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# Influence of medical stone amendment on gaseous emissions, microbial biomass and abundance of ammonia oxidizing bacteria genes during biosolids composting



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# ABSTRACT

This study aimed to evaluate the feasibility of medical stone (MS) on microbial biomass, bacteria genes copy numbers, mitigation of gaseous emissions and its correlation with analyzed parameters during the biosolids composting. Composting of the biosolids by amendment of MS 0%, 2%, 4%, 6% and 10% (on dry weight basis) was performed using a 130-L composting reactor. The results showed that with increasing the dosage of MS, the CH<sub>4</sub>, N<sub>2</sub>O and NH<sub>3</sub> emission were reduced by 60.5–88.3%, 46.6–82.4% and 38.2–78.5%, respectively. In addition, the 6–10% MS amendment enhanced the organic waste mineralization and prolonged the thermophilic phase. The abundance of ammonia oxidizing bacteria (AOB) and archaea (AOAB) were decreased during the first 28 days, but considerable increment was observed during the maturation phase which indicated that AOB and AOAB were liable for nitrification during the curing phase of composting. A significant correlation was observed among the all analyzed parameters in 6–10% MS blended treatments.

# 1. Introduction

Huge quantity of biosolids or sewage sludge (SS) is generated from the waste water treatment plant and its improper disposal may cause serious health hazardous and ecological problem ([Cai et al., 2016](#page--1-0)). Many ecofriendly strategies such as anaerobic digestion and composting, since long time are available to managed this kind of organic waste, which is fit with 3R's (Reduce, Reuse and Recycle) philosophy, and produced stabilized end product ([Villasenor et al., 2011; Maulini-](#page--1-1)[Duran et al., 2013; Awasthi et al., 2016a, 2017a](#page--1-1)). However, the recycling of high moisture contain biosolids through anaerobic digestion is one of best ecofriendly disposal methods but the digested residues are most of the time not completely stabilized. And anaerobic digestion has lead several advantages like energy conservation than other technologies but required high level of investment and proper monitoring for commercial scale, and if the process not runs efficiently that can generates less quantity of gases and then noxious odors problems in surrounding area ([Teglia et al., 2011; Zeng et al., 2012; Jiang et al., 2016](#page--1-2)). In addition, it is difficult to store and transport the un-digested residue owing to its high moisture content and odors as well as limit the direct land applications. Therefore, recycling of this un-digested residue

requires further treatment and has become urgent need for the management of organic residues of biosolids.

Composting is cost effective green technology for disposal of biosolids into available stable humus by the influence of microorganism. Although, this technology is commonly applied over the last many years by rapid increases in biosolids production and abundant agricultural resources ([Villasenor et al., 2011; Zeng et al., 2014; Malinska](#page--1-1) [et al., 2014](#page--1-1)). An infelicitous accident during conventional biosolids composting is the depletion of extensive quantity of organic nutrients through gaseous emission ([Bong et al., 2016; Awasthi et al., 2017b\)](#page--1-3) and high concentration of heavy metals, which is not only affect the agronomic value of the end product but also involves for global warming and formation of odor as well as phytotoxicity [\(Fang and Wong, 1999;](#page--1-4) [He et al., 2000; El Kader et al., 2007; Czekala et al., 2016\)](#page--1-4). However, carbon dioxide  $(CO<sub>2</sub>)$  and ammonia  $(NH<sub>3</sub>)$  emission are two important biogenic gases produced due to the rapid mineralization of organic matters but high moisture content reduced the total aerobic microbial abundance and thus decreased the rate of organic matter (OM) mineralization, and produced bulk of  $CH_4$  and  $N_2O$  gases. Simultaneously, out of total gaseous generation during the composting, only 24% CH4 and 3%  $N_2O$  alone while major amount of (63%) is  $CO_2$  emitted,

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respectively ([Beck-Friis et al., 2000; Fukumoto et al., 2003; Sanchez-](#page--1-5)[Monedero et al., 2010; Wang et al., 2016\)](#page--1-5); but  $CH_4$  and  $N_2O$  global warming effect 30 and 210 times higher than  $CO<sub>2</sub>$  ([Sommer and Moller](#page--1-6) [2000; Luo et al., 2013; Sanchez-Garcia et al., 2015\)](#page--1-6). Consequently, SS normally has low C/N ratio that were negatively influenced the composting and also involved to higher GHGs emission. Although, various additives such as biochar, wood ash, fly ash, kaoline, zeolite, lime, phosphogypsum, struvite salt and Ca-bentonite ([Fang and Wong, 1999;](#page--1-4) [Wong et al., 2009; Li et al., 2012; Luo et al., 2013; Wang et al., 2016;](#page--1-4) [Awasthi et al., 2017a](#page--1-4)), and bulking agents like higher dosage of biochar, wood dust and agricultural wastes ([Ermolaev et al., 2014; Santos](#page--1-7) [et al., 2016](#page--1-7)) have been widely used for the mitigation of  $CH<sub>4</sub>$  and N<sub>2</sub>O emission, and nitrogen conservation during the various kind of organic waste composting, but knowledge concerning about medical stone (MS) amendment into SS composting is limited.

Therefore, it is hypothesized that MS addition into biosolids composting may have many benefits. Because micro porous-structure of MS has leads to provide optimum aeration for rapid biodegradation of OM and that could not only enhanced the microbial activities but act as biofilter to reduce the gaseous emission and nitrogen losses. Beside this, MS amendment adequate to buffer the pH and reduced the mobility and bioavailability of heavy metals during the bio-oxidative phase of composting [\(Wang et al., 2016\)](#page--1-8). At present, from an extensive literature was searched and conclude that no earlier investigation described about carbon and nitrogen conservation through mitigation of  $CH<sub>4</sub>$  and  $N<sub>2</sub>O$ emission and its relation of microbial biomass of carbon and nitrogen for biosolids composting employing MS. Hence, it is interesting to examine the feasibility of MS amendment for mitigation of GHGs and NH<sub>3</sub> emission during the biosolids composting and resulting maturity indexes as well as the evolution of gene copy numbers of total bacteria (TB), ammonia oxidizing bacteria (AOB), total archaea bacteria (TAB) and ammonia oxidizing archaea bacteria (AOAB) microorganism involved in this process.

## 2. Materials and methods

## 2.1. Raw materials collection and processing

The biosolids and wheat straw (WS) used in this study were collected from the Yangling sewage water treatment plant and Northwest A & F University, China. The MS was used as additive and purchase from Shijiazhuang Jiacheng Building Materials Co. Ltd., China. The biosolids and WS (bulking agent) were mixed at 1:1 ratio (dry weight basis) to achieved the ∼55% moisture content and ∼25 C/N ratio, while 1.0 kg of plastic spheres (non-biodegradable) were also mixed with initial feedstock to adjust the 0.5 kg/L bulk density. The physicochemical characteristics of raw materials are already provide in our previous study ([Awasthi et al., 2017a\)](#page--1-9), while MS pH (8.73  $\pm$  0.02), moisture content (1.02  $\pm$  0.04), and total organic matter (TOM), total organic carbon (TOC) and total Kjeldahl nitrogen (TKN) are not detectable.

## 2.2. Experiment design and compost sample collection

The composting experiment was carried out in a series of 130-L invessel reactors. Details of the setup and operation of the composting process were provided in our previous report [\(Awasthi et al., 2017b](#page--1-10)). Five treatments were performed to assess the impact of MS-2%, 4%, 6% and 10% (on biosolids dry weight basis) for mitigation of gaseous emission and production of matured compost. The biosolids without any MS blended was used as control for comparison purpose. The ∼100-L of composting mixture was then loaded into the each composter for the present experiment, as described above. Prior to feedstock loading, the biosolids residue and WS were manually mixed thoroughly combined with additive to ensure the homogeneity. Next, the composting mass from the reactor was taken out and mixed

properly in a separate container on day 0, 3, 7, 10, 14, 21, 28, 35 and 42; while periodically moisture content of the composting mixture was readjusted to ∼55% on turning days. On each mixing days (0, 3, 7, 10, 14, 21, 28, 35 and 42), ∼250.0 g compost samples were taken, which were split into two parts and preserved at 4 and −20 °C, respectively. The composting mass temperature was observed every day four times (6 h) to evaluate the progressiveness of the process and ventilation according to our previous study ([Awasthi et al., 2016a\)](#page--1-11).

# 2.3. Gaseous emission and compost analysis

The gaseous emission (NH<sub>3</sub>, CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O) were determined according to our earlier study [Awasthi et al. \(2016a\).](#page--1-11) The pH, EC, moisture content, TOM, TKN and TOC were analyzed as per [TMECC](#page--1-12) [\(2002\).](#page--1-12) The compost microbial biomass of carbon (MBC) and nitrogen (MBN) were quantified employing the chloroform fumigation-incubation and extraction method ([Jenkinson and Powlson, 1976\)](#page--1-13).

#### 2.4. Molecular microbiology sampling and analysis

Fresh compost samples collected on day 0, 3, 7, 14, 21, 28, 35 and 42 were used to extract DNA using a DNA extraction kit (Fast DNA SPIN Kit for Soil, Omega Biotek, Inc.) as per manufacturer's directions. The extracted DNA was quantified using a spectrophotometer (Nano Drop 2000, Thermo, Japan), while DNA concentration and purity were observed on 1% agarose gels. Based on the concentration, DNA was diluted to 1.0 ng/μL using Milli-Q water. The PCR was performed using special bacterial universal primers 515F (5′- GTGCCAGCMGCCGCGGTAAT-3′) and 806R (5′-GGACTACHVGGG TWTCTAA-3′), and a GC-clamp. All PCR reactions were carried out with Phusion® High-Fidelity PCR Master Mix (New England Biolabs). Touchdown PCR was used to amplify bacterial and archaea 16S V3 variable regions as follows; the PCR mixture (final volume 50 μL) contained 20 μL Premix Ex Taq (Takara Biotechnology), 0.4 μL of each primer (10  $\mu$ M), 4  $\mu$ L of five-fold diluted template DNA (1–10 ng) and 25.2 μL-sterilized water. Polymerase chain reaction (PCR) was performed according to [Sun et al. \(2016\)](#page--1-14), while finally PCR products were purified using Qiagen Gel Extraction Kit and PCR Clean-up system (Qiagen, Germany). The concentrations of DNA in the PCR products were fluorometrically estimated employing the Qubit dsDNA HS Assay Kit (Invitrogen, Carlsbad, CA, USA), while the sequences were identified using by the help of Novogene (Miseq platform, Illumina, San Diego, CA, USA), Beijing, China. The total abundance of the groups of selected bacteria was analyzed according to [Wang et al. \(2017\).](#page--1-15) The target gene data was revealed as mean slop of the gene copy number per 1.0 kg of the total feed stock (copies·kg<sup>-1</sup>-TS).

#### 2.5. Statistical analysis

The average value of three replicates of each analysis were reported and the data were deal on the basis of two-way analysis of variance (ANOVA), while multiple comparison tests were also conducted to identified the least significance difference at  $p = 0.05$  values employing SPSS v.21 software package for windows. The redundancy analysis (RDA) was implemented to identify the correlation of among the all physiochemical properties, gaseous emission and biological parameters during the biosolids composting by the using of Canoco 5 software.

# 3. Results and discussion

#### 3.1. Effect of medical stone amendment on maturity indexes

The temperature is one of the important criteria to assess the overall composting process, at which plenty of the microbiological bio-oxidative reactions take place during composting. From a biological flyspeck, three composting passed through three main phases: first temperatures Download English Version:

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