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A Novel Method for Contributing to Composting Start-up at Low Temperature by Inoculating Cold-adapted Microbial Consortium

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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.

Keywords: bio-heat; cold-adapted microbial consortium (CAMC); composting start-up; low temperature

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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 & 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, 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 & 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 composting plant production. Previous study showed that cold-adapt strains could keep high activity at low temperature (Margesin & Schinner, 2001). Therefore, to study if the cold-adapt strains can also play important role in the complex compost environment at low temperature ($< 20\text{ }^{\circ}\text{C}$) 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 strains *Pseudomonas fragi* (KY283110), *Pseudomonas simiae* (KY283111), *Clostridium vincentii* (KY283112), *Pseudomonas jessenii* (KY283113) and *Iodobacter fluviatilis* (KY283114).

2.2. Sample strategy and composting operation

Composting pile 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.

Table 1

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.

The composting experiments were performed in a rectangular reactor, which was made of polystyrene foam plastics. The capacity was 56.7L (length: 360mm; width: 350mm; height: 450mm), 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 1×10^8 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.

The PCR amplification of the 16S rRNA V3 region was performed using the bacterium primer 341f (5'-CCTACGGGAGGCAGCAG- 3'), 534r (5'-ATTACCGCGGCTGCTGG-3') and a GC clamp (5'-CGCCCGGG-GCGCGCCCCGGGCGGGGCGGGGGCACGGGGGG- 3'). Each PCR mixture system (25µl) contained: 3 µl of Ex Taq buffer ([Takara Bio, Japan](#)), 2 µl of deoxyribonucleotide triphosphate (dNTP) ([Takara Bio, Japan](#)), 2 µl of primer pair, 100 ng template DNA and 0.5 µl of Ex Taq DNA polymerase ([Takara Bio, Japan](#)), the rest reaction was treated with Q-water. PCR was programmed as follows: (i) denaturation at 94 °C for 4 min, (ii) 30 touchdown cycles of denaturation at 94 °C for 30 s, annealing at 65 s for 30 s, extension at 72 °C for 1 min, (iii) followed by a final extension at 72 °C for 10 min.

2.3.2. DGGE analysis

The bacterial community composition of composting was monitored by DGGE technique ([Vallaeys et al., 1997](#)). The bands of DGGE were scanned by 312nm UV image analysis system and Quantity one software ([version 4.5, Bio-Rad laboratories, USA](#)). And then the bands were cut down for PCR amplification. The reaction of PCR was the same as above. The amplified segments were purified with the cycle-pure kit ([OMEGA Bio, USA](#)) and cloned into the T1-sample vector per the peasy-T1 cloning kit

(TRAN Bio, China). The connected products were transformed into *Escherichia coli* DH5 α for the blue-white screening. The positive clones could grow on the Luria-Bertani (LB) medium containing ampicillin. The positive clones were collected into the LB liquid medium cultured until OD₆₀₀ 0.8. Finally, all bacterial fluid containing target gene segments were sent for sequencing by Invitrogen (Shanghai, China). The sequencing results were blasted in National Center of Biotechnology Information (NCBI).

2.4. Organic matter analysis

The hydrolysable non-cellulose carbohydrate, lipids and protein was analyzed by the method as previously described (Alef & Nannipieri, 1995). About 1 g (dry weight, removed the maize straw) sample was hydrolyzed with 12 M sulfuric acid solution. The filtrate was analyzed with the phenol-sulfuric acid method to analyze the non-cellulose carbohydrate content. The lipids were extracted with a mixture of chloroform and methanol (2:1, V/V) under N₂ circumstance for 16 h at room temperature. Lipids were washed and dried, the amount of the lipids was then determined by difference. The protein content of the samples was estimated from total nitrogen content ($N \times 6.25$) as obtained by Kjeldahl method. The volatile solids (VS) content was measured by heating samples at 550°C for 7h in muffle furnace. And biodegraded volatile solids(BVS) was calculated by the VS content (Yang et al., 2013). All the determinations for the organic components were carried out by triplicate.

2.5. Calculations

2.5.1. The heat energy generation calculations

The heat energy generated by the microbes during composting was determined by the biodegradable organic matter and the calorific value of the compost materials (H) using Eq. (1) (Ahn et al., 2007).

$$Q_{bio} = \Delta M \cdot H \quad (1)$$

where Q_{bio} (kJ/h) is the amount of biological heat, ΔM (kg) is the dry weight of consumed biodegradable organic matter, H (MJ/kg) is the calorific value of the biodegradable organic matter. The calorific value (H) of food waste was 21 MJ/kg (Komilis et al., 2012) in this study.

2.5.2. The heat consumption in the composting

Heat balance of the compost system was determined by heat energy production from the organic substrates degradation, sensible heating of compost system, conductive and radiation consumption, convective consumption, turning consumption and latent heat consumption of water evaporation under different temperature (Bach et al., 1987).

Radiation consumed heat were usually 5% or less, even to be negligible in the compost models (Ahn et al., 2007; Van Lier et al., 1994). The heat balance equation during composting could be described by Eq. (2):

$$Q_{bio} \approx Q_i + Q_c + Q_g + Q_t + Q_l \quad (2)$$

where Q_i (kJ/h) is the sensible heating of compost system, Q_c (kJ/h) is the conductive heat consumption, Q_g (kJ/h) is the convective heat consumption, Q_t (kJ/h) is the turning heat consumption, Q_l (kJ/h) is the latent heat consumption of water evaporation during composting.

The heat consumed for the temperature increasing of the compost materials were calculated using Eq. (3):

$$Q_i = (M_{water} \cdot c_{water} + M_{solid} \cdot c_{solid}) \cdot \Delta T_m \quad (3)$$

where M_{water} and M_{solid} (kg) are mass of water and solid of compost materials, respectively; ΔT_m (°C) is the change of piles temperature in a time period, c_{water} and c_{solid} (kJ/(m²·°C)) are the specific heat capacity of the water and solid, respectively. The

specific heat capacity of the water is 4.18 kJ/kg·°C (Noel & Ring, 1992). The specific heat capacity of the solid is 1.92 kJ/(kg·°C) (Ma et al., 2011).

The heat consumption of the reactor walls determined by Eq. (4) (Bach et al., 1987):

$$Q_c = U \cdot A \cdot (T_m - T_a) \quad (4)$$

where U is the coefficient of heat transfer (0.39×10^{-3} kJ/(h·m²·°C)) (Persson et al., 1979), A is the surface area of reactor interior wall (0.77m²), T_m (°C) is the piles temperature, T_a (°C) is the ambient temperature.

The convective heat consumption by the gases passed through the piles was given as Eq. (5) (Ahn et al., 2007):

$$Q_g = G \cdot (H_{out} - H_{in}) \quad (5)$$

where G is the air flow rate (kg/h), H_{out} and H_{in} are the enthalpy of the outlet gas and inlet air (kJ/kg), respectively. H_{out} and H_{in} are determined by Eq. (6):

$$H_{out, in} = (1.01 + 1.88x_{out, in}) \cdot T_m + 2491x_{out, in} \quad (6)$$

where $x_{out, in}$ is the absolute humidity of outlet and inlet gas (kg/kg).

The latent heat consumption of the water vapor from composting is given by Eq. (7):

$$Q_l = G \cdot Q_e \cdot (x_{out} - x_{in}) \quad (7)$$

where Q_e (kJ) is the enthalpy evolution of water vaporization that calculated by Eq. (8) (Bach et al., 1987):

$$Q_e = -2.5T_m + 2502 \quad (8)$$

The heat by the piles turning consumed during composting is given by Eq. (9) (Li et al., 2015):

$$Q_t = M_{water} \cdot c_{water} \cdot (T_m - T_{m-n}) + M_{solid} \cdot c_{solid} \cdot (T_m - T_{m-n}) \quad (9)$$

where T_{m-n} (°C) is the piles temperature after the n^{th} turning.

2.5.3. Economic calculations

The economic calculations consisted of economic cost and organic fertilizer earnings. The economic cost was mainly from the saving of the electricity, which the price was 0.08 US \$ per kW h and the power of the external heating equipment was 5kW. If the density of the organic wastes was 2.0 kg/m³ and 1kg compost raw materials produced about 0.3 kg organic fertilizer, the organic fertilizer was produced 29400 kg each year. Moreover, the price of the organic fertilizer is 0.1 US \$ per kg.

2.6. Statistical analysis

The data was analyzed using SPSS 21.0 and ORIGIN pro 9.2. The relative intensity of DGGE bands were calculated by Quantity one (Version 4.5). The relationship between species and environmental factors were analyzed using Redundancy analysis (RDA) with Monte Carlo permutations from CANOCO pro 5.0. The temperature and degradation of the organic substances were regarded as environmental factors data. The significant level of differences in this study was set at $p < 0.05$.

3. Results and Discussions

3.1. Evolution of Temperature

Temperature is a crucial indicator during the composting, it can affect composting efficiency and microbial activity (Zhao et al., 2016). Evolution of the temperature in T and CK was illustrated in Fig.1. Some images of composting temperature were shown in Supplementary Fig.S2. The ambient temperature fluctuated around 10 °C. The temperature increased rapidly after inoculated CAMC in T, but the temperature trend of CK was relatively stable. For T, the piles temperature was start-up rapidly and reached to 20 °C at 37 h of composting. At temperature of 20 °C, the most mesophilic microorganisms could grow normally (Madigan et al., 1997). When the microbes were at their adaptable temperature (20 °C), a large amount of heat could be generated in a

short time owing to the activities of microbes in composting, resulting the increase of piles temperature. After the temperature reached to 45.4 °C, the piles temperature began to fall. For CK, the evolution of temperature fluctuated from 9.3 to 16.7 °C, and did not pass start-up period. In this study, CAMC can be used as a catalyst to increase temperature and accelerate composting start-up.

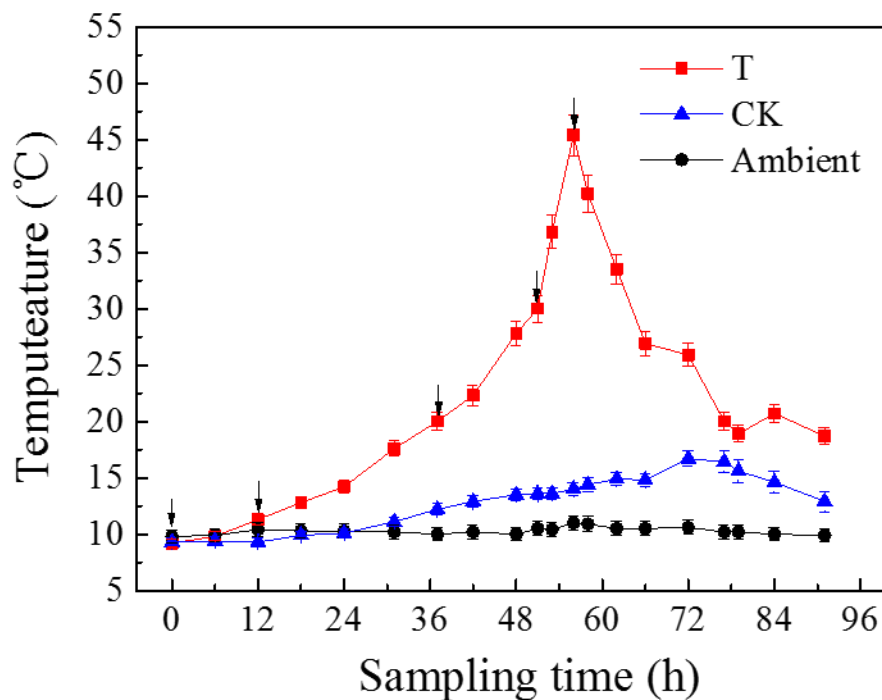


Fig.1 Temperature evolution during composting. The arrows are represented for the sampling times in composting. All values represent means \pm SD (n=3).

3.2. Microbial community composition

Temperature and the bacterial community composition could be influenced with each other (Ratkowsky et al., 1982). The DGGE patterns showed the 16S rRNA PCR-amplified products of bacterial community composition during the whole temperature increasing process (Fig.2). Twenty-two different bands were detected in the DGGE profile. Bands 3, 4, 5, 7, 11 belongs to CAMC. Bands 3, 4, 5, 7 existed in both

treatments T and CK, indicating that they were the members of indigenous microorganism during composting. However, it was found that the brightness of bands 3, 5 and 7 in treatment T were higher than these in CK. Band 5 appeared in treatment T from 0 to 56 h, while it just appeared in CK from 0 to 51 h. Also, band 11 was special to treatment T at 12 h. These results suggested that the abundance and content of bands 3, 4, 5 7 and 11 were enhanced in T caused by CAMC. In addition, the abundance of some bands (2, 9,14,15 and 16 etc.) were decreased in treatment T. Bands 1 and 13 were just detected in treatment T, while bands 10 and 12 only occurred in treatment CK. Oppositely, bands 21, 22 existed in both T and CK from 0 to 56 h, which indicated these two species were less influenced by CAMC. In addition, NMDS analysis (Supplementary Fig.S3) demonstrated that significant difference existed in bacterial community structures of treatment T and CK, and the composting stages difference in microbial structure was more notable in treatment T and less dramatic in CK except for 56 hours. These results suggested that cold-adapted microbial consortium inoculants significantly reshaped the indigenous microbial communities, which was probably due to the temperature limitations (Nedwell, 1999), which caused the competition among microbial populations (de Bertoldi et al., 1983), the changes of nutritional component in composting system or the generation of secondary metabolites from inoculants (Insam et al., 1996). The influence degree of each factor needs further study. Also, it was found that the diversity of microbial communities in treatment T decreased, but the brightness of dominate microorganisms (3, 4, 6 and 7, etc.) significantly increased from hour 37 to 56. In this period, the temperature of treatment T increased quickly and passed the start-up period. Therefore, after inoculating CAMC, the temperature increased when the piles were composted at low ambient temperature. It showed that the CAMC could survive in

the surrounding and had high activity at low temperature during composting.

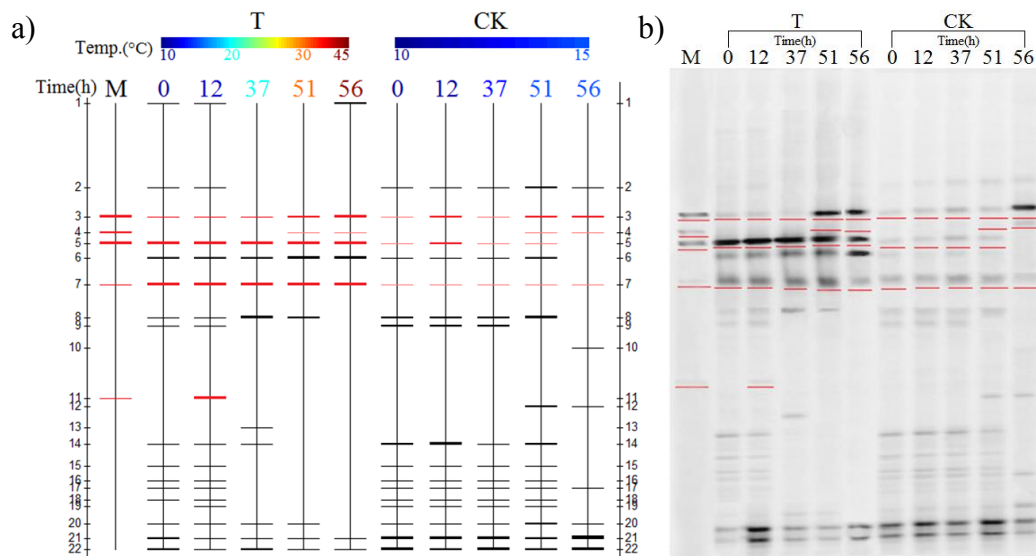


Fig.2 DGGE profile of bacterial community in CK and T. (a) The diagram of DGGE gel; (b) the real photo of DGGE gel. M is the Marker produced by inoculated CAMC. The red band of 3, 4, 5, 7 and 11 denoted inoculated CAMC.

To better comprehend the difference in bacterial diversity between treatments T and CK, recognizable bands were excised and sequenced (Supplementary Table S2). Fig. 3a and b showed the bacterial communities of treatment T and CK at the phylum level and class level. At the phylum level, *Firmicutes*, *Proteobacteria* and *Actinobacteria* were the main phyla in both T and CK. The species from these phyla were ubiquitous during most aerobic composting (Partanen et al., 2010b), and they had been found in nearly all related studies. The inoculation (bands 3, 4, 5, 7, 11) were clustered within the *Proteobacteria* and *Firmicutes*. *Proteobacteria* and *Firmicutes* were widely found in low temperature environment (Peeters et al., 2011; Perreault et al., 2007). It was also found that the phyla *Proteobacteria* and *Firmicutes* presented dominated roles in T, which respectively accounted for 45.8–67.2% and 36.9–19.4% of the total 16S rRNA sequences. It not only indicated that phyla *Proteobacteria* exhibited strong tolerance to

the low temperature, but also showed that the *Proteobacteria* was an effective phylum for potential organic degradation capacity during the composting at low temperature. And *Actinobacteria* occupied 17.3–13.3% of 16S rRNA sequences. However, *Firmicutes*, *Proteobacteria* and *Actinobacteria* were accounted for 39.9-46.4%, 47.3-53.6% and 12.9-0% in CK. It is worthwhile to note that *Firmicutes* phylum was gradually reduced, meanwhile, *Actinobacteria* phylum disappeared at 56h (14.9 °C) in CK.

At the class level (Fig. 3c and d), *Bacilli*, *Gammaproteobacteria*, *Corynebacteriales*, *Clostridia* and *Alphaproteobacteria* were detected in both CK and T. The class *Bacilli*, *Clostridia* and *Gammaproteobacteria* were common in the composting, and these species might contribute to the degradation of organic substances (Partanen et al., 2010a; Wakase et al., 2008). For T, *Bacilli*, *Corynebacteriales*, *Clostridia*, and *Alphaproteobacteria* abundances decreased from 14.4% to 2.9%, 17.3% to 13.3%, 22.5 to 16.4% and 9.2% to 3.8%, respectively. However, the abundances of *Gammaproteobacteria* cluster (including bands 3, 4, 5) increased from 36.6% to 63.4%. Based on above analyses, it could be inferred that class *Gammaproteobacteria* played important role in organic matter degradation no matter under low or high temperature condition (Martínez-Rosales & Castro-Sowinski, 2011; Tuyet et al., 2015). In addition, *Betaproteobacteria* was only existed at 12 h in T, with abundance of 3.24%. For CK, the abundances of *Bacilli*, *Corynebacteriales* and *Alphaproteobacteria* were not significantly changed before 51 h (14.6 °C). Afterwards, the abundances at the class level began to change at 51 h. The abundances of *Alphaproteobacteria* and *Clostridia* increased to 29.5 % and 34.4 %, whereas the *Corynebacteriales* were disappeared at 56 h (14.9 °C). These results showed that low temperature would inhibit the richness of

dominant microbial communities related to organic matter degradation (Lu et al., 2012).

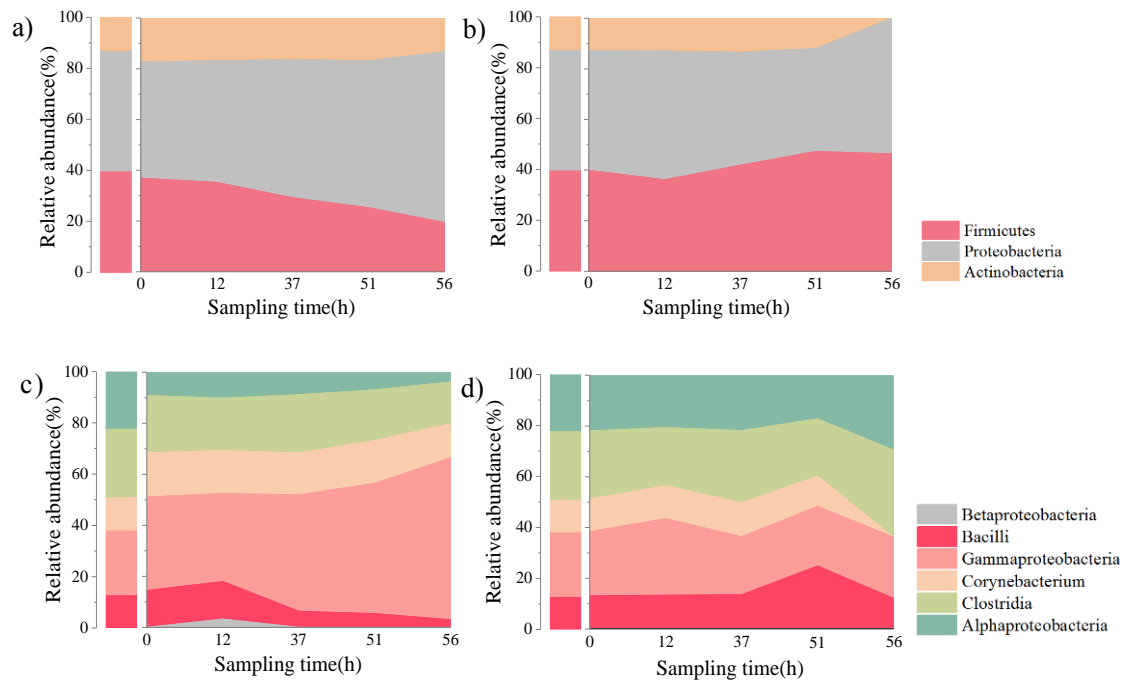


Fig.3 Taxonomic classification of the 16S rRNA gene read at (a) phylum level of T; (b) phylum level of CK; (c) class level of T; (d). class level of CK.

3.3. Correlation between the microorganisms and environmental variables

Redundancy analysis (RDA) was used to explain the correlation between CAMC, indigenous microorganisms and environmental factors (Dong & Reddy, 2010). As shown in Fig. 4, the change of the species-variables, e.g. temperature, degradation rate of the protein (DPR), hydrolysable carbohydrate (DCR) and lipids (DLR), explained by the first two axes which accounted for 73.81% and 11.77% on the bacterial DGGE fingerprints of T. In CK, the first two axes accounted for 72.12% and 24.16%. In general, the correlation was determined by the angle between the arrow of species and variables in RDA model (Van Dobben et al., 2001). Each specie had its own dependence while in the different matter substances and variables (Blagodatskaya & Kuzyakov, 2008) It was found that the correlation of CAMC and environmental factors were

significantly different between treatment T and CK. For T, bands 3, 4, 5, 7 presented significant positive correlations with temperature, DPR, DCR and DLR in T. Band 11 had no significant positive correlation with environmental factors. And band 11 probably played a key role in synergistic effect with other inoculants for organic substances degradation in CAMC. Band 11 might produce some intermediate metabolites that were utilized by other microorganisms. Band 11 might promote organic matter degradation indirectly in composting, this condition was also shown by (Tuomela et al., 2000). For CK, the degree of positive correlation between band 3 and temperature, DCR were lower than that in T. Band 4 had more significant positive correlation with DCR and DPR in CK. No significant positive correlation was detected between band 5 with temperature, DPR, DCR and DLR in CK. Lower positive relationship was found between band 7, DCR and DPR, and higher positive relationship between band 7 and DLR. In addition, the correlation between indigenous microorganisms and environmental factors was changed by CAMC. For an example, bands 2, 10, 12, 20, 21, 22 had significant positive correlation with temperature, DPR, DCR and DLR in CK, while no significant positive correlation was found between these bands with environmental factors in T. Similarly, band 6 had significant positive correlation with environmental factors in T, while the correlation between band 6 and environmental factors was changed in CK. It was obviously found that the species amounts, which had significantly correlation with environmental factors, was fewer in T than these in CK. The similar phenomenon was observed in the study of Zhang et al. (Zhang et al., 2016). It was reported that different microbes would compete with each other for nutrient substances such as protein, hydrolysable carbohydrate and lipid used for microbial growth (Gómez et al., 2016). So, the decrease of species amount related to

environmental factors would provide a more favourable growth environment for CAMC. And then bands 3, 4, 5 and 7 became the dominant species in T, contributing to the degradation of organic substances and production of heat.

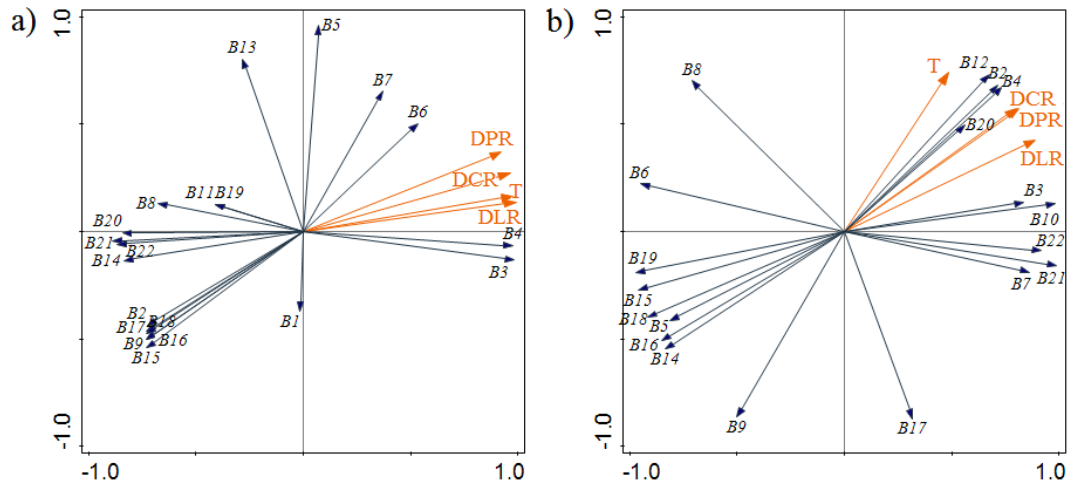


Fig.4 RDA analysis of bacterial community composition in relation to temperature and organic matter degradation. (a) inoculation during composting as T; (b) without inoculants during composting as CK. (Note: DPR = degradation of protein rate, DCR = degradation of hydrolysable carbohydrate rate, DLP = degradation of lipids rate. B1 - B22 indicated the bands given in Fig.2).

3.4. The heat energy generation origin form degradation of protein, hydrolysable carbohydrate and lipids

At the initial of composting, the decomposition of organic substances such as protein, hydrolysable carbohydrate and lipids by the microbes could produce bio-heat, leading to the increase of piles temperature during composting (Sánchez-Monedero et al, 1999).

The DPR, DCR and DLR were significantly higher in T than these in CK during composting ($p < 0.05$) (Fig.5a), suggesting that CAMC significantly enhanced biodegradation activity of microorganisms. The maximum values of DPR, DCR and DLR in T were $(2.30 \pm 0.12) \%$, $(3.06 \pm 0.15) \%$ and $(1.63 \pm 0.08) \%$ on hour 12, 56 and 51,

respectively. As shown in [Fig. 5b](#), the accumulative degradation of protein (ADP), hydrolysable carbohydrate (ADC) and lipids (ADL) in T increased greater and quicker than these in CK. This was especially true for hydrolysable carbohydrate, of which the accumulative degradation increased to $(9.13 \pm 0.46) \%$ in T. And ADP and ADL increased to $(7.22 \pm 0.36) \%$ and $(4.21 \pm 0.21) \%$, respectively. While the ADC, ADP and ADL just increased to 1.9 % approximately in CK.

The total energy production was determined by Eq. (1). in the compost system from hour 0 to 56. The energy production was in T (32107.3 kJ) and CK (9708.4 kJ). Moreover, the energy produced by degradation of protein, hydrolysable carbohydrate and lipids was 31803.6 kJ in T, which was 3.25 times higher than the total heat in CK (9760.2 kJ) during composting. Also, the total energy production in the whole compost system was closed to the energy produced by degradation of protein, hydrolysable carbohydrate and lipids. This result was similar with previous studies ([Yang et al., 2013](#); [Zhao et al., 2010](#)). For T, the maximum value of heat (11967.9 kJ) was produced by lipids degradation ([Fig.5c](#)), accounted for 37.6% of the total heat production. The heat values of protein and hydrolysable carbohydrate degradation by microorganisms were 11554.6 kJ and 8281.0 kJ, respectively. For CK, the heat produced by the three organic substances in CK was in the order ([Fig.5d](#)): hydrolysable carbohydrate (2391.7 kJ) < protein (3593.7 kJ) < lipids (3774.9 kJ). These results indicated that inoculation could accelerate the degradation of protein, hydrolysable carbohydrate and lipids, leading to the heat production used for composting self-heating. In addition, it was observed that 10612.6 kJ heat, which was produced from 0.413kg protein, hydrolysable carbohydrate and lipids with the biodegradation rate of 8.92%, which was required for the increase of pile temperature from 9.2 to 20°C to pass the start-up period.

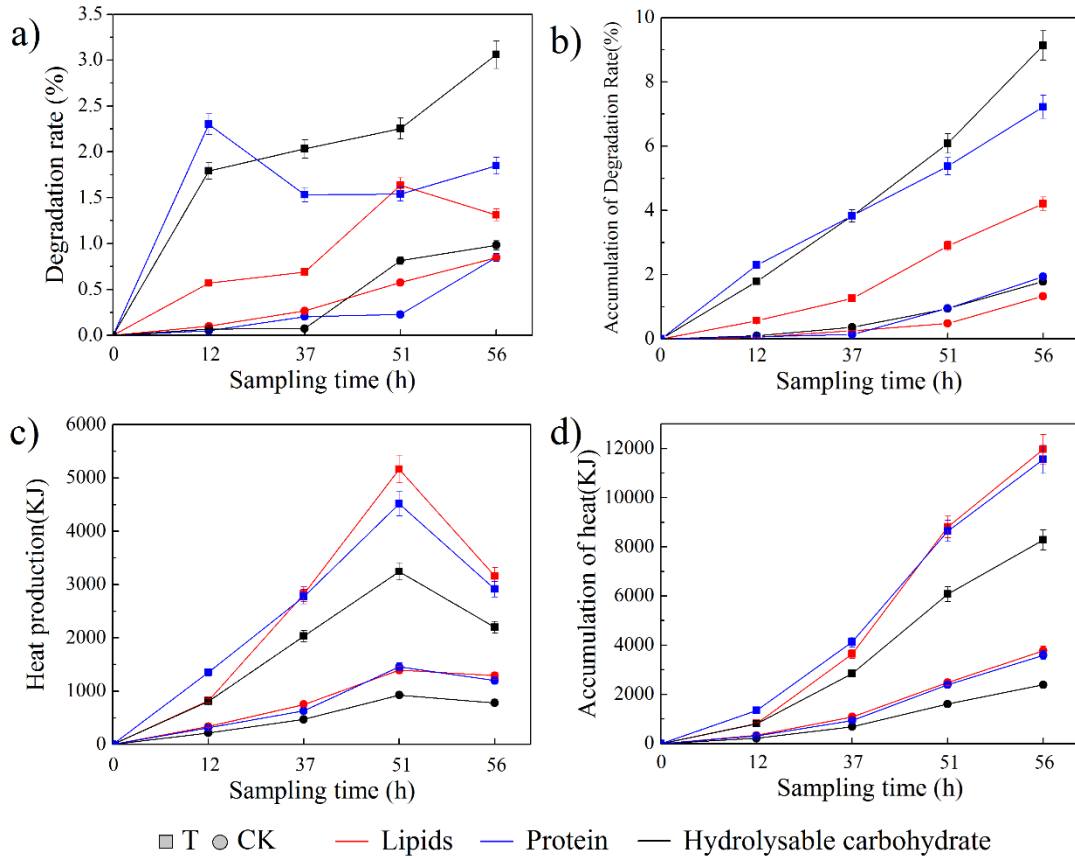


Fig.5 Changes and accumulation of degradation rate of the protein, hydrolysable carbohydrate, lipids and heat production in different treatment trials during composting. (a) Degradation of the protein, hydrolysable carbohydrate, lipids; (b) Accumulation of degradation rate of the protein, hydrolysable carbohydrate, lipids; (c) Heat production by degradation of protein, hydrolysable carbohydrate, lipids; (d) Accumulation of the heat production. The arrow pointed inoculation in different stages of composting. All values represent means \pm SD (n=3).

Supplementary Fig.S4 and Table S3 showed the change of the heat consumption through the main approaches including composting conductions, materials, conductivity and the latent heat of evaporation during composting. In the system, the total heat consumed was 31427.6 kJ and 9564.5 kJ in T and CK, which were approximate to the amount of bio-generated heat. Although the amount of heat consumption was

significantly different between T and CK, the proportion of various heat consumption approaches were similar, which were consistent with the results of Li et al. (Li et al., 2015). For T, 4.88% of total heat consumption was applied for compost materials heating (Q_i). Also, the heat was consumed 56.47 % in inlet air (Q_g) and 37.17 % in latent heat consumption of the water vapor (Q_l), which were the main consumption approaches. This was consistent with the previous result (Ahn et al., 2007). If the heat consumption of Q_g and Q_l could be recycled for the piles temperature increasing, it was beneficial to accelerate compost process. Therefore, designing and modifying compost reactors, which could effectively decrease the loss of Q_g and Q_l , were necessary. Meanwhile, compost turning consumed 1.47% heat. From these results, it can be concluded that improving the heat recovery and utilization were more meaningful in compost process.

3.5. A new reactor of application bio-heat was proposed in actual production

In order to make better use of the bio-heat produced from cold-adapt microorganisms (CAMC) in start-up period under low ambient temperature circumstance, a new type of compost reactor is designed according to the results in this study (Fig. 6). Reactor body is like a ‘matreshkas’. It consists of a main fermentation chamber and microbial heating chamber. The microbial heating chamber is wrapped the main fermentation chamber. In order to conveniently supply organic matter, we suggest that the dimension of the total reactor is 2.5-3.0 m length, 1.5-2.0 m width, 1.5-2.0 m height. The dimension of the main fermentation chamber is 1.5-2.5 m length, 0.5-1.0 m width, 0.5-1.0 m height. The significant reactor volume is 5-10 m³. The main fermentation chamber is primarily for organic wastes fermentation. CAMC is inoculated in microbial heating chamber for biological heating. Meanwhile, the microbial heating chamber could provide the heat

for the main fermentation chamber used for the increase of pile temperature. In this study, the piles temperature reached 45.4 °C in hour 56 and then gradually declined, which was due to the nutrients reduction for microbial growth. Based on these results, a method of fed-batch is suggested to use for ensuring the CMAC growth and activity in microbial heating chamber to provide continuous heat for main fermentation chamber. For an example, when the temperature in microbial heating chamber reaches to 40 ± 2 °C, it suggested to take out half of the materials and add new materials to microbial heating chamber. And then these materials taken out from the microbial heating chamber could be put into the main fermentation chamber. Fed-batch could solve the problem that nutrition exhaust, leading to the microbial activity slow down and temperature fall in the microbial heating chamber. Also, this method could provide continuously heat for main fermentation chamber.

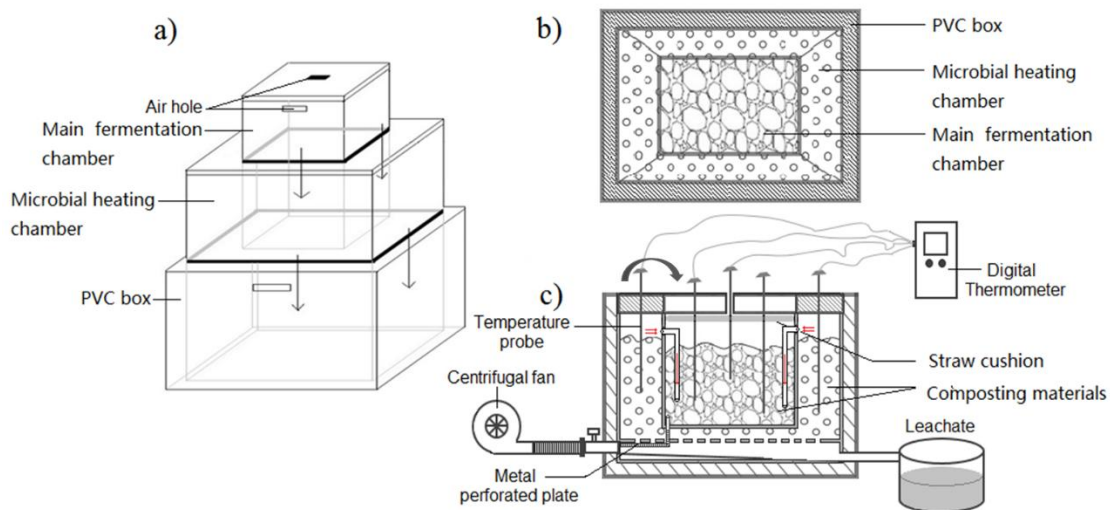


Fig.6 Diagram of self-heating reactor. (a) 3D diagram of self-heating reactor, (b) vertical view of self-heating reactor, (c) front view of self-heating reactor. The dimension of the total reactor is (length \times width \times height) 2.5-3.0 \times 1.5-2.0 \times 1.5-2.0 m. The dimension of the main fermentation chamber is (length \times width \times height) 1.5-2.5 \times 0.5-1.0 \times 0.5-

1.0 m. The significant reactor volume is 5-10 m³. When the temperature reaches 40±2 °C, half of the materials are taken out and the new materials are added to microbial heating chamber. And then these materials taken out from the microbial heating chamber could be put into the main fermentation chamber for further fermentation. The core temperature in microbial heating chamber is kept at above 20 °C at low temperature.

There are several advantages by using the reactor: firstly, it increases organic wastes removal efficiency and heat availability. Secondly, it uses clean and renewable bio-heat without needing an external heat source. Then, it could avoid further pollution to the environment, reduce the cost and save time. Finally, it could provide the piles temperature, which most strains could adapt for composting start-up. Thus, it would effectively enhance the success rate of compost in the cold region. Based on the result of lab-scaled start-up experiment, we can estimate a 10 m³ reactor could consume about 30000kJ – 35000kJ energy in each batch for the piles temperature increasing. Meanwhile, the reactors can economize 3504 US \$ each year, and it can produce 2940 US \$ of the organic fertilizer each year.

4. Conclusions

To reduce the challenges encountered during low temperature composting, we developed a novel biological method for inoculating CAMC. The pile inoculated with CAMC successfully passed start-up period at low temperature. The CAMC contributed to organic compounds degradation and heat generation. Moreover, the structure and succession of bacterial community were affected by inoculating CAMC. Additionally, the correlation among bacterial community, temperature and organic substrates degradation was changed during composting. Based on these results, a new reactor was

designed to apply the bio-heat produced by CAMC. This study would provide a biotechnology support to ensure normal start-up of winter composting.

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