Bioresource Technology 238 (2017) 39–47

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

# A novel method for contributing to composting start-up at low temperature by inoculating cold-adapted microbial consortium

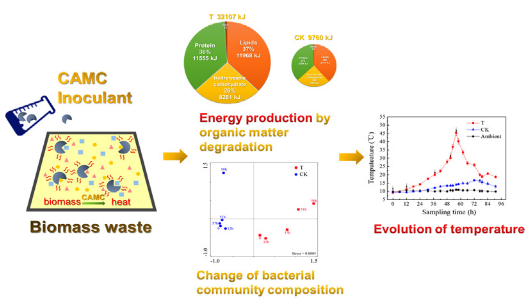
Xin-Yu Xie [a], Yue Zhao [a], Qing-Hong Sun [a], Xue-Qin Wang [a], Hong-Yang Cui [a], Xu Zhang [a], Yan-Jie Li [b], Zi-Min Wei [a,*]

[a] College of Life Science, Northeast Agricultural University, Harbin 150030, China
[b] Heihe Branch of Heilongjiang Academy of Agricultural Sciences, Heihe 164300, China

## HIGHLIGHTS

- CAMC has high activity of degradation biomass waste at low temperature.
- CAMC can change the bacterial community composition and succession of composting.
- Inoculated CAMC contribute to start-up in winter composting.
- The energy produced by CAMC would be a clean heat supply source for heating-up.

## GRAPHICAL ABSTRACT




T: Pile with cold-adapted microbial consortium (CAMC); CK: Control treatment.

## ARTICLE INFO

*Article history:*
Received 10 February 2017
Received in revised form 6 April 2017
Accepted 8 April 2017
Available online 11 April 2017

*Keywords:*
Bio-heat
Cold-adapted microbial consortium (CAMC)
Composting start-up
Low temperature

## ABSTRACT

Low temperature climate presented a technical challenge to start-up composting in northern region of China. This study investigated if the cold-adapted microbial consortium (CAMC) could promote composting start-up at low temperature. In this work, the CAMC was inoculated when food waste was composted at 10 °C. The results showed that inoculating CAMC accelerated the piles temperature effectively, the piles passed through the start-up period within 37 h. Moreover, the inoculants could enhance the abundances of dominant strains related to organic matters degradation rate. Redundancy analysis (RDA) indicated that the relationships among indigenous bacteria, organic substrates degradation and temperature evolution were influenced by the inoculants. Furthermore, the heat generation value and degradation rate of the hydrolysable carbohydrate, lipids and protein were significantly enhanced with CAMC inoculated. This work demonstrated that inoculating CAMC was beneficial to composting self-heating, it provided a novel biotechnology support to ensure the normal start-up of winter composting.


## 1. Introduction

Aerobic composting is a widely accepted approach to dispose the organic waste, it could provide humic substance to improve fertility of soil and some basic nutrients for plant (Elango et al., 2009). Composting is a biological self-heating process. The heat produced by microorganisms is tightly coupled with the metabolic reactions of microbes (Bayne-Jones and Rhees, 1929). Microorganisms act as the energy transducer during the composting. They transform the energy stored in the biomass (such as municipal solid wastes, wood wastes, industrial residues, animal wastes etc.) into energy (Demirbas, 2004). The energy produced by the organism is called bio-energy (McKendry, 2002), it is one of the renewable energy source. A portion of the bio-energy is used for maintaining microbial activity, while the rest of the energy presents as heat to increase the piles temperature and accelerate the normal proceeding of composting process. During composting,

* Corresponding author.
*E-mail addresses:* weizimin@neau.edu.cn, weizm691120@163.com (Z.-M. Wei).

http://dx.doi.org/10.1016/j.biortech.2017.04.036
0960-8524/

the bio-energy produced by microbial degradation can be estimated. The energy produced from hydrolysable carbohydrate, protein and lipids degradation by microorganisms were approximately 17.2 MJ/kg, 23.4 MJ/kg and 39.3 MJ/kg (Haug, 1993), respectively. Therefore, the microorganisms played vital roles for the composting temperature increasing (Strom, 1985).

However, in the north of China, the ambient temperature can decline to a low level in winter. The low temperature condition presents a technical challenge to the normal operation of the composting. When the piles and ambient temperature were 20 °C downward, the microbial metabolism process was significantly slowed down or even stopped (Tateda et al., 2002). Low microbial activity led to low heat energy production which resulted in the piles temperature difficult to increase. Consequently, it was hard for the composting to start-up (Zhao et al., 2012). To ensure normal operation of composting, some methods for piles heating-up have been widely studied, such as natural gas and biogas heating (Xu et al., 2010), winter cover cropping (Dabney et al., 2001) and electric heating (Masse and Masse, 2001). These heating techniques aim at enhancing the microbial activity in the composting under the low ambient temperature condition. However, these methods are not energy-saving and economical in the practical composing plant production. Previous study showed that cold-adapt strains could keep high activity at low temperature (Margesin and Schinner, 2001). Therefore, to study if the cold-adapt strains can also play important role in the complex compost environment at low temperature is of high interest for the practical production and application.

Cold-adapted strains can survive in cold environments normally, which is due to their own adaptive capacity coping with cold stresses (De Maayer et al., 2014). As a result, they have attracted increasingly attention with their higher catalytic enzymes at cold ambient temperatures, which could provide huge biotechnological applications for the practical production (Cavicchioli et al., 2011; Gerday et al., 2000). We hypothesize that cold-adapt strains may be able to help the composting to pass the start-up period under the low ambient temperature condition. However, there have been few studies on this topic, this study aimed to investigate the cold-adapt strains in the start-up period of composting for ensuring the normal operation of composting under the low ambient temperature. Therefore, further investigation of cold-adapt strains in the composting under low ambient temperature is of high interest for the practical production and application.

Previous study showed the benefits of inoculating microorganisms in the composting (Kinet et al., 2015). A major contribution that inoculating microorganisms in the composting is to increase the production of bio-energy to help the composting pass the start-up period rapidly under the low ambient temperature. Therefore, the effectiveness of the cold-adapted microorganisms as the heat-catalyzer should be validated to ensure that they can enhance the temperature of the piles and start up the composting. (i) Study the effect of the cold-adapted microbial consortium (CAMC) on the start-up period of composting as well as the production of energy at 10 °C, (ii) investigate the correlation between bacterial communities, degradation of organic matter and production of heat energy, (iii) further understand the application of the energy produced by CAMC. It is expected that the goal of this study is to provide a new technique for the application of CAMC in the start-up of composting in cold region.

## 2. Material and methods

### 2.1. Preparation of the inoculum

The inoculum of composting in this study was a compound of cold-adapted microbial consortium (CAMC) that obtained from environmental microbiology laboratory of Northeast Agricultural University (NEAU). The CAMC was inoculated in LB medium (10 g peptone, 5 g yeast extract, and 10 g NaCl in 1 L distilled water). The CAMC comprised of stains *Pseudomonas fragi* (KY283110), *Pseudomonas simiae* (KY283111), *Clostridium vincentii* (KY283112), *Pseudomonas jessenii* (KY283113) and *Iodobacter fluviatilis* (KY283114).

### 2.2. Sample strategy and composting operation

Composting plie was conducted with food waste (FW) and maize straw. The FW and maize straw were collected from the campus canteen and grain garden of NEAU in China. Then the raw materials were homogenized by a blender (BL-70, BALING Co., China) according to 1:1 ratio (dry weight). The fresh and dry weight of mixture materials were 17.2 kg and 6.0 kg. The change of the fresh and dry weight was presented in Supplementary Table S1. The raw materials were stored at 4 °C for further use. The basic characterization of the raw materials was shown in Table 1.

The composting experiments were performed in a rectangular reactor, which was made of polystyrene foam plastics. The capacity was 56.7L (length: 360 mm; width: 350 mm; height: 450 mm), the schedule drawing of the reactor was shown in Supplementary Fig. S1. The reactor was bound with 50 mm-thick glass wool for heat preservation. A baffle with 2-mm holes was placed above the reactor bottom to support the compost materials and to promote aeration. The dry air was pumped (4 L/(h·kg)) by air pumps (S-20, SAIER Co., China). The wheat stalk was covered above the compost materials to reduce the water vapor condensation and heat consumption. The change of the temperature in the composting was recorded using a high sensitivity digital thermometer (18105a, Shenzhen TOOLWELL Co., China).

The whole composting experiments were operated in the cold chamber where the temperature was 10 °C. Two trials of composting experiments were carried out, the analyses of the two treatments were carried out by triplicate. The first trial (T) was inoculated the CAMC. The amount of the inoculants was at the level of 1% in dry weight, and the content of the bacteria were $1 \times 10^8$ CFU/ml. For the control trial (CK), no microbes were inoculated in the composting. The samples collected according to the change of the piles temperature (0 h, 12 h, 37 h, 51 h, 56 h collected). The piles were turned at sampling port every in this study.

### 2.3. Microbiological analysis

#### 2.3.1. DNA extraction and PCR amplification

Total bacterial DNA of compost samplings were extracted using the soil DNA kit (Omega Biotek, Inc.). The quality of the extracted DNA was checked by a gel image analysis system (Tanon-4500, SHENZHEN TANON Co., China). Total bacterial DNA were stored at −20 °C for further use.

**Table 1**
Basic characterization of the compost raw materials.

| Raw materials | TKN ($g/kg^{-1}$) | TOC ($g/kg^{-1}$) | pH | C/N | MC (%) |
|---|---|---|---|---|---|
| Food waste | 45.3 | 504.6 | 5.87 | 14.5 | 66.7 |
| Maize straw | 6.8 | 463.3 | 6.41 | 60.2 | 5.71 |

TKN, total kjeldahl nitrogen; TOC, total organic carbon; MC, moisture content.