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Effects of sucrose amendment on ammonia assimilation during sewage sludge composting

Liqiang Meng [a,b,c], Weiguang Li [a,*], Shumei Zhang [b,c], Chuandong Wu [a], Ke Wang [a]

[a] School of Municipal and Environmental Engineering, Harbin Institute of Technology, 150090 Harbin, China
[b] Institute of Microbiology, Heilongjiang Academy of Sciences, 150010 Harbin, China
[c] Institute of Advanced Technology, Heilongjiang Academy of Sciences, 150020 Harbin, China

HIGHLIGHTS

- Sucrose amendment can promote ammonia assimilation.
- Sucrose decreased ammonia emission by 24.7–31.1%, and 3% sucrose gave the least ammonia emission.
- Sucrose increased ammonia assimilating bacteria population 2.5–3.5 fold.
- The activities of key enzymes associated with ammonia assimilation were enhanced by sucrose.

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ABSTRACT

The aim of this study was to evaluate the laboratory-scale composting of sewage sludge and pumice mixtures that were amended with sucrose. The variation in temperature, pH, NH_4^+-N, ammonia emission, bacterial community, ammonia assimilating bacteria (AAB) populations and enzymatic activity related to ammonia assimilation were detected. The addition of sucrose increased the AAB population by 2.5–3.5 times, reduced ammonia emission by 24.7–31.1% compared with the control treatment, and promoted the growth of *Bacillus* and *Wautersiella*. The activities of glutamate dehydrogenase (GDH), glutamate synthase (GS) and glutamine synthetase (GOGAT), were enhanced by the addition of sucrose. GDH made a substantial contribution to ammonia assimilation when the ammonia concentration was high ($\geqslant$1.5 g/kg) in the thermophilic phase. The GS/GOGAT cycle played an important role at low ammonia concentrations ($\leqslant$1.1 g/kg) in the cooling phase. These results suggested that adding sucrose to sludge compost could promote ammonia assimilation and reduce ammonia emission.

1. Introduction

Sewage sludge is an inescapable by-product of wastewater treatment (Bialobrzewski et al., 2015). With improvements and the increased capacity of municipal wastewater treatment in China, tremendous quantities of sewage sludge are produced. Composting is a simple and low-cost technique for converting sewage sludge into a safe and usable product. Nitrogen is one of the most important nutrients in sludge, and it can be recycled for agricultural application (Wang et al., 2011). However, approximately 40–80% of nitrogen is lost via ammonia emission during the sludge composting process (Nakhshiniev et al., 2014). Therefore, ammonia emission control is a key factor in reducing nitrogen loss.

Ammonia emission is mostly a result of microbial activity. Nitrogen in composting is transformed by microorganisms through ammonification, assimilation, nitrification and denitrification. Ammonia assimilation by ammonia assimilating bacteria (AAB) is one of the key processes in nitrogen transformation (Sasaki et al., 2004). Ammonia can be converted to bio-nitrogen, which is immobilised in compost through assimilation by AAB. Accordingly, the population of AAB has an important impact on ammonia assimilation and immobilisation during composting. However, AAB populations and their effect on ammonia assimilation during sludge composting have received very little research attention.

Three key enzymes, glutamate dehydrogenase (GDH), glutamine synthetase (GS) and glutamate synthase (GOGAT), participate in ammonia assimilation via two classic routes (GDH and GS/GOGAT). In the GDH route, GDH not only catalyses NH_4^+-N

* Corresponding author at: School of Municipal and Environmental Engineering, Harbin Institute of Technology, Box No. 2602, 73 Huanghe Road, Harbin, Heilongjiang 150090, China. Tel.: +86 18904512510; fax: +86 451 86283003.
E-mail address: hitlwg@126.com (W. Li).

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and α-ketoglutarate in the formation of glutamate, but also catalyses the decomposition of glutamate into NH_4^+-N and α-ketoglutarate. In the GS/GOGAT route, GS catalyses the synthesis of glutamine from NH_4^+-N and glutamate with hydrolysis ATP (Miller and Maier, 2014), while GOGAT catalyses glutamine's production of glutamate and a-ketoglutarate along with the reduction of NADH (Van den Heuvel et al., 2004). Therefore, the activities of GDH, GS and GOGAT are closely related to the formation of bio-nitrogen in composting systems. However, few studies have investigated the influence of the enzymatic activity of GDH, GS and GOGAT on ammonia assimilation during composting.

Previous research has reported that carbon source amendment, such as biochar, glucose, sucrose and triacylglycerol, reduces nitrogen loss during the composting of sewage sludge (Li et al., 2013b; Malińska et al., 2014; Matsumura et al., 2010; Wu et al., 2015). However, the relationship between carbon source amendment and ammonia assimilation is not clear.

The purpose of this study is to investigate the effect of sucrose amendment on ammonia assimilation in sewage sludge composting. Temperature, pH, NH_4^+-N, NH_3 emissions and bacterial community were measured, and the quantity of AAB was monitored. Furthermore, the activity of GDH, GS and GOGAT was recorded throughout the composting process.

2. Methods

2.1. Feeding materials and composting process

Dewatered sewage sludge was collected from a municipal wastewater treatment plant in Harbin, China. Fresh pumice was used as an inorganic bulking agent, and sucrose was introduced as the carbon source. A mixture of 8000 g of dewatered sewage sludge and 4000 g of pumice was divided into four equal parts. Sucrose was added to the mixtures at the mass ratios of 1%, 3% and 5% (sucrose/sludge), hereafter denoted as 1% SC, 3% SC, 5% SC, respectively. No sucrose was added to the control (0% SC). The characteristics of the raw materials are shown in Table 1.

Composting treatments were conducted for 20 days in four separate but identical reactors, which had an inner diameter of 300 mm and a height of 600 mm. Fresh air was pumped via an air pump into each reactor from the bottom through perforation plates. The aeration rate was controlled at 0.4 L/min by a flow meter during the composting process. The exhaust gas was passed through an Erlenmeyer flask containing 500 mL of 2% boric acid, and the ammonia was captured. The four composting treatments were conducted in four reactors. To prevent heat loss and simulate the composting process, the four composting reactors were placed in the same water bath. The water bath temperature was always maintained at 2–4 °C below that of the control treatment (Li et al., 2013a; Mason and Milke, 2005) and monitored with temperature sensors.

2.2. Physical and chemical analysis

The pH of the sludge samples was measured using a pH meter to evaluate 1 g of sample dissolved in 10 mL of distilled water (Rihani et al., 2010). The concentrations of ammonia in the exhaust gas were measured every two days. The ammonia was trapped by a boric acid solution and measured by titration (Li et al., 2013a). NH_4^+-N was extracted in a 2 mol/L KCl solution and measured first with the Indophenol Blue method and then by colorimetry (Li et al., 2012).

Table 1
The characteristics of the raw materials.

	pH	Moisture (%)	C/N
Sewage sludge	7.31 ± 0.25	79.36 ± 0.63	6.94 ± 0.35
Pumice	7.15 ± 0.64	0.83 ± 0.39	–
Sucrose	6.94 ± 0.82	1.23 ± 0.21	–

2.3. DNA extraction and high-throughput sequencing of 16S rRNA

The total genomic DNA was extracted using a PowerSoil DNA isolation kit (MoBio Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer instructions. The concentration and quality of DNA were determined by NanoDrop ND-2000c (Theromo Fisher Scientific, Waltham, MA, USA). The extracted DNA was amplified using universal primers with a forward primer (5′-GTACTCCTACGGG AGGCAGCAG-3′) and reverse primer (5′-GTGGACTACHVGGGTWTCTAAT-3′) (Yu et al., 2015), which target the hypervariable V3–V4 regions of the bacterial 16S rRNA gene. The PCR mixture (25 μL) contained 1 × PCR buffer, 1.5 mM $MgCl_2$, 0.4 μM of a dNTP mixture, 1.0 μM of each primer, 0.5 U of Taq DNA Polymerase (TaKaRa, China) and 10 ng of genomic DNA from the sludge. The PCR amplification procedure included an initial denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 30 s and then a final 10 min extension stage at 72 °C. The PCR products were purified with a PCR Product Purification Kit (iNtRON, Gyeonggi-do, Korea) and quantified with a Qubit 2.0 Fluorometer (Life Technologies, Shanghai, China). Equal molar amounts of the PCR products from each sample were pooled and prepared using a TruSeq DNA kit (Illumina, USA). The samples were then sequenced using the PE300 protocol on the Illumina MiSeq according to the manufacturer instructions (Illumina, San Diego, CA, USA).

2.4. Population analysis of ammonia-assimilating bacteria

The AAB population was detected by plate counting, using ammonia as the sole nitrogen source. The medium contains 0.5% glucose, 0.01% $Fe(NH_4)_2H(C_6H_5O_7)_2$, 0.025% NH_4Cl, 0.05% $MgSO_4 \cdot 7H_2O$, 0.05% NaCl, 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 diluted with 10 mL of sterile water and was shaken at 100 rpm for 1 h. Then, the composting suspension was diluted with sterile water at 10^{-1}–10^{-6}. Each diluted solution (100 μL) was spread onto the plate. The plates were incubated at 37 °C for 3–5 days until visible colonies appeared. Each solution was plated 3 times, and the AAB were counted according to the formula: CFU/g composting sample = average colony number × dilution × 100.

2.5. Preparation of enzyme extracts

The enzymes were extracted from the sludge samples by ultrasonic lysis (Hristozova et al., 2008). One gram of sludge sample was suspended into 10 mL of 0.05 M Tris buffer (pH 8.0) and lysed with an ultrasonic disrupter system VCX605 (Sonics, America) in an ice-water bath at 5 ± 1 °C (operated for 3 s at 100 W, for 30 circles, 10 s intervals). The sludge suspension was centrifuged at 14,000×*g* for 20 min at 4 °C. The supernatant was collected and filtered through a 0.45 μm sludge filter membrane, and the enzymatic activity was immediately measured in the filtrate.

2.6. Enzyme activities analysis

The activity levels for GDH (EC 1.4.1.2), GS (EC 6.3.2.1) and GOGAT (EC 1.4.7.1) were detected by a GDH Activity Assay Kit (Sigma–Aldrich Co., America), GS Assay Kit (Nanjing jianchengtech Co., China) and GOGAT Activity Detection Kit (Solarbio Co., China), respectively. The reaction rates of each enzyme were evaluated on