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Effect of different extra carbon sources on nitrogen loss control and the change of bacterial populations in sewage sludge composting

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ABSTRACT

The aim of this study was to evaluate the effect of different extra carbon sources on nitrogen loss control and the change of bacterial populations in sewage sludge composting. Four amendments, glucose, sucrose, starch and cellulose, were applied into the composting experiments conducted in the lab-scale reactor. The variation in temperature, pH, nitrogen content and bacterial population related to nitrogen transformation were detected during the 33 days composting. The addition of glucose, sucrose and starch reduced nitrogen loss and denitrifying bacteria population while increased the population of ammonifying, ammonia assimilating and nitrifying bacteria. Nitrogen loss was reduced by 24.7%, 46.3% and 26.5% in glucose, sucrose and starch treatments, respectively. The population of ammonifying, ammonia assimilating and nitrifying bacteria increased by 6.8–22.7%, 2.1–19.4%, 3.3–16.0%, respectively. Sucrose was the optimal amendment due to the lowest nitrogen loss (15.87%), the highest population of ammonia assimilating (7.0 $\log_{10}$ cfu/g) and nitrifying bacteria (5.6 $\log_{10}$ cfu/g). The results suggested that adding carbon sources in sludge composting could reduce nitrogen loss through improving the population of ammonifying, ammonia assimilating, nitrifying bacteria and reducing the population of denitrifying bacteria.

1. Introduction

Sewage sludge is a byproduct of wastewater treatment process. With the rapid increase of sewage sludge production in China, sewage sludge disposal has attracted increasing attention (Wu et al., 2015). Composting is an effective technique for converting sewage sludge to a stabilized and nutrient-rich compost suited for agricultural application (Baddi et al., 2004; Wang et al., 2011; Cho et al., 2013). Nitrogen content is a critical parameter for compost quality. However, approximately 40%-80% nitrogen is lost mainly via ammonia emission during composting (Bakhtiyor et al., 2014). Such massive nitrogen loss reduced the quality of compost and led to air pollution.

Nitrogen loss is mainly a result of microbial activity related to nitrogen transformation. Ammonifying, ammonia assimilating, nitrifying and denitrifying bacteria are mainly responsible for nitrogen transformation. Organic nitrogen can be transformed into NH_4^+-N by ammonifying bacteria. NH_4^+-N can be converted into NO_3^--N by nitrifying bacteria or converted into bio-nitrogen immobilized in composted materials by ammonia assimilating bacteria. NO_3^--N can be converted into N_2/N_2O released from compost by denitrifying bacteria. Accordingly, the population of ammonifying, nitrifying, denitrifying and ammonia assimilating bacteria at different stages of aerobic composting have an important effect on nitrogen transformation and nitrogen loss during composting process. However, most research focused on the bacterial community and population in composting (García et al., 2010; Tian et al., 2013; Kim et al., 2014). Little attention was paid to study the population of ammonifying, ammonia assimilating, nitrifying and denitrifying bacteria and their effect of on nitrogen transformation and nitrogen loss.

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Nitrogen loss is related to temperature, pH, C/N ratio, aeration and moisture (Nakasaki et al., 1993; DeLaune et al., 2006; Jiang et al., 2011; Waszkielis et al., 2013; Bakhtiyor et al., 2014). C/N ratio is one of the main factors influencing nitrogen loss in composting (Barrington et al., 2002). Previous research has reported that the increase of C/N ratio by extra carbon sources amendment, such as glucose, sucrose, sawdust and straw, could reduce nitrogen loss by descreasing ammonia emission (Nakasaki et al., 2001; Liang et al., 2006; Torkashvand, 2009; Jiang et al., 2011; Li et al., 2013). However, the microbial mechanism responsible for this effect is not clear.

In recent decades, culturable method is widely used to isolate and enumerate microorganisms from composts (Ryckeboer et al., 2003; Sasaki et al., 2005; García et al., 2010). Accordingly, in this study, the populations of ammonifying, ammonia assimilating, nitrifying and denitrifying bacteria at different stages of aerobic composting were detected by cultivable method. The variation in temperature, pH and total nitrogen was detected. The purpose of this study is to investigate the effect of different carbon sources on nitrogen loss control and the change of bacterial population related to the nitrogen transformaton, and evaluate the relationship between these bacterial population and nitrogen loss. This study will provide practical information for the exploitation of a compost amendment reducing nitrogen loss in sludge sewage composting.

2. Materials and methods

2.1. Lab–scale composting

Sewage sludge was obtained from Wenchang wastewater treatment plant in Harbin, China. The sewage sludge contained 71.63% water, 38.11% volatile solid, 54.31% MLVSS, 18.85% carbon and 2.3% nitrogen. The C/N ratio was 8.06 and pH value was 7.29. A mixture of 3300 g of sewage sludge and 2640 g of pumice was divided into five equal piles. Pile 1 was the control without extra carbon source. Pile 2 to pile 5 were treatment experiments with the addition of glucose, sucrose, starch and cellulose, respectively at the mass ratio of 4% (carbon source/sludge). Composting treatments were conducted for 33 days in five separate but identical reactors. The reactor has an inner diameter of 100 mm, height of 300 mm and a volume of 2.5 L. Three holes were drilled on the top of the reactor for aeration, temperature detection and exhaust gas collection, respectively. Fresh air was pumped to the bottom of the reactor after passing through a 250 mL Erlenmeyer flask containing NaOH solution to remove CO_2. The exhaust gas was collected by a 250 mL Erlenmeyer flask containing 50 mL 2% boric acid to capture the ammonia. To prevent heat loss, five composting reactors were placed in the same waterbath. The waterbath temperature was always maintained 2–4 °C below that of the control treatment. The samples for detecting pH and bacterial population were collected at 1, 3, 5, 7, 9, 13, 18, 23 and 32 days from each Pile. Temperature was measured daily. The initial characteristics of five piles are presented in Table 1.

2.2. Bacterial population analysis

Culturable microorganisms were enumerated by inoculating the appropriate medium. Ammonifying, nitrifying and denitrifying bacteria were evaluated using the different medium (medium A for ammonifying bacteria, medium B for nitrifying bacteria and medium C for denitrifying bacteria) by the Most Probable Number method (Greenberg et al., 1992). Ammonia assimilating bacteria was enumerated using medium D by plate counting. Media A consists of 0.5% peptone, 0.05% KH_2PO_4, 0.02% K_2HPO_4, 0.05% $MgSO_4{\cdot}7H_2O$. Media B contains 0.1% CH_3COONa, 0.01% $(NH_4)_2SO_4$, 0.05% K_2HPO_4, 0.25% $MgSO_4{\cdot}7H_2O$, 0.25% NaCl, 0.05% $FeSO_4{\cdot}7H_2O$, 0.05% $MnSO_4{\cdot}4H_2O$. Media C contains 0.2% KNO_3, 0.1% KH_2PO_4, 0.1% K_2HPO_4, 0.01% $MnSO_4{\cdot}4H_2O$, 0.02% $MgSO_4{\cdot}7H_2O$, 0.5% $Na_3C_6H_5O_7{\cdot}2H_2O$. Media D contains 0.5% glucose, 0.025% NH_4Cl, 0.01 $Fe(NH_4)_2H(C_6H_5O_7)_2$, 0.05% NaCl, 0.05% $MgSO_4{\cdot}7H_2O$, 0.01% $MnCl_2{\cdot}4H_2O$, 0.1% K_2HPO_4, 0.32% KH_2PO_4, and 1.5% agar (Sasaki et al., 2005).

One gram of composting sample was suspended into 10 mL sterile water and shaken for 1 h. Then the composting suspension was diluted with sterile water at 10^{-1}–10^{-6}. For ammonifying, nitrifying and denitrifying bacteria, each diluted solution (1 mL) was inoculated into 5 mL of the medium A, B and C, respectively. Each treatment repeated 5 times. The medium A and B were incubated at 30 °C for 7 days and medium C was incubated at 30 °C for 14 days. The presence of ammonifying and nitrifying bacteria was detected by the apparition of orange and blue colour after the addition of Nessler's reagent and sulphuric diphenylamine, respectively (Greenberg et al., 1992). The presence of denitrifying bacteria was detected by the production of gas bubble. For ammonia assimilating bacteria, each diluted solution (100 μL) was spread onto the plate containing medium D. The plates were incubated at 30 °C until visible colonies formed. Each solution repeated 3 times.

The population of ammonifying, nitrifying and denitrifying bacteria was counted according to the formula: cfu/g composting sample = quantitative index from MNP list × dilution times of first number × 10. Ammonia assimilating bacterial population was counted according to the formula: cfu/g composting sample = colony number × dilution times × 100.

2.3. Physical and chemical analysis

Temperature was measured by the thermometer every day at the same point. pH was measured by pH meter (826PH, Metrohm, Switzerland) after dissolving 1 g compost sample in 10 mL distilled water. The total organic carbon was determined according to WalkleyeBlack wet combustion method (Walkley and Black, 1934). The total N was determined by elemental analyser (Vario EL, Germany). All data were presented by using mean ± standard deviation. Means were calculated as an average of three replicates of each treatment. The standard deviation of these values were calculated using Excel (Microsoft, 2003).

3. Results and discussion

3.1. Temperature

Temperature is a key parameter in monitoring the ccomposting process and microbial activity. Fig. 1 shows the temperature change during the composting process. No obvious difference was observed among treatments. The variation of temperatures was divided into three phases. Firstly, the temperature rose rapidly to above 50 °C within 5 days. Secondly, the temperature remained at high level (>50 °C) for about 7 days. Finally, the temperature decreased gradually to the ambient level. The addition of carbon sources had no significant effect on temperature during composting process except reaching high temperature 1 day ahead in comparison to control treatment. One possible reason is that the composting condition with extra carbon sources is favorable for the growth of some bacteria, which results in the generation of heat and the increase of composting temperature. Similar result was observed by other researchers and it has been stated that biochar amendment could shorten the time reaching high temperature at the start of composting (Steiner et al., 2010; Krystyna et al., 2014).