

# Evolution of Biochemical Parameters During Composting of Urban Wastes

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An experiment was performed for evaluating the most suitable biochemical parameters to measure dynamics of substrate transformation during composting of the organic fraction derived from pre-selected urban wastes and wood bark mixture. Changes of chemical (organic C, hydrosoluble sugars, total and mineral N, humified fraction, volatile acids and phenolic compounds) and biochemical (microbial respiration, biomass C,  $qCO_2$ , dehydrogenase, catalase, urease activities, FDA, and BIF) parameters were monitored for 120 days. Limited changes in organic C, total nitrogen, and humification characteristics were observed during composting. Dehydrogenase and catalase activities, BIF and FDA showed small changes during composting. Urease activity and, with some limitations regarding the early stages of composting, microbial respiration and  $qCO_2$ , were found to be the most suitable parameters to measure dynamics of substrate transformation during composting of pre-selected urban wastes.

## Introduction

Measuring compost stability has become a necessary step to determine the suitability of compost for different uses. Various physical (temperature, humidity, color, texture), chemical (pH, organic matter content and forms), biological (phytotoxicity, microbial community type and level), spectroscopic and thermal (Fourier transform infrared, differential scanning calorimetry) parameters have been proposed to evaluate the progress of the composting process (Saviozzi *et al.* 1992; Senesi and Brunetti 1996; Barberis and Nappi 1996; Adani *et al.* 1997; Fisher 1998; Grego *et al.* 2000; Outmane *et al.* 2000; Tomati *et al.* 2000; Belete *et al.* 2001; Eggen and Vethe 2001). Some biochemical parameters, such as enzyme activities (Gomes *et al.* 1998; Benitez *et al.* 1999), amount of microbial biomass (De Nobili *et al.* 1996), or microbial respiration (Lasaridi and Stentiford 1998; Butler *et al.* 2001; Eggen and Vethe 2001; Wu and Ma 2001), have been recently monitored for the evaluation of the course of composting. However, the conclusions of the studies are often diverging and, consequently, there is widespread acceptance of the need for a better evaluation of the dynamics of substrate transformation during composting.

The aim of the present study was to establish suitable biochemical parameters to measure dynamics of substrate transformation during composting of preselected urban wastes.

## Materials and Methods

The organic fraction derived from preselected urban wastes and wood bark mixture was composted for 120 days. The composting process involved two physically separated phases: a first stage of accelerated biooxidation lasting 21 days and a second stabilizing phase in which the product was windrowed for 99 days. During the first phase, material was loaded along the short side of a horizontal reactor, moved forward by means of screws installed on a travelling bridge and discharged at the opposite side. Continuous aeration, supplied by a forced ventilation system from the bottom, maintained oxygen level greater than 10%. In the second phase, the product was piled outside in a triangular configuration roughly 1.30 m high, 4 m wide and 5.50 long. The windrows were turned by a loading shovel.

### Sampling Procedures

To evaluate biomass transformation, representative samples for analysis were collected three times during the first 21-d processing (after 3, 10, 21 d) and five times during the 99-d stabilizing phase (after 41, 55, 69, 90 and 120 d of the whole composting period). Samples of about 1 kg were obtained from eighteen bulked and mixed subsamples (each of about 0.1 kg) taken from three positions near the top, middle and bottom. All the samples were lyophilized, crushed and screened through 0.5 mm sieves.

### Chemical Analyses

Total organic C (TOC) was determined after removing carbonate-C (Nelson and Sommers 1982) by dry combustion (induction furnace 900CS, Eltra). Total N was determined by the Kjeldahl procedure. The  $\text{NO}_3^- + \text{NO}_2^-$  and  $\text{NH}_4^+$  contents were measured by the Bremner MgO-Devarda alloy method using 2 N KCl extracts (sample/solution = 1:20).

Humic substances (TEC) were extracted by 0.1 M NaOH +  $\text{Na}_4\text{P}_2\text{O}_7$  (sample/solution 1:100). After centrifugation at 5000 g for 30 min, the supernatant was filtered and the raw fulvic acid and humic acid (HA) fractions separated by acidifying to pH 2 with  $\text{H}_2\text{SO}_4$ . Isolation, separation and purification of "true" fulvic acid (FA) were made by adsorption onto insoluble polyvinylpyrrolidone (PVP), as suggested by Lowe (1975). According to the standard procedure described by Sequi *et al.* (1986), the humification index (HI) was calculated by dividing the nonhumified fraction (NH) by the HA + FA fractions. Total and humic C in any fraction were determined by  $\text{Cr}_2\text{O}_7^{2-}$ -oxidation.

On the soluble fraction, obtained by shaking sample and water in a 1:50 ratio for 1 hour, the following analyses were performed: sugars by the phenol-sulfuric acid reaction (Dubois *et al.* 1956); volatile acids content by direct titration (Di Lallo and Albertson 1961); phenolic compounds according to the method of Kuwatsuka and Shindo (1973).

### Biochemical Analyses

The biomass-C content was determined according to Vance *et al.* (1987) through extraction of organic C from fumigated and unfumigated soils by 1 N  $\text{K}_2\text{SO}_4$ ; the organic C was then measured using dichromate digestion as described by Jenkinson and Powlson (1976); an extraction efficiency coefficient of 0.38 was used to convert the difference in soluble C between the fumigated and the unfumigated soil in microbial biomass C (Vance *et al.* 1987). The hydrolysis rate of fluorescein diacetate (FDA) was estimated, as reported by Swisher and Carrol (1980), by determining at 490 nm the concentration of hydrolyzed FDA. The dehydrogenase activity was measured according to the method described by Casida *et al.* (1964). The catalase activity was measured by the method of Beck (1971). The urease activity was determined according to Tabatabai (1982).

The biological index of fertility (BIF) was calculated as follows:  $(\text{DHA} + k \text{ CA}) / 2$ , where k is a proportionality coefficient (0.01) (Stefanic *et al.* 1984).

### Respirometry

The respiration rate of the compost was monitored in an aerobic incubation procedure over 60 days, through the measurement of  $\text{CO}_2$  evolution: 25 g (dw) of the samples collected at 3, 21, 55, 90 and 120 days were adjusted to approximately 50% moisture, and preincubated in bags placed in a chamber at 37°C and 100% humidity for 24 hours. Then, samples were placed in 250-ml glass containers closed with rubber stoppers, moistened at 60% of the maximum water holding capacity and incubated at  $25 \pm 1$  °C. Glass vials holding 20 ml of 0.5 M NaOH to trap the evolved  $\text{CO}_2$  were placed in the above containers. The excess alkali was back titrated with standard 0.5 M HCl after precipitating the carbonate with a 1.5 M  $\text{BaCl}_2$  solution. Daily opening of the bottles to replenish the NaOH for  $\text{CO}_2$  absorption prevented any inhibition of decomposition owing to lack of  $\text{O}_2$ .

The microbial metabolic quotient ( $q\text{CO}_2$ ), defined as specific respiration of the microbial biomass, was calculated from basal respiration values at the steady state of respiration (Anderson and Domsch 1993) by the formula:  $\text{mg CO}_2\text{-C/mg biomass-C/h}^{-1}$  (Schnürer *et al.* 1985). Biomass C content was measured at the 60<sup>th</sup> day of incubation.

### Statistical Analysis

Results are the means of determinations made on three replicates. Data was compared through the analysis of variance by ANOVA test (Snedecor and Cochran 1978). The means were compared by using least significant difference values calculated at the 5% level.

Results of respirometry ( $\text{CO}_2$  evolution) are the means of two replicates. The mean coefficient variation, i.e., the standard deviation as a percentage of the average of two values, was always lower than 5%.

### Results and Discussion

Temperature (Figure 1) during the first two days of composting followed the typical pattern of many composting systems, with a rapid increase from 20 to 48 °C. Successively, during the 21-d period deputed to accelerate biooxidation, the temperature showed an anomalous decrease and remained almost constant at about 40 °C. Only in the second phase, in which the product was standing on pile, heat was generated and the temperature raised rapidly to about 60 °C. The thermophilic phase lasted for about 20 days. As expected, in the last mesophilic phase the temperature decreased, approaching ambient levels.

Changes of organic carbon content (Figure 2) indicate a low level of organic matter evolution. Only about 6% of the organic C present in the starting material was mineralized by the 55<sup>th</sup> day of composting, probably because the high amount of volatile acids and phenolic compounds (Saviozzi *et al.* 1992) (Table 1) caused a lag-phase in the microbial activity. Successively, the breakdown of the or-

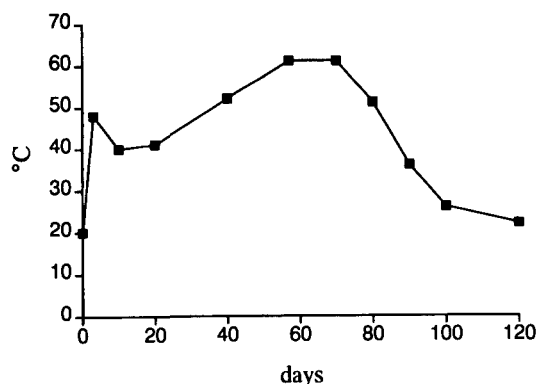


FIGURE 1. Temperature during composting process.

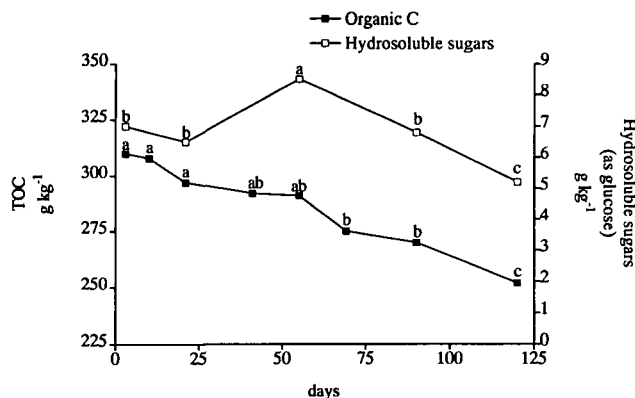


FIGURE 2. Changes in the contents of total organic carbon and soluble sugars during composting process. Different letters indicate the differences at a 5% probability level according to LSD/F multiple range test.

ganic carbon proceeded more rapidly, so explaining the simultaneous increase of temperature. The initial content of hydrosoluble sugars (Figure 2) was almost one fifth lower than that reported by Saviozzi *et al.* (1992) for urban compost. In accordance with the findings of Eggen and Vethe (2001), the shortage of these easily degradable compounds may have contributed to the latency in CO<sub>2</sub>-C evolution observed in the early phase of composting (Figure 4). The need to obtain simple soluble sugars could have forced microorganisms to breakdown more resistant polysaccharides, shown by the increase of hydrosoluble sugars as the composting progressed. The successive decrease of hydrosoluble sugars, after about two months, coincided with the increase in temperature, confirming that such compounds represent an easily available source of energy for microorganisms.

The initial values of total nitrogen content of about 23 g kg<sup>-1</sup> (Figure 3), higher than the average value (12 g kg<sup>-1</sup>) reported for municipal waste compost by He *et al.* (1995) from several U.S. sources, increased by about 13% during composting. Such

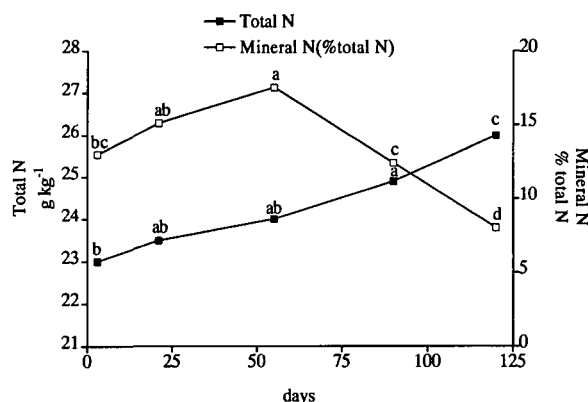


FIGURE 3. Changes in the contents of total and mineral nitrogen during composting process. Different letters indicate the differences at a 5% probability level according to LSD/F multiple range test.

TABLE 1.

Changes in volatile acids, phenolic compounds and humus characteristics during composting.

Composting Period (Days)	TEC g kg <sup>-1</sup>	C <sub>HA1</sub> g kg <sup>-1</sup>	C <sub>FA1</sub> g kg <sup>-1</sup>	C <sub>HA</sub> +C <sub>FA</sub> g kg <sup>-1</sup>	HI	Volatile Acids (as acetic acid) g kg <sup>-1</sup>	Phenolic Compounds (as coumaric acid) g kg <sup>-1</sup>
3	114a	77ab	16a	93ab	0.23a	36a	4a
21	113a	94a	13a	107a	0.15b	29ab	4a
55	108a	80ab	14a	94b	0.15b	23b	2b
90	105a	75b	14a	89b	0.18b	22b	2b
120	108a	79b	13a	95b	0.17b	14c	1b

TEC = Total extractable carbon; C<sub>HA</sub> = humic acid carbon; C<sub>FA</sub> = fulvic acid carbon; HI = humification index. Values followed by different letters indicate the differences at a 5% probability level according to LSD/F multiple range test.

an increase may be due to a concentration effect resulting from degradation of nonnitrogenous organic matter, which originates a loss of weight and, therefore, a relative increase of concentration. This trend is in agreement with previous reports for municipal waste compost (Iannotti *et al.* 1994). Due to the deamination of organic compounds (Diaz-Burgos *et al.* 1993), the mineral fraction of total N, initially 13%, increased to a level of 17.5% after 55<sup>th</sup> day of composting. Successively, mineral N began to decline to 8% of total N, due to the progressive inclusion into humic aromatic structures. The trend for mineral N seen during composting coincides with the patterns reported by Cegarra *et al.* (1986) for city refuse compost.

Table 1 also reports the changes which took place in the humic content (TEC) examined four times during composting. Many authors report the formation of humic substances during composting (Chen *et al.* 1996; Singh and Amberger 1996), but others (Roletto *et al.* 1985; Chefetz *et al.* 1998) affirm that no quantitative increase in humic substances occurred. Table 1 shows that during composting, values of TEC remained constant, probably because of balance between synthesis and degradation of the newly formed humic materials or scarce humification activity. The lack of a definite trend of the other humification parameters (Table 1) seems to confirm the second hypothesis.

Temporal trends in the contents of volatile acids and phenolic substances (Table 1) were typical of those reported by others for urban waste composting (Hirai *et al.* 1983; Levi-Minzi and Riffaldi 1988; Saviozzi *et al.* 1992), who found a fall in the volatile acid and phenolic compounds levels occurring in the water extracts of ripe samples. At the end of composting, the levels of the compounds in the product were about 60% (volatile acids) and 75% (phenolic substances) lower than in the starting material. The high

level of volatile acids and phenolic compounds found in the starting material (Saviozzi *et al.* 1992) could have inhibited microbial activity, causing a temporary lag-phase in the decomposition of the organic matter.

Microbial biomass analysis can help to characterize successional changes related to both physical and chemical changes in the substrate. Temperature is the main driving force of succession but it also interacts with other environmental regulators such as pH, availability of organic C and energy source. The lowest level of microbial biomass C was observed in the 21-d sample (Table 2), in the phase in which the temperature declined. The highest levels were reached in the thermophilic phase and maintained during the mesophilic phase, in agreement with the findings of De Nobili *et al.* (1996). As only small changes in the amount of biomass C were found during composting, the biomass C does not appear a good indicator of compost stability.

Generally, the intensity of respiration depends on the speed of microbial biomass metabolism and therefore it is inversely related to compost stability. Respiration intensity is high in the early phase of composting when easily degradable organic substances induce fast multiplication of microorganisms and it declines with time. Figure 4 shows that the respiration of the 3-d sample was constantly inhibited while the 21 and 55-d samples showed higher respiration rates with only a lag-phase in the first period of incubation of about 30 and 15 days, respectively. As observed by Eggen and Vethe (2001), compost samples with low hydrosoluble C resulted in an extended lag phase before detectable respiration occurred. The observed low respiration activity (sample of 3-d) and the delay in the start of respiration (samples 21- and 55-d) may be due to the low amount of hydrosoluble sugars (Figure 1) and/or to the high level of toxic compounds such as volatile acids and phenolic compounds in the

TABLE 2.

Microbial biomass C, cumulative CO<sub>2</sub>-C evolved in 60 days from samples of different maturity and microbial metabolic quotient (qCO<sub>2</sub>) during composting.

Composting Period (Days)	Biomass C (g kg <sup>-1</sup> )	CO <sub>2</sub> -C (g kg <sup>-1</sup> )	qCO <sub>2</sub> (mg CO <sub>2</sub> -C/mg *biomass C/h <sup>-1</sup> )
3	10.8a	8	3.8x10 <sup>-4</sup>
21	7.1b	27	2.7x10 <sup>-3</sup>
55	11.8a	66	2.3x10 <sup>-3</sup>
90	11.3a	91	1.8x10 <sup>-3</sup>
120	11.0a	45	9.5x10 <sup>-4</sup>

Values followed by different letters indicate the differences at a 5% probability level according to LSD/F multiple range test. \*The biomass C content for qCO<sub>2</sub> calculation was measured at the 60th day of incubation (see Materials and Methods section).

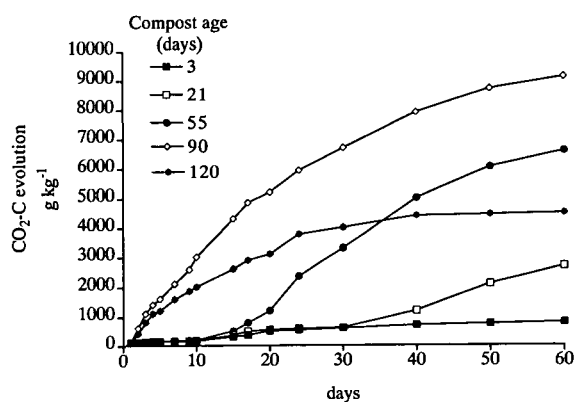


FIGURE 4. Cumulative CO<sub>2</sub>-C evolution from compost at different degrees of stability.

starting material (Table 1). The use of microbial respiration for monitoring the substrate quality during composting could be limited by the presence of anomalous trends especially in very immature samples. Due to the decrease of inhibiting volatile acids and phenolic compounds observed from the 21-d to the 55-d samples (Table 1), as a consequence of either volatilization to the atmosphere or further biologically mediated conversion to less soluble and/or phytotoxic compounds (Hänninen and Lilja 1994; Ayuso *et al.* 1996), the delay in starting of respiration was shorter in the 55-d sample than in the 21-d sample. This is confirmed by the decrease in the same period of hydrosoluble sugars which have been used as substrate by microorganisms. As a result of the very low amount of toxic compounds, the 90-d sample showed a high initial rate of respiration without a lag-phase. As expected from a composting process where the product becomes more stable with time, the 120-d sample shows, from the first measures, low values of CO<sub>2</sub> evolution, slightly diminishing during incubation. It is interesting to note that the cumulative respiration between day 30 and 60 for the 90-day sample is lower than the cumulative CO<sub>2</sub>-C evolution between day 0 and 30 for the 120-day sample (about 2300 g/kg and about 4000 g/kg of CO<sub>2</sub>-C, respectively), when it would be expected to be similar values because of the same age of both samples, (120-150 days). This is explained probably because in the 120-day sample microorganisms have evolved strategies for breaking down the irregular structure of the lignin polymer to access the cellulose and hemicellulose, which can be used as a source of C for respiration.

Generally, the trends in respiration rates of compost at different ages correspond well to the changes of the conventional chemical and physical parameters observed during composting (Wu *et al.*, 2000). However, the respiration method, commonly used by many end-users for monitoring compost quality, could be better used by applying it only after the early phase of the process.

The ratio of evolved CO<sub>2</sub>-C to biomass C content (qCO<sub>2</sub>) provides a measure of the efficiency of microbial populations in utilizing organic C and is currently employed as biological index of soil organic matter quality (Wardle and Ghani 1995; Saviozzi *et al.* 2001). In composting, it could be tested as monitoring parameter of organic matter stabilization. Table 2 shows that qCO<sub>2</sub> was low in initial stage of composting, increased almost 10 fold at the 21-d and declined thereafter over the remaining 100 days. The greatest value of qCO<sub>2</sub> in the 21-d sample results from both the increase in respiration rate, i.e. in the CO<sub>2</sub>-C evolution, and the simultaneous decline of biomass C. The qCO<sub>2</sub> value of 9.5 × 10<sup>-4</sup> mg CO<sub>2</sub>-C mg biomass C<sup>-1</sup> h<sup>-1</sup> in 120-d sample, lower than that of 90-d sample but in presence of the same amount of biomass C, indicates less respiratory activity of the microbial biomass in the last stage of composting and, consequently, a higher microbial efficiency (Anderson and Domsch 1993). Note that such a value is of the same order of magnitude of that reported for organic matter of mature soils by Trinchera *et al.* (2001). There is still a need to evaluate qCO<sub>2</sub> with respect to the significance of the produced results; in particular, the anomalous low values of microbial respiration observed during the early stages of composting (Figure 4, Table 2) negatively affected qCO<sub>2</sub>. This represents a limitation for the use of qCO<sub>2</sub> for monitoring compost quality in immature samples. However, this parameter seems to give useful information on the different phases of the process.

In parallel with the respirometric test which provides an overall estimation of compost degradability, other biochemical tests for measuring evolution of the biomass in compost could be selected. Dehydrogenase has proven to be an indicator of changes in organic matter during the composting period (Forster *et al.* 1993). Catalase, constitutive in aerobically-growing organisms, shows a linear activity, directly proportional to the microorganism population density. Table 3 shows that dehydrogenase activity remained practically constant during composting. Only at the 55<sup>th</sup> day of composting, corresponding to the thermogenesis

TABLE 3.  
Changes in enzyme activities during composting

Composting Period (Days)	DHA μg-TPF g <sup>-1</sup> h <sup>-1</sup>	CA % O <sub>2</sub> g <sup>-1</sup> 3 min <sup>-1</sup>	BIF	FDA μg fluorescein g <sup>-1</sup> 2 h <sup>-1</sup>	UR μg N-NH <sub>4</sub> <sup>+</sup> g <sup>-1</sup> 2h <sup>-1</sup>
3	2.5ab	1.7ab	1.3ab	21.7a	445.4b
21	2.2ab	0.7b	1.1ab	27.7a	3.6c
55	1.2b	1.0ab	0.6b	23.0a	1243.4a
90	4.2a	1.9a	2.1a	20.2a	494.4b
120	4.1a	2.0a	2.1a	25.4a	450.1b

DHA = dehydrogenase activity; CA = catalase activity; BIF = Biological Index of Fertility; UR = urease activity; FDA = hydrolysis of fluorescein diacetate. Values followed by different letters indicate the differences at a 5% probability level according to LSD'F multiple range test.

stage, dehydrogenase activity was lower than in the following stages. Probably, at thermophilic temperature large numbers of mesophiles are viable, as assessed by the amount of biomass C, but not necessarily active. A similar result was observed for the catalase activity, suggesting that the two enzyme activities are not feasible indicators of the state and evolution of the organic matter under composting.

As far as the biological index of fertility (BIF) is concerned, Stefanic *et al.* (1984) reported values from 0.9 to 17.2 in a series of soils. If values of compost (Table 3) are referred to soil BIF as baseline level, the index attained very low values from 0.6 to 2.1. Although the increase of values from the 55<sup>th</sup> day could indicate a trend towards a better quality of biochemical characteristics of the material, the small differences over time of this parameter do not put in evidence enough the changes through composting.

The rate of hydrolysis of fluorescein diacetate (FDA) is considered to be an index of the overall hydrolytic enzyme activity in soil (Schnürer *et al.* 1985) due to a variety of enzymes including proteases, esterases and lipases. In composting, hydrolases are mostly involved in the early phases of the process because of the great amount of easily degradable substrate (Diaz-Burgos *et al.* 1993). In our study, the FDA test showed practically uniform values during the entire composting period (Table 3) and for this reason it cannot be considered a good indicator of compost stability.

Better indications were found by analyzing urease, an enzyme active in the final stage of degradation of N substances (Nannipieri *et al.* 1990). The trend of urease activity (Table 3) was characterized by some fluctuations, i.e. a repeatedly decrease and increase of values resulting from changes in the biochemical activity during composting. The initial high value of urease activity is probably due to the large amount of total nitrogen content (Table 1). The lowest value of 21-d sample indicates the blocking of enzyme synthesis when toxic levels of ammonia accumulate outside the cell, or inhibition of enzyme activity by ammonia (Mobley and Haussinger 1989). Usually, when urease reaches a minimum, a resynthesis begins (Nannipieri *et al.* 1978). This can explain the rise in urease activity observed after about two months of composting, when proteins or their degradative products have been substantially hydrolyzed and microorganisms are affected by the presence of substrate urea. The decrease of urease activity towards the final phase of composting may be due to an excess of the enzyme concentration which can determine an inhibition of activity (Ladd and Buler 1975). Globally, the urease activity exhibited an ability for monitoring changes in composting process.

## Conclusions

On the basis of the obtained results, it can be concluded that composting of preselected urban refuse was influenced by a latency in microbial biomass and the delay in the start of thermophilic and mesophilic phase activity in the early phase of the process. During the 120 day period, there was a low level of organic matter evolution, indicated by the limited changes of organic C, total nitrogen, as well as of the humification characteristics. However, the decrease of volatile acids and phenolic compounds with time, associated with a decrease towards constantly low values of microbial respiration rate, suggests the reaching of an acceptable level of compost maturity.

The initial phase of composting, either immediately or at most within a short space of time, was characterized by a decrease of biochemical parameters characteristic of biological activity. Dehydrogenase and catalase activities, BIF and FDA show only small changes under composting and do not enable us to monitor dynamics of substrate transformation. Although cumulative microbial respiration and  $qCO_2$  showed some limitations for measuring the dynamics of substrate transformation during the initial stages of composting, together with urease activity they were found to be sufficiently suitable for assessing changes occurring during composting.

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## References

- Adani, F., P.L. Genevini, F. Gasperi and G. Zorzi. 1997. Organic matter evolution index (OMEI) as a measure of composting efficiency. *Compost Science & Utilization*, 5 (2): 53-62.
- Anderson, T.H. and K.H. Domsch. 1993. The metabolic quotient for CO<sub>2</sub> ( $qCO_2$ ) a specific activity parameter to assess the effect of environmental conditions, such as pH, on the microbial biomass of forest soils. *Soil Biology Biochemistry*, 25: 393-395.
- Ayuso, M., J.A. Pascual, C. García and T. Hernández. 1996. Evaluation of urban wastes for agricultural use. *Soil Sci. Plant Nutr.*, 42 (1): 105-111.
- Barberis, R. and P. Nappi. 1996. Evaluation of compost stability. In: de Bertoldi, M., P. Sequi, B. Lemmes and T. Papi (eds.). *The Science of Composting*, Blackie Academic & Professional, London, pp.175-184.
- Beck, T.H. 1971. Die Messung der Katalaseaktivität von Boden. *Z. Pflanzenernähr Bodenkd.*, 130: 68-81.
- Belete, L., W. Egger, C. Neunhauserer, B. Caballero and H. Insam. 2001. Can community level physiological profiles be used for compost maturity testing? *Compost Sci-*

- ence & Utilization, 9 (1): 6-18.
- Benitez, E., R. Nogales, C. Elvira, G. Masciandaro and B. Ceccanti. 1999. Enzyme activities as indicators of the stabilization of sewage sludges composting with *Eisenia foetida*. *Bioresource Technology*, 67: 297-303.
- Butler, T.A., L.J. Sikora, P.M. Steinhilber and L.W. Douglass. 2001. Compost age and storage effects on maturity indicators of biosolids compost. *Journal Environmental Quality*, 30: 2141-2148.
- Casida, L.E. jr, D.A. Klein and T. Santoro. 1964. Soil dehydrogenase activity. *Soil Science*, 98: 371-376.
- Cegarra, J., A. Vasquez, F. Costa, A. Lax and E. Morgan. 1986. Changes undergone by some characteristics of organic wastes during the composting process. In: de Bertoldi M., M.P. Ferranti, P. L'Hermite and F. Zucconi (eds). *Compost: production, quality and use*. Elsevier Appl. Sci., London, 776-780.
- Chefetz, B.F., F. Adani, P.L. Genevini, F. Tambone, Y. Hadar and Y. Chen. 1998. Humic-acid transformation during composting of municipal solid waste. *Journal Environmental Quality*, 27: 794-800.
- Chen, Y., B. Chefetz and Y. Hadar. 1996. Formation and properties of humic substance originating from composts. In: de Bertoldi M., P. Sequi, B. Lemmes and T. Papi (eds). *The Science of composting*. Blackie Academic & Professional, London, pp. 382-393.
- De Nobili M., M.T. Baca, F. Fornasier and C. Mondini. 1996. Ninhydrin reactive nitrogen of CHCl<sub>3</sub> fumigated and non-fumigated compost extracts as a parameter to evaluate compost stability. In: de Bertoldi M., P. Sequi, B. Lemmes and T. Papi (eds). *The Science of composting*. Blackie Academic & Professional, London, pp. 255-261.
- Diaz-Burgos, M.A., B. Ceccanti and A. Polo. 1993. Monitoring biochemical activity during sewage sludge composting. *Biology Fertility Soils*, 16: 145-150.
- Di Lallo, R. and O.E. Albertson. 1961. Volatile acids by direct titration. *J.W.P.F.C.*, 14: 356-364.
- Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, 28: 350-356.
- Eggen, T. and O. Vethe. 2001. Stability indices for different composts. *Compost Science & Utilization*, 9 (1): 19-26.
- Fischer, J.L. 1998. Avoidance of biorisks of composting by thermohygieneization: influence of the type of system and management on the occurrence of the potentially pathogenic mold *Aspergillus fumigatus* and fecal indicator bacteria. Ph. D. thesis, University of Neuchâtel.
- Forster, J.C., W. Zech and E. Würdinger. 1993. Comparison of chemical and microbiological methods for the characterization of the maturity of composts from contrasting sources. *Biology Fertility Soils*, 16: 93-99.
- Gomes, A.F., J.S. Lima and T.C.N. Martinez. Aspectos epidemiológicos, amonificação e catalase na avaliação da maturidade de composto orgânico In: XVII seminário estudantil de pesquisa, 1998, Salvador. Anais do XVII Seminário Estudantil de Pesquisa. UFBA, 1998. v.1. p. 456.
- Grego, S., M. Mezzetti, G. Bucci, D. Corradini and E. Minzione. 2000. Changes in the apolar organic fraction through the composting process. *Compost Science & Utilization*, 8 (2): 116-123.
- Hänninen, K. and R. Lilja. 1994. Humification during the composting of slaughter wastes. In: Senessi, N. and T. Miano (eds.). *Humic Substances in the Global Environment and Implications on Human Health*, Elsevier Science B.V., pp.1265-1272.
- He, X.T., T.J. Logan and S.J. Traina. 1995. Physical and chemical characteristics of selected U.S. municipal solid waste composts. *Journal Environmental Quality*, 24: 543-552.
- Hirai, M., V. Chanyasak and H. Kubota. 1983. A standard measurement for compost maturity. *BioCycle*, 24: 54-59.
- Iannotti, D.A., M.E. Grebus, B.L. Toth, L.V. Madden and H.A.J. Hoitink. 1994. Oxygen respirometry to assess stability and maturity of composted municipal solid-waste. *Journal Environmental Quality*, 23: 1177-1183.
- Jenkinson, D.S. and D.S. Powlson. 1976. The effects of biocidal treatments on metabolism in soil. V. A method for measuring soil biomass. *Soil Biology Biochemistry*, 8: 209-213.
- Kuwatsuka, S. and H. Shindo. 1973. Behavior of phenolic substances in the decaying process of plants. I. Identification and quantitative determination of phenolic acids in the rice straw and its decayed products by gas chromatography. *Soil Sci. and Plant Nutr.*, 19, 219-226.
- Ladd, J.N. and J.H.A. Butler. 1975. Humus-enzyme system and synthetic organic polymer enzyme analogues. In: Paul, E.A. and A.D. McLaren (eds). *Soil Biochemistry*. Marcel Dekker, New York, pp. 143-194.
- Lasaridi, K.E. and E.I. Stentiford. 1998. A simple respirometric technique for assessing compost stability. *Water Research*, 32: 3717-3723.
- Levi-Minzi, R. and R. Riffaldi. 1988. Chemical differences between fresh and composted municipal wastes. *Agricoltura Mediterranea*, 118: 273-281.
- Lowe, L. E. 1975. Fractionation of acid soluble components of soil organic matter using polyvinyl pyrrolidone. *Canadian Journal Soil Science*, 55: 119-126.
- Mobley, H. and R.P. Hausinger. 1989. Microbial ureases: significance regulation and molecular characterization. *Microbiological Review*, 53: 85-108.
- Nannipieri, P., B. Ceccanti, S. Cervelli and P. Sequi. 1978. Stability and kinetic properties of humus-urease complexes. *Soil Biology Biochemistry*, 10: 143-149.
- Nannipieri, P., S. Greco and B. Ceccanti. 1990. Ecological significance of the biological activity in soil. In: Bollag, J.M. and G. Stotzky (eds). *Soil Biochemistry*. Marcel Dekker, New York, pp. 293-355.
- Nelson, D.W. and L.E. Sommers. 1982. Total carbon, organic carbon and organic matter. In: Page, A.L. et al. (eds). *Methods of Soil Analysis, Part 2, Am. Soc. Agron.*, Madison, Wisconsin, pp. 539-594.
- Outmane, A., M.R. Provenzano, M. Hafidi and N. Senesi. 2000. Compost maturity assessment using calorimetry, spectroscopy and chemical analysis. *Compost Science & Utilization*, 8 (2): 124-134.
- Roletto, E., R. Chiono and E. Barberis. 1985. Investigation on humic substances from decomposition poplar bark. *Agricultural Wastes*, 12: 261-271.
- Saviozzi, A., R. Levi-Minzi, R. Riffaldi and A. Benetti. 1992. Evaluating garbage compost: 2. Water extract and decomposition rate in soil. *BioCycle*, 33 : 72-75.
- Saviozzi, A., R. Levi-Minzi, R. Cardelli and R. Riffaldi. 2001. A comparison of soil quality in adjacent cultivated, forest and native grassland soils. *Plant and Soil*, 233: 251-259.
- Schnürer, J., M. Clarholm and T. Rosswall. 1985. Microbial biomass and activity in an agricultural soil with differ-

- ent organic matter contents. *Soil Biology Biochemistry*, 17: 611-618.
- Senesi, N. and G. Brunetti. 1996. Chemical and physico-chemical parameters for quality evaluation of humic substance produced during composting. In: de Bertoldi M., P. Sequi, B. Lemmes and T. Papi (eds). *The Science of composting*. Blackie Academic & Professional, London, pp. 195-212.
- Sequi, P., M. De Nobili, L. Leita and G. Cercignani. 1986. A new index of humification. *Agrochimica*, 30: 175-179.
- Singh, C.P. and A. Amberger. 1996. Chemical characteristics of humic substances extracted from wheat straw compost. In: Clapp C.E., M.H.B. Hayes, N. Senesi and S.M. Griffith (eds). *Humic substances and organic matter in soil and water environment: characterization, transformation and interaction*. International Humic Substances Society, Inc. University of Minnesota, St. Paul, USA, pp. 441-451.
- Snedecor, G.W. and W.G. Cochran. 1978. *Statistical methods*. The Iowa State University Press. Ames, Iowa, USA.
- Stefanic, G., G. Eliade and I. Chirnogeanu. 1984. Researches concerning a biological index of fertility. In: Nemes, M.P., Kiss, S., Papacostea, P., Stefanic, G., Rusan, M. (Eds.), *5th Symposium on Soil Biology* (Rumanian National Society of Soil Science), Bucharest, Rumania, 35-45.
- Swisher, R. and G.C. Carrol. 1980. Fluorescein diacetate hydrolysis as an estimator of microbial biomass on coniferous needle surfaces. *Microbial Ecology*, 6: 217-226.
- Tabatabai, M.A. 1982. Soil Enzymes. In: Page, A.L., R.H. Miller, D.R. Keeney (Eds), *Methods of Soil Analysis*, part 2. Chemical and Microbiological Properties. ASA and SSSA, Madison, Wisconsin. pp. 903-947.
- Tomati, U., E. Madejon and E. Galli. 2000. Evolution of humic acid molecular weight as an index of compost stability. *Compost Science & Utilization*, 8 (2): 108-115.
- Trinchera A., F. Pinzari and A. Benedetti. 2001. Should be able to define soil quality before "restoring" it? Use of soil quality indicators in Mediterranean ecosystems. *Minerva Biotecnologica*, 13: 13-18.
- Vance, E.D., P.C. Brookes and D.S. Jenkinson. 1987. An extraction method for measuring soil microbial biomass carbon. *Soil Biology Biochemistry*, 19: 703-707.
- Wardle, D.A. and A. Ghani. 1995. A critique of the microbial quotient (qCO<sub>2</sub>) as a bioindicator of disturbance and ecosystem development. *Soil Biology Biochemistry*, 27: 1601-1610.
- Wu, L., L.Q. Ma and G.A. Martinez. 2000. Comparison of methods for evaluating stability and maturity of biodolids compost. *Journal Environmental Quality*, 29: 424-429.
- Wu, L. and L.Q. Ma. 2001. Effects of sample storage on biosolids compost stability and maturity evaluation. *Journal Environmental Quality*, 30: 222-228.

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