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Effect of Microbiological Inocula on Composting of Cow Manure

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An inoculum containing lignocellulolytic microbial consortium WCS-6, *Trichoderma harzianum* and *Saccharomyces cerevisiae* were applied to a mixture of cow manure and corn stalk (1:1). A number of composting parameters were monitored under low temperature condition (below 10 °C). It was found that the temperature reached the thermophilic phase (over 45 °C) quickly within 2 days and remained above 50 °C for about 10 days for inoculated treatment. Inoculation can significantly increase the TN, TP, TK and available potassium contents, and promote the degradation of lignocellulosic biomass. We found that the C/N ratios and GI changed rapidly at the beginning of the compost. The C/N ratio reached 18.5 on the 35th day and the GI was 83.4% at the end of the process for inoculation. Therefore, inoculation by microbial consortium would be effective in the composting process at low ambient temperature.

Key words: Cow manure, Inocula, Lignocellulolytic microbial consortium, Low temperature, Windrow composting.

China is a large animal husbandry. With rapid development of large-scale intensive livestock production, the output of livestock manure has been growing. These livestock farming solid wastes contain plenty of organic matters, such as nitrogen and phosphorus, which could be used to improve soil fertility (Liu *et al*., 2011). In the eastern area of China, the prevailing intensive animal husbandry is characterized by high productivity. Such high productivity generates a huge amount of manure residues, which has become a serious environmental and economic problem due to the long-term low-temperature climate.

Among the different proposals to lessen the negative effect mentioned above, composting seems to be one of the most interesting due to its eco-compatibility and relatively easy operational procedures (Liu *et al*., 2011). During the composting process, readily degradable organic substrate present in the refuse is mainly transformed into more stable, complex organic forms through the activities of successive microbial populations (Bustamante *et al*. 2008). The final product of composting consists of transformed, slowly-degradable compounds, intermediate breakdown products and the cell walls of dead microorganisms, which are classified together as humic substances(Wei *et al*., 2007). These stabilised materials could be applied to land as the fertilizer or soil conditioner(Zeng *et al*., 2007). They can be used as a source of organic matter, nutrients and living organisms, as well as providing plant growth regulators and properties. Additionally, they can enhance soil physical characteristics including cation exchange capacity, soil aeration and structure, buffer capacity and water holding capacity (Suárez-Estrella *et al*., 2007).

The main concerns for composting are the shortening of composting period and the improvement of compost quality. Microbial

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inoculum can be considered as an effective technology for acceleration of the composting process (Ming *et al*., 2008).

Recently, many studies have been carried out to study the effect of inoculation during composting (del Carmen Vargas- García *et al*., 2006; Lei *et al*., 2000; Liu *et al*., 2011; Ming *et al*., 2008; Sarkar *et al*., 2010; Suárez-Estrella *et al*., 2007; Tiquia *et al*., 1997; Wei *et al*., 2007; Zeng *et al*., 2010). Some studies have demonstrated that microorganisms present in the microbiological inocula promote the decomposition of organic wastes (del Carmen Vargas- García *et al*., 2006; Liu *et al*., 2011; Ming *et al*., 2008; Sarkar *et al*., 2010; Suárez-Estrella *et al*., 2007; Wei *et al*., 2007; Zeng *et al*., 2010). Notwithstanding, negative effect on composting were also observed in other studies (Lei *et al*., 2000; Tiquia *et al*., 1997). The ineffective performance of inoculation due to the weak adaptability of seed strains with artificial culture, and the potential function competition existed between seed strains and autochthonous communities (Ming *et al*., 2008). These studies were carried out at room temperature (above 20 ^{o}C), but the influence of microbial inoculation on composting which were conducted under low temperature condition (below 10 ^{o}C) would be desirable.

In this work, the impact of bacterial inoculum, including lignocellulolytic microbial consortium WCS-6, *Trichoderma harzianum* Rifai Strain TXL051 and *Saccharomyces cerevisiae* on the composting process were studied under low temperature condition (below 10 ^{o}C) in order to shorten the operation time and thus reduce the composting space and production costs. Different parameters including Hemicellulos, Cellulos, Lignin, different forms of C, N, P and K, as well as the germination index (GI) were investigated during windrow composting of cow manure.

MATERIALS AND METHODS

Microorganisms

The lignocellulolytic microbial community WCS-6 (Wang *et al*., 2011), *Saccharomyces cerevisiae*, and *Trichoderma harzianum* Rifai Strain TXL051 was used. The WCS-6 community was composed of *Bacillus thermoamylovorans*, *Paenibacillus barengoltzii*, *Proteobacterium* sp., *Pseudoxanthomonas taiwanensis*, *Rhizobiaceae* sp., *Bacillus* sp., *Beta proteobacterium* sp., *Petrobacter succinimandens*, *Tepidiphilus margaritifer*, *Ureibacillus thermosphaericus*, uncultured bacterium clone, uncultured *Clostridium* sp. clone, *Clostridium thermobutyricum*, and *Clostridium thermosuccinogenes* (Wang *et al*., 2011). Before inoculated, the complex microorganisms were cultivated by PCS (peptone cellulose solution) agar (0.1% yeast extract, 0.5% peptone, 0.2% $CaCO_3$, 0.5% NaCl, 0.5% filter paper, 2.0% agar, pH 7.0). Stock cultures of *Saccharomyces cerevisiae*, and *Trichoderma harzianum* Rifai Strain TXL051 were separately maintained on malt extract agar slants at 4 °C. The microorganism suspensions were prepared in sterile distilled water and their concentration was measured and adjusted to 1×10^{8} CFU ml^{-1}. The WCS-6, *Saccharomyces cerevisiae*, and *Trichoderma harzianum* Rifai Strain TXL051 were mixed in the ratio of 8:1:1 (w/w) for composting inoculation.

Experimental setup and sampling

Composting experiments were conducted at the composting site of Harbin Jingjing Agricultural Science and Technology Development Co., Ltd., situated in northeast Heilongjiang, China. The composting process was undertaken from October 25 to December 24, 2011. Take the cattle manure and corn stalk as composting raw materials, the main characteristics of the starting materials are presented in Table 1. The compost mixtures were prepared by mixing cow manure with corn stalk using the volume ratio of 1:1 and arranging them in trapezoidal heaps (area, 1.5 m^2; height, 1.0 m). The initial moisture content was adjusted to 60-65% (w/w) and a suspension was used as a 0.25% (v/v) inoculum. To provide some aeration, the mixture was turned every 3 days until the end of the process. A control was also run under similar condition without the introduction of the microbiological inocula.

Samples were taken at various stages of the composting process (0, 3, 6, 10, 15, 20, 25, 35, 45 and 60 days). Each sample was obtained by mixing subsamples from three different locations in the stacks. A portion of the samples was dried, ground and sieved (<1 mm) for analysis, while another part of the samples were placed in sterilized bags and stored at -20 ^{o}C.

Analysis

The temperature in the center of the composting materials was monitored every day using digital electronic thermometer. Total nitrogen (TN) and total phosphorus (TP) were determined according to the Kjeldahl procedure (Page, *et al*., 1982) and the method of Kitson (Kitson *et al*., 1944), respectively. NH_4^+-N and NO_3^-N were measured separately by Kjeldahl method (Sparks *et al*., 1996). The content of inorganic phosphorus was determined by using the Molybdenum-Antimony colorimetric method. Total potassium (TK) and available potassium were analyzed by atomic absorption spectroscopy. Total organic carbon (TOC) content was determined by the potassium dichromate method. C/N ratio was determined from the values of TOC and TN. The contents of hemicellulose, cellulose and lignin were determined as described by Watanabe *et al*. (1993).

The distilled water extract of the compost samples (1:10 compost: water ratio, w/v) were shaken for 2 h. About 3.0 mL of the extracts were added into Petri dishes with a filter paper until the filter paper was completely submerged. Twenty Chinese cabbage seeds were then placed on the filter paper. The germination percentages with respect to the control and root lengths were measured after incubating the covered Petri dishes in the dark at 25 °C for 48 h. The germination index (GI) was calculated by Eq. (1) (Zucconi *et al*., 1981).

$$GI = G_i R_b L_e / L_c \quad ...(1)$$

where G_i, L_e, and L_c are the percentage of germinated seeds with respect to the control, the mean total root length of the germinated seeds and the mean root length of the control, respectively.

Significant differences among the values of each parameter studied during composting were calculated by SPSS 19.0 software.

RESULTS AND DISCUSSION

Temperature evolution during composting

Earlier studies have shown that temperature is one of the key parameters used to monitor the composting process. It is well known that temperature evolution is associated to many of the biological reactions during composting, as well as is related to the capacity of the process to reduce the pathogen contents (Bustamante *et al*., 2008). The experiments were conducted in winter with an ambient temperature of -22–8 °C (Fig. 1). It was found that the temperature profiles of the microbiological inocula compost and natural

Table 1. Basic properties of compost materials

Composting material	Moisture (%)	pH	Total C ($g \cdot kg^{-1}$)	Total N ($g \cdot kg^{-1}$)	Total P ($g \cdot kg^{-1}$)	total K ($g \cdot kg^{-1}$)	C/N
Cattle manure	71.5	7.7	358.2	15.7	6.1	5.3	22.8
Corn stalk	9.4	8.1	418.2	8.2	3.2	17.5	51.0

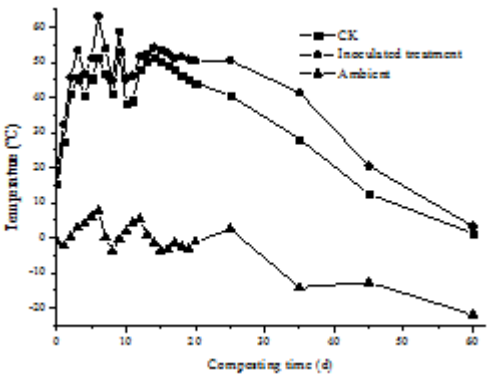

Fig. 1. Changes in Temperature during composting.

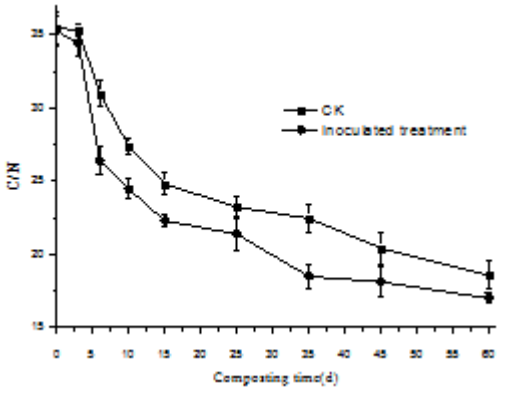

Fig. 2. Changes of C/N during composting.

Table 2. Changes in Nutritional components during compositing

Properties	Treatment	Composting time (days)								
		0	3	6	10	15	25	35	45	60
Total N (g/kg)	CK	14.3^{Aa}	16.4^{Ab}	20.7^{Ad}	18.7^{Ac}	17.6^{Ab}	17.0^{Ab}	17.1^{Ab}	16.5^{Ab}	15.1^{Aa}
	Inoculated treatment	14.4^{Aa}	19.2^{Bcd}	21.9^{Ae}	19.9^{Ad}	19.7^{Bd}	19.4^{Bcd}	18.0^{Abc}	17.4^{Ab}	16.5^{Bb}
NH_4^+-N (g/kg)	CK	0.47^{Ac}	0.95^{Ae}	1.01^{Ae}	0.97^{Ae}	0.65^{Ad}	0.48^{Ac}	0.38^{Ab}	0.31^{Aa}	0.29^{Aa}
	Inoculated treatment	0.48^{Ab}	0.97^{Ad}	0.95^{Ad}	0.60^{Bc}	0.47^{Bb}	0.21^{Ba}	0.21^{Ba}	0.20^{Ba}	0.18^{Ba}
NO_3-N (g/kg)	CK	0.08^{Aa}	0.16^{Ac}	0.06^{Aa}	0.13^{Ab}	0.12^{Ab}	0.19^{Ad}	0.36^{Ae}	0.38^{Aef}	0.41^{Af}
	Inoculated treatment	0.08^{Aa}	0.19^{Bb}	0.08^{Aa}	0.10^{Aa}	0.07^{Ba}	0.08^{Ba}	0.46^{Bc}	0.55^{Bd}	0.60^{Bf}
Total P (%)	CK	0.51^{Aa}	0.52^{Aa}	0.53^{Aa}	0.55^{Aab}	0.57^{Ab}	0.58^{Ab}	0.59^{Ab}	0.59^{Ab}	0.59^{Ab}
	Inoculated treatment	0.51^{Aa}	0.54^{Aa}	0.59^{Bb}	0.66^{Bc}	0.66^{Bc}	0.67^{Bc}	0.68^{Bc}	0.69^{Bc}	0.71^{Bc}
Total K (%)	CK	1.10^{Aa}	1.19^{Ab}	1.18^{Ab}	1.20^{Ab}	1.22^{Ab}	1.21^{Ab}	1.21^{Ab}	1.22^{Ab}	1.30^{Ab}
	Inoculated treatment	1.10^{Aa}	1.20^{Ab}	1.25^{Bc}	1.30^{Bd}	1.31^{Bd}	1.32^{Bd}	1.40^{Be}	1.39^{Be}	1.40^{Be}
Available K (%)	CK	0.62^{Aa}	0.67^{Ab}	0.72^{Ac}	0.77^{Acd}	0.77^{Acd}	0.78^{Ad}	0.78^{Ad}	0.79^{Ad}	0.80^{Ad}
	Inoculated treatment	0.61^{Aa}	0.85^{Bb}	0.90^{Bbc}	0.89^{Bbc}	0.90^{Bbc}	0.92^{Bc}	0.90^{Bbc}	0.92^{Bc}	0.94^{Bc}
Ratio of inorganic to total P	CK	48.0^{Ad}	46.2^{Ad}	46.6^{Ad}	45.1^{Ad}	42.0^{Ac}	37.9^{Ab}	35.2^{Aab}	32.7^{Aa}	32.2^{Aa}
	Inoculated treatment	48.1^{Af}	43.5^{Ae}	38.9^{Bd}	33.7^{Bc}	32.8^{Bc}	30.3^{Bb}	28.9^{Bb}	26.1^{Ba}	24.6^{Ba}
Hemicellulos (%)	CK	10.0^{Ad}	8.4^{Ac}	7.3^{Ab}	6.7^{Ab}	6.7^{Ab}	6.5^{Ab}	4.5^{Aa}	4.1^{Aa}	4.3^{Aa}
	Inoculated treatment	10.0^{Ah}	8.5^{Ag}	6.2^{Bf}	5.7^{Bef}	5.5^{Be}	4.5^{Bd}	3.7^{Bc}	2.9^{Bb}	2.2^{Ba}
Cellulos (%)	CK	21.8^{Af}	21.1^{Ae}	20.8^{Ae}	19.5^{Ad}	18.6^{Ac}	17.7^{Ab}	17.1^{Aa}	17.0^{Aa}	17.0^{Aa}
	Inoculated treatment	21.8^{Ag}	20.2^{Af}	19.4^{Be}	18.5^{Bd}	17.6^{Bc}	16.7^{Bb}	16.4^{Ab}	16.1^{Bab}	15.7^{Ba}
Lignin (%)	CK	3.9^{Aa}	4.5^{Ab}	5.6^{Ac}	6.1^{Ad}	6.4^{Ade}	6.6^{Ae}	5.7^{Ac}	5.5^{Ac}	5.5^{Ac}
	Inoculated treatment	3.8^{Aa}	4.7^{Ab}	6.6^{Be}	6.6^{Ae}	5.4^{Bd}	5.3^{Bcd}	5.2^{Acd}	4.9^{Bbc}	4.8^{Bbc}

Note: Different capital letters mean significant difference ($P<0.05$) among treatments of the same property. Different small letters mean significant difference ($P<0.05$) among treatments of composting time.

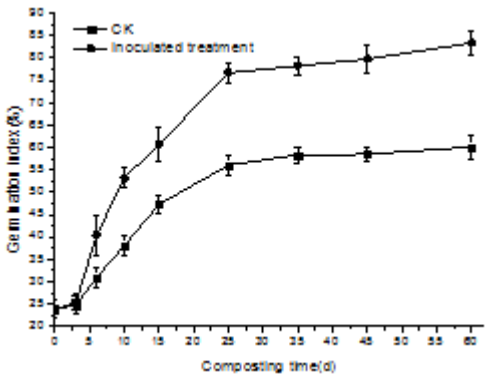

Fig. 3. Changes of GI during composting.

compost had a similar pattern. The temperature reached the thermophilic phase (over 45 °C) quickly within 2 days and remained above 50 °C for about 10 days for inoculated treatment (Fig. 1). This is in accordance with the temperature changes occurred in a composting system, initial rise followed by stabilization and drop (Zeng *et al*., 2010). As displayed in Fig. 1, it was observed that the natural compost reached the thermophilic temperature values slower and had a shorter thermophilic phase than inoculated treatment. It has been reported that more heat output probably was the result of biological activity in compost (Zeng *et al*., 2010). Our results indicated that the microbiological inocula could start temperature rapidly and prolong

the time of high temperature process (Liu *et al*., 2011).

Chemical composition evolution during composting

Changes in the chemical composition of the different compost treatments during the composting period are given in Table 2. It was observed that the contents of TN and NH_4^+-N increased firstly and then decreased in all runs. The NO_3^--N content also increased firstly and then decreased during the first fermentation phase, while rapidly increased during the second fermentation phase. The TN and NO_3^--N contents in inoculated treatment were significantly higher than those of natural compost. In contrast, the NH_4^+-N content in inoculated treatment (0.18 g/kg) was significantly lower than that of natural compost (0.29 g/kg). It is generally believed that the decrease in NH_4^+-N and increase in NO_3^--N content is a sign of compost maturity. Moreover, it has been stated that when the NH_4^+-N content is less than 0.4 g/kg, the compost is mature (Riffaldi *et al*., 1986). In our experiment, the NH_4^+-N contents decreased below 0.4 g/kg at the 25th day for inoculated treatment (0.38 g/kg) and the 35th day for natural compost (0.21 g/kg), respectively. These implied that the microbiological inocula could improve the TN content, promote the conversion of NH_4^+-N to NO_3^--N, and speed up the composting process.

The variation tendencies of the contents for TP, TK and available potassium are basically similar during composting in all runs, showing ascendant trend. It can be seen that the TP contents increased from 0.51% to 0.59% for inoculated treatment and increased from 0.51% to 0.69% for natural compost, respectively. The contents of TK and available potassium were 1.3% and 0.8% at the 60th day for natural compost, while were 1.4% and 0.94% for inoculated treatment. This was attributed to the decay of organic matter, the loss of carbon as carbon dioxide emissions and the decrease of dry matter as water evaporation, resulting in the concentration of phosphorus and potassium (Vuorinen *et al*., 1997). With the increase of temperature, the microbial activity was improved during the composting process. So the high microbial activity promoted the adsorption of inorganic P, which results in the decrease of inorganic P and increase of organic P. Therefore, the ratios of inorganic to total P for inoculated treatment and natural compost decreased gradually during the composting period. The TP, TK and available potassium contents in inoculated treatment were significantly higher than those of natural compost at the end of composting. While the ratio of inorganic to total P for inoculated treatment were significantly lower than those for natural compost. These results indicated that microbiological inocula could promote concentration of phosphorus and potassium, and speeded up the conversion of inorganic P to organic P.

The concentration changes of the main components of the lignocellulosic fraction during composting are shown in Table 2. The most evident changes were observed for cellulose and hemicellulose in all runs during the composting. The cellulose and hemicellulose contents in inoculated treatment were significantly lower than those of natural compost at the end of composting. These indicated that microbiological inocula could promote the degradation of lignocellulosic biomass.

However, the lignin content remained at a similar level throughout the process in the two runs (Table 2). Generally, lignin did not decrease markedly until the late phase of composting process due to its resistance to microbial attack (Zeng *et al*., 2007). It has been stated that the intricate association existed between lignin and hemicellulose hinders the enzymatic action and prevents the biodegradation process (Suárez-Estrella *et al*., 2007). In this work, the lignin content in inoculated treatment at the end of the process was significantly lower than that of natural compost. Results suggested that the presence of the inocula led to lower lignin content than that determined in control.

The C/N ratio has traditionally been considered as an indicator of compost maturity. It has been reported that the compost is mature when the C/N ratio range is from 15 to 20 (Liu *et al*., 2011). In our experiment, the C/N ratio decreased below 20 in all runs during the two months composting (Fig. 2). The initial C/N ratio of about 35 was reduced to <25 in the 15 days of composting. This could be due to carbon loss and water evaporation which can be linked to volume reduction that occurred in the first 15 days.

Nevertheless, some differences could be observed in the two runs. It can be seen that the C/N ratios decreased rapidly at the beginning of the compost and reached the value of 18.5 and 18.6 on the 35th and 60th day for inoculated treatment and natural compost, respectively. This observation suggested that microbiological inocula could promote the decrease of the C/N ratio during the composting process.

Germination assay

Previous study has been reported that the germination index in compost or compost extracts is one of the most sensitive parameters for evaluating the toxicity and the degree of compost maturity (Liu *et al*., 2011). If the GI is above 50%, the compost is judged to be nonphytotoxic. While if the GI is above 80%, the compost has a degree of sufficient maturity(Zeng *et al*., 2007). The GI profiles of two composts had a similar pattern and the GI values were at low levels during the beginning of composting (Fig. 3). The GI was 83.4% and 60.1% for inoculated treatment and natural compost at the end of the process, respectively. These showed that all composts were nonphytotoxic and the inoculated treatment was almost mature. It was also apparent that GI in inoculated treatment mounted up to above 50% after 10 days of composting, while 25 days were needed for natural compost. All the results suggested that the phytotoxicity from inoculated treatment was lower than that from natural compost and the maturity degree of inoculated treatment was significantly higher than that of natural compost. Therefore, microbiological inoculation can be a useful tool to reduce the maturation time of cow manure compost, and to improve the quality of compost by achieving a higher maturity degree and a lower phytotoxicity level.

CONCLUSION

The effect of inoculation on the composting of cow manure was explored under low temperature (below 10 ^{o}C). We found that inoculation can start temperature rapidly, prolong the periods of high temperature, improve the TN, TP, TK content, promote the conversion of NH_4^+-N to NO_3^--N, accelerate the lignocellulose degradation, reduce the phytotoxicity level, and increase the maturity degree.

Therefore, the inoculum containing lignocellulolytic microbial consortium WCS-6, *Trichoderma harzianum* and *Saccharomyces cerevisiae* may be a useful tool in composting processes when the ambient temperature is low (below 10 ^{o}C). Fortunately for general public health, a large number of pathogens and hazardous materials were treated through composting. Even in cold areas, this inoculum could activate the compost pile and speeds the process. Due to the low-cost, easy-operated, simple, and large capacity of disposal and not having second pollution, the composting method of the present study can use as home composting or large-scale composting in cold areas. Additionally, the compost product can be marketed to "wholesalers" (farms, landscaping companies, etc.) or be packaged for retail sale to the general public.

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