STUDY OF ENZYME ACTIVITY CHANGING PATTERN IN LIVESTOCK MANURES COMPOSTING


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Abstract. Aerobic static composting was conducted for 21 days with cattle, pig and chicken manure along with the leavening agent of 10% corn stalk. In the composting process, the changing trend of parameters: temperature, pH and water content and quantities of microorganism including bacteria, fungi and actinomycetes were studied. It showed that the composting treatment of the three types of livestock manure entered the high temperature period of 45 °C on the 6th day and reached the maximum temperature on the 15th day. The number of days with temperature above 55 °C was 6, 6 and 9 d, which all satisfied the maturity standards. At the end of the composting process, the total nutrient content of pig manure was the highest (6.33%), the total nutrient content of cattle manure was the lowest (5.04%). Throughout the composting process, the changes of activities of urease and invertase were the same with a decreasing trend as a whole. However, the activity of catalase increased with the process of composting. At the end of the composting reactions, concentrations of catalase, urease and invertase were the highest with corresponding values of 62.18 ± 0.45/0.002 mol•L⁻¹•g⁻¹, 54.28 ± 8.35 mg•g⁻¹•d⁻¹ and 11.28 ± 0.14 mg•g⁻¹•d⁻¹, respectively.

Keywords: livestock manures, aerobic compost, process parameters, enzyme reaction, biochemical reactions

Introduction

Since 1978 onward, the industries related to livestock and poultry breeding have increased exponentially (Awasthi et al., 2018). Their rapid development produced benefit economic benefits, improved human food structure and nutrition comprehensively with enhanced life quality (Wang et al., 2013). However, the unprocessed livestock manure originated from the breeding industries creates huge environmental challenge. In comparison with the traditional dispersed feeding system, extensive feeding decreases the growth cycle of livestock and poultry. Extensive feeding increases the yield and reduces the breeding cost significantly (Yasuda et al., 2017). However, it has been proved that intensive breeding results in the separation of planting and breeding. Thus, the environmental pollution created by animal husbandry is severe. Nowadays, livestock manure has been considered as one of the three non-point sources of environmental pollution.
There are stocks of livestock manure in China with a wide range of environmental effects. However, the utilization level of livestock manure is low (Xi et al., 2011). With the low utilization efficiency, the promotion of resource utilization out of livestock manure has been neglected. To overcome this problem, researches are urgently needed targeting the livestock manure stocks arising. Livestock manure is the main agricultural waste with large quantity and wide distribution. It is urgent to solve the current utilization status of livestock manure as well as to find the optimum utilization pathway in different districts of China (Zhang et al., 2011; Feng et al., 2015). Recent studies on the microbial aerobic conversion processes of compost have been focused on the single livestock manure as raw material. There has been no report on the enzyme activities involved in the composting using raw materials of different livestock manure. The present study aimed to compare the changing pattern of enzyme activities in composting with different livestock manures. The research could provide theoretical evidence and technical support for organic fertilizer production to the local entrepreneurs.

**Experiments and methods**

**Materials**

Cattle manure, pig manure and chicken manure used in the composting process with livestock manures were procured at a breeding farm near Shenyang Aerospace University in Shenbei New District, Shenyang, Liaoning. Corn stalks were also provided by the same breeding farm. Corn stalks were cut into pieces of 2-4 cm (length) by the knife mill before use.

The physicochemical properties of composting materials are shown in Table 1.

<table>
<thead>
<tr>
<th>Raw materials</th>
<th>Water content (%)</th>
<th>pH</th>
<th>Total carbon (%)</th>
<th>Total nitrogen (%)</th>
<th>Total potassium (K₂O%)</th>
<th>Total phosphorus (P₂O₅%)</th>
<th>C/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle manure</td>
<td>68.9</td>
<td>7.64</td>
<td>38.32</td>
<td>1.78</td>
<td>1.48</td>
<td>1.45</td>
<td>21.53</td>
</tr>
<tr>
<td>Pig manure</td>
<td>73.4</td>
<td>7.35</td>
<td>39.41</td>
<td>1.80</td>
<td>1.58</td>
<td>1.78</td>
<td>21.89</td>
</tr>
<tr>
<td>Chicken manure</td>
<td>70.8</td>
<td>7.28</td>
<td>41.15</td>
<td>2.99</td>
<td>1.52</td>
<td>2.98</td>
<td>13.76</td>
</tr>
<tr>
<td>Corn stalk</td>
<td>21.5</td>
<td>7.19</td>
<td>54.82</td>
<td>1.04</td>
<td>0.98</td>
<td>0.46</td>
<td>52.71</td>
</tr>
</tbody>
</table>

**Experimental schemes**

An aerobic static composting was conducted using the composting materials of three livestock manures, including cattle manure (R1), pig manure (R2) and chicken manure (R3). The 21 d composting reaction was conducted with six self-designed aerobic composting boxes among which two composing boxes were considered as a group to contain a single type of livestock manure.

The results of every parameter data in the experiments were taken as an average. Additives of 10% corn stalk adjusted the porosity and organic matter content of the livestock manure. In the whole composting process, different parameters like temperature, oxygen concentration, carbon dioxide concentration, density were monitored. Besides, the gas produced during the composting process was treated using a
bio-filter. The authors compared and analyzed the change in every parameter and the law of enzyme activities in the composting process with different livestock manures.

**Experiment apparatus**

The self-designed composting box was used in the composting experiment. The experiment apparatus of the composting is shown in Figure 1.

![Figure 1. Experimental apparatus used in the aerobic composting with livestock manures. 1. The vent of the composting box. 2. Back-ejecta nozzle of leachate. 3. Thermometer. 4. Composting box. 5. The gas outlet of bio-filter. 6. Thermometer. 7. Bio-filter. 8. Discharge pipe of leachate. 9. Grid plate. 10. Inlet pipe. 11. The flow meter. 12. Fan](image)

The livestock manure was mixed evenly and sampled to determine the density, pH value, conductivity, moisture content, organic content and ash content of raw materials. Then in proportion to add additives (straw), full mixing, sampling, determination of mixed materials of the above indicators.

Fill the compost box with the mixed materials, press gently to ensure the material is even, then weigh and measure the height of the material in the box and the diameter of the box. Seal the box with bolts, insert thermometer, add insulation layer, put the box on the reaction rack, connect the air pump and flow meter, and breathe air into the reactor. The first data was recorded from the time the air was pumped in; temperature was recorded every 2 h, O₂ and CO₂ concentrations in the compost bin and biofilter were recorded every 4 h.

**Analysis method**

**Determination of total solid content (TS)**

The determination of TS aims to enable the evaporation of material under the condition of 110 °C until the material is completely in the dry state. With the measurement of precise balance, the weight of dry crucible was recorded as m₁. Then the experimental materials were weighed together with precise balance, and the weight was denoted as m₂. Crucible was dried until its mass is no longer changed, and the mass was recorded as m₃.

The equation for the total solid content is as follows (Zhang et al., 2011):
Determination of volatile solids (VS) content

According to the definition of volatile solid, under the condition of 650 °C, high temperature heat comes out of that part of the solid. When the sample is heated and cooled, the portion of the lost weight is the volatile solid, and the non-volatile residue is called ash (expressed as a percentage or g/L). The drying samples were put in the muffle furnace under the high temperature of 550 °C for 3 h (Feng et al., 2015). Subsequently, the samples were removed for half an hour cooling after being weighted, and the average quality was recorded as \( m_1 \). The calculation of volatile solid can be expressed as:

\[
TS = \frac{m_3 - m_1}{m_2 - m_1} \times 100\%
\]

(Eq.1)

where: VS represents the volatile solid content of the sample, %;

\( M_1 \) is the mass of the crucible, g;

\( M_2 \) represents the total mass of the sample and crucible before drying, g;

\( M_3 \) represents the total mass of the sample and crucible after drying (before putting it into the muffle furnace), g;

\( M_4 \) represents the total mass of sample and crucible after putting into a muffle furnace, g.

Determination of pH value

The samples were tested in a special centrifugal tube, and after centrifugation for 30 minutes, the supernatant was removed for pH determination. PH meter was used to measure the supernatant and record the data.

Determination of enzyme activity

The KMnO\(_4\) titration method was used to determine the enzyme and catalase activities in soil. The results were demonstrated by the quantity of KMnO\(_4\) consumed in ml by unit composting weight with the unit of 0.002 mol•L\(^{-1}\)•g\(^{-1}\)•20 min\(^{-1}\) (Seal et al., 2012). The determination of urease activity was done via colorimetric method utilizing phenol sodium and sodium hypochlorite. The results were demonstrated by the milligram quantity of NH\(_4^+\)-N per 100 g composting with the unit of mg•g\(^{-1}\)•d\(^{-1}\) (Gray F, 2015). The determination of invertase was carried out with the 3,5-dinitrosalicylic acid method and the results were demonstrated by the milligram quantity of glucose produced by 1 g composting in 1 d with the unit of mg•g\(^{-1}\)•d\(^{-1}\) (Margaritis et al., 2017).

Results and discussion

The change of temperature, pH and water content in the composting process with different livestock manure

Temperature changes

Composting is a biochemical process dominated by microorganisms. In this process, the temperature is an important index that affects both the microbial activity and the microbial...
community structure. The duration of the high-temperature stage in the composting process largely determines the composting effect and the quality of the composting product (Chen, 2012; Jieying et al., 2017).

Figure 2 shows the temperature changes during the composting processes of three types of manures. For all three types of manure, the composting process exhibited the high-temperature period (45 °C) at the same time (6 days), and they were subjected at these conditions for 15 days. R1, R2 and R3 reached the highest temperature on the 15th day, with values of 56.1 °C, 58.3 °C and 59.3 °C, respectively. Among them, R3 (chicken manure) treatment stayed at the high-temperature stage for the longest time, while the high-temperature periods for R1 (cattle manure) and R2 (pig manure) treatments were relatively short. After 18 days, all piles entered the cooling stage after lowering for three days, during which the temperature dropped to 45–47 °C at the end of the composting.

During the composting process, the temperature increase of the pile is an accumulative result of the heat generated by the microbial metabolism; therefore it reflects the metabolic intensity of microorganisms and the conversion rate of the composting substances (Jieying et al., 2017). If the temperature is too low or the high-temperature stage is too short, the pest eggs and weed seeds cannot be effectively erased. On the other hand, if the temperature is too high, the production efficiency of the enterprise is compromised, and some medium-temperature microorganisms that mainly decompose cellulose and lignin may not be able to survive, thus affecting the composting process. Research (Wei et al., 2018) suggested that the preferred high-temperature for the solid organic waste composting is 60–65 °C, and that it needs to be maintained for more than 7 days. In this experiment, the composting treatments of the three types of manures showed a rapid heating feature, and the high-temperature (55 °C) duration was 16–18 days.

![Figure 2. Temperature changes of the three manures of composting](image)

Changes in pH during composting of the livestock and poultry manure

Figure 3 shows the changes in pH during the composting of the livestock and poultry manures. For all three types of manures, the pH increased initially and decreased later. At the end of the composting process, the pH showed an increase again. Until day 3, the pH change of R1 was 7.27-7.55. In addition, the corresponding pH change of R2 was 7.29-7.65. Moreover, the pH change of R3 was 7.33-7.87. These changes were mainly
due to the decomposition of amino acids in the manure, as deamination of the amino acids could cause a slight increase of pH in the whole piles. From day 4, the pH of all the composting reactions started to drop. The lowest pH of R3 (chicken manure) compost was 6.07 during the whole composting process. In addition, the lowest pH of R1 was 6.07 which appeared on day 15. Besides, the lowest pH of R2 was 6.31 which appeared on the day 12. Such low pH was mainly due to the decomposition of the organic matter in the compost material of livestock and poultry that produced organic acids, organic acids led to a decrease of pH. It is worth noting that the organic matter from the chicken manure is more susceptible to hydrolysis and acidification, thus the pH value of R3 decreased to a highest degree.

![Figure 3. Changes in pH of three kinds](image)

On day 15, starting from 6.17 (R1), 6.31 (R2) and 6.07 (R3), the pH values of all the composting reactions began to rise again, during the high-temperature stage, the organic acids in the piles were efficiently decomposed by the thermophilic bacteria to produce CO₂, and the decrease of organic acid content, therefore, led to a rise in pH. At the end of composting, the pH values of the three piles were 6.99 (R1), 6.89 (R2), and 7.04 (R3). These change patterns was due to the oxidative decomposition of the decomposable organic matter in the piles. The produced nitrogen (ammonium) accumulated continuously, causing an increase in the pH of the piles. At the same time, the increase of pH gave rise to the volatilization of ammonium nitrogen; therefore, at the end of composting, the pH values of all the manures were in the range of 6.9–7.1.

Changes in moisture content during composting of manures

*Figure 4* shows the changes in moisture content during the composting of the livestock and poultry manure. For all three manure types, the initial moisture percentages were 64–67%. The moisture content reduced, as the composting process started, and the percentage of the moisture content in all piles in the end was 30–32%. R1 (cattle manure) showed a significant moisture loss (about 35%). The period with the largest decrease in the moisture content was the high-temperature stage, probably due to the high metabolic activity of the microorganisms during the heating-stage and high-temperature stage. In particular, during the high-temperature stage, the thermophilic bacteria dominated the microbial community, and at this stage, they rapidly utilized and degraded the organic matter. Consequently, the demand for oxygen significantly
increased. Because of the cold incoming air and the hot outgoing air, the water saturation of the outgoing air increased significantly, resulting in a faster use of water and more marked evaporation. At the end of the composting experiment, the moisture percentages were 30.11 (R1), 32.22 (R2) and 30.01 (R3). If too much water evaporates during the high-temperature period, the moisture in the late composting period and the rate of decomposing can significantly reduce, thus prolonging the composting cycle. Therefore, appropriate water replenishment needs to be applied when the moisture percentage in the maturity period is less than 20%.

Moisture content and pH are the two important indices of the composting process. Moisture can dissolve the organic matter during composting, participate in microbial metabolism, and regulate the compost temperature. Neither a high moisture content, nor low moisture content is beneficial to the survival of the microorganisms. If the moisture content is too high, it indicates that the ventilation and permeability are poor, and this can lead to partial hypoxia of the pile and produce acid odor. Furthermore, it is not conducive to the export of nutrients, thus resulting in an economic loss. On the other hand, a dry environment restricts the metabolism of the microorganisms. Studies have shown that the optimum moisture percentage in the early stage of composting is 50–65%, and in the later stage of composting, in order to maintain the speed of composting and shorten the composting cycle, the moisture percentage should not be less than 20%. The optimal pH for composting is 5.5–8.5. It is interesting that the initial moisture content and the pH measured in this experiment are in the optimal range.

![Figure 4. Change in moisture content of the three manures of three kinds of composting](image)

**Figure 4. Change in moisture content of the three manures of three kinds of composting**

**The change of C-N-P-K content in the composting process with different livestock manure**

The total nutrient of N+P$_2$O$_5$+K$_2$O is an essential index for the quantity of organic fertilizer (Awasthi et al., 2015). There are three pathways of nitrogen transformation in the composting process. The first one is the emission of ammonia gas to the atmosphere, resulting in a loss of nutrients from the composting system. The second one is the transformation of nitrogenous compounds to nitrate or nitrite by nitrifying bacteria or nitrite bacteria, respectively. The third one is the assimilation of nitrogen by microorganisms. The content of total nutrients (N + P$_2$O$_5$ + K$_2$O) shows an increasing trend in the composting process due to two reasons. The first reason is the emission of the organic state released and transformed to the inorganic state due to the
decomposition of organic matters in the composting process. The second reason is the quantity of organic carbon decreased due to the functions of microorganisms which caused an increase in the concentration effect. Thus, the total nutrient content increased (Zhuotong et al., 2018; Wang et al., 2017, 2014). According to Table 2, the total nutrient content of pig manure was the highest with the value of 6.33% and that of the cattle manure was lowest (5.04%).

According to the Organic Fertilizer Industry Standards (NY525-2012) issued in 2012, the mass fraction of organic matters in the qualified products should be larger than 45%. In addition, the mass fraction of the total nutrients (N + P₂O₅ + K₂O) should be larger than 5%. Besides, the mass fraction of water content should be smaller than 30%. Besides, values of pH in the qualified products should be in the range of 5.5-8.5. According to Table 2, all the four composting products from the present study satisfied the requirements of Organic Fertilizer Industry Standards (NY525-2012) (Jain et al., 2018).

<table>
<thead>
<tr>
<th>The composting products</th>
<th>TN/%</th>
<th>TP/%</th>
<th>TK/%</th>
<th>(N+P₂O₅+K₂O)/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1 (cattle manure)</td>
<td>1.93±0.18</td>
<td>1.57±0.19</td>
<td>1.54±0.19</td>
<td>5.04</td>
</tr>
<tr>
<td>R2 (pig manure)</td>
<td>2.25±0.18</td>
<td>2.41±0.15</td>
<td>1.67±0.12</td>
<td>6.33</td>
</tr>
<tr>
<td>R3 (chicken manure)</td>
<td>2.14±0.15</td>
<td>1.83±0.16</td>
<td>1.78±0.19</td>
<td>5.75</td>
</tr>
</tbody>
</table>

The enzyme activity changes in the composting process with different livestock manure

Composting is a biochemical process where decomposition and transformation of organic matters to stable humus take place. The process follows the utilization of the secreted enzymes in the growth and reproduction processes as well as metabolic activities of microorganisms. So, different enzymes are involved in the whole composting process (Hannum et al., 2017). Enzymes are composed of apoenzyme and the cofactor has characteristics of specificity, high efficiency and diversity. In the present study, the relationships between the enzyme activity change of catalase, urease and invertase and the quantity of microorganisms were systematically compared. For the purpose, composting process with four livestock manure was investigated targeting to provide theoretical evidence for the composting mechanism.

The enzyme activity change of catalase in the composting process with different livestock manure

Catalase demonstrates the intensity of the degree of composting oxidation, because catalase can decompose hydrogen peroxide produced in the composting process and thus decrease its poisoning effect to microorganism (Ukalska-Jaruga et al., 2018).

Figure 5 showed the changing trend of the enzyme activity of catalase in the composting process with different livestock manure. It showed that the initial concentration of catalase in the initial composting reaction of R1 (cattle manure) was 48.21 ± 1.11 mol·L⁻¹·g⁻¹. Besides, the initial concentration of catalase of R2 (pig manure) was 45.04 ± 0.85 mol·L⁻¹·g⁻¹. Moreover, the initial concentration of catalase of R3 (chicken manure) was 44.29 ± 0.65/0.002 mol·L⁻¹·g⁻¹. With the process of the
composting, the concentration of catalase gradually increased. At the end of the reaction, the concentration of catalase were in descending order of R1 (cattle manure) > R2 (pig manure) > R3 (chicken manure) with corresponding values of 62.18 ± 0.45, 55.21 ± 0.58 and 54.28 ± 0.14/0.002 mol•L⁻¹•g⁻¹, respectively. The main reason for the gradual increase of catalase concentration in the composting process with livestock manure was the increase of biochemical metabolism activity. This activity was carried out by the thermophilic bacterium as a replacement for the mesophilic bacterium. The latter bacterium happened to be the active microbial flora with the increase of the pile temperature. Besides, the temperature was also an important factor which influenced enzymatic reactions. Thus, the concentration of catalase increased gradually with the process of reactions.

![Figure 5. Activity change in catalase](image)

The enzyme activity change of urease in the composting process with different livestock manure

Figure 6 shows the changing trend of the enzyme activity of urease in the composting process with different livestock manure. Urease could catalyze the transformation and decomposition of urea to ammonia and CO₂ as well as promote the transformation and decomposition of nitrogenous organic matter. Thus, it was the key index to evaluate the transformation velocity of nitrogen in the composting process. It showed that the initial urease concentrations in the primary reaction period of the composting were 258.47 ± 8.35, 249.78 ± 3.98 and 4261.87 ± 4.28 mg•g⁻¹•d⁻¹ for R1 (cattle manure), R2 (pig manure) and R3 (chicken manure), respectively. This illustrates the high activities of microorganisms in the initial composting period which secreted urease rapidly to decompose and transform susceptible nitrogenous organic matters. With the process of the reaction, urease activity became passive with the increase in temperature and demonstrated negative correlation with the change of temperature (Jain et al., 2018; Hannum et al., 2017). The urease content decreased gradually due to the decrease of the mesophilic microorganism quantities. The decrease of urease activities and the slowdown of decomposition and mineralization of nitrogenous organic matters occurred when the process entered into the high temperature period of the composting. At the end of the reaction, the urease concentrations in the pile were in a descending order of R1 (cattle manure) > R3 (chicken manure) > R2 (pig manure) with values of 54.28 ± 8.35, 51.24 ± 1.25 and 50.24 ± 5.36 mg•g⁻¹•d⁻¹, respectively.
The change of invertase activity in the composting with different livestock manure

Figure 7 shows the changing trend of invertase activity in the composting with different livestock manures. Results show that the changing trends of invertase and catalase were the same. The initial catalase concentrations of the composting with R1 (cattle manure), R2 (pig manure) and R3 (chicken manure) were 50.28 ± 1.21, 42.27 ± 1.25 and 44.54 ± 1.01 mg•g⁻¹•d⁻¹, respectively. Results indicate that the high activity of microorganism in the initial period of composting, which secreted invertase rapidly to decompose and transform susceptible nitrogenous organic matters (Li et al., 2016; Kazemi et al., 2016). With the process of the reaction, the invertase content in the pile decreased gradually, mainly due to the decrease in quantities of mesophilic microorganisms. Therefore, a decrease of invertase activities and the slowdown of the decomposition and mineralization rate of nitrogenous organic matters also occurred when the reaction phases entered into the high-temperature period of the composting. At the end of the reaction, the urease concentrations were in descending order of R2 (pig manure) > R3 (chicken manure) > R1 (cattle manure) with values of 11.28 ± 0.14, 10.94 ± 1.11 and 10.45 ± 0.97 mg•g⁻¹•d⁻¹, respectively (Teutscherova et al., 2017; Sharma et al., 2017).
Conclusions

The composting treatment of three livestock manure entered the high-temperature period (45 °C) on the 6th day. Besides, their temperatures reached the maximum of 56.1, 58.3 and 59.3 °C, respectively. The number of days with temperature above 55 °C were 6, 6 and 9 d, respectively. Among them, the treatment of R3 (chicken manure) had the most days which belonged to the high temperature period, whereas the treatment of R1 (cattle manure) and R2 (pig manure) had relatively fewer days for belonging high-temperature period. All of them could satisfy the requirement of maturity standards.

pH values of the composting process with three livestock manure demonstrated the trend of initial increase, then decrease and final increase rally. pH of the R3 composting (chicken manure) was the lowest in the whole composting process. The value was 6.07 on the 15th day. Besides, pH values recorded were in the range of 6.9-7.1 at the end of the composting. Furthermore, the change of water content of three composting manures demonstrated the decreasing trend with the decreasing amplitude of 34-36%.

At the end of the composting with livestock manure, the total nutrients of pig manure composting were the highest with the value of 6.33%. The total nutrients of cattle manure composting were the lowest with the value of 5.04%.

Different types of enzymes were involved in different stages in the composting process. In the whole composting system, the activity change of urease and invertase was same with a decreasing trend as a whole. However, the catalase activity increased gradually with the process of composting. At the end of the composting reaction, the concentrations of catalase, urease and invertase were the highest in the piles of R1 (cattle manure), R1 (cattle manure) and R2 (pig manure), respectively, with corresponding values of 62.18 + 0.45/0.002mol•L⁻¹•g⁻¹, 54.28 ± 8.35 mg•g⁻¹•d⁻¹ and 11.28 ± 0.14 mg•g⁻¹•d⁻¹.

Recommendation

The paper compared the changing pattern of enzyme activities in composting with different livestock manures added 10% straw as bulking agent and provided theoretical evidence for temperature, pH, moisture content and nutrition parameters. Future studies should focus on the change of microbial population and the correlation between microbial quantity and enzyme activity, so as to further analyze the mechanism of microbial enzymatic reaction and study the biochemical reaction activity of livestock and poultry manure compost.

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