Waste Management 33 (2013) 1451–1460

Contents lists available at SciVerse ScienceDirect

Waste Management

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

Biotests for environmental quality assessment of composted sewage sludge

Anu Kapanen [a], Minna Vikman [a,*], Johanna Rajasärkkä [b], Marko Virta [b], Merja Itävaara [a]

[a] VTT, P.O. Box 1000, Tietotie 2, FI-02044 VTT, Finland
[b] University of Helsinki, Food and Environmental Sciences, P.O. Box 66, FIN-00014 Helsinki, Finland

ARTICLE INFO

Article history:
Received 27 September 2012
Accepted 22 February 2013
Available online 27 March 2013

Keywords:
Sewage sludge
Compost
Organic contaminant
Biodegradation
Ecotoxicity
Bioreporter

ABSTRACT

The quality of sewage sludge-based products, such as composts and growth media, is affected by the contamination of sewage sludge with, potentially, hundreds of different substances. Therefore, it is difficult to achieve the reliable environmental quality assessment of sewage sludge-based products solely based on chemical analysis. In the present work, we demonstrate the use of the kinetic luminescent bacteria test (ISO 21338) to evaluate acute toxicity and the Vitotox™ test to monitor genotoxicity of sewage sludge and composted sewages sludge. In addition, endocrine-disrupting and dioxin-like activity was studied using yeast-cell-based assays. The relative contribution of industrial waste water treated at the Waste Water Treatment Plants led to elevated concentrations of polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and polychlorinated dibenzo-p-dioxins and -furans (PCDD/F) in sewage sludge. The effect of elevated amounts of organic contaminants could also be identified with biotests able to demonstrate higher acute toxicity, genotoxicity, and potential for endocrine-disruptive properties. Additional extraction steps in kinetic luminescent bacteria test with DMSO and hexane increased the level of toxicity detected. Composting in a pilot-scale efficiently reduced the amounts of linear alkylbenzensulphonates (LASs), nonylphenols and nonylphenolethoxylates (NPE/NPs) and PAH with relative removal efficiencies of 84%, 61% and 56%. In addition, decrease in acute toxicity, genotoxicity and endocrorine-disrupting and dioxin-like activity during composting could be detected. However, the biotests did have limitations in accessing the ecotoxicity of test media rich with organic matter, such as sewage sludge and compost, and effects of sample characteristics on biotest organisms must be acknowledged. The compost matrix itself, however, which contained a high amount of nutrients, bark, and peat, reduced the sensitivity of the genotoxicity tests and yeast bioreporter assays.

1. Introduction

A total of ten million tons (dry matter) of sewage sludge is produced in Europe every year, and of this, an annual amount of 160,000 tons is produced in Finland (Stamatelatou et al., 2011; EC, 2008; Laturnus et al., 2007). Sewage sludge and products derived from sewage sludge such as soil conditioners contain beneficial nutrients and organic matter, but they also contain heavy metals, pathogens and organic contaminants. According to the Sewage Sludge Directive 86/278/EEC, the safe use of municipal sewage sludge is an essential objective of European legislation. The European Commission is reviewing the Sewage Sludge Directive and a working document draft on sludge and biowaste is available (WD, 2010). The Working Document on Sludge and Biowaste (WD, 2010, 2000) sets the frame for monitoring the quality of sewage sludge in Europe. It is not legally binding but provides a basis for discussions. In addition, the disposal and recycling of sewage sludge in Europe, as well as the content of heavy metals and organic pollutants in sewage sludge, have been discussed in several reports (Amlinger et al., 2004; Andersen, 2001; Brändli et al., 2004; Erhardt and Pruess, 2001; Gawlik and Bidoglio, 2006; EC, 2008).

Of European countries German, Denmark, Sweden, France and Austria have set national limit values for organic contaminants in sewage sludge. At EU-level the limit values have not been defined yet but are under discussion (Table 1). Major organic contaminants found from municipal sewage sludge include adsorbable organic halogens (AOX), linear alkylbenzensulphonates (LAS), nonylphenols and nonylphenolethoxylates (NP and NPE), di-ethylhexylphthalate (DEHP), polyaromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB), and polychlorinated dibenzo-p-dioxins and -furans (PCDD/F) listed in Working document on sludge 3rd draft (Andersen, 2001; Müller, 2003; Gawlik and Bidoglio, 2006; Amlinger et al., 2004; Abad et al., 2005; Schowanek et al., 2004; Marttinen et al., 2003; Jensen and Jepsen, 2005; Samsøe-Petersen, 2003; Stevens et al., 2001; Koch et al., 2001). Polybrominateddiphenyl ethers (PBDE), tetrabromobisphenol A (TBBPA), and hexabromocyclododekane (HBCD), known as

---

* Corresponding author. Address: VTT Technical Research Centre of Finland, P.O. Box 1000, Tietotie 2, FI-02044 VTT, Finland. Tel.: +358 20 722 7155; fax: +358 20 722 7071.
E-mail address: Minna.Vikman@vtt.fi (M. Vikman).

0956-053X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.wasman.2013.02.022

Table 1
Amount of organic contaminants in sewage sludge samples A and B, in compost samples from pilot composting with sewage sludge A and in compost samples from two field scale composting plants (1 and 2). Degradation % of organic contaminants after 26 days and 124 days of composting. Suggested limit values for organic contaminants in the EU in sewage sludge used as soil improvers (WD, 2000, 2010).

	A	B	Pilot composting		Field scale			WD, 2000	WD, 2010
			26 d	124 d	1	2a	2b		
	mg kg^{-1} dw		mg kg^{-1} dw (Degradation%)		mg kg^{-1} dw			mg kg^{-1} dw	
AOX	260	170	nd	nd	75	80	nd	500	–
LAS	1500	1300	110 (83%)	<50 (92%)	50	240	310	2600	–
DEPH	57	110	20 (20%)	11 (56%)	2	38	16	100	–
NPE	15.2	8.9	2.0 (69%)	2.6 (61%)	<0.6	26.0	47	50	–
Of which NP	13.3	7.8	2.0 (66%)	2.2 (62%)	0.2	23	46		
PAH-16[a]									
PAH-9[b]	8.0	0.5	2.3	0.6	<0.5	1.2	<0.3	6	6
	5.8	0.3	1.8 (28%)	0.4 (84%)	<0.3	0.9	<0.3		(2)[e]
PCB[c]	1.4	0.04	0.7 (−17%)	0.2 (67%)	0.02	0.06	0.06	0.8	–
PCDD/F[d] (ng I-TEQ kg^{-1} dw)	3.8	0.3	nd	nd	3.2	3.6	nd	100	–

[a] EPA-PAH-16: Naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, bentzo[a]antracene, chrysene/trifenyyli?, benzo[bjk]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, dibenzo[ah]antracene, benzo[ghi]perylene.

[b] PAH-9: calculated according to WD (2000).

[c] PCB-7; PCB 28,PCB 52, PCB 101, PCB 118, PCB 153, PCB 138, PCB 180.

[d] PCDD/F;2,3,7,8-TCDD; 1,2,3,7,8-PeCDD; 1,2,3,4,7,8-HxCDD; 1,2,3,6,7,8-HxCDD; 1,2,3,7,8, 9-HxCDD; 1,2,3,4,6,7,8-HpCDD; 1,2,3,4,6,7,8,9-OCDD, 2,3,7,8-TCDF; 1,2,3,7,8-PeCDF; 2,3,4,7,8-PeCDF; 1,2,3,4,7,8-HxCDF; 1,2,3,6,7,8-HxCDF; 2,3,4,6,7,8-HxCDF; 1,2,3,7,8,9-HxCDF; 1,2,3,4,6,7,8.HpCDF; 1,2,3,4,7,8,9-HpCDF; 1,2,3,4,6,7,8,9-OCDF.

[e] Orbenzo[a]pyrene.

bromated flame retardants (BFRs), are one of the emerging contaminant groups in sewage sludge (Cincinelli et al., 2012; Hale et al., 2006; de Wit, 2002).

The disposal and handling of ever-increasing amounts of sewage sludge challenge the established treatment technologies targeted at preventing harmful effects on human health, agricultural land, and the environment. Composting is one of the most efficient organic waste treatment technologies to reduce the amount of biodegradable organic waste and potentially biodegradable organic contaminants present in sewage sludge. The amount of LAS, NP, NPE, DEHP, and some PAHs, has been successfully reduced by composting (Stamatelatou et al., 2011; Amir et al., 2005; Ramírez et al., 2008; Marttinen et al., 2004; Oleszczuk, 2007; Cheng et al., 2008; Poulsen and Berster, 2010). On the contrary, PCB and PCDD/F persist throughout the composting process (Abad et al., 2005). Evaluation of the quality of sewage sludge and composted sewage sludge is challenging. It cannot be evaluated based only on chemical analysis, while it has been shown to carry more than 500 organic chemicals (Harrison et al., 2006; Eriksson et al., 2008). The synergistic effects of several harmful compounds can only be evaluated with biotests which measure biological responses of living organisms. The most commonly used application of biotests to evaluate the toxicity of sewage sludge, composted sewage sludge, or soil amended with either sewage sludge or compost, seems to be tests based on the determination of the inhibitory effect of samples on the light emission of the *Vibrio fischeri* (Alvarenga et al., 2007; Lopez et al., 2010; Mantis et al., 2005; Fuentes et al., 2006). The kinetic luminescent bacteria test has been found to be well suited for determining the acute toxicity of coloured samples, e.g. compost and composted sludge (Kapanen and Itävaara, 2001; Kapanen et al., 2007; Kapanen et al., 2009). Other biotests applied in ecotoxicity assessment of sewage sludge and sewage sludge amended soils include *Daphnia magna* mortality, earthworms, springtails, phytotoxicity tests, and ostracod mortality and growth inhibition (Domene et al., 2011; Natal-da-Luz et al., 2009; Oleszczuk, 2007; Oleszczuk, 2010; Ramírez et al., 2008; Hamdi et al., 2006). Not much attention, however, has been paid to the compatibility of the test method with the sewage sludge or compost samples.

Sewage sludge may also contain genotoxic chemicals such as benzo[a]pyrene, fluoranthene, fenantrane, and chrysene, however information pertaining to the biotests used for the detection of the genotoxicity of sewage sludge is scarce. Ames tests, and SOS-Chromotests, have been applied for genotoxicity assessment of sewage-sludge (Pérez et al., 2003; Renoux et al., 2001). Furthermore, the genotoxic potential of sewage sludge amended soil has been studied with plants by Amin (2011). In addition to the evaluation of acute toxicity or genotoxicity, the endocrine disruption potential (Svenson and Allard, 2004; Leusch et al., 2006; Murk et al., 2002; Engwall et al., 1999; Hernandez-Raquet et al., 2007) and the presence of dioxin-like compounds (Suzuki et al., 2004; Suzuki et al., 2006) in sewage and sewage sludge have also been studied with biotests. When Murk et al. (2002) used three different assays, ER-Calux, YES, and ER-binding, to predict the oestrogenic potencies in wastewater and sludge, ER-binding assay was the most sensitive of the applied tests. In the present work we used recombinant receptor transcription assays that use yeast cells with response element-regulated reporter genes are commonly used in the first stage of *in vitro* screening of chemicals. Yeast tests can be useful in the first stage of screening, as they are easy and inexpensive to perform compared with tests using mammalian cells (OECD, 2001), which require laborious and costly cell culturing. Furthermore, yeast cells are more resistant to environmental contaminants than mammalian cells, which is an important advantage when measuring complex environmental samples.

There remains a great deal of concern regarding the amount of different toxic chemicals present in sewage sludge; thus, the expanding monitoring needs for an increase in the amount of known chemicals are well acknowledged (Eriksson et al., 2008; Gawlik and Bidoglio, 2006). This has brought about an increasing demand for more affordable, reliable, and fast methods for monitoring the environmental fate of sewage sludge. Biotesting methods could be more widely used to complement the currently established chemical analyses employed in sewage sludge quality evaluation. However, there is a lack of information about the suitability of biotesting in environmental fate assessment of sewage sludge and composted sewage sludge.

In this study, we conducted a pilot composting experiment to study the efficiency of composting in reducing the load of organic contaminants and ecotoxicity of sewage sludge targeted for agricultural or landscaping applications. The aim of this study was to evaluate the acute toxicity, genotoxicity, endocrine disruptive, and dioxin-like activity in sewage sludge and composted sewage sludge by using biotests. We focused on evaluating the potential and limitations of biotests in evaluation of ecotoxicity of sewage