ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed ( Group -I) Journal Volume 11, S.Iss 6, 2022

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

**[1]Esha Yadav, [2]Akhilesh Kumar, [3]Avinash Singh, [4]Megha Shukla, [5]Divya Mishra, [6]Anam Taufik, [7]Janey Alam**

Department of Life Sciences, Axis Institute of Higher Education, Kanpur, Uttar Pradesh, India

**Abstract**

This study investigates the optimization of fermentation processes for converting cattle manure into bio-fertilizer using complex microbial agents. The focus is on improving the efficiency and effectiveness of the fermentation process to enhance the nutrient content and quality of the resulting bio-fertilizer. Various parameters, including microbial composition, fermentation time, temperature, and aeration, are examined to determine their impact on the fermentation process. The results indicate that optimizing these parameters can significantly improve the quality of the bio-fertilizer produced, offering a sustainable and eco-friendly solution for managing cattle manure and enhancing soil fertility.

**Keywords: Cattle manure, bio-fertilizer, fermentation, complex microbial agents, optimization, nutrient content, sustainable agriculture**

## 1. Introduction

Cattle manure is a significant by-product of livestock farming, often posing environmental challenges if not managed properly. Traditional methods of manure disposal can lead to nutrient runoff, greenhouse gas emissions, and pollution of water bodies. Converting cattle manure into bio-fertilizer through fermentation offers a sustainable alternative, improving soil fertility and reducing environmental impact. Fermentation, facilitated by microbial agents, is a natural process that breaks down organic matter into simpler compounds. The efficiency of this process depends on various factors, including the type of microorganisms used, fermentation conditions, and the properties of the manure. This study aims to optimize these factors to enhance the fermentation process and produce high-quality bio-fertilizer.

## 2. MATERIAL AND METHODS

### 2.1 Material for fermentation

The fermenting ingredients used were rice husk powder and fresh animal dung. Table 1 displays the primary physical and chemical characteristics.

| 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 |

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

 

**2.2 To prepare complex microbial agents in the first fermentation**

The following steps were taken:

1. **Isolation and Preservation**: Strains of *Trichoderma viride*, *Aspergillus oryzae*, *Bacillus subtilis*, and *Pseudomonas* sp. were isolated and preserved in the laboratory.
2. **Culturing and Expansion**:
   - Each strain (*Trichoderma viride*, *Aspergillus oryzae*, *Bacillus subtilis*, and *Pseudomonas* sp.) was separately cultured and expanded step by step to produce solid microbial agents.
3. **Preparation of Mixed Microbial Agents**:
   - The cultured solid microbial agents of *Trichoderma viride*, *Aspergillus oryzae*, *Bacillus subtilis*, and *Pseudomonas* sp. were mixed in the ratio of 2:1:1:2, respectively, to produce the complex microbial agents.

This process ensures a balanced and effective mixture of microbial strains for the first fermentation.

**2.3 Complex Microbial Agent Preparation for the Second Fermentation**

Three different bacteria were cultivated in liquid at 180 rpm and 30°C: Azotobacter choococcum, which could fix nitrogen; Bacillus megaterium, which could dissolve phosphorus; and Bacillus mucilaginosus, which could dissolve potassium. To make solid agents, the cell concentration was measured and then combined 1:1 with sterilized bran. This was done when the cell concentration reached 109CFU/mL. To create mixed microbial agents, the solid agents of Bacillus megaterium, Bacillus choococcum, and Bacillus mucilaginous were combined in the same proportion.

**2.4 The First Fermentation Design**

The material was piled to a height of 0.8 meters, a width of 1 meter, and a length of 2 meters in order to conduct a static composting experiment. A single factor test was used to examine inoculation, moisture content, C/N ratio, and turning frequency. Temperature and germination indexes were taken into consideration as assessment indices in this study in order to determine the ideal range of four parameters. The orthogonal test was used to evaluate four factors before determining the ideal fermentation settings. with a surrounding temperature of roughly 30 °C.

**2.5 The Second Design for Fermentation**

After the initial fermentation reached 45 °C, a second fermentation was carried out by adding Azotobacter choococcum, Bacillus megaterium, and Bacillus mucilaginosus. This combination released potassium, dissolved phosphate, and fixed nitrogen, and it was turned once a day to maintain a temperature that was favorable for bacterial growth and reproduction. The secondary fermentation process's terminal time was ascertained by testing a viable count of bacteria.

**2.6 Determination of the Indicators**

A glass thermometer was used to measure the temperature. The moisture content and C/N ratio were measured using the potassium dichromate-sulfuric acid digestion method [16] and the organic research method [15]. This is how the seed germination index was examined: A petri plate with filter paper on it was filled with thirty rape seeds. Each petri dish was filled with 5 mL of fermentation product extracts, and all of the dishes were stored in seed germination boxes at 25 °C for 96 hours. Distilled water was used as a control. After that, the following formula was used to measure and compute the root length and germination rate. Germination index percentage is calculated as follows: (length of seed root in test group × seed germination rate in test group)/(seed).

## 3 RESULTS

### 3.1 Moisture Content's Effect on the Fermentation of Cattle Manure

The effect of moisture content on the germination index and fermentation temperature is shown in Table 2 and Figure 1.

- **Optimal Moisture Content for Fermentation**:
  - When the moisture content reached 60%, the temperature of the material increased, reaching a maximum of 68 °C (as shown in Figure 1).
  - This high temperature could be maintained for 8.5 days.
  - All treatments met the hygiene standards set by the Chinese Ministry of Agriculture industry standards (NY / T 394-2000).
- **Effects of Excessive Moisture**:
  - When the moisture content increased beyond 60%, the fermentation temperature gradually declined.
  - This decline was due to excessive moisture limiting the contact between aerobic microorganisms and oxygen.
- **Germination Index**:
  - The germination index, as shown in Table 2, was maintained above 80% when the moisture content was between 60% and 80%.

In summary, maintaining the moisture content at 60% is optimal for cattle manure fermentation, ensuring both high temperatures necessary for effective fermentation and a high germination index, while adhering to industry hygiene standards. Excess moisture, however, can hinder the process by limiting oxygen availability to aerobic microorganisms.

### 3.2 The Effect of C/N Ratio on the Fermentation of Cattle Manure

Table 3 and Figure 2 illustrate how the carbon to nitrogen (C/N) ratio affects the temperature during fermentation and the germination index. The material's temperature rose and reached a maximum of 65 °C when the C/N ratio reached 30:1 (Figure 2). It is possible to sustain this high temperature for 8.5 days. Every treatment complied with the industry standards for hygiene established by the Chinese Ministry of Agriculture (NY/T 394-2000). When the C/N ratio was 30:1, the germination index, as seen in Table 3, remained above 86.6%.

### 3.3 Turning Times' Effect on the Fermentation of Cattle Manure

The effect of turning frequency on the germination index and fermentation temperature is shown in Table 4 and Figure 3.

- **Temperature Trends**:
  - Regardless of the turning frequency, the temperature trends in the fermentation process were similar, including a heating period, a high-temperature period, and a cooling period.
  - When the turning frequency was once every 3 days, the maximum temperature reached 65°C (as shown in Figure 3).
  - This increase in temperature is due to the improved ventilation rate from turning, which facilitates aerobic microbial activity.

- **Effects of Extensive Turning**:
    - Extensive turning, while improving aeration, can also cause heat loss, potentially reducing the efficiency of the fermentation process.
- **Germination Index**:
    - The germination index, as shown in Table 4, could be maintained above 80% when the turning frequency was either once every 3 days or once a day.

In summary, turning the cattle manure once every 3 days optimizes the fermentation process by maintaining high temperatures necessary for effective fermentation and ensuring a high germination index. However, excessive turning can lead to heat loss, which might negatively affect the process.

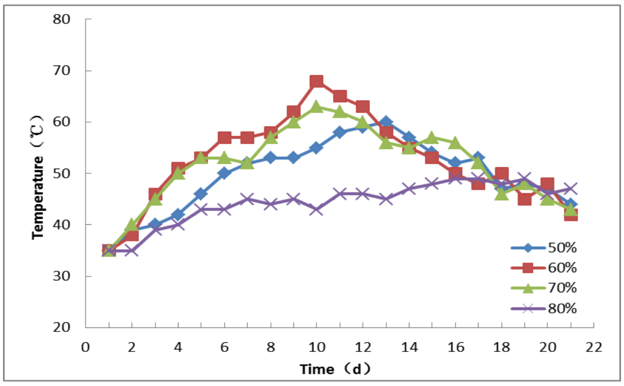


**Fig. (1). Impact of fermentation temperature on moisture content.**

**Table 2. The germination index and moisture content.**

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

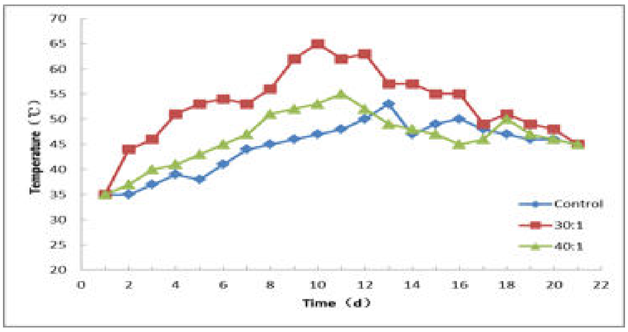


**Fig. (2). Temperature of fermentation as a function of C/N ratio.**

**Table 3. C/N ratio's impact on the germination index.**

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

### 3.4 The Effect of Immunization on the Fermentation of Cattle Manure

The effect of inoculation on the germination index and fermentation temperature is shown in Table 5 and Figure 4.

- **Temperature Increase**:
  - As observed in Figure 4, the temperature increased rapidly with an increase in the rate of inoculation.
  - This suggests that higher inoculation rates enhance microbial activity, leading to a quicker rise in fermentation temperature.
- **Germination Index**:
  - The germination index, as shown in Table 5, was maintained above 80% when the inoculation amount was between 2‰ and 3‰.
  - This indicates that this range of inoculation is effective for maintaining a high germination index during fermentation.

In summary, increasing the inoculation rate effectively raises the fermentation temperature and maintaining an inoculation amount between 2‰ and 3‰ ensures a germination index above 80%, promoting effective cattle manure fermentation.

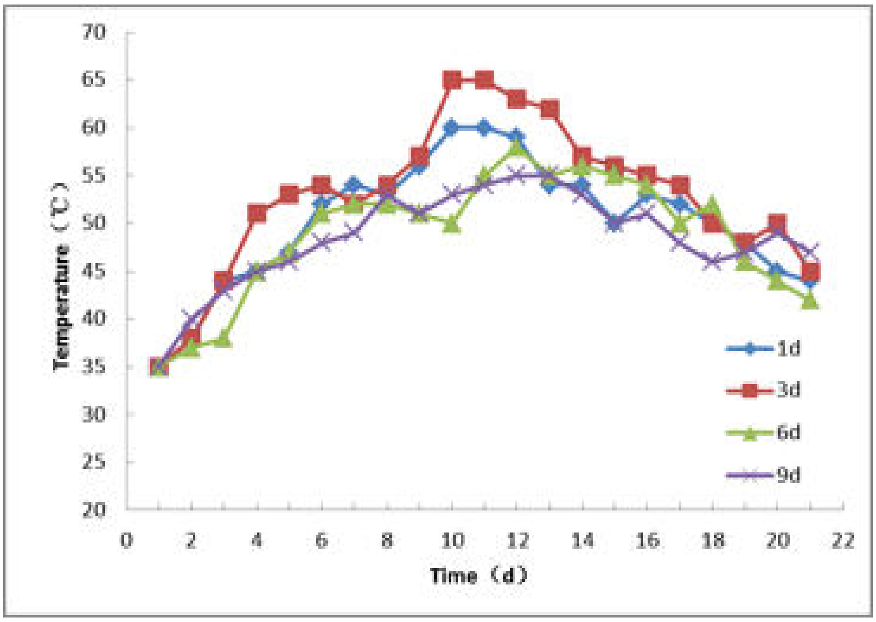


**Fig. (3). The temperature of fermentation is affected by rotating frequency.**

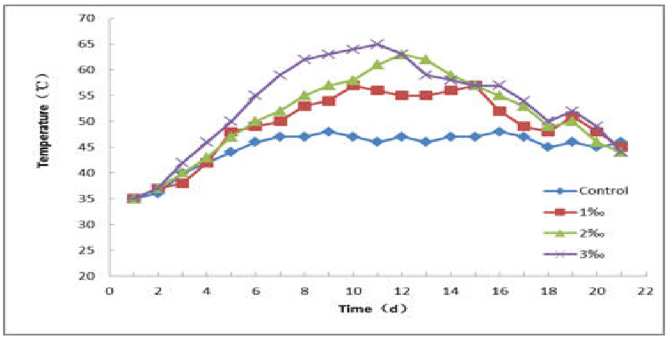


**Fig. (4). The impact of inoculation on the temperature of fermentation.**

 

The Identification of Ideal Fermentation Conditions To optimize the fermentation process, four parameters were investigated using a three-level orthogonal experiment. Table 6 presents the results, which comprise the following:

- Elements Examined: Moisture content (A) and the C/N ratio (B)
- D: Inoculation Rate;
- C: Turning Frequency;
- Analysis of Results: It was discovered using range analysis that there was a D > B > A > C relationship between the primary and secondary components. This implies that the complicated microbial inoculants had the biggest impact on the fermentation process (Factor D).
- **Optimal Fermentation Conditions**:
  - The optimal conditions were determined to be A2B1C2D3, which corresponds to:
    - Moisture content of 70%
    - C/N ratio of 20:1
    - Turning frequency of once every three days
    - Inoculation rate of 3‰
- **Outcome**:
  - Under these optimal conditions, the germination index could reach 91.3%.

In summary, the most influential factor in the fermentation process was the complex microbial inoculants. The optimal conditions for fermentation were a moisture content of 70%, a C/N ratio of 20:1, turning the material once every three days, and an inoculation rate of 3‰, resulting in a high germination index of 91.3%.

**Table 4. Impact of frequency of turning on index of germination.**

| **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. Influence of vaccination on the index of germination.**

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

**Table 6. The orthogonal experiment's results.**

| **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

To maximize the fermentation of bio-organic fertilizer, single factor testing and orthogonal testing were employed in this investigation. The ideal conditions included a 70% moisture content, a 20:1 C/N ratio, a once-every-three-day rotation frequency, and a three-fold inoculation. In these conditions, following the initial fermentation, the germination index may rise to 91.3%. In order to increase soil fertility, various microbial agents were added for the second fermentation. Following the second fermentation, there was an increase in the germination index to 98.8%. When everything was perfect, the cattle manure heated up quickly and maintained the highest temperature for a considerable amount of time. Furthermore, at high maturity, fermentation cycles did not decrease. The criteria and theoretical underpinnings required for the synthesis of bio-organic fertilizer are provided by this work.

## REFERENCES

[1] Montes F, Meinen R, Dell C, *et al*. SPECIAL TOPICS—mitigation of methane and nitrous oxide emissions from animal operations: II. A review of manure management mitigation options. J Anim Sci 2013; 91(11): 5070-94.

[2] Guo D-J, Wu H-S, Ma Y, Chang Z-Z. Study on the Amount of Manure and Urine Excreted by Sheep and Rabbits in Intensive Pasture. J Ecol Rural Environ 2011; 1: 010.

[3] Heuer H, Schmitt H, Smalla K. Antibiotic resistance gene spread due to manure application on agricultural fields. Curr Opin Microbiol 2011; 14(3): 236-43.

[4] Bouwman L, Goldewijk KK, Van Der Hoek KW, *et al*. Exploring global changes in nitrogen and phosphorus cycles in agriculture induced by livestock production over the 1900–2050 period. Proc Natl Acad Sci USA 2013; 110(52): 20882-7.

[5] Holden S, Lunduka R. Do fertilizer subsidies crowd out organic manures? The case of Malawi. Agric Econ 2012; 43(3): 303-14.

[6] Chakraborty A, Chakrabarti K, Chakraborty A, Ghosh S. Effect of long-term fertilizers and manure application on microbial biomass and microbial activity of a tropical agricultural soil. Biol Fertil Soils 2011; 47(2): 227-33.

[7] Müller C, Johansson M, Salomonsson AC, Albihn A. Effect of anaerobic digestion residue *vs* livestock manure and inorganic fertilizer on the hygienic quality of silage and haylage in bales. Grass Forage Sci 2014; 69(1): 74-89.

[8] Elsaidy N, Abouelenien F, Kirrella GA. Impact of using raw or fermented manure as fish feed on microbial quality of water and fish. Egypt J Aquatic Res 2015; 41(1): 93-100.

[9] Erhart E, Siegl T, Bonell M, *et al*. Fertilization with liquid digestate in organic farming-effects on humus balance, soil potassium contents and soil physical properties. EGU General Assembly Conference Abstracts 2014.

[10] López I, López R, Santamaría P, Torres C, Ruiz-Larrea F. Performance of malolactic fermentation by inoculation of selected Lactobacillus plantarum and Oenococcus oeni strains isolated from Rioja red wines. Vitis 2015; 47(2): 123.

[11] Jiang J, Liu X, Huang Y, Huang H. Inoculation with nitrogen turnover bacterial agent appropriately increasing nitrogen and promoting maturity in pig manure composting. Waste Manag 2015; 39: 78-85.