Bioresource Technology 102 (2011) 7975–7983

Contents lists available at ScienceDirect

Bioresource Technology

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

Evolution of clostridia and streptomycetes in full-scale composting facilities and pilot drums equipped with on-line temperature monitoring and aeration

Aija Rainisalo, Martin Romantschuk, Merja H. Kontro *

Helsinki University, Department of Environmental Sciences, Niemenkatu 73, 15140 Lahti, Finland

ARTICLE INFO

Article history:
Received 16 March 2011
Received in revised form 30 May 2011
Accepted 30 May 2011
Available online 12 June 2011

Keywords:
Clostridia
Streptomycetes
Composting
Full-scale facilities
Pilot drums

ABSTRACT

The evolution of sporulating bacteria in full-scale composting facilities with online temperature monitoring has been poorly studied, although organic matter recycling increases. We analysed *Clostridium perfringens* and sulphite-reducing clostridia (SRC) by cultivation, and streptomycetes by real-time PCR in five full-scale, temperature-monitored and aerated composting processes, and two pilot-scale drum composters. Facilities composted woodchips, sawdust, peat, or bark amended sludge or source-separated biowaste. Streptomycetes genes of 0.21–110 $\times$ 10^7 copies/g feed increased fast to 0.019–33 $\times$ 10^9 copies/g, and then were equal or decreased. SRC of 0.06–2.2 $\times$ 10^7 cfu/g feed decreased to 0–600 cfu/g, with regrowth in two facilities. End products were clean of *C. perfringens*, detected in sludge composts. Although processes contained large quantities of spore-forming bacteria, in the best facilities end products had the high quality. Temperature (>55 °C, >2d) was not related to the end compost quality, but relations between waste and bulking agent qualities, aeration, and processing time should be better controlled.

1. Introduction

The municipal solid waste management is under the development towards the increased recycling of organic wastes to soil fertilizer, instead of incineration, or use in landfills blamed for pollution incidents and enhanced greenhouse gas production. The soils progressively lose organic matter in the intensive cultivation and through water runoff. In the municipal waste management, the sewage sludge, manure, yard wastes, and municipal, source-separated and sorted organic wastes are transported for the anaerobic or aerobic processing (Giusti, 2009; Epstein, 1997). The aerobic microbial waste treatment called composting efficiently transforms organic material to humus-like substances concomitantly with hygienization of harmful microbes. The composting proceeds from the active mesophilic decomposition generating heat to the thermophilic phase, and finally in the curing phase the less susceptible carbon fractions are mineralised into a stable humic product (Epstein, 1997). The increase in the organic material flow to composting has resulted in the construction of large process units with sometimes inadequately developed stages, leading to odour emissions under anaerobic conditions, low process temperatures, and immature end products without hygienization.

The industrial, full-scale composting facilities typically have closed tunnel or drum process units for the initial phases of composting, while the curing of compost occurs in windrows. In the beginning of composting, the dense waste material is usually mixed with the bulking agent to enhance the oxygen flow into the compost mass, and in the end of the process the coarse bulking agent is removed by sieving. The typical bulking agents include wood chips, sawdust, bark, and peat. The temperature within the compost mass determines the rate of biological processes, plays the selective role in the evolution and succession of microbial communities, and is related to the hygienization of harmful microbes (Giusti, 2009; Epstein, 1997). According to the health rules concerning animal by-products not intended for human consumption, a composting plant must be equipped with a closed composting reactor having temperature monitoring without bypass possibility. The maximum compost particle size is 12 mm and minimum temperature in material 70 °C for 60 min. The other standardised process parameters may be provided when the applicant ensures minimisation of biological risks (European Commission, 2002, 2006).

The composting process often involves activities of microorganisms presenting health risks to the general population. The workers in composting facilities are exposed to, e.g. bioaerosols, airborne microbes and spores, microbial volatile organic compounds and mycotoxins (Epstein, 1997; Poulsen et al., 1995). According to hygiene requirements for processing animal by-products, *Clostridium perfringens* must be absent from 1 g of product directly after the heat treatment (European Commission, 2002,

* Corresponding author. Address: Helsinki University, Department of Ecological and Environmental Sciences, Niemenkatu 73, 15140 Lahti, Finland. Tel.: +358 9 19120309; fax: +358 9 19120331.
E-mail address: merja.kontro@helsinki.fi (M.H. Kontro).

0960-8524/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2011.05.087

2006). C. *perfringens* causes gas gangrene and food poisoning in humans, and several enterotoxemic diseases in domestic animals. The virulence of *C. perfringens* is related to the toxin-production (Petit et al., 1999). The genus *Clostridium* comprises mainly motile, Gram-positive, anaerobic, and rods-shaped bacteria, which are widely distributed, including the soil and gastrointestinal tract of animals and human. The sulphite-reducing clostridia (SRC) reduce sulphite to sulphide, the property being used in their selective enumeration (NMKL, 1994).

Streptomycetes from the class Actinobacteria are Gram-positive, filamentous, and saprophytic bacteria commonly found in soil, rhizosphere, and compost. They have a relatively complex life cycle; secrete hydrolytic enzymes involved in the degradation of complex organic compounds like cellulose or starch; and produce a variety of medically and/or industrially useful secondary metabolites including antibiotics (Kutzner, 1986; Baccella et al., 2002). The cytotoxic spores of streptomycetes have induced inflammatory responses in macrophages *in vitro*, and have been associated with the farmer's lung disease (Roussel et al., 2005; Hirvonen et al., 2001). *Streptomyces* spp. may be related to infections (Kapadia et al., 2007).

The spores of clostridia and streptomycetes have survived the temperature of 70 °C (Miwa et al., 2002; Mocé-Llivina et al., 2003; Ryckeboer et al., 2003). *C. perfringens* has commonly been detected in the end of composting (Briancesco et al., 2008; Brinton et al., 2009; Lasaridi et al., 2006; Pourcher et al., 2005; Wéry et al., 2008). SRC have not been eliminated in pilot-scale aerated piles, although the reduction efficiency of SRC increased under the aeration (Bustamante et al., 2008). The occurrence of streptomycetes has been poorly studied (Ryckeboer et al., 2003; Vargas-Garciá et al., 2007). Processes have only seldom been equipped with closed, temperature-controlled, and aerated bioreactors (Wéry et al., 2008). Therefore, we determined the numbers of SRC and *C. perfringens* by cultivation, and *Streptomyces* spp. 16S rDNA levels by SYBR Green real-time PCR in early stages of five biowaste composting processes in four full-scale composting facilities and two pilot drums equipped with on-line temperature monitoring and aeration. The hypothesis of the investigation was that clostridia are hygienized, and streptomycetes amounts approach the numbers commonly found in the environment in an efficient composting process. The noll hypothesis was that no changes occur in spore-forming bacteria. The additional objectives of the investigation were to clarify process stage, temperature, waste and bulking agent combinations that enable the high microbial quality of the compost after the early stages of biowaste composting in the closed, temperature-monitored composting reactors.

2. Methods

2.1. Composts

The composts were collected from early stages of process in four full-scale composting facilities (Table 1, Fig. 1). Facility A composted anaerobically fermented sludge from the waste-water treatment, facility B sludge, and facilities C and D crushed source-separated biowaste. Bulking agents used in the input compost included woodchips, peat, bark, and sawdust in the ratios presented in Table 1. In facility C, additional samples were collected when woodchips and peat (1:1, vol/vol) were bulking agents. In facility C, a drum composter of 160 m^3 was first half-filled with the compost material, the duration of compost mass in drum being 3–4 days. The drum rotated periodically, more frequently in the daytime than at night. Between periods of rotation, the drum was aerated by pumping air through nozzles beneath the compost mass, and air space above the mass was constantly replaced by pumping. This compost was then fed to a tunnel composter, which was the first stage of composting in facilities A, B and D. In the tunnels, the aeration beneath the compost maintained the oxygen percentage above 14–15% in the exhaust air above the compost in facilities A, B and D, while in facility C the outflow oxygen was not followed. Tunnels varied between 300 and 700 m^3 in volume, and were half-filled during the composting. The composting time in tunnels was 10 days in facility B, 14–21 days in facility C, and 7 days in each of two (facility D) or three (facility A) tunnels. In facility B, the compost from tunnel was maintained in windrows for 10 days, and in facility D for 90 days. In facilities A and B, the material from tunnels and indoor windrow, respectively, was sieved. The sieved compost was regarded as ready for use, e.g. in landfills or gardening.

In the pilot-scale drum composters of 5 m^3 inside volume (outer diameter 1.6 m, length 4 m) (Rumen Ltd., Lahti, Finland), the volume of compost mass was 2.5–3 m^3. The composters were filled at the front end of drums by twin screws. In drum 1, all large particles were also crushed during feeding. The source separated municipal biowaste, collected at the Päijät–Hämeen Jätehuolto waste treatment station (Lahti, Finland), and wood chips were mixed in the ratio of about 2:3 (vol/vol), and fed to the composter, the ratio of fresh feed to the processed compost mass being about 1:1 (vol/vol) (Table 1, Fig. 1). The compost material in the loading end of the drum was mixed with the incoming material and transferred towards the end part, while the oldest compost mass was removed with the screw transporter from the end part of the drum. The composter was aerated at 40 m^3/h, and rotated at 0.25 rpm for 8–10 min/h. The aeration was above the compost in drum 1 and through the compost mass in drum 2.

Samples were collected from sludge (facilities A and B), compost material mixed with bulking agent (all facilities), loading and unloading ends of drums (facility C and pilot drums), tunnels (all facilities), windrows (facilities B and D), and sieved windrows (facilities A and B) (Table 1, Fig. 1). Three parallel ten litre samples were collected approximately 50 cm below the compost surface, mixed carefully, and sieved through a 2 cm steel sieve. A pooled sample from three separate samplings was stored at 4 °C until it was cultivated within 24 h, and the dry weight was determined in triplicate from weight loss of 1–3 g of compost after drying at 105 °C for 16 h. Samples were stored at −70 °C for DNA-based analyses.

2.2. Bacterial cultivations

SRC were cultivated on ferri ammonium citrate and disodium disulphite medium (Oxoid, Hampshire, England) for 1–2 days at 37 °C (NMKL, 1994). *C. perfringens* was cultivated on tryptose sulphite cycloserine agar (Difco, Sparks, MD, USA) for 24 h at 37 °C (NMKL, 1997). The cultivations were done in Ramboll Analytics Ltd. (Lahti, Finland) using methods accredited by Finnish Accreditation Service according to SFS-EN ISO/IEC 17025 guidelines. The limit of detection (LOD) was <100 cfu/g for *C. perfringens*, and <10 cfu/g for SRC.

2.3. Real-time SYBR Green PCR assays

The template DNA was isolated in duplicate from 50 mg of compost (wet weight) using FastDNA SPIN Kit for Soil (Qbiogene, Carlsbad, CA USA) according to the manufactures instructions, with the following modifications. The sodium phosphate and MT buffers were added; samples were processed for 30 s in FastPrep instrument (Holbrook, NY); heated at 65 °C for 20 min to inactivate DNAses; and centrifuged. In the preparation of quantification standards, this supernatant was spiked with *Streptomyces griseus* subsp. *griseus* DSM 40236 (German Collection of Microorganisms and Cell