Bioresource Technology 110 (2012) 144–152

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Nitrification and microbiological evolution during aerobic treatment of municipal solid wastes

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ARTICLE INFO

Article history:
Received 3 November 2011
Received in revised form 20 January 2012
Accepted 24 January 2012
Available online 1 February 2012

Keywords:
Aerobic treatment
Ammonia emissions
Municipal solid wastes
Nitrification
Nitrogen

ABSTRACT

Nitrification is a key step in the nitrogen cycle in various ecosystems. In this study, the nitrogen dynamic and the evolution of groups of microorganisms were studied during aerobic treatment of fine organic fraction of municipal solid wastes. Mineralization of organic nitrogen exhibited two phases and resulted in two ammonia emissions peaks. The nitrogen balance indicated the onset of nitrification only during the maturation stage, which was confirmed by the accumulations of both nitrite and nitrate and the nitrous oxide emissions in this period. A significant development of ammonia-oxidizing bacteria correlated to the onset of nitrification. On the contrary, ammonia-oxidizing archaea were less abundant and declined through treatment. Identification of these ammonia oxidizers indicates that the *Nitrosomonas europaea/eutropha-like* ammonia oxidizing bacteria were responsible for ammonia oxidation instead of other groups of ammonia oxidizers during aerobic treatment of fine organic fraction of municipal solid wastes.

1. Introduction

Aerobic treatment of organic wastes, typically named composting, is a conventional and centuries-old agricultural technique (Gajalakshmi and Abbasi, 2008). It transforms crop residues, animal feces, organic municipal wastes and industrial organic by-products to composts, which are appreciated and used in agriculture land as soil conditioners and bio-fertilizers. Agricultural uses make the nitrogen (N) content an important reference to the quality of composts.

During composting of organic wastes, the mineralization of some organic nitrogen (N_{org}) containing materials such as proteins, amino acids and urea releases considerable free ammonium (NH_4^+/NH_3). For this reason, the mineralization of N_{org} is also termed as ammonification. This transformation is however bilateral since part of NH_4^+/NH_3 can be immobilized in turn by biomass to synthesize N_{org} (Paredes et al., 2000). Since the ability of ammonification is generally greater than that of immobilization, it causes an accumulation of NH_4^+/NH_3. The accumulated NH_4^+/NH_3 have therefore the potential to be stripped into the atmosphere with the aeration flow. The N losses from composting are mainly due to ammonia (NH_3) emissions, which account, respectively for 24–33% and 46.8–77.4% of the initial N of household wastes and manures (Beck-Friis et al., 2001; Martins and Dewes, 1992). These emissions cause meanwhile series of environmental problems because of their odor, toxicity and contribution to eutrophication and acid rains (Paoli et al., 2010).

Recent studies suggested that nitrification may occur during the composting process (De Guardia et al., 2010; Kowalchuk et al., 1999). The increase of nitrate (NO_3^-) or nitrite (NO_2^-) concentration in the composts proved the onset of nitrification (Eklind and Kirchmann, 2000). However, amounts of NO_3^- and NO_2^- (NO_x^-) accumulated in the composts were often too low to complete the default of N balance during composting. It was suggested that the anoxic niches existing in the composting pile allow the onset of denitrification (De Guardia et al., 2010). Denitrification transforms NO_x^- to N_2 and contributes to another kind of N loss during composting.

Nitrification is one of the key processes involved in the N cycle. In various ecosystems, ammonia-oxidizing bacteria and archaea (AOB and AOA, respectively) oxidize NH_3 to nitrite (NO_2^-), whereas NO_2^- is further oxidized to nitrate (NO_3^-) by nitrite-oxidizing

Abbreviations: AOA, ammonia-oxidizing archaea; AOB, ammonia-oxidizing bacteria; AMO, ammonia monooxygenase; DM, dry matter; FOFMSW, fine organic fraction of municipal solid wastes; KN, Kjeldahl nitrogen; NOB, nitrite-oxidizing bacteria; N_{org}, organic nitrogen; NO_x^-, nitrite and nitrate; NXR, nitrite oxidoreductase; OM, organic matter; PAO, potential ammonia oxidation; PCR, polymerase chain reaction; PR, pall rings; rDNA, ribosomal deoxyribonucleic acid; TA, total archaea; TB, total bacteria; TC, total carbon.

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doi:10.1016/j.biortech.2012.01.135

bacteria (NOB). The major grouping of AOB belongs to the subclass *Beta-proteobacteria* and comprises genera such as *Nitrosomonas*, *Nitrosococcus*, *Nitrosospira*, *Nitrosovibrio* and *Nitrosolobus* (Spieck et al., 2005). Only a few species of marine *Nitrosococcus* belong to the *Gamma-proteobacteria*. All currently know AOA belong to the new phylum *Thaumarchaeota* (Brochier-Armanet et al., 2008; Leininger et al., 2006). Due to their slow growth rate, several molecular tools have been developed to detect these microorganisms in the environment. These tools are based on the amplification by polymerase chain reaction (PCR) of the 16S rDNA or the structural genes (*amo*A, B or C) of ammonia monooxygenase (AMO) (Purkhold et al., 2000). Combined with real-time PCR, they allow the quantification of AOB and AOA in various environments (Maeda et al., 2010; Martens-Habbena et al., 2009). Since AOB and AOA represent usually the minority in the environment, the specific function trait (structural *amo*A gene) is considered as a more effective tool for analyzing and quantifying these microorganisms (Rotthauwe et al., 1997).

Unlike AOB, NOB are more scattered phylogenetically. The genera *Nitrobacter, Nitrococcus* and *Nitrospina* belong, respectively to the *Alpha*, *Gamma* and *Delta* subclasses of the *Proteobacteria*, whereas the *Nitrospira* represents the *Nitrospirae* phylum (Spieck et al., 2005). In the genus *Nitrobacter*, the key enzyme of NO_2^- oxidation is the nitrite oxidoreductase (NXR), whose catalytic subunit is encoded by the *nxr*A and *nxr*B genes. The *Nitrobacter* were investigated in various environments by using specific primers targeting these functional genes (Poly et al., 2008). For the genus *Nitrospira*, specific primers targeting their 16S rDNA gene have been designed and used for their quantification in wastewater treatment plants (Dionisi et al., 2002). Different species of AOB and NOB may coexist and contribute to successful oxidation of NH_3 to NO_3^- (Lydmark et al., 2007).

Besides bacteria and archaea, fungi are another kind of decomposer for which the role and development are not clear in the composting process. It is believed that the composting self-heating pile may reach a temperature too extreme for their survival (Bonito et al., 2010). They would thus be eliminated during the thermophilic stage and recovered when the temperature decreases (Gajalakshmi and Abbasi, 2008). However, fungi may play an important role during the maturation stage because of their ability to decompose some slow biodegradable materials like cellulose and lignin (Gajalakshmi and Abbasi, 2008). Moreover, some fungi exhibit heterotrophic nitrification activity linked to lignin degradation (De Boer and Kowalchuk, 2001). Their role involved in N transformations during composting needs to be considered.

In order to deal with the N losses and NH_3 emissions from aerobic treatment, understanding the N dynamic and studying the microorganisms involved in N transformations are indispensable. The first objective of the present study was to investigate the N dynamic during aerobic treatment. The second objective was to evaluate the onset and the flow of nitrification, as well as the development of nitrifying microorganisms. The fine organic fraction of municipal solid wastes (FOFMSW) mixed with bulking agents were treated in aerobic conditions until the end of the maturation stage (50 days). The N dynamic and the evolution of groups of microorganisms during treatment were investigated. The responsible ammonia oxidizers were identified.

2. Methods

2.1. Substrates and experimental devices

Fine organic fraction of municipal solid wastes (FOFMSW) were collected in the composting site of Launay Lantic in France (15,000 tons year^{-1}). Kitchen wastes, papers, cardboards, textiles and plastic films are mixed in this site. Mixtures are firstly incised and pre-degraded for 4 days in two rotary tubes. Pre-degraded mixtures are then sent to mechanical treatments to remove impurities like metals and glasses and then sieved at 10 mm. Finally, the sieved mixtures are mixed with green wastes to compost in windrows for 3 months. FOFMSW used in this study were collected at the output of sieving before mixing with green wastes. The physical and chemical characteristics of the initial FOFMSW are presented in Table 1.

The experimental device consisted of a group of 10 air-tight cylindrical cells (see S1 in Supplementary materials). Each cell was immersed in a water-bath whose temperature was regulated by a thermostat. The real time temperature of wastes was monitored by a thermal probe inserted into the middle of the cell. The interior volume of the cell was approximately 10 l. The incoming flow of compressed air was firstly adjusted by a flow meter and passed through a volumetric gas meter. The volumetric gas meter was recorded every 24 h to calculate precisely the applied flow (L h^{-1}). The temperature and humidity of compressed air were measured by a humidity and temperature probe. Wastes were loaded on a grid 10 cm above the bottom of the cell. Below the grid, a glass screen homogenized the incoming flow. The air passed through the wastes and went to the outlet at the top of the cell. Outlet gas flow bubbled successively in two sulfuric acid traps (150 ml, 4% H_2SO_4) which could completely absorb NH_3 emissions. The H_2SO_4 traps were changed every day. The incoming flow of compressed air and the outlet flows of 10 cells were alternately analyzed by gas analyzers. The concentration of oxygen (O_2) was measured using a paramagnetic analyzer (Maihak, multor 640) and those of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) were measured by an infrared detector (Uras 14, ABB). O_2 consumption, CO_2, CH_4 and N_2O productions were calculated by multiplying the dry molar air flow by the differences of the concentrations between the incoming compressed air and the outlet flow from each cell.

2.2. Cells preparation and experimental plan

The purpose of this experiment was to monitor the aerobic treatment of FOFMSW for a period of 50 days using 10 identical cells (C1–C10).

For each cell, 1600 g FOFMSW were mixed with 512 g of bulking agents and homogenized manually. Two hundred grams of distilled water were added to bring the initial moisture of the mixture to around 60%. Bulking agents used in this study were Ø 16 mm polypropylene pall rings (PR). They ensured a favorable aeration condition. Material polypropylene was chosen because it does not absorb water or N materials and neither contains microorganisms. The introduction of PR thus did not influence the N balance during treatment. The total volume of the mixture was nearly 10 l. The temperature of wastes was controlled constantly at 35 °C to avoid inhibition of nitrification at high temperatures (Grunditz and Dalhammar, 2001). The aeration rate was adjusted to 78 L h^{-1}. It was around 150 L h^{-1} per kg initial organic matter (L h^{-1} kg^{-1} OM_0). Once the wastes were loaded, the cells were enclosed and aerated. The cells were then immersed in the bath and maintained at 35 °C.

Every 5 days, one cell among the 10 cells was stopped. The residues of this cell were separated from PR and stored for further analyses. Residues of other cells were turned. Two hundred grams of distilled water were added to keep the materials humid.

2.3. Physical and chemical analyses of solid samples

Dry matter (DM) content was measured by drying fresh samples at 80 °C until the loss of mass in 24 h was inferior to 0.5%. Or-