# Inoculation used in rapid composting: screening and application

**Ren Li [1], Li Ji [2,P] Cheng Xu Y. [3], Shang XiaoY. [4], Wang Ding M. [5], Wang Li Y. [6]**
*(1) Ren Li, Beijing Voto Sky And Land Biotechnology Limited Company, 100193, Beijing, CN*
*(2) Li Ji, CAU-Department of Ecology and Ecological Engineering, College of Resources and Environmental Engineering, 100193, Beijing, CN*
*(3) (4) (5) (6) CAU- College of Resources and Environmental Engineering, 100193, Beijing, CN*
*Corresponding author: liji@cau.edu.cn

## Abstract
By using the methods of Low-temperature and high-temperature enrichment, pour plate count, hydrolysis analysis, enzyme activity analysis for primary screening and the degradation ratio of organic substance, growth rate for secondary screening. Then the three hypothermal and three thermophilic bacterium strains were isolated and screened. Isolated strains were made single and complex agent for composting test. Compost test of hypothermal and thermophilic bacterium were made independently. Results showed that inoculated low-temperature microorganisms could improve heating rate in the early of the compost and thermophiles could grow quickly and had high enzymatic activity in hot environment .The complex microbial inoculants had more advantages than single one.
**Key words** hypothermal and thermophilic bacterium, screening, composting

## Introduction
Composting is a controlled biological process that uses natural aerobic process to increase the biological decomposition rate of organic materials (Huag, 1980; Sanderson, 1974) Composting is one essential method for utilizing organic solid wastes. Microbial inoculation is often performed for effective and successful composting, although its effect is controversial (Finstein and Morris, 1975). The most convenient method for inoculation is the use of functional microorganisms as agent. For example, the application of *Phanerochaete chrysosporium* and *Trichoderma reese* can increased temperature during the process and improved the degradation of cellulose in compost processes (Irigoyen, 2011) .But in compost process, problems were difficult to deal with heating slowly in the cold environment and holding high temperature for a short time in high temperature environment. It is expected that the use of low-temperature functional microorganisms and functional hypothermia as inoculants solve the above problems to rapid composting.

## Material and Methods
### *1. Samples*
18 samples were taken from the south and north in China. They were taken from rice soils, soil from tropical rain forest, compost material, compost samples in low temperature and high temperature phase, domestic sewage and so on.

### *2. Isolates and Screening*
**Enrichment culture** 18 soil samples were used for screening. Soil (10g) was placed into conical flask (250ml) containing 100 ml of the screening medium with beef extract peptone with shaking at 15□ and 70□. Subculture (6 times) in the same medium was carried out 5d by adding 5 ml of broth to fresh medium.Microorganism from samples were incubated in 15□ and 70□, respectively.

**Primary screening** Single-colony isolation was performed using cellulose, protease and amylase plates. All colonies grown on the plates were tested for their cellulose-degrading, protease-degrading and amylase-degrading activities illiquid culture and a colony that showed organics–degrading activity was stored at -4□.

**Secondary screening** The degradation ratio of organic substance and growth rate of strains were measured (Zhou.D.Q, 1984). A colony that showed high degradation ratio of organic substance and growing fast was stored in 20% glycerol at -80□.

### *3. Indoor compost experiment*

**Experimental materials**

The compost was made from chicken manure and sawdust. The initial water content was adjusted to 65□, C/N ratio was regulated to between 25 and 30.Then single strain and complex microbial inoculants were inoculated in compost devices□bubble box ) with raw material respectively.

**Table 1 Physical and chemical properties of tested materials**

| Raw material | Organic carbon (%) | TN | C/N | Water |
|---|---|---|---|---|
| Chicken manure | 28.15 | 2.05 | 13.73 | 52 |
| Saw-dust | 52.65 | 0.29 | 181.55 | 16.4 |

**Experimental design**

The hypothermal bacterium compost experiments set seven treatments and the thermophilic bacterium compost experiments set three treatments. The treatments were as follows (The treatments □ was not made compost test owning to its low growing rate).

□Inoculating single microbial agent with the weigh ration of 3/1000

□Inoculating complex microbial agent with the weigh ration of 3/1000

□ Inoculating VT1000 agent with the weigh ration of 3/1000(provided by VOTO company)

□Control without adding any agent

**Results**

### *1. Isolation and screening of functional bacterial strains from the original microflora*

Three hypothermal bacterium strains B5-16, B6-4, B6-15 and three thermophilic bacterium strains HN-5, ZC-1C, 5d-6B were isolated by degradation ability of cellulose, protein and starch simultaneously. Cellulase activity of hypothermal bacterium strain B6-15 was 24.94U/mL, higher than the others. Highest protease activity of stain was B6-4 which was 11.85U/mL Amylase activity of strain B5-16 was 71.61U/mL, higher than the others. Cellulase, protease and amylase activity of the thermophilic bacterium strain HN-5 was 7.308, 13.296 and 76.136 U/mL respectively, higher than the others. Degradation ratio of cellulose, protein and starch were 17.94%, 15.39% and 42.55% respectively.

**Table 2 Results of enzyme activity**

| Strain | Cellulase activity □U/mL□ | Protease activity □U/mL□ | Amylase activity □U/mL□ |
|---|---|---|---|
| B5-16 | 5.94 | 8.33 | 71.6 |
| B6-4 | 5.65 | 11.85 | 59.57 |
| B6-15 | 24.94 | 0.50 | 4.22 |
| HN-5 | 7.308 | 13.296 | 76.136 |
| ZC-1C | 7.003 | 9.557 | 75.574 |
| 5d-6B | 14.378 | 2.119 | 55.829 |

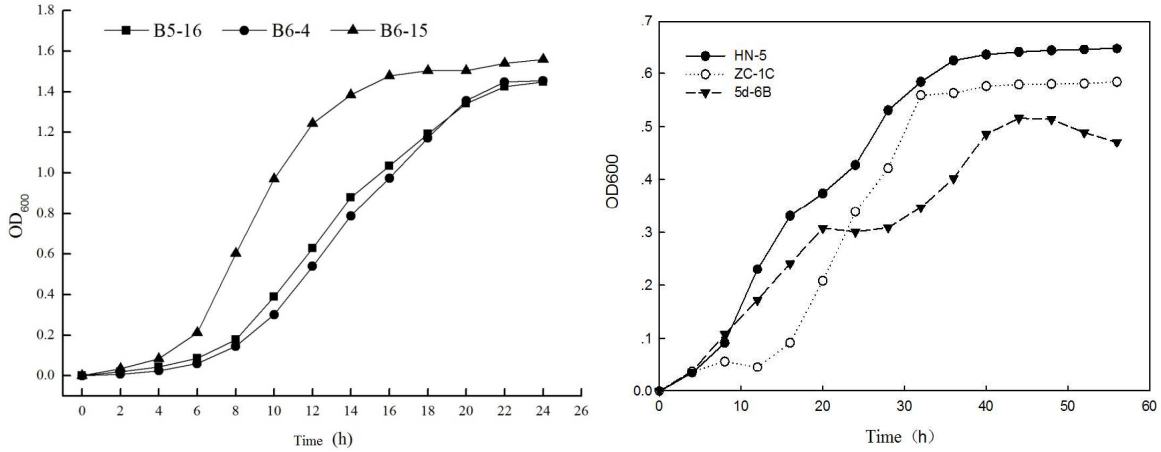


**Figure 1. Growth curve of hypothermal and thermophilic bacteria**

***2. Indoor compost experiment analyse***

The pile temperature change is the macroscopic reflection of the composting process, and is also an important factor influencing microbial activity and the composting process(Li G X, 2000).The temperature increasing is resulted from the heat accumulation produced by intensive metabolism of the composting material( Zhang K Q, 2004). Inoculating the complex microbial agent under the low temperature could accelerate the degradation of the organic matter, make the compost produce heat quickly and keep the proper high temperature to kill the pathogen, worm eggs and grass seeds and it could also act as dehydrate. Then the decreased temperature could facilitate the formation of humic acid and release of nutrient (He H X, 2007). During the composting, in single agent and complex agent, the temperature reached to above 60□ on the 1st day and maintained for about 4 days. However, the temperature of the control reached to 54□ on the 1st day (Fig 1). During the composting, in single agent and complex agent, the temperature reached to above 65□ on the 1st day and maintained for about 4 days. However, the temperature of the control reach to 33-53□ from the 1st day to 5th day(Fig 2).The results show that that inoculating the microbial agent under the low temperature had obvious effects on increasing the temperature and shortening fermentation period. The complex microbial inoculants had a more effective effect than single bacterial strain treatments.

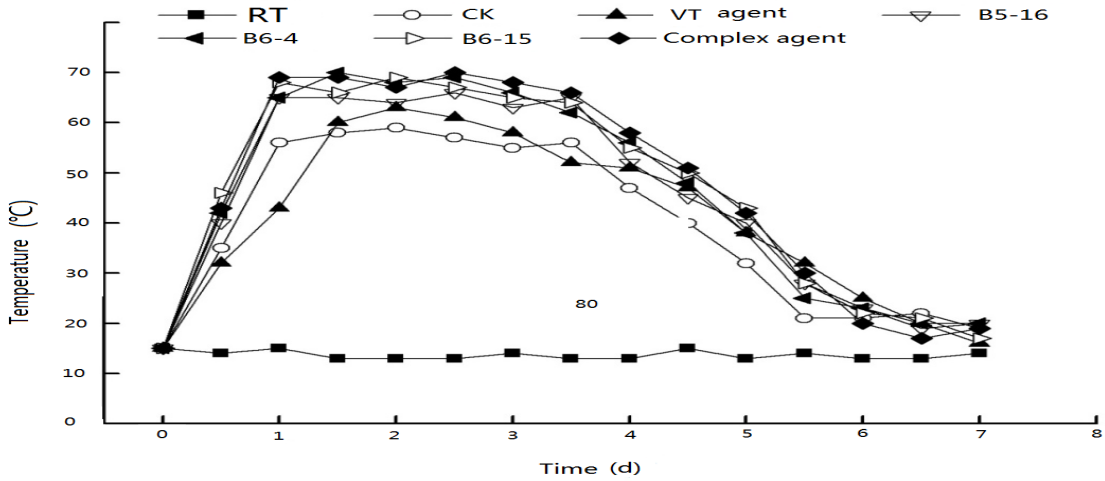


**Figure 2. Change of the temperature in the composting process under different treatments of hypothermal bacterium**

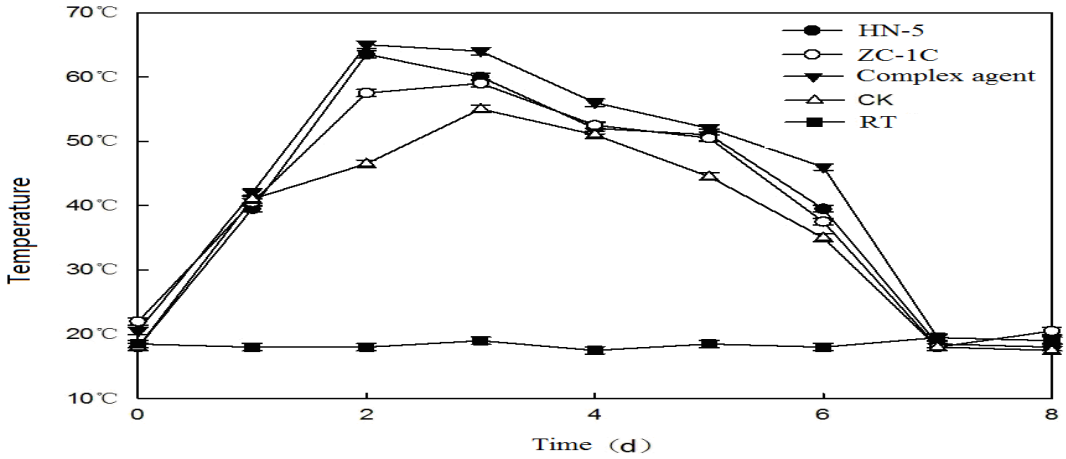


**Figure 3. Change of the temperature in the composting process under different treatments of thermophiles bacterium**

## Conclusion and perspectives

Low-temperature microorganisms can growth and breed quickly in the cold environment. Inoculated low-temperature microorganisms can improve heating rate in the early of the compost. Thermophiles can grow quickly and have high enzymatic activity in hot environment. Inoculated thermophiles can heat quickly and valid control pathogens in the composting process. Iinoculate the microbial agent can promote fermentation and maturation , promote metabolisms of microorganisms, raise the composting temperature, speed up the composting process They have wide application prospects in the field of solid waste treatment.

## References

[1] R.T.Huag. Compost Engineering: Principal and Practice. Ann Arbor Science Publishers, Inc., Michigan, USA, l980.
[2]K.C.Sanderson, W.C.Mart in. Performance of woody or nonmetals in municipal compost medium undermine fertilize regimes, Hortic.Sci .3(1974) 242-243.
[3] M.S. Finstein, M.L. Morris, 1975.Microbiology of municipal solid waste composting, Adv. Appl. Microbiol., 19, 113–151.
[4] Irigoyen, F.Pachec, M .Sesma, 2011. Turning, microbial inoculation and use of Eisenia foetida in household organic waste composting, Journal of Agricultural Science and Technology A 1, 734-738.
[5]Zhou D Q, Microbiology Laboratory manual [M]. Shanghai: Science and Technology Press, 1986.
[6]Li G X. Zhang F S. Solid waste and organic fertilizer production [M].Beijing: Chemical Industry Press, 2000:25-49.
[7] Zhang K Q, Gao H Y. The disposal of livestock and poultry industry pollutants [M]. Beijing: Chemical Industry Press, 2004.
[8]He H X, Xu F H, Zhao X F, et a1.Effects of inoculated complex microbial agent to cattle manure on compost temperature and microorganism under low temperature [M].Journal of Northeast Agricultural University, 2007, 38(1):54-58.

## Acknowledgment

The authors would like to acknowledge Professor Li and his team, including Cheng Xu Y., Shang XiaoY. , Wang Ding M., Wang Li Y. and so on. They paid their hard work.