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Effect of Microbial Consortia on the Composting of Pig Manure

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Abstract: In this study, we investigated the effect of microbes on the composting of pig manure. *Geotrichum candidum*, *Yarrowia lipolytica*, *Bacillus coagulans*, *Bacillus stearothermophilus* and *Nocardia* sp. were isolated from fresh pig feces. Fifteen different combinations of these microorganisms were added to a mixture of pig manure and sawdust for composting. An orthogonal, fractional factorial experimental design consisting of five factors and two levels was used. Statistical analysis indicated that *Geotrichum candidum* plus *Bacillus stearothermophilus* was the most effective combination. The results indicated that the addition of microbes to feces raised the maximum temperature of composting from 67-72°C and elevated the available nutrients by 9.5%.

Key words: Pig feces, compost, microbial consortia, quality of compost, waste, China

INTRODUCTION

The amount of pig manure is increasing dramatically due to the intensive development of feeding pig farm in China, recently which requires increase capacity for its treatment and making pig manure harmlessness and usefulness have become an important issue (Ferreira *et al*., 2003; Eriksson *et al*., 2005). Composting is used widely for safer disposal of pig manure and increased utilization of organic waste. During composting, organic wastes are decomposed into more environmental friendly and stable components. However, conventional composing processes lead to the production of odor due to the use of indigenous microbes (Said-Pullicino *et al*., 2007) and need to be further improved. Besides optimization of the composting condition, in order to accelerate the pig manure degradation and improve the organic fertilizer quality, the selection and application of microbial strain in composting has become a research focus (Tsui and Roy, 2007).

Recently, adding microorganisms to speed up composting and increase the nitrogen content in the waste to improve the microbiological degradation (Vargas-Garcia *et al*., 2007; He *et al*., 2008; Kavitha and Subramanian, 2007; Tiquia *et al*., 1997) is actively investigated. Hastein *et al*. (2001) investigated the prevailing physico-chemical conditions and microbial community of municipal waste composting by Denaturing Gradient Gel Electrophoresis (DGGE) and 16S rDNA analyses. Meanwhile, Aoshima *et al*. (2001) discussed suitable microflora of household composting. Most of these studies have focused on a single nutritional indicator such as total nitrogen or total phosphorus. Few studies on the effects of microbes on the composting waste from animal husbandry with a complete evaluation of nutrient status and the availability of total nitrogen, phosphorus, potassium and other nutrients are carried out. In this study, we examine the effects of microbes on the composting of pig manure and show that adding microbes increases the efficiency of composting and available nutrients in composting wastes.

MATERIALS AND METHODS

Pig feces were collected from the Hefei Huajie Pig Breeding Farm, Anhui, China. The Carbon Nitrogen ratio (C/N) was 16.7 and the moisture content was 60.4% in the pig feces. The organic matter, total carbon, total nitrogen, total phosphorus and total potassium content were 69.87, 40.55, 2.45, 5.03 and 2.27%, respectively in the pig feces. Sawdust was taken from the Hefei Wood Processing Factory, Anhui, China. The C/N was 565.2 and the moisture content was 49.8% in the sawdust. The organic matter, total carbon and total nitrogen contents were 99.38, 57.65 and 0.10%, respectively in the sawdust. Diameter of the sawdust was <0.9 mm.

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Table 1: Orthogonal experimental results (days 25)

Treatments	GC	YL	BC	BS	NS	Available N (%)	Available P (%)	Available K (%)
0	1.00	1.00	1.00	1.00	1.00	0.49	0.81	2.10
1	1.00	1.00	1.00	1.00	2.00	0.53	0.88	2.14
2	1.00	1.00	2.00	2.00	1.00	0.52	0.85	2.04
3	1.00	1.00	2.00	2.00	2.00	0.50	0.90	2.07
4	1.00	2.00	1.00	2.00	2.00	0.51	0.89	2.16
5	1.00	2.00	2.00	1.00	1.00	0.53	0.91	2.20
6	1.00	2.00	1.00	2.00	2.00	0.52	0.82	2.19
7	1.00	2.00	1.00	2.00	1.00	0.54	0.81	2.20
8	2.00	1.00	2.00	1.00	2.00	0.48	0.82	2.24
9	2.00	1.00	2.00	1.00	1.00	0.50	0.88	2.18
10	2.00	1.00	1.00	2.00	2.00	0.47	0.89	2.17
11	2.00	1.00	1.00	2.00	1.00	0.50	0.84	2.21
12	2.00	2.00	1.00	1.00	1.00	0.50	0.87	2.23
13	2.00	2.00	1.00	1.00	2.00	0.52	0.88	2.17
14	2.00	2.00	2.00	2.00	1.00	0.52	0.87	2.27
15	2.00	2.00	2.00	2.00	2.00	0.48	0.90	2.22
Available N								
$Total_1$	4.14	3.99	4.07	4.06	4.10	-	-	-
$Total_2$	3.97	4.12	4.04	4.05	4.01	-	-	-
Range	0.17	-0.13	0.03	0.01	0.09	-	-	-
Available P								
$Total_1$	6.87	6.87	6.80	6.94	6.84	-	-	-
$Total_2$	6.95	6.95	7.02	6.88	6.98	-	-	-
Range	-0.08	-0.08	-0.22	0.06	-0.14	-	-	-
Available K								
$Total_1$	17.10	17.15	17.41	17.42	17.43	-	-	-
$Total_2$	17.69	17.64	17.38	17.37	17.36	-	-	-
Range	-0.59	-0.49	0.03	0.05	0.07	-	-	-

1, 2 represents the inoculation content of 0 and 0.3%, respectively ($R_{GC}>R_{YL}>R_{NS}>R_{BS}>R_{BC}$)

Microbial isolation and cultivation: Pig feces samples containing microbes were plated at 1:1000, 1:10000 and 1:100000 dilutions on pig excrement culture medium and beef extract-peptone culture medium, incubated at 25°C initially and then at 50°C for microbial isolation and purification. Using API assay, appraisal system and VITEK appraisal card (BioMerieux, France), the identities of the purified isolates were determined. Microbes were then enriched by inoculating detached colonies in a medium containing bran, sawdust and nutrient fluid at a mass ratio of 5:5:15 followed by continuous cultivation till the microbes reached 10^9 colonies g^{-1} of medium. The enriched cultures were then used as the zymogen for the composting of pig manure. The microbes isolated from the pig feces 7B1, 7B3, a1-a3 were *Geotrichum candidum* (GC), *Yarrowia lipolytica* (YL), *Bacillus coagulans* (BC), *Bacillus stearothermophilus* (BS) and *Nocardia* sp. (NS), respectively.

Testing procedures: Pig excrement and sawdust were mixed at a wet mass ratio of 20:1 in the original sample. The water content was adjusted to 65-75%. The mixed pig manure and sawdust was divided into 16 parts of 400 kg each and mixed with corresponding microorganisms as shown in Table 1. For treatment, 0.3% (w/w) of chosen microbes was inoculated. The stacking was kept in the same fermentation field and turned to allow air circulation every 4 days. Every 5 days, one sample from each stacking was taken for experimental test. Five-factor and two-level orthogonal, fractional factorial experimental design (Table 1) were performed to determine the most suitable microbes and the optimal combination of microbes to enhance the quality of compost. Temperature was measured using digital thermometers that were placed in the reactors. The temperature probes were placed 30 cm deep into the surface of the composting. Temperatures were measured at noon every day. Moisture, total and available nitrogen, total and available phosphorus, total and available potassium were determined according to the national standard method with the vacuum dry method, Kjeldahl determination, colour comparison method of vanadium-ammonium molybdate and flame photometry, respectively.

Statistical analysis: All data from the experiments were analyzed using the ANOVA procedure of SAS randomized designs. Statistical differences among treatments were assessed using the Duncan's multiple-range test. Significant difference was accepted when $p<0.05$ and highly significant difference was accepted when $p<0.01$.

RESULTS AND DISCUSSION

In these experiments, the composting temperature increased rapidly during the 1st 2 days and remained high (T>50°C) for an additional 19 days (Fig. 1). Although, the rate of the temperature increase in treatment groups was

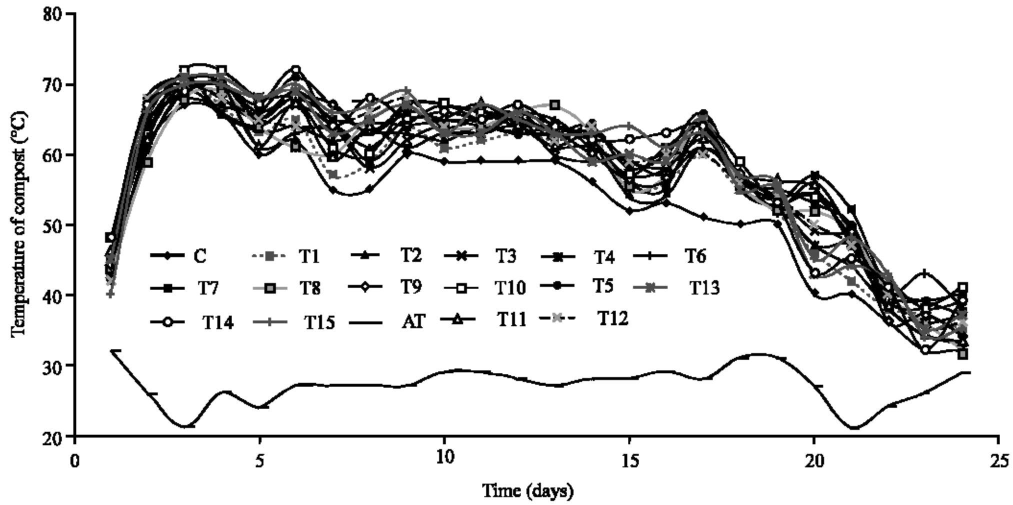

Fig. 1: The temperature change in the compost process

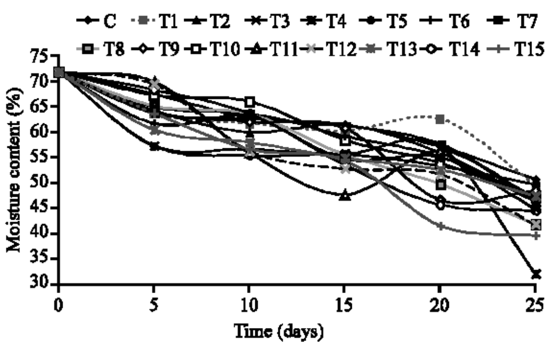

Fig. 2: The moisture change in the compost process

similar to that of the control (no exogenous microorganisms added into compost), the maximum temperatures in the treatment groups were higher than in the control (Fig. 1). The results, we obtained were consistent with Liu *et al.* (2003) indicating that adding microorganisms accelerated composting maturation. The water contents in the treatment groups were generally lower than in the control (Fig. 2). The moisture change trend was converse with the temperature (Fig. 1 and 2) which indicated that there was an osculation relationship between temperature and water content. The moisture of the compost was therefore related to temperature, porosity and ventilation rate as reported by Zhang *et al.* (2002).

Temperature was the primary factor affecting composting water content at the same porosity and ventilation rate. According to these results (Fig. 1), adding microorganisms significantly increased the composting maximum temperature and accelerated water evaporation which was beneficial for improving the quality of the compost and reducing the energy

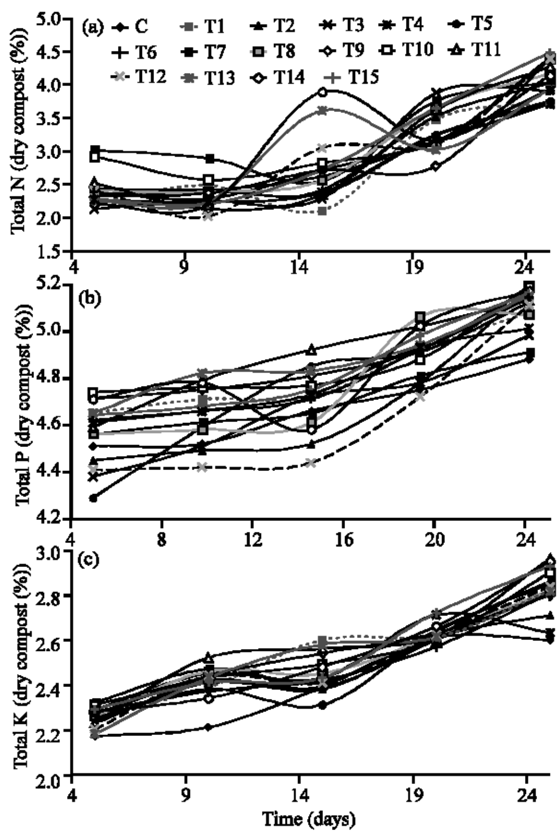

Fig. 3: a) Total nitrogen; b) phosphorus and c) potassium k changes during compost

consumption for desiccation. Consistent with previous results obtained by Zhou *et al.* (2004), total nitrogen content in the compost decreased at first and then increased in all the treatments except for treatments 12-14 (Fig. 3a-c). The reasons for the increases in total nitrogen of treatments 12-14 at the 5th day were not clear based on the current results and require further studies. The decline of total nitrogen from 5-10 days (high-temperature phase, Fig. 1) was likely due to high microbial activities, during which the loss rate of nitrogen was greater than that of dry matter. On the contrary after the composting phase, the organic matters have been used to produce CO_2 but the loss of nitrogen was less. Therefore, the total nitrogen content became higher (Vuorinen and Saharinen, 1997). In addition, sawdust has a strong adsorption capacity for NH_3 (Bhamidimarri and Pandey, 1996). All these factors likely contributed to the higher total nitrogen content in the reactor system after composting.

Both the total phosphorus and total potassium contents increased in all the treatment groups. Because phosphorus and potassium were retained while carbon (CO_2), hydrogen (H_2O), oxygen and nitrogen (NH_3) were lost with the exit gas, phosphorus and potassium were concentrated in the compost. Because of the higher moisture content in the controls at the end of the compost, the total phosphorus and total potassium contents in the treatment groups were higher than those in the control groups at the end of the composting which were similar to the consumption of carbon and nitrogen in the treatment groups (Fig. 2). The nutrients in the organic wastes were decomposed in the process of composting during which the content of available N, P and K increased significantly. Nitrogen is one of the most important factors to assess the nutrient level.

Some nitrogen in the compost was used to generate NH^{+}_{4}-N and some was evaporated in the form of NH_3. In this study, sawdust was used as the filler material that expedited the transformation from NH^{+}_{4}-N to NO_3-N (Bhamidimarri and Pandey, 1996). At the end of the fermentation, the available nitrogen in each treatment group increased (Fig. 4a-c). The available nitrogen in each treatment group increased gradually. The available nitrogen contents in most treatment groups were higher than those in the control groups at the end of the treatment. The available potassium in the treatment groups was higher except for treatment 2 and 3. The addition of exogenous microorganisms effectively increased the available nutrient contents and accelerated the production of high quality compost from manure. Table 1 showed that total available nutrition content (together of available N, P and K) of all treatments after the addition of exogenous microorganisms were higher than control. The contents of treatment 5 (YL+BC), 12 (GC+YL), 14 (GC+YL+BC+BS) and 15 (GC+YL+BC+BS+NS) were four highest among all treatments (>3.6%). Only two exogenous microorganisms

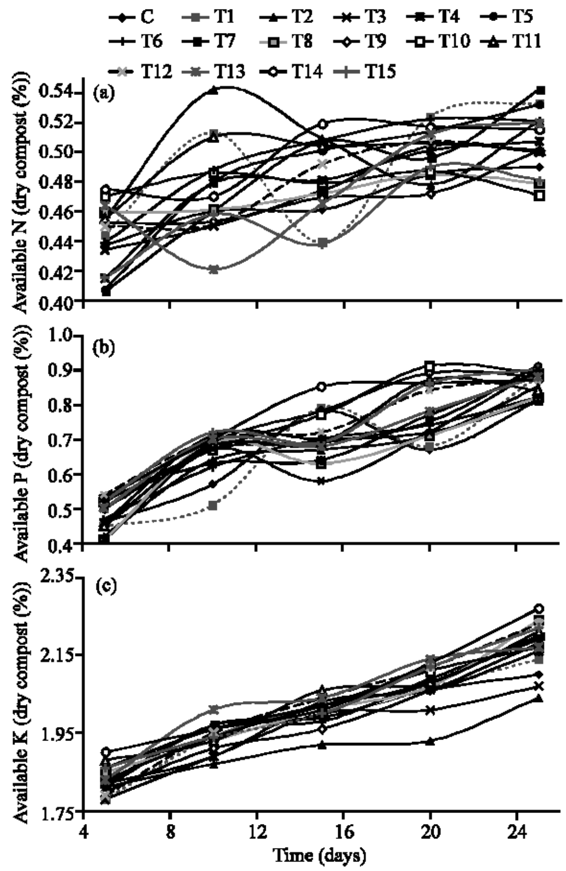

Fig. 4: a) Available nitrogen; b) phosphorus and c) potassium content changes during compost

Table 2: Variance analysis of available N

Sources	DF	Square sum of deviations	Variance	F	p
GC	1	0.00180625	0.00180625	6.66	0.0274
YL	1	0.00105625	0.00105625	3.89	0.0767
BC	1	0.00005625	0.00005625	0.21	0.6586
BS	1	0.00000625	0.00000625	0.02	0.8824
NS	1	0.00050625	0.00050625	1.87	0.2018

Table 3: Variance analysis of available P

Sources	DF	Square sum of deviations	Variance	F	p
GC	1	0.00040000	0.00040000	0.33	0.5780
YL	1	0.00040000	0.00040000	0.33	0.5780
BC	1	0.00302500	0.00302500	2.50	0.1449
BS	1	0.00022500	0.00022500	0.19	0.6755
NS	1	0.00122500	0.00122500	1.01	0.3381

were added to treatment 5 and 12 which was less than what was added in treatment 14 and 15. Therefore, it is clear that BC+YL and GC+YL were the better combinations. Because *Geotrichum candidum* and *Bacillus stearothermophilns* had the highest R values (Table 1), they were considered the most suitable inoculums to enhance the content of available nutrients (Table 2-4). Table 2 and 4 showed that *Geotrichum*

Table 4: Variance analysis of available K

Sources	DF	Square sum of deviations	Variance	F	p
GC	1	0.02175625	0.02175625	10.82	0.0082
YL	1	0.01500625	0.01500625	7.46	0.0211
BC	1	0.00005625	0.00005625	0.03	0.8705
BS	1	0.00015625	0.00015625	0.08	0.7861
NS	1	0.00030625	0.00030625	0.15	0.7046

candidum significantly enhanced the available nitrogen content ($p<0.05$) and available potassium content ($p<0.01$) while *Yarrowia lipolytica* significantly enhanced the available potassium content ($p<0.05$). However, no microorganism significantly enhances the available phosphorus content (Table 3).

CONCLUSION

We isolated five strains of microbes from pig feces and investigated their effects on the composting of pig manure. We found that adding suitable microorganisms increases the composting temperature and the available nutrient content in the compost and reduces the water content of the compost which are beneficial for the industrial production of organic fertilizer. *Geotrichum candidum* (GC) plus *Yarrowia lipolytica* (YL) and *Bacillus coagulans* (BC) plus *Yarrowia lipolytica* (YL) were the most suitable combinations among the microbes tested in this study for improving the quality of the compost.

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