

Evolution of Microbial Biomasses C and N during the Composting of Municipal solid Wastes

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Abstract: Problem statement: The aim of this study was mainly focused on the evolution of microbial biomasses C and N during the composting of municipal solid wastes. **Approach:** The carbon and the nitrogen of the microbial biomass (B_C and B_N) were studied using the fumigation-extraction method. **Results:** The dynamics of the B_C/B_N ratio, index of the chemical composition of the whole microbial population suggested a shift in the composition of microbial populations during the process from prevailing bacteria and actinomycetes to prevailing fungi. **Conclusion/Recommendations:** Microbial characterization of composting is of importance for the optimization of the process and the quality of the end product.

Key words: Compost substrates, microbial biomass, fumigation-extraction, microbial population

INTRODUCTION

The composting of organic waste has been defined as a controlled microbial aerobic decomposition process and therefore information on the microbial component of the composting substrates could be expected to give valuable information on the factors influencing the process, on its optimization and on the quality of the end product (Jedidi *et al.*, 2004; Mondini *et al.*, 1997; 2002).

These methods are based on the concept of soil microbial biomass as introduced by Jenkinson (Paul, 2006). Determination of microbial biomass in soil is currently based on the so-called fumigation-extraction method (Vance *et al.*, 1987b). It is possible to determine the soil microbial Carbon (C), Nitrogen (N), Phosphorus (P), Sulphur (S) (Vance *et al.*, 1987b) and ninhydrinreactive N (Amato and Ladd, 1988; Joergensen and Brooks, 1990; Mergulhao *et al.*, 2010) in the extracts. The microbial biomass plays a crucial role in the carbon cycle via the organic matter humification-mineralization processes. It also presents a determinant role in the nitrogen cycle with transformation of the organic matter into mineral elements. In fact, chloroform fumigation lyses the cells of micro-organisms and makes part of the cytoplasm extractable with various reagents (K_2SO_4).

The evaluation of microbial biomass content could be investigated by direct methods, such as the counting

of micro-organisms (Paul and Johnson, 1977), or by several indirect methods, such as the measurement of cellular compounds or the fumigation-extraction approach (Vance *et al.*, 1987a). Many authors have investigated the carbon and nitrogen content of the microbial biomass with the later approach.

Several authors have applied the fumigation-extraction technique with compost substrates (De Nobili *et al.*, 1996; Hellmann *et al.*, 1997; Horwath and Elliot, 1996; Mondini *et al.*, 1997). It is important to note that the present study was quite different from theirs in several respects. Firstly in the fumigation-extraction technique, we used a Kjeldahl distillation to measure the nitrogen ratio which is relatively simple, easy and more reproducible than the ninhydrin reactive nitrogen method, as used by De Nobili *et al.* (1996) or Mondini *et al.* (1997). Moreover, the study represents a first report of an evaluation of the microbial biomass in Tunisian compost under the local climate conditions and specific waste content. These two parameters make the present study an original contribution to compost microbial biomass evaluation.

However, the application of the microbial biomass technique to composting substrates requires special care to obtain realistic and reproducible results, because of the particular properties of compost substrates, such as the fast changes in physicochemical and microbiological properties, the high spatial heterogeneity, the coloration of the extracts and the

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problems related to representative sampling and storage of samples.

The aim of this study was mainly focused on the evolution of microbial biomasses C and N during the composting of municipal solid wastes.

MATERIALS AND METHODS

Preparation of windrows: The study was performed in the composting plant of Beja located 100 km from the north of Tunis, using two types of windrows: (i) the first windrow W1 constituted with 100% of municipal solid wastes and (ii) the second windrow W2 composed by weight of 60% of municipal solid wastes and 40% of dried sewage sludge.

Municipal solid wastes from the Erriadh city of Beja were pre-selected at source (household pre-selection with average physical-chemical characteristics, humidity = 60%, organic matter = 30% dry weight and C/N = 32) and subjected to an accurately manual sorting in the plant, weighted and put on the composting platform, in the form of windrows of 7.5×3×1.5 m (length×width×height, respectively). Stabilized sewage sludge from anaerobic digestion of the urban wastewater treatment plant of Beja was integrated in the process at the dry state (with average physical-chemical characteristics, humidity = 30%, organic matter = 65% dry weight and C/N = 12.5) and primarily for the cover of windrow W2.

Temperature and humidity were controlled daily. When the mean temperature recorded in the different depths (depths 20, 40 and 60 cm) of the pile averaged 55°C (using a thermo-couple iron-constantan type J), the windrow was turned and watered. These operations of turning and watering with tap water were performed almost twice monthly in considering ambient temperature.

Microbial Biomass determination: Solid waste microbial C and N biomasses were evaluated using the fumigation-extraction method (Sparling and West, 1988). Duplicate samples (10 g) of the treated and non-treated compost (control) were fumigated with ethanol-free CHCl₃ for 24 h at 25°C in a dessicator. The fumigated and non-fumigated compost samples were extracted for 60 min with 0.5 M K₂SO₄ 0.5 N and then filtered through a Whatman filter paper. Organic C was quantified by the potassium dichromate oxidation method (Jenkinson and Powlson, 1976) and titrated with unreduced dichromate.

All results were evaluated on an oven-dry sample basis and are the mean of six replicates.

The amounts of soluble C in the fumigated and unfumigated compost extract are used to determine biomass C expressed as:

$$B_C = (C_f - C_{nf}) / K_{ec}$$

Where:

C_f = The C in the fumigated extract

C_{nf} = The C in the unfumigated extract

K_{ec} = The proportion of the microbial C that is extracted from the compost

Voroney *et al.* (1991) suggests a K_{ec} of 0.35 as a general value of microbial C extraction efficiency determined initially for soils because a standardization of the fumigation-extraction technique for compost is not yet established.

Fumigation-extraction was first adapted for use with soil microbial biomass. As the present study required the adaptation of this technique to compost it was necessary to use microbial C and N coefficients of extraction efficiency.

Biomass N is calculated as the flush of N from a fumigated compost less that extracted from unfumigated compost:

$$B_N = (N_f - N_{nf}) / K_{en}$$

Where:

N_f = The total N in the fumigated soil extract

N_{nf} = The N in the unfumigated extract

K_{en} = The efficiency of extraction of organic microbial N and inorganic N from compost

Brooks *et al.* (1985) suggest an extraction efficiency of 0.68 determined initially for soils.

RESULTS

Characteristics of compost: The fluctuation of temperature recorded in the two windrows during the composting process showed the three classic steps (Fig. 1).

Mesophilic phase: Mesophilic microorganisms in waste windrows tended to increase during the first 25 days of the composting process, while the temperature increased gradually to reach 25-45°C as a consequence of biodegradation of organic compounds.

Thermophilic phase: This important step occurred between 30 and 130 days of the composting process, during which the temperature exceed the tolerance limit of mesophilic microorganisms (≤45°C). The passage to the thermophilic phase for the two piles could be attributed to high numbers of indigenous microorganisms which attack rapidly the soluble, the readily degradable compounds and the high content of available nutrients and the relatively small size of organic fraction particles. As the temperature rises above about 40°C, the mesophilic microorganisms become less competitive and are replaced by others that are thermophilic.

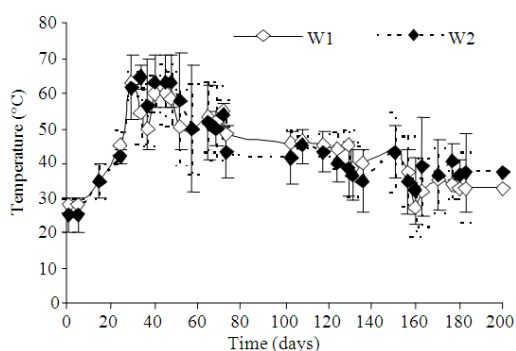


Fig. 1: Progress of temperature over time in windrows W1 and W2 ± Standard deviation, n = 3

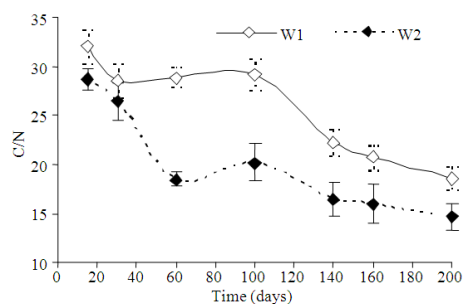


Fig. 2: Progress of C/N ratio over time in windrows W1 and W2

Cooling phase: The temperature began to decrease after the 19th week. This decrease resulted from a beginning depletion of organic matters. During this phase, the C/N ratio in the two windrows tended to stabilize (Fig. 2). By the end of fifth month of composting, average temperatures inside the two windrows marked a real decrease with values of approximately 33°C. The temperature remained no change in spite of watering and turning of the windrows.

In this study, the values of C/N ratio appeared generally higher in the windrow W1 than in the windrow W2, these values decrease from 32 at the beginning of the composting process to 18.6 at the end of the process for windrow W1 and from 28.5-14.6 for windrow W2 (Fig. 2). The use of sewage sludge can explain this relatively feeble C/N ratio recorded throughout the composting process in windrow W2. Indeed, sewage sludge is known by their high nitrogen content (C/N varied between 5-10) as compared to the one generally registered in municipal solid waste of Beja (C/N varied between 25-35). Many authors reported that a C/N ratio below 20 is indicative of an acceptable maturity (Jimenez and Garcia, 1991).

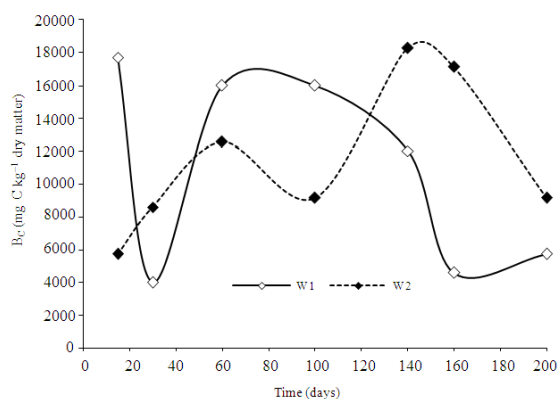


Fig. 3: Progress of B_C over time in windrows W1 and W2

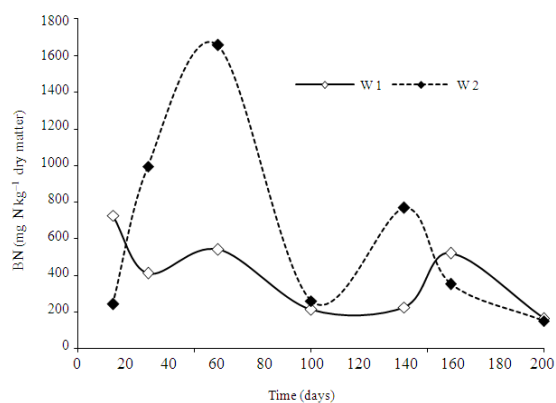


Fig. 4: Progress of B_N over time in windrows W1 and W2

Evolution of microbial biomass B_C and B_N: The dynamics of B_C (Fig. 3) showed a close resemblance to that of B_N (Fig. 4). There was an initial phase of increase in the size of the microbial biomass reached after about 60 days from the beginning of the process. Thereafter the content of microbial biomass tended slightly to decrease. This behaviour has been reported in many researchers study (Garcia *et al.*, 1992; Hellmann *et al.*, 1997; Insam *et al.*, 1996; Klamer and Baath, 1998; Tiquia *et al.*, 1996). In W2, the addition of sewage sludge stimulates microbial activity and creates an increase in the amount of B_C and B_N.

Evolution of B_C/B_N ratio: The B_C/B_N ratio represents the biological activity balance of the compost and a high B_C/B_N ratio is equivalent to a high microbial heterogeneity, diversity and synonym of an important biological activity (Belete *et al.*, 2001; Jedidi *et al.*, 2004;

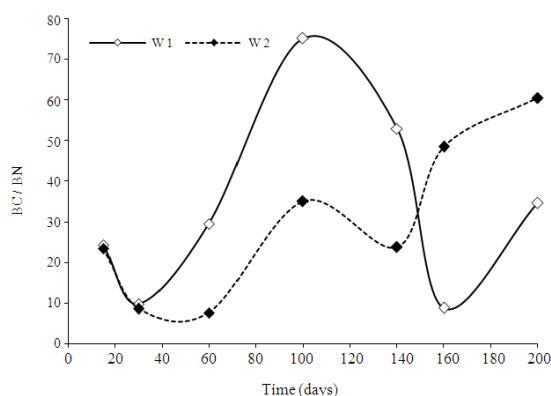


Fig. 5: Progress of B_c/B_n ratio over time in windrows W1 and W2

(Metting, 1993) According to Fig. 5, this ratio showed a real increase during the thermophilic phase in the two windrows. A minimal value corresponding to the beginning of the cooling phase is recorded and followed by a clear increase of this B_c/B_n ratio during the maturation phase.

DISCUSSION

This behaviour is probably related to the availability of readily decomposable substrates; in fact when organisms are presented with a substrate they normally multiply rapidly until the substrate is nearly exhausted, when numbers reach a maximum (Joergensen and Brooks, 1990). Thereafter, with the exhaustion of these substances caused by the intense microbial activity and by ongoing humification, the microbial biomass decreased. A similar trend of microbial biomass during composting was reported by (Hellmann *et al.*, 1997; Horwath and Elliot, 1996; Insam *et al.*, 1996).

Initially the fumigation-extraction method introduced by Vance *et al.* (1987b) was used in the evaluation of the soil microbial biomass. He used the chloroform fumigation to lyses the cells of the microorganisms and K₂SO₄ for their extraction. Now, the use of this method is expanding, with use for either soils (Chaussod *et al.*, 1992; Insam *et al.*, 1996) (followed the evolution of the microbial biomass in a Venezuelan tropical soil), different types of composts (De Nobili *et al.* (1996), who studied the microbial biomass in green compost and municipal solid waste compost; Mondini *et al.* (1997) who worked on rewetted compost) and in compost-amended soils (Lalande *et al.*, 1998). However, the standardization of a technique of FE from compost is not yet established but it is noted that most authors used almost the same

methodology for carbon extraction but they differed in the nitrogen extraction. Whereas some used a ninhydrin-reactive N method the Kjeldahl method was used in the present study. The principle is the same and this method was well adapted to our compost substrates and gave accurate results that confirmed the results obtained using the ninhydrin-reactive N method.

Populations of bacteria and actinomycetes are reported to have a protoplasmic C/N ratio of 5; whereas fungi have a ratio of 10 (Metting, 1993). The results suggested a change from an initial population in which bacteria and actinomycetes were prevalent towards a final community structure in which the number of fungi had increased. These results were in good agreement with the general dynamics of compost microbial populations reported by literature (Rosen *et al.*, 1997; Hellmann *et al.*, 1997; Metting, 1993; Paul and Clark, 1996). Generally there is an early thermophilic attack by bacteria and actinomycetes that converts easily degradable substrates such as sugars and proteins, whereas fungi are reported to be the major organisms in the following part of the process when cellulose, hemicellulose and lignin are the available substrate and humification takes place.

The value of 0.35 for K_{ec} and 0.68 for K_{en} were used by Jedidi *et al.* (Jedidi *et al.*, 2004) and they reported high values of B_c/B_n ratio. The same results in a field study revealed a high B_c/B_n ratio. Hence, we recommend the utilization of 0.45 for K_{ec} and K_{en}, as reported initially by Jenkinson *et al.* (2004) for several types of soil.

The chloroform fumigation-extraction method has two principal limitations: (i) the determination of K_{ec} and K_{en} depends on soil micro-organism composition and (ii) this method did not give any idea about the microbial community composition. For this reasons the DNA quantification method has been compared with the chloroform fumigation-extraction in different soils (Bailey *et al.*, 2002; Leckie *et al.*, 2004; Marstorp *et al.*, 2000) and has been proposed as an alternative method of chloroform fumigation-extraction to measure soil microbial biomass (Marstorp *et al.*, 2000).

CONCLUSION

The microbial biomass content measured by fumigation-extraction is based on the assumption that microbial biomass is a unique functional entity. Application of this concept to compost samples from different stages of the process has shown the following results.

The dynamics of the B_c/B_n ratio suggest a shift in microbial populations during the process, from a phase in which bacteria and actinomycetes are dominant to a subsequent phase of fungi predominance.

The results of the present research suggested that application of microbial biomass concept to composting substrates is valuable because of useful information on the composting process. Furthermore, the fumigation-extraction technique is easy, inexpensive and does not require sophisticated instrumentation. On the other hand considering the microbial population as a whole presents some limitation, because it is not possible to discriminate between microbial populations. For this reason, it seems that deeper information on the microbiological aspects of the composting process could be reached by integrating information on size and activity of the whole microbial biomass with information obtained with techniques able to estimate the size and the functional ability of the different microbial populations.

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