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# MICROBIAL COMMUNITY DYNAMICS AND STABILITY ASSESSMENT DURING GREEN WASTE COMPOSTING

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**ABSTRACT**

Green waste (leaves, grass and shredded twigs) was composted for 5.5 months in a wooden composting bioreactor, of 1 $m^3$ capacity, using both passive convective aeration, facilitated by the design of the bioreactor, and manual turning. Temperature was measured daily, while samples were analysed for moisture content, volatile solids, pH value and electrical conductivity, all of which showed a typical variation for the type of composted material and process.

For the assessment of compost stability, a simple automated respirometric technique was applied (SOUR test), which utilised a dissolved oxygen probe to measure changes in the oxygen concentration in an aqueous compost suspension, under conditions ensuring optimum microbial activity and maximum reaction rates. Results were compared with other stability tests, such as the dehydrogenase activity and the germination index (GI) of cress seeds and correlated with the changes in population size of different microbial groups (total aerobic and spore forming heterotrophs, ammonium- and nitrite- oxidising bacteria, actinomycetes, filamentous fungi, yeasts and cellulolytic bacteria).

Both the SOUR test and the GI indicated increasing compost stability with processing time and gave significant correlation with compost age and with each other. SOUR reached a value of 4 mg $O_2$ $g^{-1}$VS $hr^{-1}$ at the end of the active composting phase (after 3 weeks) and declined gradually to below 1 mg $O_2$ $g^{-1}$VS $hr^{-1}$, a value indicative of stable composts, after about 3 months of processing. GI increased from 30% for the raw material, a value indicating high phytotoxicity, to about 80% at the end of the active phase and fluctuated around this value thereafter. Dehydrogenase activity fluctuated during the process, with the highest values being measured during the active composting phase. However, high values were also recorded towards the end of the maturation phase, following a long period of stabilisation at low values. The parameter did not correlate with compost age or SOUR and GI and thus did not seem suitable for monitoring green waste compost stability.

Although at lower levels compared to other substrates, such as manures and biowaste, the population size of different microbial groups did not seem to be a limiting factor in green waste composting. The numbers of most microbial groups increased after the end of the active composting phase, indicating that microorganisms multiplied rapidly as temperatures felled, although for some groups population counts declined again towards the end of the maturation period, possibly indicating the exhaustion of specific substrates.

**KEYWORDS**: green waste composting, stability, maturity, SOUR, respiration, germination index, dehydrogenase activity, plant trials, grass, *Festuca arundinacea sp*.

## 1. INTRODUCTION

As the landfill directive 99/31/EC sets stringent requirements for the diversion of the biodegradable fraction of municipal waste from the landfill, composting of green waste is increasingly regarded as an attractive option for the partial achievement of this target [1]. However, for composting to have any future as an important component of an integrated waste management system its product, the compost, should be able to find its way to the market, a prerequisite that could only be achieved if compost quality is quantifiable and can be measured and certified though appropriate assurance systems. Compost stability constitutes an important, and probably the most controversial, aspect of overall compost quality in terms of definition and evaluation [2]. In this context, it is important to better understand the dynamics of the process and assess the rate and the degree of the organic matter decomposition (compost stability), in order to facilitate the design of efficient systems and produce composts that may find their way into the markets [2,3].

The composting process involves the aerobic exothermic microbial decomposition of the initial substrate, which results to dynamic changes in temperature, moisture content, oxygen concentration and nutrient availability. These factors, in their turn, strongly affect the structure and diversity of the microbial community, microbial activities and the physical and chemical characteristics of the substrate [4]. Therefore, monitoring of the microbial succession may provide important information for the effective management of the composting process and the appearance of certain groups of microorganisms is believed to reflect the degree of stabilisation of the organic matter during the process [5].

A variety of methods have been used so far to investigate microbial community dynamics during composting, ranging from the traditional plating and identification of culturable microorganisms [5-9] to more recent techniques measuring ATP content and enzyme activities [10,11] and the quinone profiles [12]. In the last few years, novel methods that give an indication of the microbial community composition without culturing of organisms on agar media, have been developed, such as the direct analysis of phospholipid fatty acid patterns (PLFA) and of extractable DNAs or rRNAs [5,9,13]. However, in spite of the developments in the new molecular techniques, no single method has proven to be most reliable for monitoring microbial communities in environmental samples and the traditional techniques are still considered useful in environmental microbiology [5,14].

Compost stability is an important aspect of compost quality, determining compost nuisance potential, nitrogen immobilization and leaching and phytotoxicity [15,16]. If insufficiently stabilized compost is applied to land, it can create anaerobic conditions in the rhizosphere or induce phytotoxicity, mainly caused by organic acids present during the early stages of composting process [17]. In spite of its importance, there is still no universally accepted parameter for the determination of compost stability and a wide range of physical, chemical and biological parameters have been proposed. The most promising methods seem to be those based on compost microbial activity, usually measured through the respiration activity (e.g. $AT_{4,}$ Dynamic Respiration Index, SOUR) or the self-heating potential (Rottegrade), which are increasingly described in compost quality standards [2,15,18].

The microbial community dynamics in green waste composting and their associations with the rate and the degree of the organic matter stabilisation are not well understood. Moreover, it is unclear how these parameters influence the phytotoxicity of the compost and its effect to plants. Therefore, the aim of this work was to monitor changes in the microbial community structure during green waste composting, using traditional plate counting techniques and the dehydrogenase activity, an enzyme activity which in soils is believed to provide a good index of the total microbial activity [19], in conjunction with the typical physical and chemical parameters characterising the process and global indicators of the microbial activity, such as the respiration rate, in order to gain an insight into the dynamics of the process and assess the degree of compost stability. These changes were related to the effect of compost water extracts on the germination of cress seeds

## 2. MATERIALS AND METHODS

Green waste (leaves, grass and small shredded brunches) were collected from the gardens of Harokopio University in Kallithea, in the period from September to October 2003. The material was stockpiled in the open, until a sufficient quantity was collected to fill a wooden composting bioreactor of $1m^3$ capacity (1m x 1m x 1m). During the preliminary stockpiling, the volume of the material was reduced by a factor of about three, resulting into a bulking density of 340 kg $m^{-3}$ for the starting composting mix. The composting box was placed in a glass greenhouse and passive convective aeration was facilitated by its design, including the position of a perforated tube in its middle. The material was also turned manually, about every three weeks. If needed, water was added during turning (on day 23 and 82, 45 and 30 l respectively). Samples were obtained from the homogenised material after each turning. Composting lasted for 5.5 months, from 4/11/03 until 15/5/04.

Moisture and organic matter (OM) content were determined through the weigh loss at 105$^oC$ and 550$^oC$ respectively [18]. Electrical conductivity (EC) and the pH value were determined according to FCQA [18]. Phytotoxicity, a parameter partly related to stability, was calculated as the Germination Index (GI) of *Lepidium Sativum* seeds (Agrocementi Ltd, Athens), using a modification of the method proposed by Zucconi *et al.* [17]. Twenty five seeds were placed on 5 layers of filter paper pads wetted with 5 ml of 1:10 compost aqueous extract, in petri dishes incubated in dark, at 25 $^oC$ for 48 hours. Tap water was used as control. The dehydrogenase activity was analysed using a modification of the common colorimetric method [19], in which 0.5 g dw of compost are used. All aforementioned analyses were performed in triplicates. Compost stability was determined in duplicates, using a modification of the SOUR test (Specific Oxygen Uptake Rate), which measures the maximum rate of oxygen consumption in an aqueous compost suspension of 10g compost (wet weight) in 500 ml d $H_2O$, at ambient temperature [6].

The total aerobic culturable mesophilic bacteria (total bacteria) were determined by the dilution plate count technique on nutrient agar. The plates were incubated for 3 days at 30$^oC$ [20]. For the enumeration of spore forming bacteria the same substrate was used but the samples were pre-incubated in a water bath at 80 $^oC$ for 10 min [20]. Cellulolytic bacteria were determined according to Hendricks *et al.* [21] and actinomycetes according to Clark [22]. The number of viable yeasts and filamentous fungi was measured by plating appropriately diluted suspensions onto Sabouraud-Dextrose Agar supplemented with 30 μg/ml streptomycin, incubated at 30 $^oC$ for 2 days. Nitrifying bacteria (*Nitrosomonas* spp. and *Nitrobacter* spp.) were determined using the Most Probable Number method [23]. Three replicate dilution series were prepared for all microbiological analysis. The Sigma Stat 2.03 software was used for the analysis of the results.

## 3. RESULTS AND DISCUSSION

The temperature variation (Figure 1) was typical of green waste composting [11]. The thermophilic phase, with temperatures above 45$^oC$ lasted for a bit over 3 weeks, during which period temperatures as high as 63 $^oC$ have been recorded. After day 26, the temperature dropped dramatically, reaching ambient levels by day 30. Thereafter neither compost turning nor moisture correction resulted to any temperature increase.

Moisture content (MC) was maintained above 50% until day 100, after which the material was allowed to dray naturally, reaching a MC of 40% at the end of the process. Volatile solids declined rapidly from 83% to 69% during the thermophilic phase and thereafter exhibited a very low decrease to about 65% after a total of 143 days of processing. The compost was slightly alkaline while its electrical conductivity was low and remained around 2.0 dS $m^{-1}$ throughout the process.

The succession of different microbial groups is illustrated in Figure 2. The indigenous population of total heterotrophic mesophilic bacteria (Figure 2a) in the fresh material was fairly high for this type of material, at $3.4x10^9$ CFU $g^{-1}$ (dw), possibly due to the gradual collection and stockpiling of the green waste, which may have allowed some decomposition to begin before day 0. The compost was not sampled during the themophilic phase, thus the possible drop of population at peak temperatures, commonly reported in the literature [5-7,11,20] has escaped analysis. During curing the populations of total mesophilic bacteria increased and

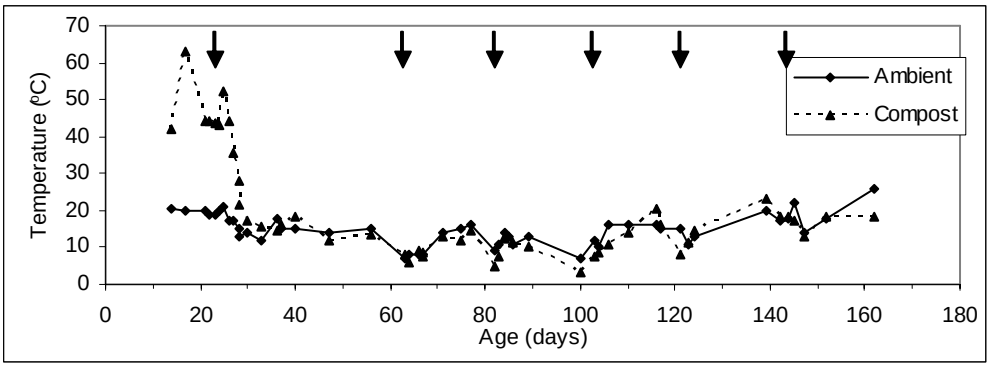


*Figure 1.* Temperature variation with composting time. Arrows indicate compost turning

were highest after three to four months of processing, at $4.5x10^{10}$ and $1.4x10^{10}$ CFU $g^{-1}$ (dw) respectively. Spore forming bacteria (Figure 2a) were detected throughout the entire process but their population increased 100-fold, from $3.0x10^{6}$ CFU $g^{-1}$ (dw) in the raw material to $3.4x10^{8}$ CFU $g^{-1}$ (dw) on day 63, about a month after the end of the thermophilic phase, through the selective pressure that high temperatures exercise to the microbial population, and remained high thereafter, in agreement with similar studies [9].

As high temperatures favour cellulose degradation, cellulolytic bacteria (Figure 2b) demonstrated a high count at the end of the thermophilic phase ($3.7x10^{8}$ CFU $g^{-1}$ (dw)), their numbers slightly declining during the curing phase, in accordance with [5]. This decline could be attributed to the fact that during curing the cellulose may become inaccessible to enzymatic attack because of low water content or association with protective substances such as lignin [5]. Cellulolytic bacteria in the fresh material were not determined.

The numbers of actinomycetes and fungi (Figure 2b,c) begun to increase after day 23, when compost temperature started to decline, as these organisms are not temperature resistant, in accordance to the literature [5-7,11,13]. Actinomycetes increased from $3.0x10^{7}$ CFU $g^{-1}$ (dw) at the end of the thermophilic phase to $1.1x10^{9}$ CFU $g^{-1}$ (dw) at day 63 and remained high throughout curing. Actinomycetes utilise complex organic compounds and their population tends to increase in the later stages of composting. Their appearance as a grey-white growth at the surface of the material is often considered as an indication of compost maturing.

Fungi (Figure 2c) are also believed to be involved in the decomposition of cellulose and lignocellulosic compounds of the compost [5]. In this study, they increased from $4.8x10^{6}$ CFU $g^{-1}$ (dw) at the end of the thermophilic phase to $4.3x10^{7}$ CFU $g^{-1}$ (dw) after about three months of processing and remained at this level thereafter. Yeasts population increased slightly after the thermophilic phase, but declined again after about three months of processing, possibly due to the pH increase [8]. Fungi and yeasts can survive the thermophilic phase as spores or are re-inoculated into the compost from the environment or the cooler material at the edges of the bioreactor.

Nitrifying bacteria (Figure 2d) were not detected in the raw material and only appeared when the temperature declined, as these organisms are not thermotolerant. Ammonium oxidizing bacteria (*Nitrosomonas* spp.) increased for two months after the active composting phase, from $6.3x10^{3}$ MPN $g^{-1}$ (dw) at day 23 to $1.1x10^{6}$ MPN $g^{-1}$ (dw) at day 103, remained at the same level for about a month and declined thereafter, probably due to depletion of reduced forms of nitrogen. Nitrate oxidisers (*Nitrobacter* spp.) reached their highest population by day 121, continuing to feed on the nitrates produced by the *Nitrosomonas* spp. This variation of the nitrifying bacteria indicates that after three to four months of processing, nitrites have been transformed into nitrates, which is considered as an indicator of a high degree of compost stabilisation [4,11,24].

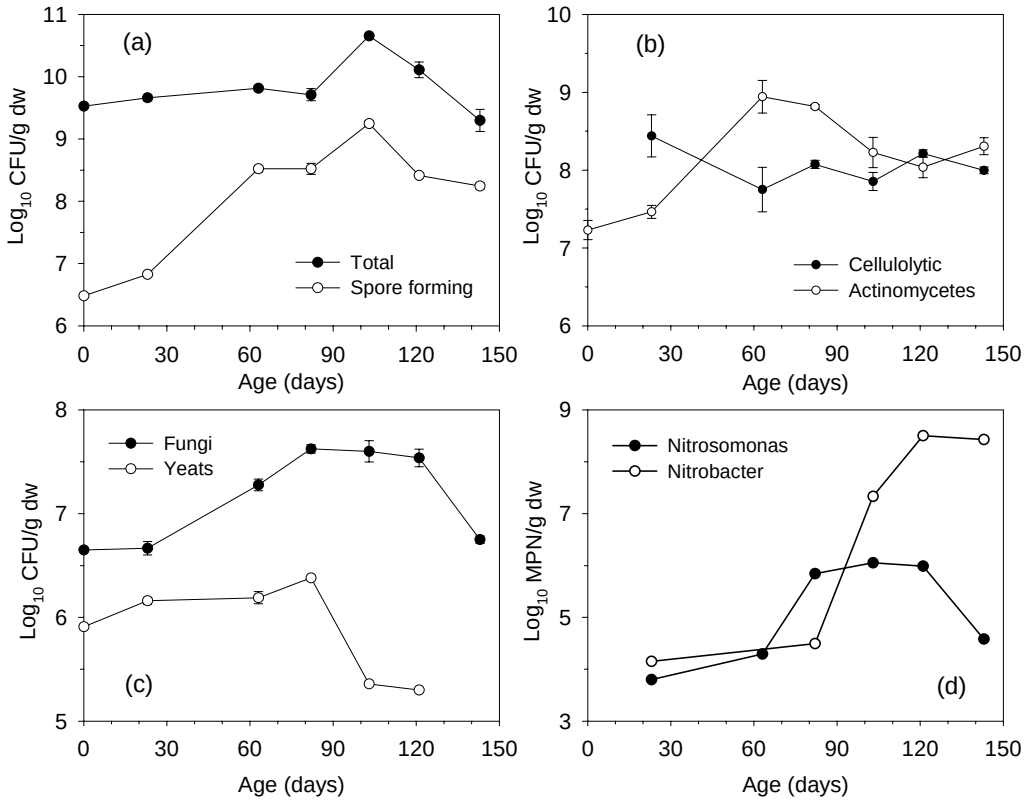


*Figure 2.* Dynamics of the population of different microbial groups during composting of green waste. (a) Total and spore forming bacteria; (b) Cellulolytic bacteria and actinomycetes; (c) Fungi and yeasts; and (d) Nitrosomonas and nitrobacter (these organisms were determined with MPN-method). Bars in Figures (a)-(c) represent standard error (n=3)

The respiration rate (SOUR test – Figure 3) increased at the beginning of the active composting phase, as complex compounds are broken down to simpler, more easily degradable ones, reaching a value of 4 mg $O_2$ $g^{-1}$VS $hr^{-1}$, and gradually declined thereafter, to 0.5 mg $O_2$ $g^{-1}$VS $hr^{-1}$, a value indicative of stable composts. The parameter correlated well with compost age (r=-0.85, p=0.02), a result repeatedly observed with a wide range of materials [2,16]. Furthermore, measuring oxygen consumption of an aqueous compost suspension, instead of a solid matrix, as in most respiration tests, offers certain advantages as it ensures that: the test is not affected by variations in the matric water potential of the samples; there is immediate contact between the substrate and micro-organisms leading to maximum reaction rates; and the gas-liquid barrier for oxygen diffusion at the solid particles surface is omitted.

Dehydrogenase activity has been used as a measure of total heterotrophic microbial populations in soils and composts and has been proposed as an indicator of compost stability [10,11]. However, in this study dehydrogenase activity fluctuated during the process, with high values being observed both at the end of the thermophilic phase and during the last stages of maturation (Figure 3). The parameter did not correlate with compost age, a result which merits further investigation. The GI of the fresh substrate was 35% indicating a fairly high level of phytotoxicity. However, GI increased by the end of the thermophilic phase to 80% and fluctuated around this value thereafter (Figure 3), indicating an increasing stabilisation of the organic matter with the corresponding metabolism of phytotoxic substances [15-17].

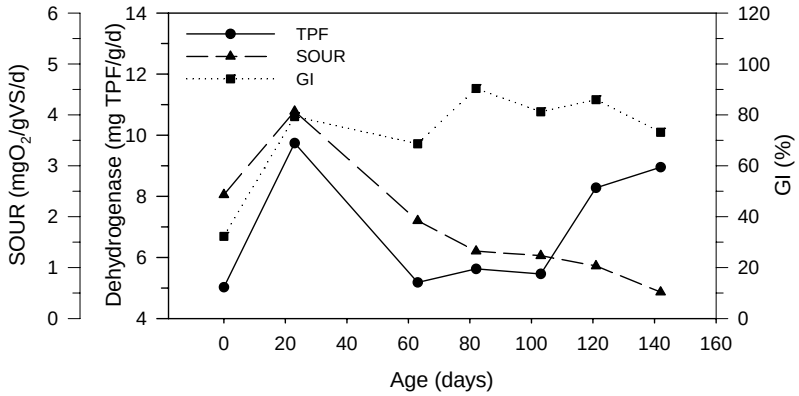


*Figure 3.* Variation of SOUR, dehydrogenase activity and GI with composting time

## 12. CONCLUSIONS

Results of the microbial community dynamics analysis suggested that, although at lower levels compared to other substrates such as manures and biowaste, the population size of different microbial groups was not a limiting factor in green waste composting. The numbers of most microbial groups increased after the end of the thermophilic phase, although for some groups population counts declined again towards the end of the maturation period, possibly indicating the exhaustion of specific substrates. Overall, microbial community succession reflected well the changes during the composting process.

Both the SOUR test and GI were useful as indicators of the advancement of the composting process and the stabilisation of the compost, and so were the dynamics of the nitrifying bacteria population. In contrast, the enzyme activity examined (dehydrogonase) did not seem to reveal any information for the stabilisation of the substrate, a result requiring further research as it contradicts findings of other studies.

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