African Journal of Biotechnology Vol. 10(67), pp. 15163-15169, 31 October, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.2559
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Screening of complex thermophilic microbial community and application during municipal solid waste aerobic composting

Yang Mingyan[1]*, Zeng Xianlai[2], Tianjia[3] and Zhang Xiaoqi[3]

[1]School of Environ Science and Engineering, Chang'an University, Xi'an, 710061, P. R. China.
[2]Environmental Management College of China, QinHuangDao, 066004, P. R. China.
[3]Shaanxi Microbiology Institute, Xi'an, 710043, P. R. China.

Accepted 21 September, 2011

Two thermophilic bacteria HP83 and HC181 with fast growth and good decomposition ability of protein and cellulose were separated from 48 samples such as landfill, composting and animal manure by identical medium and activity assaying. Complex microbial community HP83 and HC181 were applied during municipal solid waste aerobic composting that was carried out in a composting reactor under controlled conditions. The comparison of inoculation and no-inoculation ones were examined through inspection of temperature, volatile organic compounds (VOC), NH_4^+N /NO_3^-N, main composting period and other physical parameters. The result show that compared with the no-inoculation one, the inoculation composting period was shortened by four days; the maximum temperature was increased by 10°C and the time to get to peak temperature was shortened by two days. Organism degradation was effectively accelerated and the volume and weight reduction were superior to the no-inoculation one.

Key words: Thermophilic bacteria, screen, complex microbial agency, municipal solid waste (MSW), aerobic composting.

INTRODUCTION

Composting is a viable technology to treat the organic fraction of municipal solid waste (MSW), because it can help to divert more than 80% of the total waste and contributes to reduce the quantity of MSW to be incinerated or land-filled and leads to enormous savings in costs of waste collection, transport and disposal (Erasmo et.al., 2009, Kumar and Goel, 2009).

However, there are still some problems associated with the natural fermentation processes that need to be overcome, such as long composting time, odorous gas and low efficiency (Makaly et al., 2000).

Thus, in recent years, many researches such as inoculation composting have been made to enhance the composting efficiency and production quality (Wei, 2000) and various biological studies have been carried out to identify the major microbiological agents responsible for biodegradation (Taiwo and Oso, 2004). For example, Shin et al. (1999) studied the enhancement of composting efficiency by seeding solid and liquid inoculation. The result suggests that, seed inoculation is applicable to the on-site treatment, because it could initiate composting reaction efficiently without initial lag period. Tiquia (1996) implemented an experiment to determine the physical and chemical changes occurring during inoculation composting process. Gu and Xu, (1995) inoculated two bacterial strains with fast growth and good decomposition ability of coarse fiber to the fermented refuse compost and the result show that, the temperature of inoculation compost rose faster and higher than that of no-inoculation compost and when the high temperature was kept long, the numbers of epiphyte and cellulose catabolic bacteria increased, and the content of humus was improved by 21 to 26%.

Such researches had shown that, microbial inoculation agent is an effective practical technique for waste disposal because it could raise the composting speed, enhance the composting efficiency and promote organic

*Corresponding author. E-mail:yangmingyan67@163.com. Tel: 0086-029-82357038.

solid waste being turned into high quality biological organic fertilizer (Akihito, 1998; Davis, 1992).

However, the previous researches tended to focus on single species and general temperature bacteria. Compared with general temperature bacteria, thermophilic bacteria have more fast growth speed, high enzymatic activity and organics decomposing speed and can be applied in inoculation composting technology to raise the temperature and kill creatures such as insect ovum efficiently and so on. As a result, it has the effect of quickly decomposing compost and causing no harm (Li et al., 2004).

In this paper, two thermophilic bacteria HP83 and HC181 with fast growth and good decomposition of protein and cellulose were obtained from 48 samples such as landfill, composting and animal manure by identical medium and activity assaying. The screening of the strains, the preparation of the complex microbial agent and the application during municipal solid waste aerobic composting were studied.

MATERIALS AND METHODS

Screening of strains with high activity of protease and cellulase

Samples

48 samples were obtained from landfill, composting and animal manure so on. The samples were further processed immediately after it reached the laboratory.

Culture medium

Differential medium

Protease differential medium (casein medium) contained: Na_2HPO_4 (0.38 g), KH_2PO_4 (4.5 g), NH_4Cl (1 g), casein (1 g), glucose (12 g), agar (20 g), 1000 ml H_2O, pH nature. Cellulase differential medium (Congo red CMC-Na medium) contained: $MgSO_4 \cdot 7H_2O$ (0. 5 g), CMC-Na (5.0 g), KH2 PO_4 (1.0 g), $(NH_4)_2$ SO4 (2.0 g), NaCl (0.5 g), Congo-red (0.2 g), agar (20 g), 1000 ml H_2O, pH nature.

Shaking medium

Protease shaking medium contained: soybean meal (2%), corn meal (5%), Na_2HPO_4 (0.2%), $ZnSO_4$ (0.005%), pH nature. Cellulase shaking medium contained straw powder (2.0%), yeast extract (0.25%), K_2HPO_4 (0.50%), NaCl (0.10%), $MgSO_4$ (0.02%), $(NH_4)_2SO_4$ (0.06%), pH nature.

Selection and screening method

The samples were incubated in 55°C by adding some nutrient broth for three to five days. Then, the samples were spread on the two differential medium after gradient dilution, respectively. Strains with big hydrolyzed circle were selected and transferred to the shaking medium and cultured in 55°C for 48 h. The supernatant obtained by centrifugation were used to detect the activity of protease and cellulase by the methods of Fulin- phenol and dinitrosalicylic acid (DNS), respectively.

Preparation of complex microbial agent

The bacteria with high activity of protease and cellulase were adsorbed by turf after shaking culture, respectively. The adsorptive capacity was examined by number of colony (CFU/g turf). The turf with thermophilic bacteria was mixed together in the ratio of 1:1 before use.

Municipal solid waste (MSW) aerobic composting with complex microbial community

Fermentation reactor

Fermentation reactor was provided by College of Science, Northwest A& F University (Zeng et al., 2006). The size was 1000×500×600 mm and the volume was 300 L. Asbestine was used to cover the surface to keep temperature. Leachate recycling and air system were distributed.

The time of the leachate recycle was120 s, the amount of the wind for air-blower was 0.055 m^3/min and the wind pressure was 300 Pa. Meanwhile, the air-blower was controlled automatically to make 5 min on and 30 min off. The fermentation reactor is shown in Figure 1.

Composting procedure

MSW were collected from a typical community, sorted and mixed directly with sawdust without drying. The original weight, carbon to nitrogen ratio (C/N), moisture and VOC were around 80 kg, 40:1, 85 and 65%, respectively.

Composting was performed under the following conditions: Run I (no-inoculation composting): nature composting without inoculation of complex microbial community. The composting period was kept for 15 days; Run II (inoculation composting): complex microbial community was inoculated with 2% of weight fraction and mixed thoroughly with the fermentation material. The composting period was kept for 11 days.

Physical and chemical parameter

The temperature, pH, moisture content, height and weight were monitored everyday; VOC and NH_4^+-N /NO_3^-N of composting were monitored every two days.

RESULTS AND DISCUSSION

Screening result of strains with high activity of protease and cellulase

The preliminary screening result of thermophilic bacteria

Nine strains of thermophilic protein decomposition bacteria and seven strains of thermophilic cellulose decomposition bacteria (the ratio of diameter of hydrolyzed cycle to diameter of colony ≥ 2) were

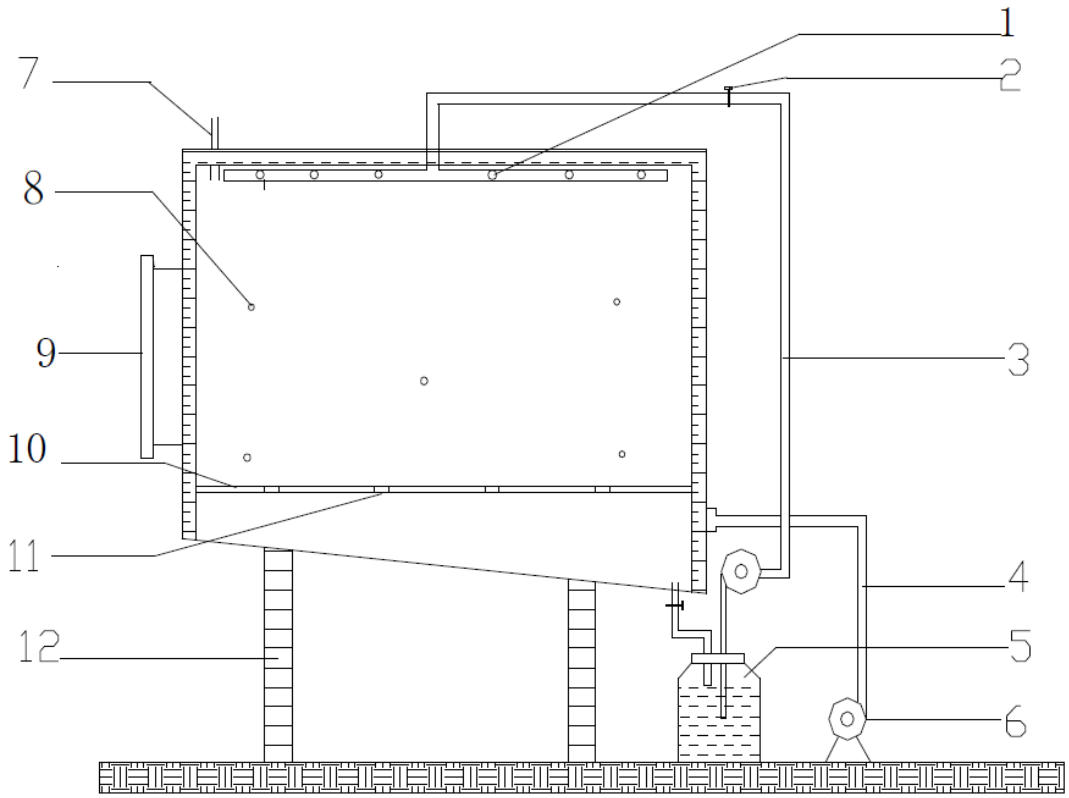

Figure 1. Side elevation of composting reactor. (1) Leachate distribution; (2) tap; (3) water pipe; (4) air pipe; (5) barrel; (6) air pump; (7) vent hole; (8) thermometer hole; (9) feed inlet; (10) sieve plate; (11) air distribution; (12) bracket.

separated from 48 samples. The result are shown in Tables 1 and 2.

The re-screening result of thermophilic bacteria

The strains with bigger hydrolized circle were transferred to the shaking medium and cultured in 55°C for 48 h. The activities of the strains were detected by Fulin- phenol and DNS methods. The result was showed as Tables 3 and 4.

Strains of HP83 and HC181 that were separated from composting and animal manure had both big hydrolyzed circle and high enzymatic activity. They were adapted to the environment of MCW easily and had fast growth speed in MCW. Thus, these two stains were selected for the preparation of complex microbial community.

The preparation of complex microbial agency

The fermentation of strain MP83 and HC181 was adsorbed by turf after shaking the culture. The adsorptive capacity was 1-3×10^9 CFU/g turf.

The result of municipal solid waste aerobic composting with inoculation of complex microbial agent

The temperature during composting process

The MSW aerobic composting depends very much on a number of factors, one of which is temperature. The temperature can reflect the changes of the activity of the microorganism. While large heaps lead to the generation of high temperature and keep for longer time, small heaps generate low temperature and keep for shorter time.

Figure 2 shows that, the inoculation composting had a faster rise in the starting temperature; a peak of about 60°C was attained in the third day when only about 80 kg waste (wet weight) was composted. Meanwhile, the no-inoculation composting reached the highest temperature of 50°C in the 5th day. The temperature of inoculation composting rose faster and higher than that of the no-inoculation composting. It indicates that, inoculation could initiate composting reaction efficiently without initial lag period. However, due to the fast reduction of volume in inoculation composting, the temperature dropped down quickly than that of no-inoculation composting.

Table 1. Preliminary screening result of thermophilic bacteria with high protease.

Number	Diameter of hydrolyzed cycle (mm)	Diameter of colony (mm)	Ratio
HP6	11.3	3.4	3.32
HP15	12.3	3.2	3.84
HP34	18.6	5.2	3.57
HP45	13.2	4.3	3.06
HP56	15.3	5.5	2.78
HP69	17.4	5.0	3.48
HP83	15.4	3.9	3.94
HP85	12.0	4.5	2.66
HP102	16.5	4.9	3.36

Table 2. Preliminary screening result of thermophilic bacteria with high cellulose.

Number	Diameter of hydrolyzed cycle (mm)	Diameter of colony (mm)	Ratio
HC68	15.3	5.1	3.00
HC156	12.3	4.5	2.73
HC157	11.2	4.0	2.80
HC181	12.7	3.2	3.97
HC183	16.2	5.1	3.18
HC193	17.5	5.2	3.37
HC195	12.0	4.3	2.79

Table 3. The re-screening result of thermophilic bacteria with high protease.

Number	Activity (U/ml)
HP6	660
HP15	776
HP34	720
HP45	848
HP56	870
HP69	960
HP83	1040
HP85	930
HP102	1060

The VOC during composting

Figure 3 shows the changes of VOC between the two composting processes. The VOC was 48% in the 11th day in inoculation and 58% in the 15th day in the no-inoculation composting, respectively. The result suggest that, the organic substance degraded faster in inoculation composting than that of the no-inoculation composting.

NO_3^-N / NH_4^+N changes during composting

The reduction of NH_4^+-N is one of the parameters that could evaluate the maturity of composting (Garcia, 1991). However, the absolute NH_4^+-N content in different material changed very differently. So Bernai et al. (1998) advised the ratio of NH_4^+N /NO_3^-N to be the parameter to reflect the progress of the composting. Figure 4 shows

Table 4. The re-screening result of thermophilic bacteria with high cellulose.

Number	Activity (U/ml)
HC68	52
HC156	40
HC157	48
HC181	63
HC183	43
HC193	54
HC195	45

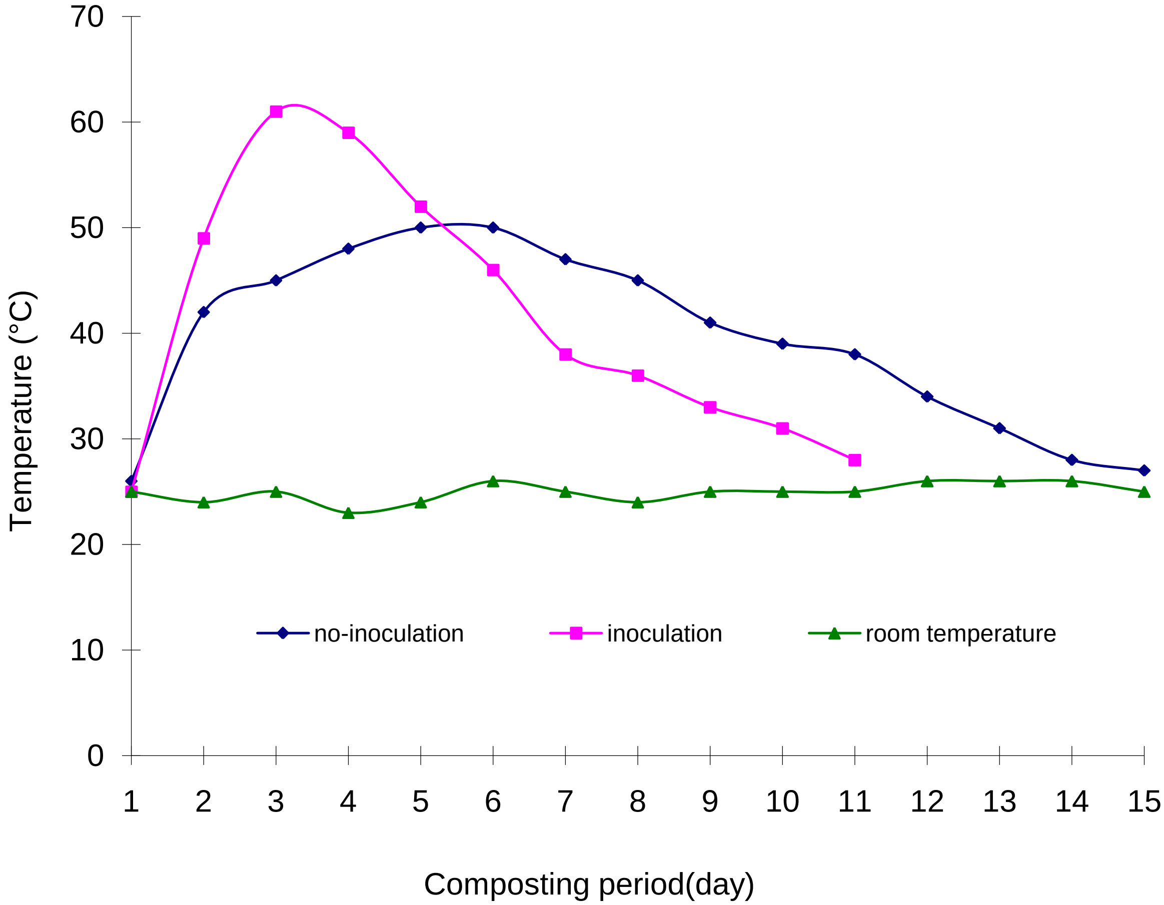

Figure 2. Temperature between inoculation and no-inoculation composting processes.

the result of NH_4^+N /NO_3^-N changes during inoculation and no-inoculation composting. The ratio of NH_4^+N /NO_3^-N reached 0.33 in the 11th day in inoculation and 0.63 in the 15th day. The result indicates that complex thermophilic microbial agent could reduce the composting period.

Other parameters during composting process

The weight, height and moisture content of waste material were tested after composting. The result is shown in Table 5. The result shows that, the weight and height of inoculation composting reduced more than that

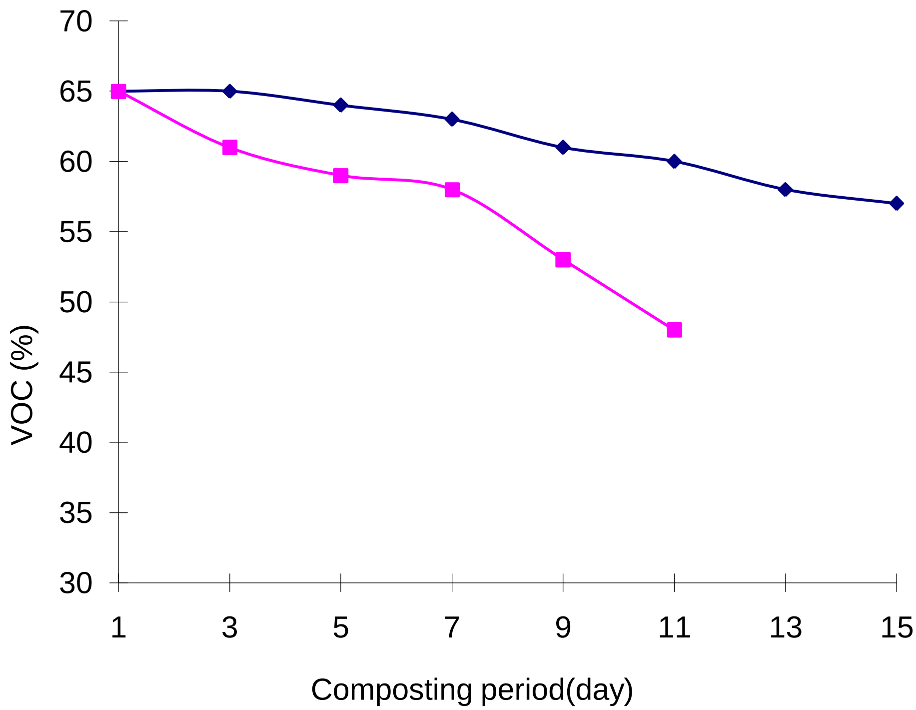

Figure 3. VOC (%) between inoculation and no-inoculation composting processes.

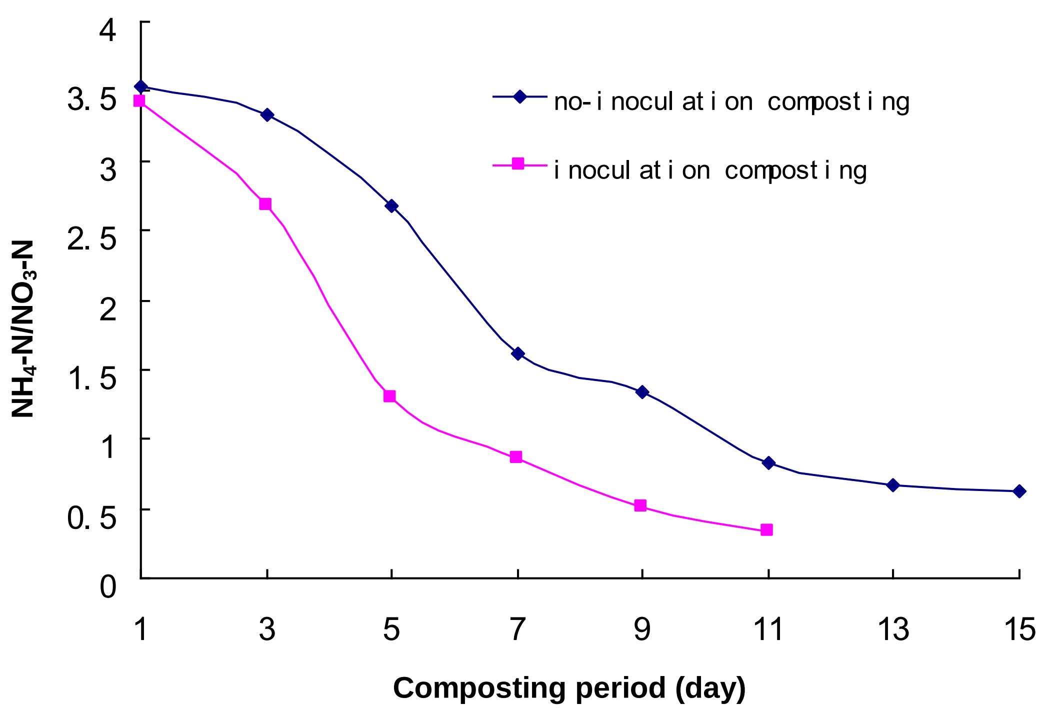

Figure 4. NH_4-N/NO_3-N between inoculation and no-inoculation composting processes.

Table 5. Physical characteristic after composting between inoculation and no-inoculation composting process.

Method	Composting period	Moisture (%)	Weight (kg)	Height (cm)
No-inoculation composting	15	58	25	10
Inoculation composting	11	55	13.5	7

of the no-inoculation composting.

Compared with no-inoculation one, with the help of thermophilic complex microbial community, the inoculation composting process was shortened by four days effectively. The maximum temperature was increased by 10°C and the time to get to the peak temperature was shortened by two days. In the same composting time, organism degradation was effectively accelerated, the volume and weight reduction were superior to the no-inoculation one.

ACKNOWLEDGEMENT

This project was supported by Shaanxi Academy of Science (2004k-13).

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