Journal of Environmental Management 215 (2018) 57–67

Contents lists available at ScienceDirect

Journal of Environmental Management

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

Research article

Effect of semi-permeable cover system on the bacterial diversity during sewage sludge composting

Tatiana Robledo-Mahón [a, b], Elisabet Aranda [a, b], Chiara Pesciaroli [a, 1], Alfonso Rodríguez-Calvo [a], Gloria Andrea Silva-Castro [a], Jesús González-López [a, b], Concepción Calvo [a, b, *]

[a] Institute of Water Research, University of Granada, Granada, Spain
[b] Department of Microbiology, Pharmacy Faculty, University of Granada, Granada, Spain

ARTICLE INFO

Article history:
Received 16 August 2017
Received in revised form
5 March 2018
Accepted 10 March 2018

Keywords:
Composting
Sewage sludge
Semi-permeable membrane cover
Enzymatic activity
Illumina MiSeq
Bacillus

ABSTRACT

Sewage sludge composting is a profitable process economically viable and environmentally friendly. In despite of there are several kind of composting types, the use of combined system of semipermeable cover film and aeration air-floor is widely developed at industrial scale. However, the knowledge of the linkages between microbial communities structure, enzyme activities and physico-chemical factors under these conditions it has been poorly explored. Thus, the aim of this study was to investigate the bacterial dynamic and community structure using next generation sequencing coupled to analyses of microbial enzymatic activity and culturable dependent techniques in a full-scale real composting plant. Sewage sludge composting process was conducted using a semi-permeable Gore-tex cover, in combination with an air-insufflation system. The highest values of enzymatic activities such as dehydrogenase, protease and arylsulphatase were detected in the first 5 days of composting; suggesting that during this period of time a greater degrading activity of organic matter took place. Culturable bacteria identified were in agreement with the bacteria found by massive sequencing technologies. The greatest bacterial diversity was detected between days 15 and 30, with *Actinomycetales* and *Bacillales* being the predominant orders at the beginning and end of the process. *Bacillus* was the most representative genus during all the process. A strong correlation between abiotic factors as total organic content and organic matter and enzymatic activities such as dehydrogenase, alkaline phosphatase, and ß-glucosidase activity was found. Bacterial diversity was strongly influenced by the stage of the process, community-structure change was concomitant with a temperature rise, rendering favorable conditions to stimulate microbial activity and facilitate the change in the microbial community linked to the degradation process. Moreover, results obtained confirmed that the use of semipermeable cover in the composting of sewage sludge allow a noticeable reduction in the process-time comparing to conventional open windrows.

1. Introduction

Wastewater-treatment plants (WWTPs) produce large amounts of sewage sludge, which are considered hazardous wastes due to the complexity, high pathogen load, heavy-metal content, and high percentage of moisture (Dumontet et al., 1999). As a consequence of the high volume produced, the treatment of these wastes can reach values of between 25 and 50% of the total WWTP costs (Neyens et al., 2004). Currently, incineration, mesophilic or thermophilic anaerobic digestion, are among the most frequently used treatments in WWTP for managing these wastes.

Due to its high organic matter content, sewage sludge has been proposed to be used as soil amendments (Raut et al., 2008). However, the addition of non-stabilized sewage sludge to soils could have negative effects related with pathogens and chemical pollution, and consequently these wastes need treatment for later use. One of these treatments includes sewage-sludge composting, an environmentally and economically sustainable alternative which

* Corresponding author. Department of Microbiology, Institute of Water Research, University of Granada, Ramón y Cajal, 4, Granada, 18071, Spain.
E-mail address: ccalvo@ugr.es (C. Calvo).
[1] Current address: Department of Biotechnology and Bioscience, University of Milano Bicocca, Milano, Italy.

https://doi.org/10.1016/j.jenvman.2018.03.041
0301-4797/

reduces the waste volume, sanitizes the product, and allows its further use as a soil organic amendment. As a result, it would be possible to recover part of the treatment costs under cost-effective operational conditions (Garrido-Baserba et al., 2015).

Composting is an aerobic microbiological process in which the macromolecules are broken down to produce a rich humic product with lower complexity, i.e. the compost (Mondini et al., 2004). The characteristics of the compost depend on many factors, such as the starting material and the operational conditions (temperature, moisture, and aeration, etc.), which directly affect microbial populations (Karadag et al., 2013; Vargas-García et al., 2010). Microorganisms and their natural enzymatic activity are essential in the composting processes. In fact, enzymatic activities such as dehydrogenase, has been suggested as indicator parameters of maturity and stabilization of compost (Castaldi et al., 2008; Tiquia, 2005). Thus, the characterization of microbial communities is useful to provide information on the progression of the process and the stabilization and maturity of the end product (Ismail et al., 2013; Karadag et al., 2013) and the study of the time course of their enzymatic activities could be a powerful tool in designing effective systems.

In recent years, composting technologies have been undergoing major changes (co-composting, vermicomposting, windrow composting) in order to make the process more cost effective (Gutiérrez et al., 2017; Malińska et al., 2017; Mengistu et al., 2018; Zhang et al., 2018). González et al. (2016) have demonstrated the environmental and economic advantages of a novel sewage-sludge composting in an aerated static windrow with semi-permeable-cover technology. This technology minimizes the environmental impact by reducing emissions from compounds and odors while accelerating the composting process by lengthening the thermophile phase.

However, the effect of this methodology on microbial populations remains uncharacterized, which is of main concern for further methodology optimization. Therefore, the aim of this work was to study enzymatic activities such as dehydrogenase, protease, ß-glucosidase, arylsulphatase, and phosphatases as indicators of microbial action, as well as to study bacterial population shifts during the process by culture-dependent and independent techniques. This information will allow assessing about the relationship between biological parameters and the efficiency of novel applied composting technologies at industrial scale by using semi-permeable cover systems.

2. Material and methods

2.1. Compost pile and sampling

The composting process was undertaken in the biosolid industrial plant "El Salao", located in Vegas Del Genil (Granada, Spain). As previously we reported by González et al. (2016), the pile was built at real scale (30 × 8 × 1.5 m) and the composting process lasted 190 days. The pile consisted of mixing sewage sludge (SS) from the WWTP Emasagra Sur (Granada, Spain), partially stabilized by mesophilic anaerobic digestion and dehydrated and mixed with pruning debris as a bulking agent (B) in a volumetric proportion of 1:3. The first stage, the "composting phase", was performed under a Gore-Tex® semipermeable cover in combination with insufflation system for 60 days. The second stage, the "maturation phase", i.e. when the cover was removed, was conducted in an open windrow system (from 60 to 190 days). Sampling was performed at 0, 1, 5, 15, 30, 60, 120, and 190 days at four random sites in the pile. To dig the desire depth and to form a composite sample, a shovel was used. After that, a sterilized hand trowel was used to collect 500 g of the compost. Each sample was placed in polyethylene bags, transported at 4 °C to the lab, and immediately used for the biological and chemical analysis. Samples for DNA extraction were frozen at −20 °C. Physico-chemical characteristic of composting samples retrieved from González et al. (2016) are given in Supplementary table S1.

2.2. Enzymatic assays

For enzymatic activities, each compost sample was air-dried (except protease) and sieved (<2 mm). Enzymatic activities were determined using a spectrophotometer Unicam 5625 UV/VIS. All the results were expressed as $\mu g^{-1}\ h^{-1}$ dry weight and represented as the result of three analytical replicates.

Dehydrogenase activity (EC 1.1.1.2) was determined according to Casida et al. (1964) from 20 g of sample by the reduction of triphenyltetrazolium chloride (TTC; Sigma–Aldrich, Germany) to triphenyl formazan (TPF; Fluka Biochemika, Switzerland) after incubation at 37 °C for 24 h in the dark. The final suspension was spectrophotometrically measured at 485 nm using a calibration curve of TPF.

Protease activity (EC 3.4.2.21–24) was analyzed using the procedure developed by Ladd and Butler (1972), based on the quantification of amino acids released after incubation of 2 h at 50 °C under shaking. The final suspension measured after 1 h at 700 nm and estimated by reference of tyrosine standard solutions curve (Scharlau, Scharlab, Spain).

Phosphomonoesterase activity was determined by measuring the acid phosphatase (EC 3.1.3.2) and alkaline phosphatase (EC 3.1.3.1) activities according to Eivazi and Tabatabai (1977) and by quantifying the *p*-nitrophenol (PNP; Fluka Biochemika, Switzerland) released from 1 g of sample. The final suspension was spectrophotometrically measured at 400 nm after 1 h of incubation at 37 °C. The values were referenced using a calibration curve made with a standard solutions of *p*-nitrophenol following Tabatabai and Bremner (1969).

The assays of β-glucosidase (EC 3.2.1.21) and the arylsulphatase activity (EC 3.1.6.1) were based on PNP quantification, following the phosphatase method but using *p*-nitrophenyl–β-D-glucopyranoside (Sigma–Aldrich, Germany), and *p*-nitrophenyl sulfate as substrate, respectively. Both were incubated for 1 h at 37 °C and measured spectrophotometrically (Eivazi and Tabatabai, 1988; Tabatabai and Bremner, 1970).

2.3. Isolation and identification of bacterial strains

The culturable aerobic heterotrophic bacteria were quantified at each sample time in triplicate. One g of each sample was diluted in 10 mL of sterile saline solution (0.9% NaCl), and then aliquots (0.1 mL) were serially diluted and spread on plates of 1/10 Triptycase soy agar (TSA, Oxoid©), made by 1/10 diluted TSA and adding 1.5% agar (Roko SA, Oleiros, Spain). Plates were incubated during 24–48 h, at 30 °C and 55 °C for mesophilic and thermophilic bacteria, respectively.

Representative dominant morphotypes for each concentration tested were selected and isolated in TSA plates until pure cultures were obtained. The isolates were preserved on TSA for short-term and in cryo-tubes with 80% glycerol at −80 °C for long-term. Each isolate was identified based on partial 16S rRNA gene-sequencing analysis and using the primer pairs FD1 (5′-AGAGTTTGATCCTGGCTCAG-3′) and RD1 (5′- AAGGAGGTGATCCAGCC -3′) (Weisburg et al., 1991). Genomic DNA extraction was carried out using the DNeasy® Blood and Tissue Kit (Qiagen, Valencia, CA), following the manufacturer's instruction. Purified DNA was eluted in a final volume of 50 μL of sterile MilliQ water and stored at −20 °C. The DNA was amplified by using a PCR Kit (Canvax Biotech, Córdoba, Spain), following manufacturer's instructions in