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Effects of a nutrient additive on the density of functional bacteria and the microbial community structure of bioorganic fertilizer

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HIGHLIGHTS

- Solid and liquid wastes were synthetically used to prepare BOF.
- CMGW prompted the metabolism of the microorganisms in compost.
- CMGW was a recommendable nutrient additive for strain F2 predominating in BOF.
- CMGW improved species richness and stability of microbial community in BOF.

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ABSTRACT

In this study, a secondary composting experiment was conducted to investigate the effects of concentrated monosodium glutamate wastewater (CMGW) as a nutrient additive on enhancing the density of functional bacteria in bioorganic fertilizer (BOF). The results showed that, as the CMGW was added, the composting mixture temperature rose more quickly and strongly, and the functional bacteria *Bacillus subtilis* F2 percentage in spores increased significantly. Furthermore, both the highest species richness value (*Rs*) of a given sample and similarity coefficient value (*Cs*) between a sample and strain F2 based on DGGE analysis were also observed in the treatment with CMGW. A similar effect of CMGW on the microbial community structure was verified by means of illumine-MiSeq sequencing. It may thus be concluded that CMGW is a recommendable nutrient additive for F2 predominating in the secondary composting process.

1. Introduction

Large amounts of chemical fertilizer and pesticide applied in agriculture have played important roles in meeting the needs of the sustainable growth of population in China over the past several decades (Fang and Meng, 2013), but have also caused a series of negative effects and potential threats on food safety and the agricultural ecological environment (Verger and Boobis, 2013). It is desired to develop an environmentally friendly alternative to partially replace the chemicals which unfortunately constitute the most common approach in present-day agriculture. Bioorganic fertilizer (BOF) is one of these substitutes that possess attraction for sustainable agriculture, due to its dual roles of microbial fertilizer and organic fertilizer (Agriculture Ministry, 2004). It is recombined with particular functional microbial strains and organic fertilizer, i.e. the compost of the residues of animals and plants, such as livestock and poultry dung and crop stalks, etc.

The commercial products of BOF in China first appeared in the 1990s. Shen's group has performed some pioneering researches in this area, and has developed a series of products with efficacies of protecting cucumber, watermelon and banana from fusarium wilt (Huang et al., 2011; Ling et al., 2013; Yuan et al., 2013), as well as preventing potato and tobacco from the infection of bacterial wilt in continuous mono-cropping systems (Ding et al., 2013; Wu et al., 2014). The Chinese BOF industry has developed rapidly in

Abbreviations: CMGW, concentrated monosodium glutamate wastewater; BOF, bioorganic fertilizer; DMT, daily mean temperature; AT, ambient temperature.

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recent years, but the qualities of its commercial products are sometimes unstable and spotty. However, available related researches have mainly focused on the applying effects of BOF (Ding et al., 2013; Dukare et al., 2011; Huang et al., 2011; Ling et al., 2013; Wu et al., 2014; Yuan et al., 2013), yet rarely on preparation technical to improve its quality.

In general, BOF was simply prepared by mixing the matured compost with a liquid microbial agent (Wu et al., 2009) or by a secondary composting process after the matured compost was inoculated with a microbial agent (Dukare et al., 2011; Wu et al., 2014; Zhang et al., 2011). Due to the fact that the density of functional strain in BOF is one of the most important quality indexes, the key technology for improving the quality of BOF is how to promote the functional strain predominating in the matured compost, which usually has a complex indigenous microbial community structure (Dukare et al., 2011) and lacks an available carbon source (Gabhane et al., 2012).

Concentrated monosodium glutamate wastewater (CMGW) from the monosodium glutamate industry is intractable to treat, due to its high concentrations of COD, ammonia and sulfate, and low pH (Yang et al., 2005). The conventional approaches, e.g. physico chemical treatments, are mostly infeasible for the environmental hazard of resultant stench (Hui, 1995; Korner et al., 2001) and consume large energy leading to high costs (Bai et al., 2004). Therefore, it is realistic to seek a more eco-friendly and economical way to deal with CMGW. Due to the fact that CMGW is rich in nutrients, such as amino acids and carbon sources, and free of heavy metal pollution (Bai et al., 2004), it may be an ideal additive to nourish the functional strain inoculated in the secondary composting for preparing BOF. In view of this assumption, a secondary compost experiment, with CMGW as a nutritional additive and *B. subtilis* F2 as a functional strain, was conducted to investigate the impacts of this wastewater on the density of functional strain and the microbial community structure of BOF.

2. Methods

2.1. Materials

The compost, used as the secondary composting matrix for preparing BOF, was prepared from cattle manure and mushroom residue via high temperature aerobic composting. It was sieved through 7 mesh and shown to contain 24.6% organic C, 2.2% TN, 1.6% P_2O_5 and 2.1% K_2O, 2.03 mg/kg Hg, 1.68 mg/kg Cd, 32.54 mg/kg Cr, 23.83 mg/kg As, and 3.86 mg/kg Pb, with moisture content of 53.0%, germination index of 209.5%, and bulk density of 0.42 g/cm^3.

The concentrated monosodium glutamate wastewater (CMGW) used as the basic nutrient resource of medium for culturing the functional strain and as the nutritional additive for preparing BOF, with a pH of 3.1 and electrical conductivity of 33.2 ms/cm, was provided by Anhui Huanyu Fertilizer Co., Ltd. (China). The wastewater contained 13.6% amino acids, 25.9% TC, 4.01% TN, 2.07% TK, 0.16% TP, 5.51% Na, 15.1% S, 0.26 g/kg Fe, 0.62 g/kg Ca, 0.93 g/kg Mg, 9.17 mg/kg Mo, 2.16 mg/kg Zn, 2.54 mg/kg Cu, and 2.29 mg/kg Ni.

A plant pathogen resistant strain, *B. subtilis* F2, isolated from a commercial organic fertilizer (Lu et al., 2007), was used as the functional bacteria for preparing the BOF. The F2 seed culture was obtained by inoculating its colony to the LB liquid medium (yeast extract 5 g/L, tryptone 10 g/L, NaCl 10 g/L, pH 7.2) for culturing for 24 h under 150 r/min and 30 °C. The F2 inoculants with 3.5×10^8 cfu/mL were prepared by means of 48 h culturing under 150 r/min and 30 °C after 2% (v/v) F2 seed culture inoculated into the culture medium of CMGW that was diluted by water 100 times in additional beef extract 2 g/L, peptone 4 g/L, $MnSO_4{\cdot}2H_2O$ 0.5 g/L, H_3BO_3 0.02 g/L, $FeSO_4{\cdot}7H_2O$ 0.1 g/L, and $MgSO_4{\cdot}7H_2O$ 0.5 g/L, and pH 7.2.

2.2. Experiment design

The 7-day secondary composting experiment was conducted using the devices for solid waste high temperature aerobic composting (patent number: ZL 201010589910X), with ventilation of 30 min/2 h and 4 L/min. The following three treatments were carried out: (1) **NW0** with neither F2 inoculants nor CMGW, (2) **IW0** with 1% (v/w) F2 inoculants but no CMGW, and (3) **IW1** with both 1% (v/w) F2 inoculants and 2% (v/w) CMGW added in the compost matrix. The pH value of CMGW was adjusted to 7.0 with NaOH before being applied. According to the aforementioned design, the secondary composting materials of each treatment were mixed thoroughly, and the mixture moisture content was adjusted with additional water to about 60%. One device was loaded with 6 kg of mixture, and each treatment was replicated three times. The mixture was sampled as soon as mixed (the 1st day sample), and sampled again after 7 days composting (the 7th day sample).

2.3. Temperature monitoring

The probe of the ZDR-40 data recorder (Hangzhou Zeda Instruments Co., Ltd., China) was placed in the center of the mixture in each device, to monitor the temperature of the composting mixture every 15 min online. The daily mean temperature (DMT) was calculated, and the ambient temperature (AT) was monitored at the same time.

2.4. Plate counting of vegetative cells and spores

The vegetative cells population of the total bacteria and F2 in the composting mixture were tested by the method of plate counting, as follows: Ten grams of mixture samples was placed into 90 mL sterilized distilled water with 20 sterilized glass beads, shaken for 30 min, then left standing for 10 min. Serial dilutions of the suspension were plated on beef extract medium (beef extract 3 g/L, peptone 10 g/L, NaCl 5 g/L, agar 20 g/L, pH 7.2). Triple plates of each dilution were incubated at 30 °C for 48 h. The spores populations were detected using the same method besides the suspensions being incubated in 80 °C water for 20 min.

2.5. DNA extraction and purification

The DNA of the mixture samples was extracted with the modified protocol described by (Howeler et al., 2003). To lyse the microbial cells, a composting sample (0.3 g) was added to a sterilized 10 mL centrifuge tube containing 1.2 g silica sand, and mixed with 0.3 mL of extraction buffer (10% SDS, 0.1 M NaCl, 0.5 M Tris–HCl, pH 8.0), 0.3 mL of phosphate buffer (0.1 M, pH 8.0), and 0.3 mL of hydroxybenzene-chloroform-isoamyl alcohol (25:24:1). Each tube was oscillated on a vortex shaker for 1 min and left to stand for 1 min, and this was repeated three times. Then the mixture was centrifuged at 5000 rpm for 2 min to collect the upper lysate in a new centrifuge tube of 1.5 mL. The lower phase was subsequently washed with 0.5 mL sterilized distilled water, and centrifuged a second time. The two lysate samples were pooled, and then centrifuged at 13,000 rpm for 5 min to remove and place the upper phase in a new 1.5 mL tube. The nucleic acids in the supernatant were precipitated by blending them slightly with 0.6 volumes of isoamyl alcohol, followed by incubation at room temperature for the next hour, then collected by centrifugation at 13,000 rpm for 20 min at 4 °C to abandon the supernatant. Next, the precipitate was rinsed with 1 mL of pre-cooled 70% ethyl alcohol and centrifuged at 13,000 rpm for 5 min at 4 °C to be collected again. The extracted