Bioresource Technology 102 (2011) 5863-5872

Contents lists available at ScienceDirect

Bioresource Technology

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

A modified static respiration assay and its relationship with an enzymatic test to assess compost stability and maturity

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ARTICLE INFO

Article history: Received 7 September 2010 Received in revised form 1 February 2011 Accepted 4 February 2011 Available online 4 March 2011

Keywords: Compost Enzymatic assay Maturity Respiration index Stability

ABSTRACT

Despite the numerous compost stability and maturity tests, no universally accepted compost stability or maturity index exists. The fluorescein di-acetate (FDA) enzymatic assay, originating from soil studies, is examined here as a potential new compost stability test, and is compared to microbial respiration and phytotoxicity indices. Thirteen composts were used in the study from different source materials. Static microbial respiration activity indices calculated were the cumulative O₂ consumptions, O₂ consumption rates, total C–CO₂ production, the respiratory quotient and the bio C/N ratio. Compost phytotoxicity was quantified via a 7-day tomato seed germination assay. Results showed that the net fluorescein release rates correlated with all stability indices. The germination index marginally correlated with the fluorescein release rates, but not with any of the other stability indices. New limits to classify composts regarding their stability were proposed.

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1. Introduction

The composting process, when used for the treatment of organic wastes, primarily aims to produce a stabilized end-product with properties that could enhance plant growth. Alternatively, the composted product could be simply added to arid soils to increase soil carbon content. Compost researchers have been struggling for years to produce a common index to characterize the quality of composts resulting from various sources. An inevitable result of that struggle was the introduction of the terms "stability" and "maturity". Maturity is a term related to the effect of composts to crops and indicates the presence or absence of phytotoxic effects. Immature composts are usually phytotoxic - mostly due to the production of organic acids (Epstein, 1997) - and may have a negative impact to plant growth. Compost stability is a stage in the decomposition of the organic matter and is, therefore, a term related to the microbial decomposition or microbial respiration activity of the composted matter (Iannotti et al., 1993). A high quality compost should be both mature and stable. However, a stable compost, i.e. one that has a low microbial respiration activity, is not necessarily mature (non-phytotoxic) as was shown by Komilis and Tziouvaras (2009).

Although numerous compost stability and maturity tests have been proposed and researched over the past decades, there is, still, no universally accepted compost stability and/or maturity index.

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As a result, researchers still seek new stability and maturity indices that would apply to composts derived from various sources.

1.1. Compost stability and maturity indices

Some of the most common compost stability tests are based on the quantification of the microbial respiration. The most common microbial respiration activity indices are the ones based on oxygen uptake (consumption) and carbon dioxide evolution, expressed either as cumulative amounts over a specific period of time or as rates. Static and dynamic tests have been used to measure oxygen consumption. Static tests are performed at the absence of continuous air flow and O₂ consumption is usually measured via pressure differences or via direct measurements of the O₂ content in air or liquid medium (Iannotti et al., 1993; Adani et al., 2003; Gea et al., 2004; Ruggieri et al., 2008; Komilis and Tziouvaras, 2009; Ponsá et al., 2009). Dynamic tests are continuous air flow systems which allow oxygen transfer through the biomass layers to cells; they require the precise measurement of the air flowrate and the O₂ content at the inlet and outlet of the test devices (Scaglia et al., 2000; Barrena-Gómez et al., 2005; Tremier et al., 2005; Adani et al., 2006; de Guardia et al., 2010).

Several respirometric methods have been developed by researchers. The AT₄ test is a German static test, performed at 20 °C, which measures stability via the cumulative oxygen uptake measured after 96 h (Binner and Zach, 1999). The Dynamic Respiration Index – DRI (Scaglia et al., 2000; CEN, 2007) is the average of 24 instantaneous oxygen consumption rates during the 24 h period of maximum aerobic activity. The Respiration Index – RI suggested



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by Barrena-Gómez et al. (2005) is a static test that measures the rate of O_2 content decline during a 90 min period. The pertinent ASTM standard (ASTM, 1996) calculates the cumulative oxygen uptake after 4 days of incubation using a dynamic air flow regime at 58 °C.

Researchers have frequently compared and correlated static with dynamic methods. Barrena et al. (2009) observed a very good correlation, and very small differences between static and dynamic indices from wastes of a mechanical – biological pretreatment. The dynamic oxygen uptake rates (OURs) have been mentioned to be two times higher than static OURs (Scaglia et al., 2000). On the other hand, Gea et al. (2004) found that a dynamic OUR can be as high as 4–5 times the static OUR for some composts, while for one compost, derived from municipal solid waste, the static and dynamic indices were similar. Ponsá et al. (2010b) also observed good correlations among static and dynamic respiration indices as well as with anaerobic biodegradation coefficients.

The present study adopts a modified static respiration test that calculates cumulative oxygen consumptions at 1, 4 and 7 days (also referred to as Cumulative Respiration Indices or CRI₁, CRI₄ and CRI₇, respectively) as well as OURs over two different time periods of maximized activity (12 and 24 h). The term static respiration index (SRI), which was introduced by Adani et al. (2003), will be used here to express the maximum rates of oxygen uptake over these periods of time (i.e. 12 and 24 h). The term "Cumulative Respiration Index (CRI)" – a term also included in Ponsá et al. (2010b) – is the cumulative amount of oxygen consumed after certain time periods (e.g. 1d, 4d, 7d) using static respirometers that are occasionally aerated.

The most commonly used compost maturity tests are the seed germination bioassays. A germination index (GI) is usually calculated as a percentage of a control using various types of seeds. In this research work, a modified procedure is used based on an ad hoc germination index. Although tomato seeds were used here, it is worth mentioning that germination indices are highly dependent on the types of seeds used (Komilis and Tziouvaras, 2009).

1.2. The fluorescein di-acetate (FDA) assay

An enzyme activity assay that was originally developed to measure microbial activity in soils is the fluorescein di-acetate (FDA) hydrolysis test. The FDA hydrolysis test (or FDA assay) was initially

Table 1

Preparation data of the 13 composts.

used by Schnürer and Rosswall (1982), as an indicator of microbial enzymatic activity in soil and litter, and was lately revised by Adam and Duncan (2001). Recent studies on compost science have used the FDA assay to examine compost biological properties (Cayuela et al., 2008; Ntougias et al., 2006, 2008).

1.3. Research objectives

Based on the literature review, there are no studies to investigate the potential correlation of the FDA assay results with compost stability and maturity indices. This gap in knowledge led to the research work presented here. Therefore, objectives of this work were:

- (i) to calculate correlations of the FDA assay results with static respiration activity indices and seed bioassay results,
- (ii) to propose limits for the classification of compost stability.

2. Methods

2.1. Source materials

Thirteen composts were collected from various parts of Greece and were transferred to the Laboratory of Solid and Hazardous Waste Management at the Democritus University of Thrace. The available information for all composts is included in Table 1. According to Table 1, a wide range of source materials was used including manures, sea weeds, household organic waste and commingled MSW. Some composts were produced by small scale producers and two (2) were received from actual Greek MSW composting facilities in Crete and Kalamata.

2.2. Compost characterization

The moisture content of the materials was measured through weight difference at 75 °C till constant weight (USDA and USCC, 2001). The dry material (DM) was ground using a commercial grinder (mesh size 2 mm). Organic matter (OM) was measured in a muffle furnace through the loss on ignition (LOI) at 550 °C for 2 h. Total C and total N contents were measured using an elemental analyzer (CE Instruments, CHNS-O Model EA-1110) (Komilis and Tziouvaras, 2009). The water holding capacity (WHC) of the

1	I				
Compost	Source materials	Location in Greece	Approximate active	Approximate	Info on compost preparation
ID ID		in diecee	composting period	curing time	
1	Cotton residues	Athens	2 months	2 years	two thermophilic stages occurred
2	Green waste	Athens	2 months	3 months	Thermophilic temperatures recorded
3	Municipal Solid Waste	Chania	6 weeks	4 weeks	In-vessel composting facility with pre-selection units
4	Municipal Solid Waste	Kalamata			End product from the old MSW composting facility in Kalamata received
					from a 5 year old curing pile. No composting and curing period provided
5	Greenhouse weeds	Preveza	3 months	6 months	Prepared in pits. Continuous flow system.
6	Goat manure and greenhouse	Preveza	3 months	6 months	Prepared in pits. Continuous flow system.
	weeds mixed				
7	Sheep manure and old compost	Preveza	3 months	6 months	Prepared in pits. Continuous flow system.
	derived from chicken manure				
8	Shredded olive tree trimmings	Kalamata	3 months	1 month	Aerated in static piles
9	Olive pulp, yard waste	Kalamata	4 months	4 months	Olive pulp (derived from a two-phase olive mill) and yard trimmings
	trimmings and sawdust				combined at approximately 1:1 wet weight ratios
10	Food waste	Athens	10–15 days	No curing	Prepared in home composting bin
11	Green waste	Kalamata		3 months	No composting period provided
12	Sea weeds ^C	Kefallonia	1 month	1 month	Prepared from 80% sea weeds and 20% cow manure and other agricultural
					products
13	Cow manure ^C	Attiki	10 months		Vermicomposting process
		area			

C: commercial product.

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