Application of Compound Microbial Preparations in Composting with Lactic Acid Fermentation Residue from Kitchen Waste

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Original scientific paper Received: February 22, 2010 Accepted: October 30, 2010

In order to utilize the lactic fermentation residue obtained from kitchen waste, composting was carried out with such residue and sawdust. Compound microbial preparations (CMPs) were added into the system to test feasibility to facilitate the composting process. The changes of physicochemical properties and microbiological index were investigated during composting with lactic acid fermentation residue added with sawdust as the conditioner. The results showed that the addition of CMPs resulted in a rapid increase in temperature of the system, and prolonged the maintaining time during high temperature phase. Compared with the system without inoculation (control group), TOC degradation efficiency could be increased by 4.5 %, NH4+-N mass fraction decreased by 71.8 % (30 d), and the NO3-N mass fraction increased by 35.8 % (30 d) in the inoculated system; furthermore, the release of ammonia could be reduced, thus the function of N maintaining and odor inhibition could be achieved. The amount of microorganisms in the inoculated group was $2 \sim 3$ orders of magnitude higher than those of the control group. The coliform could not be examined during the final phase of composting, which accorded with the hygienic criterion. The composting of lactic acid fermentation residue from kitchen waste could effectively utilize the fermentative byproduct, and prevent secondary pollution.

Key words:

Kitchen waste, fermentation residue, aerobic composting, compound microbial preparation, lactic acid

Introduction

Kitchen waste discharged from households and restaurants occupies a large proportion of municipal domestic garbage in China. For example, over 1300 tons of kitchen waste are discharged per day in Shanghai.¹ Kitchen waste is characterized by high organic content and high moisture, thus making the landfilling and incineration processes unsuitable.²

Kitchen waste could be utilized as a kind of raw material for composting due to its abundant elements and organic contents. Lee *et al.* studied the effects of food waste (FW), commercial compost (CC) and mineral fertilizer (MF) on bacterial and fungal populations, soil enzyme activities and growth of lettuce in a greenhouse.³ Xi *et al.* studied the effects of different conditioners, such as sawdust, straw, leaves, and horse manure in composting kitchen waste. Their results showed that dry horse ordure and sawdust significantly improved the structure, enhanced the moisture holding capacity, and increased the organics degradation rate.⁴ It was found that organic substrates in the stacking could be degraded rapidly when the effective microorganisms (EM) were inoculated into kitchen waste. This could speed up the composting process and shorten the maturity period to 12 days, while that of the control group would take 30 days. However, it took a larger space and longer time, and some germs could not be killed easily in the composting process. Moreover, the high salt and fat contents in kitchen waste were unfavorable to the growth of microorganisms, which restricted the development of the composting process.

Shirai⁵ developed a new system for reducing and recycling kitchen waste. In this system, kitchen waste was transported to a factory for lactic acid (LA) production. After the waste was sterilized, lactic acid bacteria (LAB) was inoculated to produce LA. The residue obtained after separating LA was utilized as high quality fertilizer. In this process, LA production from kitchen waste could be achieved, and its relevant environmental problems could be solved.⁶ Yet, little information showed the utilization of Chinese kitchen waste in the process mentioned above. To evaluate the parameters and characteristics of the process was of great importance to its application and development in China.

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In fact, some Chinese researchers have carried out studies on lactic acid production from kitchen waste. Their research results showed that lactic acid could be obtained if the kitchen waste was anaerobically stored. LA production from kitchen waste inoculated LAB was 36.9 % higher than those of the fermentation without inoculum (control).⁹ Particularly the utilization of LAB screened from kitchen waste was found to be more beneficial in LA production.7 Wang's study demonstrated that kitchen waste could also be utilized to produce glucoamylase.¹⁰ The resource technology for the lactic acid fermentation residue should be carried out since this could not only meet environmental requirements, but also obtain a value-added product. Composting is a proper option due to its high economic and environmental value. But how to accelerate the composing process and reduce the odor produced are of great importance.11 The dominant microbial population in the fermentation residue is lactic acid bacteria. In order to increase the quantity and type of microbe in the early period of composting, and thus enhance the microbial degradation activity to step into the thermophilic phase earlier, microorganisms should be inoculated into the composting substrates. The inoculation of compound microbial preparations (CMPs) was found to be a useful method, but little information is available on utilization of CMP in lactic acid fermentation residue obtained from kitchen waste in China.

In this paper, the changes of physicochemical properties and microbiological index were investigated during the composting process of lactic acid fermentation residue inoculated with CMPs. The purpose was to utilize the fermentation residue as raw material and sawdust as conditioner to raise organic pollutants biodegradation rate, shorten the composting period, reduce odor, and improve compost quality.

Materials and methods

Aerobic composting reactor

The reactor was a 30 x 50 cm cylinder (30 cm diameter, 50 cm height) with three sampling points in its different parts. The stack was stirred mechanically by a motor-driven propeller above the reactor. A perforated pipe at the reactor bottom served for ventilation. Above the pipe, a perforated plate was installed to diffuse the ventilating air. The ventilating air flowed through an air compressor and is controlled by a flow meter and a time controller. The emission gas was absorbed and then discharged via one acid absorption bottle and one alkali absorption bottle. The temperature probes were installed along different heights of the reactor to

determine the stack's temperature. The reactor had a volume of 15 L, the effective volume of 12 L. When in operation, it will mix twice a day, 10 minutes at a time. The particle size diameter was $d_p = 0.5-2$ cm. Deionized water was used to dip into the compost for pH measurement. Experimental reactor is illustrated in Fig. 1.



Fig. 1 – Schematic of aerobic composting reactor

Experimental materials

Kitchen waste was collected at No. 2 Dining room on the campus of Harbin Institute of Technology. The waste was separated manually to remove the watters unsuitable for fermentation. The waste was stored at -20 °C for future utilization. LA fermentation from kitchen waste was carried out as mentioned in the previous article.⁹ After the LA was extracted by electrolysis, the fermentation residue was obtained by the following methods. The kitchen waste fermentation broth was centrifuged (3000 rpm, 10 min), the supernatant liquor poured out, and then the residue was washed twice to remove the remaining lactic acid. The physical and chemical properties of the residue are shown in Table 1.

Conditioner: Due to the high water content and the relatively low $r_{C/N}$ ratio in the fermentation residue, the addition of sawdust as conditioner was necessary in order to adjust porosity, water content and $r_{C/N}$. The sawdust was provided from the Harbin Xiangfang Timber Mill with properties as shown in Table 1.

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	Water fraction $w/\%$	Volatile solid fraction <i>w</i> /%	pН	r _{C/N}		
fermentation residue	70.0	27.2	5.68	13.8 : 1		
amendment (sawdust)	10.2	7.0	6.86	560 : 1		

Table 1 – Characteristics of the fermentation residue and bulking agent

Inoculation preparation: CMP was obtained from International Nature Farming Research Center, Japan. It was a mixed culture of microorganisms including a predominant population of thermophilic bacteria, fermenting fungi, and a small proportion of photosynthetic bacteria, actinomycetes, lactic acid bacteria and yeast.

Initial conditions of composting

By adding the conditioner, $r_{C/N}$, water content and pH value of the composting materials were adjusted. The parameters are shown in Table 2. The ventilation flow rate was $Q = 1.6 \text{ m}^3 \text{ d}^{-1}$, ventilation time was 0.5 h every time with the frequency of once an hour. Inoculum size of the complex microbial community of all tests was w = 3 %.

Table 2 – Initial composting conditions

Moisture, $\varphi^{/\%}$	рН	r _{C/N}	Inoculum size/%	Temperature, ϑ/°C
53	6.78	30:1	3	18

Analytical methods

Measurement of the physical-chemical indexes

20 g fresh samples were taken and weighed after drying to constant mass at 105 °C. The moisture was calculated by the reducing mass method. The dry samples were then weighed after calcinations to constant mass at 600 °C to get the VS (organic matter). The TOC was calculated by the empirical formula TOC = VS/1.8. Total nitrogen was obtained by semi-micrometheel of Kjeldah method.

Water soluble organic carbon (WSOC), ammonia nitrogen, NO_3^--N : Ten grams of fermentation residue freeze-dried to constant mass were extracted by 100 mL distilled water, and oscillated for 1 h in the shaker (oscillation frequency 200 rpm). Then, the sample was filtered through a 0.45 µm-pore membrane after being centrifuged at 3000 for 10 min. The water soluble organic carbon (WSOC), ammonia nitrogen, $NO_3^{-}-N$, and total nitrogen of the filter liquor were analyzed by standard potash dichromate oxidation process, Nessler's reagent photometry, ion chromatography with the type of DIONEX 4500i, potassium persulfate oxidation-ultraviolet spectrometer method, respectively. (Kwon and Lee, 2004).

pH and conductivity: Ten grams of the fresh sample was mixed with ten times its weight of deionized water, and oscillated for 30 min, then the pH value and conductivity of the clear supernatant liquid were measured with pH meter and conductivity ity meter.

Test of the microbiological indexes

The viable count was performed using the pour plate method. Total bacteria *eumycete*, *actinomycetes*, *Bacillus coli* were counted using beef extract peptone medium, Martin medium, Gause 1 medium, and EMB medium, respectively.¹⁴

Results and discussion

Changes of the physical and chemical indicators during aerobic composting

Temperature and water content

For composting systems, temperature is an important factor that influences the physiological activity of microorganisms and the composting processes. The appropriate temperature could help compost go on wheels and obtain high quality product. The microbial decomposition of organic compost would release heat, thus making the compost temperature rise. The change in temperature during composting with time is shown in Fig. 2, revealing that the temperature first increased and then declined. The temperature for CMP added rose quickly, and reached 55 °C on the second day, the



Fig. 2 – Change of temperature and moisture ratio during composting. $\Box \in T$ (environmental temperature); $\triangle T$ (no strain) Temperature with no strain added; $\blacktriangle T$ (strain added) temperature with strain added; \circ (no strain), moisture with no strain added; $\bullet M$ (strain added), moisture with strain added.

tiptop temperature was 63 °C. The tiptop temperature for control group was 54 °C. This result was mainly attributable to the addition of CMP to increase the number of microbial organisms in the compost, which boosted the decomposition capacity of organics and made the system temperature rise rapidly. The maintaining of high temperature could satisfy the requirements for temperature above 55 °C obtained during 5~7 d. In these conditions, not only would the pathogenic bacteria be killed, guaranteeing the hygiology index of compost, but the decomposition of organics would be boosted and the compost time shortened.

The water content was influenced by two factors; the organic matter oxygenolysis could produce moisture, while ventilation made the water volatilize in the form of vapor. From Fig. 2, we can see that the water content first increased, and then declined. This was because the microbe would decompose organics and produce large amounts of water at initial composting, followed by microbial activity cut down, water produced by decomposition of organic matter reduced, and ventilation caused substantial loss of water.

Water soluble ammonia nitrogen, NO₃⁻-N, and water soluble total nitrogen

Ammonia nitrogen could be transformed into $NO_2^{-}N$ and $NO_3^{-}N$ or evaporated in the form of NH_3 by nitrifying bacteria. These mainly depended on stack temperature and pH conditions. The change curves of water-soluble $NH_4^{+}N$ and $NO_3^{-}N$ are shown in the Fig. 3.

As shown in the figure, the changing trend of NH₄⁺-N during composting first increased, and then declined. This was because the ammonia nitrogen mass fraction was low at initial composting. As time proceeded, the organic ammonification reaction occurred, which led to accumulation of ammonia content, and reached its peak at a high temperature. At this time, the nitrifying bacteria would be seriously inhibited, nitration reaction was almost impossible, the major inorganic nitrogen on high-temperature phase existed in the form of ammonium N, and NO₃-N content was very low. After the high temperature period, with the temperature cutting down, nitrification strengthened and the accumulated ammonia nitrogen converted to NO₃⁻-N, so NO₃⁻-N mass fraction increased, particularly at the later period of composting, NO₃--N fraction scaled up fast. Moreover, the organic substrate in the system, which could be used by ammonification bacterium was almost exhausted, the NH₄⁺-N also decreased, therefore, NH₄⁺-N mass fraction had a descending tendency.



Fig. 3 – Change of NH_4^+ -N, NO_3^- -N and WSTN mass fraction during composting – – WSTN with strain added; – – WSTN with no strain added; – \blacktriangle – NH_4^+ -N with strain added; – \bigtriangleup – NH_4^+ -N with no strain added; ··· • ··· NO_3^- -N with strain added; ··· □ ··· NO_3^- -N with no strain added

Fig. 3 also demonstrated that compared with control group, the NH4+-N mass fraction of the group with CMP decreased 69.6 % (6 d) ~71.8 % (30 d), and the $NO_3^{-}N$ mass fraction increased 35.8 % (30 d) during composting. This showed that CMP had a good result to preserve nitrogen and reduce dissociation, loss of NH₃ form. The change curve of water-soluble total nitrogen (WSTN) including water-soluble ammonia nitrogen, nitrate nitrogen, nitrite nitrogen and organic nitrogen was shown in the Fig. 3. From the figure, we could see that in the initial composting, a large number of readily biodegradable materials hydrolyzed rapidly, ammonification made WSTN increase rapidly. As the composting proceeded, the organic nitrogen mineralization, volatilization of some ammonia nitrogen, and re-nitrification of some parts nitrate-nitrogen reduced the WSTN mass fraction.

pH and electronics conductivity

pH is one of the important factors affecting microbial growth. For most microbes, the appropriate pH value is neutral or weak alkaline. According to Fig. 4, the pH value increased rapidly in the initial composting phase due to the rapid decomposition of nitrogen-containing organics such as protein by ammonifiers. However, in late composting phase, the pH value dropped, because ammonia nitrogen changed into nitrate nitrogen as the role of nitrifying bacteria. In the control group experiment, composting was carried out for 6 d with pH 8.5 and a significant ammonia smell released. This was because the release of ammonia was closely related to the environmental pH, when pH was less than 7, ammonia was rarely released. When pH was more than 8.5, the release of ammonia accelerated and odor occurred. Whereas the composting process with CMP (compound microbial preparation) did not produce a bad smell, which noted that CMP had good deodorizing ability. This was probably due to inhabitation and proliferation of bacteria from the CMP. In addition, the low pH and low water-soluble ammonia nitrogen mass fraction also reduced the release of ammonia.



Fig. 4 – Change of pH value and EC during composing $-\blacksquare$ – pH with strain added; $-\Box$ – pH with no strain added; $-\blacktriangle$ – EC with strain added; $-\bigtriangleup$ – EC with no strain added

Electrical conductivity reflected the ion concentration of leaching liquor, which was the content of soluble salt. Since the kitchen waste contained a high salt content, it would cause salinization of soil if directly applied to farmland. The salinity for fermentation residue was low, because the salt was removed in the liquid phase. Recent studies have shown that when the EC is less than $\kappa = 9.0 \ \mu \text{s cm}^{-1}$ it has no effect on the seed germination rate.

As shown in Fig. 4, the microorganism decomposed giant molecule to small molecular, which aroused the EC during the initial stages of composting. With composting, the microbes produce carbon dioxide, ammonia and other losses, which also arouses the increase of EC. At the telophase of composting, all of the EC was lower than $\kappa = 9.0 \ \mu s \ cm^{-1}$, which had no adverse effect on the plant.

TOC degradation rate and water soluble organic carbon (WSOC)

The change of TOC degradation rate during the composting process is shown in Fig. 5. In this figure, the microbial activity in the initial stage of composting was very high, and the TOC degradation rate quite rapid. After 30 days, the TOC degradation rate in the compost with CMP was 4.5 % higher than that of the control group, amounting to 13.28 %. This suggested that the addition of CMP helped increase the microbial biomass, enhance the organic degradation capacity, and facilitated the stability of the composting reaction process.



Fig. 5 – Change of TOC degradation ratio and WSOC during composting $-\blacksquare$ – TOC degradation rate with strain added; $-\Box$ – TOC degradation rate with no strain added; $-\blacktriangle$ – WSOC with strain added; $-\triangle$ – WSOC with no strain added

The biodegradable contents of compost could be hydrolyzed into water soluble contents through the extracellular enzymes secreted by microbes. Therefore, water soluble organic carbons served as the first primary carbon source for microbial degradation. The change curve of WSOC during composting is shown in Fig. 5. It showed that as composting proceeded, two peak values of WSOC appeared. Although during the initial stage of composting, a great proportion of organics was decomposed, which converted insoluble organic carbon into soluble carbon, but the performance of WSOC biodegradation was poor. After the adaptation period, a large mass of WSOC, as the result of hydrolization reactions of organics in the compost with the aid of extracellular enzymes secreted by a great population of microbes, was synthesized into compounds for the microorganism's physical buildup or transformed into CO₂. In the moderate temperature stage, the bioactivity of thermophilic microbes was inhibited, while that of the mesophiles was restored. So, the process of converting insoluble organic carbon into water soluble organic carbon continued, leading to the appearance of the second peak value of WSOC.

Changes of the microbial indicator during aerobic composting

Bacteria and fungi

When the composting began, the number of microorganisms increased sharply and fluctuated during the growth period, and reached the maximum number on the day 7. The number of bacteria then decreased gradually and kept stable, increased slightly on day 23, and gradually became steady. The bacteria count in the control group was similar to that of CMP added, but the first peak appeared four days later than that of CMP added, which was on day 11, the peak number was one order of magnitude less. The number of the bacteria for the control group was all less than that of CMP added during the whole composting process, and the final bacteria count was 0.5 order of magnitude less. When it came to the number and change trend of fungi, they were similar to those of bacteria, only in a lower quantity.

In the initial phase of composting, the easily degradable materials were rapidly used by microbes. The nutrient requirements and proper temperatures for different microbes varied greatly. The change of stack environment resulted in the competition between different microbes. Thus, the total number increased at the initial composting phase, but there were some fluctuations. When the easily biodegradable substances were consumed, a large number of microorganisms were dead and resulted in the decline in quantity.

As the composting proceeded, the easily degradable substances gradually weakened the competitiveness of microorganisms, while the microbes which could utilize hard degradable materials began to work, then the second peak appeared. But the second peak was far less than the first one, and the peak period for bacteria was earlier than that for fungi. Although the microbial changes in



F ig. 6 − Change of bacteria and fungi counts during composting − fungi counts with strain added (log CFU g⁻¹ dry mass); −□− fungi counts with no strain added (log CFU g⁻¹ dry mass); −▲− bacteria counts with strain added (log CFU g⁻¹ dry mass); −△− bacteria counts with no strain added (log CFU g⁻¹ dry mass)

the overall trend were similar, but the various types of microorganisms in the CMP added were higher than those of control, indicating that the addition of CMP was beneficial for the increase of microbes.

Actinomycete and change of pathogenic bacteria indicator microorganism

Actinomycetes could degrade cellulose and dissolve lignin as well. Compared with fungi, they could suffer from high temperature. For this reason, actinomyces was regarded as the dominant microbial during the mesotherm period in the composting process, even though its ability to degrade cellulose and lignin was not as powerful as fungi. In poor living conditions, actinomyces survived in the form of a spore. The change of actinomycetes counts is shown in Fig. 7.



Fig. 7 – Change of actinomycetes and coliform counts during composting. - – coliform counts with strain added (log CFU g⁻¹ dry mass); - – coliform counts with no strain added (log CFU g⁻¹ dry mass); - – actinomycete counts with strain added (log CFU g⁻¹ dry mass); $-\Delta$ – actinomycete counts with no strain added (log CFU g⁻¹ dry mass)

Compared with bacteria and fungi, the level of thermophilic actinomycetes counts at the initial stage were less, and had no peak value. When the temperature continued to rise, the thermophilic actinomycetes began to grow and breed. The amount of actinomyces of the two sets of experiments reached peak value on days 7 and 11, respectively, and the value decreased as the temperature went down.

Coliform is a kind of bacteria, and high temperature may kill coliform and other pathogenic bacteria as well. The coliform count is also shown in Fig. 7. Fermentation residue contained certain levels of coliform, but not very many. The level increased to some extent at the initial stage of composting. However, as temperature increased, the coliform counts dropped sharply and almost no coliform existed in the late stage. Its sterilizing ratio was as high as 100 %, which fits the hygiene standard of composting as well.

Conclusions

The physical and chemical properties of the composting process with lactic acid fermentation residue were investigated with and without CMP. The result demonstrated that the addition of CMP was helpful for the rapid heating of the system, maintaining high temperature steadily and endurably. Compared to the control group, the microbe quantity of the compost system increased by 1-2 orders of magnitude over the same period, which enhanced decomposition of organic matter and shortened the composting cycle time; water-solubility of NH₄⁺-N mass fraction decreased 71.8 % (30 d), water-solubility of nitrate-nitrogen mass fraction increased 35.8 % (30 d), which decreased the release of ammonia thus conserving the nitrogen in the compost, reducing odour production.

Many kinds of microbes were discovered in the composting pile, among them bacteria had the largest number, about 10^{10} cfu g⁻¹ dry materials, fungi ranked the second and actinomycetes was the least. The existence of coliform bacterium could not be detected at the later composting stage, which is consistent with the compost hygiology indexes.

The fermentation residue was a suitable composting material due to its high content of organic matter, all-round nutrition elements, and low salt and oil content. This method could realize the reuse and reduction of kitchen waste, prevent secondary pollution, but also the composting product could meliorate the soil and promote agricultural production, and save the cost of lactic acid fermentation.

ACKNOWLEDGEMENT

This research was supported by the 11th Five Years Plan for Scientific and Technological Support Program (2006BAJ04A06-03), national science foundation of China (50978028) and (51008020), national high technology development plan (863 project) (2008AA06Z341)

List of symbols

- $d_{\rm p}$ particle diameter, cm
- Q volumetric flow rate, m³ d⁻¹
- $r_{\rm C/N}$ mole ratio
- v biodegration rate, %
- w mass fraction, %, mg g⁻¹

Greek

- η degradation efficiency, %
- ϑ temperature, °C
- κ conductivity, µs cm⁻¹
- φ relative moisture, %

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