a petri dish lined with filter paper. After adding 5 mL of fermentation product extracts into the petri dish and taking distilled water as control, all the petri dishes were kept into the seed germination boxes at 25 °C for 96 h. Following this, the germination rate and the root length were measured and calculated according to the following formula:

Germination index % = (seed germination rate in test group × the length of seed root in the test group)/(seed germination rate in control group× the length of seed root in the control group) ×100%.

3. RESULTS

3.1. Influence of Moisture Content on Cattle Manure Fermentation

The effect of moisture content on germination index and on fermentation temperature is respectively shown in Table **2** and Fig. (**1**). When the moisture content reached 60%, the material warming increased with the maximum temperature (Fig. **1**) of 68 °C and the high temperature could hold 8.5d. All the treatment was in line with the Chinese Ministry of Agriculture industry standards (NY / T 394-2000) hygiene standards. When the moisture continued to increase, the temperature gradually declined, due to excessive moisture limiting the aerobic microorganisms' contact with oxygen. Meanwhile, the germination index (Table **2**) was maintained above 80% when the moisture content was between 60%-80%.

3.2. Influence of C/N Ratio on Cattle Manure Fermentation

The effect of C/N ratio on germination index and on fermentation temperature is respectively shown in Table **3** and Fig. (**2**). When the C/N ratio reached 30:1, the material warming increased with the maximum temperature (Fig. **2**) of 65 °C and the high temperature could hold 8.5d. All the treatment was in line with the Chinese Ministry of Agriculture industry standards (NY/T 394-2000) hygiene standards. Meanwhile, the germination index (Table **3**) was maintained above 86.6% when the C/N ratio was 30:1.

3.3. Influence of Turning Times on Cattle Manure Fermentation

The effect of turning frequency on germination index and on fermentation temperature is respectively shown in Table **4** and Fig. (**3**). Due to different turning frequencies, the temperature trends in the fermentation were basically the same, which experienced heating period, high temperature period and cooling period. When the turning frequency was once every 3 days, the material warming increased with the maximum temperature (Fig. **3**) being 65°C. This manifested because the turning frequency increased the ventilation rate in the fermentation. However, extensive turning could cause the heat loss. The germination index (Table **4**) could be maintained above 80% when the turning frequency was once every 3 days or once a day.

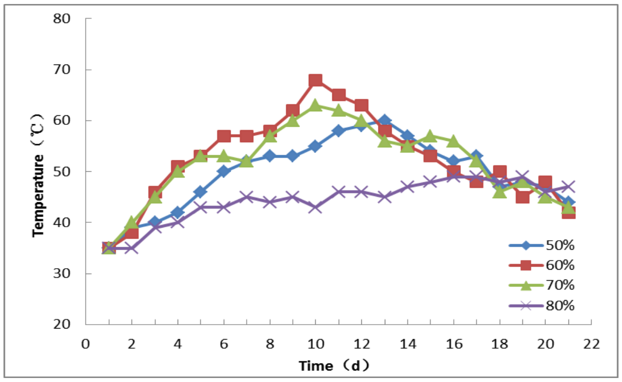

Fig. (1). Effect of moisture content on fermentation temperature.

Table 2. Effect of moisture content on germination index.

Moisture content (%)	50	60	70	80
Germination index (%)	80.8	88.6	86.6	<50

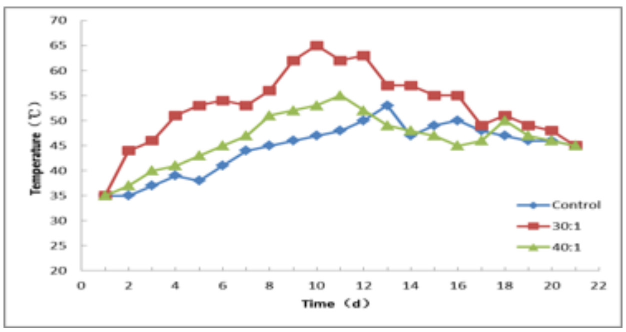

Fig. (2). Effect of C/N ratio on fermentation temperature.

Table 3. Effect of C/N ratio on germination index.

C/N	Control (20:1)	30:1	40:1
Germination index (%)	70	86.6	72

3.4. Influence of Inoculation on Cattle Manure Fermentation

The effect of inoculation on germination index and on fermentation temperature is respectively shown in Table **5** and Fig. (**4**). From Fig. (**4**), it was observed that the temperature increased rapidly with an increase in the rate of inoculation. Meanwhile, the germination index (Table **5**) could be maintained above 80% when the inoculation amount was between 2‰-3‰.

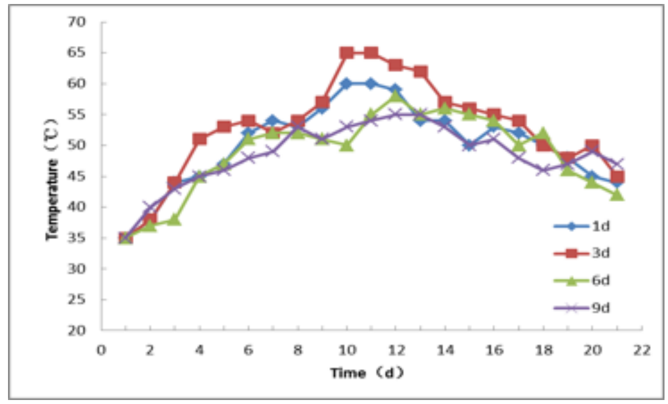

Fig. (3). Effect of turning frequency on fermentation temperature.

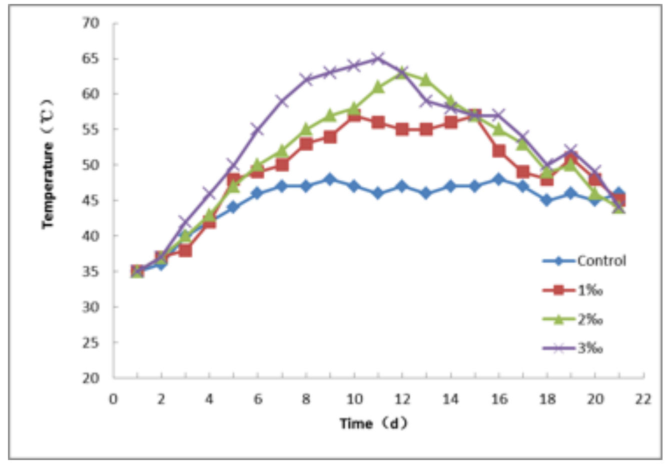

Fig. (4). Effect of inoculation on fermentation temperature.

3.5. The Determination of Optimum Conditions in the Fermentation

In order to optimize the fermentation process, four factors were analyzed and three levels orthogonal experiment were conducted according to the optimal range of factors resulting from single-factor test. The result is shown in Table **6**. By analyzing the range, the relationship between the primary and secondary factors was observed to be D＞B＞A＞C, which suggests that the complex microbial inoculants were the main source of influence on the fermentation. The optimum fermentation conditions were combined into $A_2B_1C_2D_3$, which inculded moisture content of 70%, C/N ratio of 20:1, turning frequency of once every three days, and inoculation of 3‰. Under this condition, the germination index could reach 91.3%.

Table 4. Effect of turning frequency on germination index.

Turning Frequency	Once a Day	Once Every Three Days	Once Every Six Days	Once Every Nine Days
Germination index (%)	70	86.6	72	68

Table 5. Effect of inoculation on germination index.

Inoculation	Control (without Microbe Addition)	1‰	2‰	3‰
Germination index (%)	<50	71.6	89.6	91.8

Table 6. Results of orthogonal experiment.

No.	A Moisture Content %	B C/N Ratio	C Turning Frequency	D Inoculation	Germination Index (%)
1	60	20	2	1	82.3
2	60	30	3	2	84.2
3	60	40	4	3	86.6
4	70	20	3	3	91.3
5	70	30	4	1	88.5
6	70	40	2	2	81.3
7	80	20	4	2	82.2
8	80	30	2	3	89.2
9	80	40	3	1	83.6
K1	84.367	85.267	84.267	84.800	--
K2	87.033	87.300	86.367	82.567	--
K3	85.000	83.833	85.767	89.03	--
R	2.666	3.467	2.100	6.466	--

CONCLUSION

In this study, single factor test and orthogonal test were used to optimize bio-organic fertilizer fermentation. The optimum parameters involved; moisture content of 70%, C/N ratio of 20:1, turning frequency of once every three days, and inoculation of 3‰. Under this condition, the germination index could reach 91.3% after the first fermentation. Subsequently, the second fermentation was conducted by adding complex microbial agents to improve soil fertility. After the second fermentation, the germination index was increased to 98.8%. Under the optimal conditions, the cattle manure heated up fast and the maximum temperature was achieved with a long duration. Moreover, fermentation cycles did not reduce with high maturity. This study provides necessary parameters and theoretical basis for the production of bio-organic fertilizer.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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