

Evolution of Carbon Compounds during Municipal Solid Waste Composting: Suitability of Chemical and Biochemical Parameters in Defining the Stability and Maturity of the End Product

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ABSTRACT

In this review we discuss the transformation of organic matter during municipal solid waste (MSW) composting and the suitability of chemical and biochemical parameters in defining the stability and maturity of the end product. The dynamics of chemical and biochemical parameters, such as several hydrolase activities and water-soluble fractions (i.e. water soluble carbon, water soluble nitrogen, water soluble carbohydrate) during MSW composting is discussed along with their suitability as tools for compost characterization. Besides, the evolution during the MSW process of the molecular structure of humic acid (HA), based on Fourier transform infrared (FTIR), ¹³C NMR spectroscopy and thermal analysis, is also discussed. The data presented highlight the suitability of several enzymatic activities and water soluble fractions as indicators of the evolution of the organic matter during MSW composting. However, since the extent of each enzyme activity at the end of composting depends on the starting material used, single point determinations appear inadequate for compost characterization. Moreover, the importance of the characterization of the dynamics of enzymatic activities and water-soluble fractions during the process has been emphasized. FTIR and ¹³C NMR spectra appeared useful tools reflecting the rate of change in HA structure during MSW composting. Thermoanalytical analyses (TG/DTG and DSC curves) also revealed valuable in order to characterize the organic matter during composting and distinguish between well and poorly stabilized organic matter.

Keywords: MSW compost, enzymatic activities, water soluble fractions, FTIR spectroscopy, ¹³C NMR spectroscopy, thermal analysis

Abbreviations: DSC, differential scanning calorimetry; DTG, differential thermogravimetry; FTIR, Fourier Transform-Infrared; HA, humic acid; MSW, municipal solid waste; NMR, nuclear magnetic resonance; OM, organic matter; TG, thermogravimetry; WS, water soluble

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INTRODUCTION

Soil organic matter (OM) is universally recognized to be among the most important factors responsible for soil structure, water holding capacity, pH buffering, complexing ions, crop production, and land protection from contamination, erosion and desertification (Wilson 1991; Piccolo 1996; Senesi *et al.* 2007; El Hajjouji *et al.* 2008). Crop residues and animal manure have been conventionally applied to agricultural soil as a means of maintaining, or increasing,

OM content and fertility status. However, as a result of adverse climatic conditions and unsustainable soil management practices (such as the extensive use of chemical fertilizers and the over-exploitation of the soil resource), a growing scarcity of OM has been recently recorded in soils of many Mediterranean regions. This has led to the search for new and cheap sources of OM able to replace the losses caused by mineralization and crop production (Hernández *et al.* 2006), stabilise and protect the soil, and reduce the application of chemical fertilizers.

On the other hand, recent estimates suggested that the municipal solid waste (MSW) alone exceeded (at a global level) 2 billion tonnes per year at the beginning of the new millennium (e.g. Giusti 2009). Particularly, in 2006 the 15 countries of the European Union generated 219 million tonnes of MSW (OECD 2008). The large amounts of MSW produced in the modern society, as well as their disposal, have a profound socio-economic and environmental impact. The most important European legislation dealing with organic waste recycling is the "Directive on the Biological Treatment of Biological Waste" (European Commission 2001). This Directive is aimed at promoting the biological treatment of biodegradable waste (i.e. through composting and anaerobic digestion) and help meet the Landfill Directive targets (Council Directive of the European Union 1999). Based on the "Directive on the Biological Treatment of Biological Waste" only treated biowastes would be allowed for spreading on land, except for those untreated biowastes specifically mentioned in this Directive such as vegetable plant wastes.

In principle, MSW could be applied, as organic amendment, to agricultural soils and used for the rehabilitation of degraded areas. However, the direct use of these wastes as they are may be critical because the presence of xenobiotics, pathogenic microorganisms and phytotoxic activity are quite common as the presence of bad odours.

Among the techniques of disposal, the composting of the organic fraction of MSW is an environmentally friendly and economic solution (Veeken and Hamelers 2002). The composting process can be viewed as an accelerated version of the natural processes of transformation of organic debris in soil, and can be achieved by providing optimized environmental conditions for the microorganisms that carry out the process.

During the composting process, organic compounds are biologically oxidized through the successive activities of different microbial species to a more stable and complex OM (García *et al.* 1992a). The rate and extent of these transformations are influenced by the nature of the starting materials and the conditions applied (Hernández *et al.* 2006). In particular, moisture and aeration are critical (it should be kept in mind that composting is a biological oxidation) in order for the process to start and to be maintained until the transformation of organic substrates into stabilized OM. This is a critical point since the application of unstable compost to soil may be responsible for several environmental issues. For example, Mathur and co-workers (1993) showed that unstabilized compost can induce a competition for oxygen between soil microorganisms and plant roots/seeds. In this case, these latter can possibly suffer for oxygen deprivation. Moreover, the application to soil of unstabilized compost with a high C to N ratio can indirectly induce nitrogen starvation in plants since soil microbial populations have a higher soil N demand due to the unbalanced C to N ratio in compost. Phytotoxicity issues can also arise from the application to soil of not completely stabilized OM. Several intermediate compounds of the microbial metabolism can accumulate in such a medium (e.g. phenolic compounds and organic acids) exerting toxic effects to crops (Fuchs 2002).

It is now generally accepted that the main constraints for a safe use of compost in agriculture are its degree of stability and maturity. This implies the presence in compost of stable OM and the absence of phytotoxic effects (Chen and Inbar 1993; Castaldi *et al.* 2004; Mondini *et al.* 2004; Castaldi *et al.* 2008). Stability and maturity were sometimes treated as different terms for defining the same compost property with no real distinction. However, nowadays it is generally agreed that compost stability refers to the degree of OM decomposition by microbial activities and can be evaluated by respirometric measurements (Adani *et al.* 2003) or by monitoring the evolution of chemical (e.g. HA) and biochemical (e.g. hydrolase activities) characteristics of the OM in compost (Chefetz *et al.* 1998a; Castaldi *et al.* 2008). On the contrary, the maturity concept is mostly

related to the presence or absence in compost of phytotoxic compounds produced during the active composting stage (Benito *et al.* 2005). In this regard it should be mentioned that, as stressed by Said-Pullicino *et al.* (2007), phytotoxic effects can be due, other than to the presence of organic compounds originated during composting, also by other factors such as excess of soluble salts or heavy metals. This is likely why there is not a universal method for measuring compost maturity even if several plant or seed assays are widely employed for this aim (Bernal *et al.* 1998; Wu *et al.* 2000). Besides, in most cases maturity measurement implies the combination of several parameters (Iannotti *et al.* 1993).

The identification of suitable physicochemical and/or biological parameters able to define the actual degree of OM evolution during the composting process, is not an easy task and is currently under debate (Castaldi *et al.* 2008). The identification of such parameters requires a deep knowledge of the factors conditioning the transformation and evolution of the OM during the different composting processes.

Many parameters have been proposed for the evaluation of compost stability and maturity, with varying degrees of reliability and technical complications. These include physicochemical properties, such as C/N ratio, quantification of humified organic substances, water soluble carbon and cation exchange capacity (Grigatti *et al.* 2004; Castaldi *et al.* 2005; Zmora-Nahum *et al.* 2005; Albrecht *et al.* 2008), as well as biological properties. Microbial respiration and enzyme activities are amongst these latter the more commonly applied (Adani *et al.* 2003; Mondini *et al.* 2004; Tiquia 2005; Albrecht *et al.* 2008; Castaldi *et al.* 2008).

Besides, some spectroscopic techniques, such as Fourier Transform-Infrared (FTIR) (i.e. Chefetz *et al.* 1998a), ¹³C Nuclear Magnetic Resonance (¹³C NMR) (i.e. Chefetz *et al.* 1998b; Chen 2003), mono-, two-, and three dimensional fluorescence (i.e. Senesi *et al.* 1996; Provenzano *et al.* 2001; Wei *et al.* 2008), electron spin resonance (i.e. Mikki *et al.* 1997) spectroscopy, and thermal analyses (i.e. Blanco and Almendros 1994; Provenzano *et al.* 2000) were widely used in the past and nowadays by several researchers to characterize MSW composting and end-products.

Given the complexity of the biological events occurring during composting, the different factors governing the process and the heterogeneity of the starting mixtures, it is not surprising the occurrence of results that are sometimes contradictory. The number of chemical and biological changes occurring during composting and the number of methods used to monitor these parameters (Ayuso *et al.* 1996; Melis and Castaldi 2004; Castaldi *et al.* 2005) have resulted in a certain difficulty to agree on a single method for the assessment of the stability and maturity of composts derived from organic wastes (Benito *et al.* 2003; Wang *et al.* 2004; Castaldi *et al.* 2008). On these bases, it is understandably difficult to build up a general and complete model describing the OM evolution during composting and the need of gathering further scientific data related to the process is indeed urgent.

Generally, most of the physical and chemical methods, microbiological tests, plant assays and humification indexes currently in use for the evaluation of compost stability and maturity are empirically-based criteria which are often not self-consistent. On the other hand, a general index of maturity, independent of the source material or the conditions applied during composting, would be really helpful for the consistent production of high quality compost and would have major practical and economical relevance.

In the following pages we review the transformations of OM during the MSW composting process and discuss the suitability of several physicochemical and biochemical parameters in defining the stability and maturity of the end product. In particular, the dynamics of several hydrolase activities, total organic carbon, humic carbon, and water-soluble fractions, during MSW composting will be reviewed and their effectiveness as quality-evaluation tools

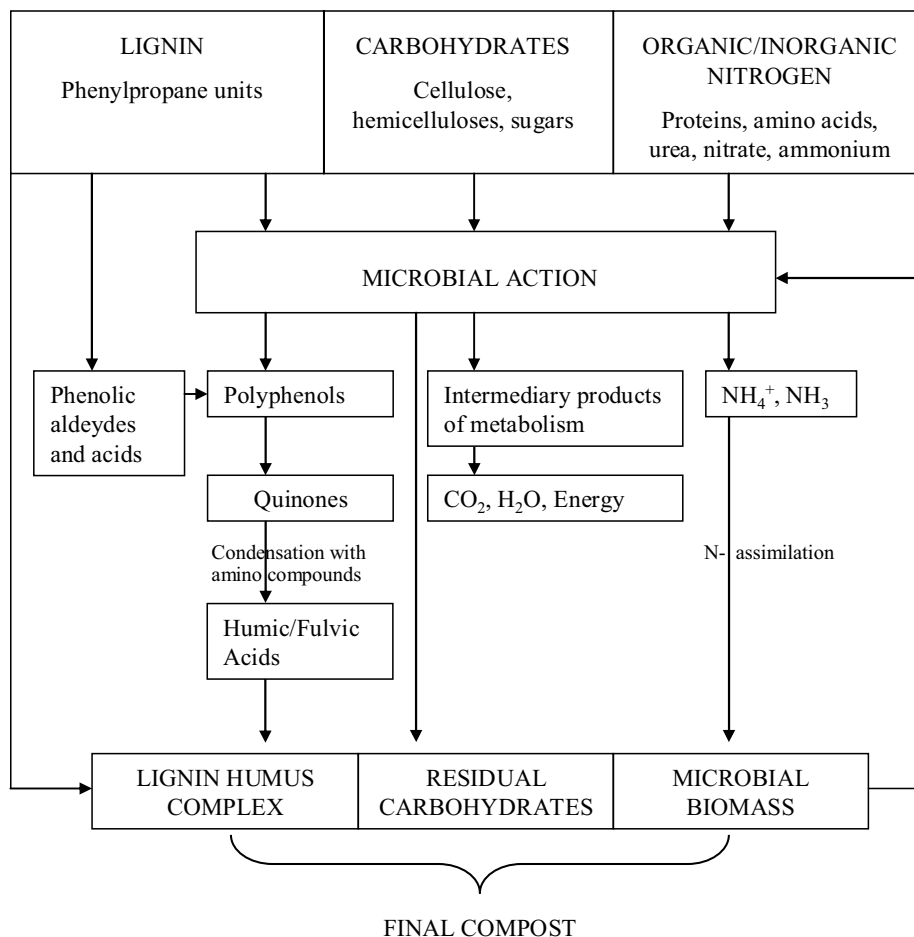


Fig. 1 Simplified carbon degradation pathway during municipal solid waste composting.

discussed. Besides, special emphasis will be put on the evolution of the molecular structure of HA during the process as revealed by thermal analysis, Fourier Transform-Infrared (FTIR) and ^{13}C Nuclear Magnetic Resonance (^{13}C NMR) spectroscopy.

THE COMPOSTING PROCESS

Composting is a bio-oxidation process carried out by diverse microbial populations under aerobic conditions. This process basically involves the degradation of organic residues of plant and animal origin and their conversion into a stabilized product. It is largely a microbiological process based upon the activities of several bacterial groups and fungi (de Bertoldi *et al.* 1983). The main product arising from the composting process is rich in humus and plant nutrients whereas carbon dioxide, water, and heat are common by-products (Gajalakshmi and Abbasi 2008; **Fig. 1**). During the composting process, aerobic microorganisms metabolize simple and more complex organic compounds to provide a more stable OM, which chemically and biologically resembles humic substances (**Fig. 1**). The rate and extent of these transformations depend on both the available substrates as well as on the conditions of the process (Gajalakshmi and Abbasi 2008). In particular, the effectiveness of the composting (i.e. the mineralization and humification of the starting materials) is largely dependent upon the environmental conditions present within the composting system, such as oxygen tension, temperature, moisture, material disturbance and the quantity and quality of OM. All these factors contribute to provide a suitable environment for the activities of the different microbial populations involved in the process.

The composting process can be divided into four phases: (i) an initial (first) mesophilic phase (temperature range 10-42°C), which may last for a few hours or a couple of

days; (ii) a thermophilic phase (active phase, temperature range 45-70°C), which may last for a few days, several weeks or even months (particularly for wood wastes); (iii) second mesophilic phase during which mesophilic microorganisms, often dissimilar from those present in the first mesophilic phase, re-colonize the substrate; and (iv) the maturation (or curing) and stabilization phase which can last from some weeks to several months. The first two phases of the process are characterized by an intense microbial activity leading to the decomposition of easily biodegradable organic compounds and stabilization of organic residue. The lasting (i.e. humification) phases are characterized by the conversion of part of the remaining organic material into humic-like substances (Chen and Inbar 1993; Castaldi *et al.* 2005).

Temperature is most likely the dominant parameter controlling microbial activity during the process (McKinley *et al.* 1985). The high metabolic activity of microorganisms at the beginning of the process is responsible for a rapid increase of the temperature that, in the thermophilic phase (70-80°C), strongly selects for thermophilic bacteria. High temperatures in this phase are helpful to eliminate weed seeds and control microbial pathogens (Boulter *et al.* 2000). The slowing down of temperatures in the following phases promotes a re-colonization of the composting matrix with mesophilic microorganisms that continue the decomposition process. In these latter phases (second mesophilic and curing phases) the breakdown of recalcitrant components occurs. This is usually accompanied by a shift in the microbial community towards mainly eumycetes and actinomycetes that are able to degrade cellulose, hemicellulose and lignin (Boulter *et al.* 2000).

EVOLUTION OF CHEMICAL AND BIOCHEMICAL PARAMETERS DURING THE MSW COMPOSTING PROCESS

pH

At the beginning of the composting process the pH should be within tolerable limits for microbial growth, e.g. bacteria generally have an optimum pH range for growth between 6 and 8.0 and below pH 5.0 bacterial bio-control agents are inhibited (Hoitink *et al.* 1997). Fungi and actinomycetes generally can grow optimally in a wider range of conditions (pH ranges between 5.5-8.0 and 5.0-9.0 respectively) (Goodfellow and Williams 1983; Boulter *et al.* 2000). Indeed, the pH of the composting piles clearly affects the potential for beneficial bacteria to effectively colonize the compost piles. The pH is also an important indicator of the aeration level during composting. Well-aerated composting piles generally show higher pH values compared to piles in which anaerobic conditions are prevailing (Boulter *et al.* 2000).

It has been shown by several authors that a pH drop (between 0.2-0.8 units) generally occurs at the beginning of the process (Said-Pullicino *et al.* 2007; Gómez-Brandon *et al.* 2008) as a consequence of the bacterial activity that metabolize complex organic compounds to produce intermediates such as organic acids. The pH drop during the very early stages of composting has been also attributed to partial anaerobic conditions that can occur in the pile at the beginning of the process, and can result in the formation of organic acids (El Hajjouji *et al.* 2008). As aerobic conditions are provided through forced aeration and/or turning of the composting mass the pH generally increases as a consequence of organic acids degradation (Said-Pullicino *et al.* 2007). Several authors, i.e. Beck-Friis *et al.* (2003), also pointed out that the change of mesophilic to thermophilic conditions during MSW composting is accompanied by a pH change from slight acidic (<6) to alkaline (8-9). This pH increase during the active phase of the composting has been explained by some authors as an alkalization of the mixture subsequent to the release of ammonia through the mineralization of organic compounds and consequent release of alkaline ions (Hellmann *et al.* 1997; Said-Pullicino *et al.* 2007; Gómez-Brandon *et al.* 2008; Raut *et al.* 2008). However, there is not a general agreement on this. For example, El Hajjouji *et al.* (2008) attributed the pH increase in the active phase of composting to a reduced level of carbon compounds with $-\text{COO}^-$ and/or $-\text{OH}^-$ groups due to microbial degradation and/or condensation-polymerisation reactions involving these moieties (El Hajjouji *et al.* 2008).

At the end of composting the pH values are generally higher than 7. Alkaline pH values in the end product can be used as an empirical indicator of compost maturity since organic acids of low molecular weight are generally phytotoxic, and not expected to be present in mature and stable compost. For example, Said Pullucino *et al.* (2007) and Komilis and Tziouvaras (2009) reported that the pH of MSW and lignocellulosic residues increased during the process reaching values above 8 at maturity. This could have significant implications on the fertility and productivity of soils amended with such a compost, as well as on the growth of pH-sensitive plants.

Total Organic Carbon (TOC)

The stabilization of organic wastes which is realized through the composting is mainly based on biodegradation of the total organic carbon (TOC). Indeed, compost stability could be expressed as a function of the degradation of TOC (Cooperband *et al.* 2003) and this parameter could be helpful in the monitoring of the process and evaluation of compost quality. Generally, the degradation of OM during composting depends on the materials used and the rate of TOC decomposition is strongly related to the microbial activity in the composting mixture (Zmora-Nahum *et al.* 2005). For

instance, mixtures of MSW and materials with high lignin content showed a slower OM degradation (because of lignin's resistance to degradation) compared to MSW composting alone. In this latter pile the reduction of the OM content (in the same period) was more than 70% of the initial values (Sánchez-Monedero *et al.* 1999).

During the first month of MSW composting a rapid decrease of the TOC concentration is commonly recorded (Fig. 2): during this time period, encompassing the first mesophilic and the "active phase", thermophilic and mesophilic bacteria decompose the easily available and degradable organic compounds producing CO_2 , H_2O , and heat. Subsequently, the rate of OM degradation decrease and OM maturation and stabilization generally take place. During this second period or "curing phase" the TOC values generally do not vary significantly and reach values between 17-27% dry matter (dm) (Fig. 2) (Melis and Castaldi 2004; Said-Pullicino *et al.* 2007; Barrena *et al.* 2009).

Nitrogen forms

A large majority of studies showed that significant losses of total organic nitrogen occur during MSW composting (Fig. 2). These losses vary depending on several factors that regulate the composting process such as aeration, moisture content, temperature and C/N ratio (De Gioannis and Muntoni 2007). Barrington and co-workers (2002) quantified N-losses during composting ranging between 19.3 and 61.5% of dry matter (dm) whereas Martin and Dewes (1992) estimated N-losses between 9.6 and 19.6% dm. These losses mostly occurred through leaching during the first phase of the aerobic treatment. However, increases of organic N during the process were also reported by some authors. For instance, De Gioannis and Muntoni (2007) showed an increase of the concentration of total organic nitrogen during MSW composting, especially during the intense bio-oxidative phase (thermophilic phase). This was explained as a likely consequence of the hydrolysis of complex nitrogenous compounds present in the biodegradable fractions of the composting wastes. Other authors explained such increase as a concentration effect due to weight reduction of the composting pile (Bustamante *et al.* 2008).

For what it concerns the inorganic N, some researchers observed significant increases of ammonium nitrogen (N-NH_4^+) during the active phase of the process indicating an intense mineralization of organic N-compounds, active OM transformation, and hence the presence of a still unstable substrate (Zucconi and de Bertoldi 1987; Bernal *et al.* 1998; Tognetti *et al.* 2007; Gómez-Brandon *et al.* 2008). N-NH_4^+ is an important N-source for microorganisms, which rapidly incorporate it into different organic compounds (assimilation) increasing the total microbial biomass. The rate of nitrogen assimilation by microbial populations depends on the rate of C assimilation and microbial C/N ratio, and varies during the process (De Gioannis and Muntoni 2007). The rate of microbial nitrogen uptake during MSW composting is known to reach a peak within the first 20-30 days of the process, while it drops down during the following phases when fungi and actinomycetes proliferate (De Gioannis and Muntoni 2007; Said-Pullicino *et al.* 2007).

During the active phase of MSW composting an important part of the total nitrogen loss is due to ammonia volatilization and/or immobilisation processes, which are favoured by high temperatures and high pH values (between 7.7-8.2) (García *et al.* 1991). Sánchez-Monedero *et al.* (2001) reported N losses due to ammonia volatilization of 41% dm during MSW composting. A decrease and a stabilization of the N-NH_4^+ concentration has been recorded by several authors at the end of the thermophilic phase (Fig. 2) (Tognetti *et al.* 2007; Castaldi *et al.* 2008). The decrease to near-zero concentrations of N-NH_4^+ at the end of the process is likely due to a progressive utilization of N-NH_4^+ by nitrifying bacteria. During the nitrification process nitrifying bacteria oxidize the N-NH_4^+ to N-NO_3^- (nitrate) via N-NO_2^- (nitrite). This process is usually carried out at mat-

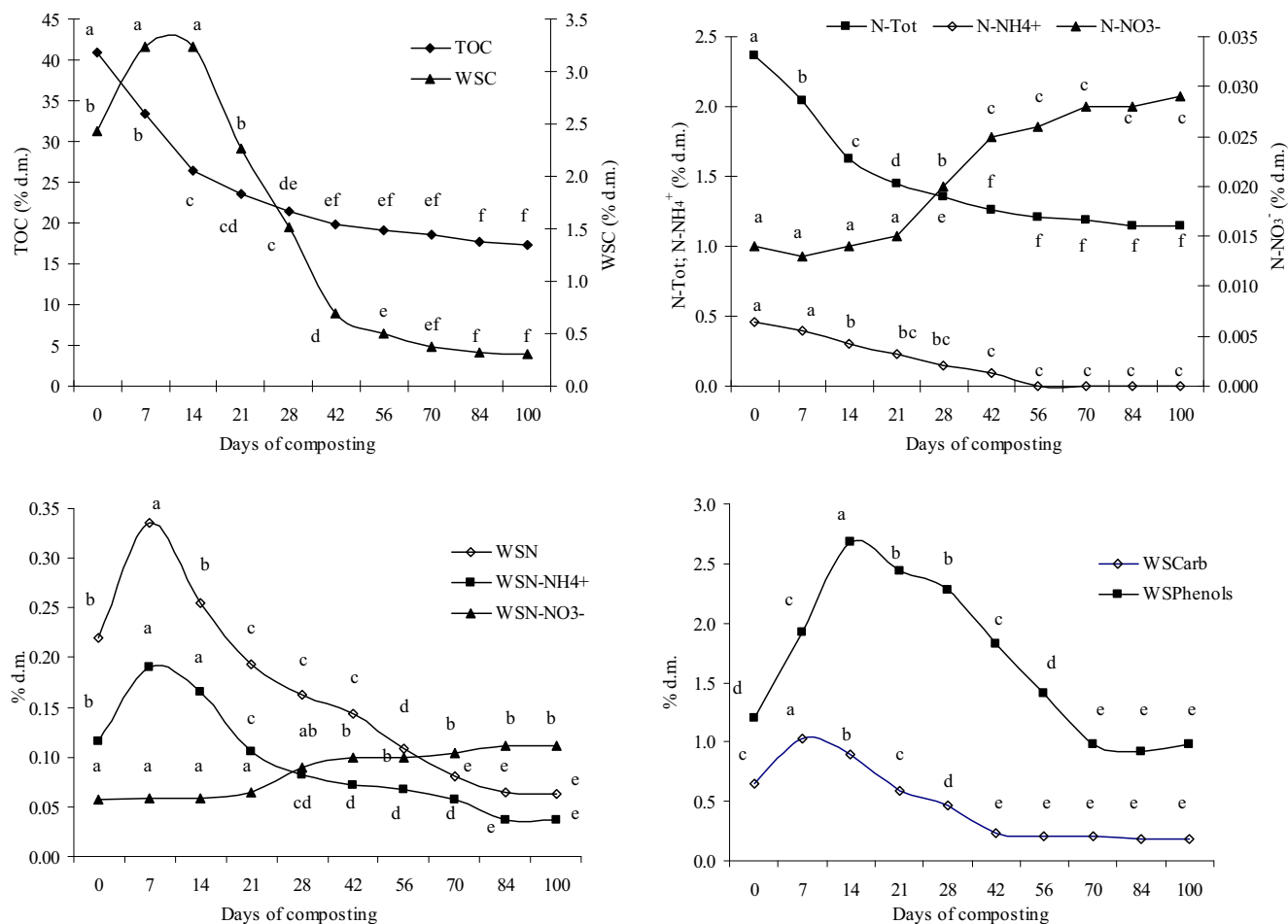


Fig. 2 Evolution of some chemical parameters (TOC; WSC; TN; N-NH₄⁺; N-NO₃⁻; WSN; WSN-NH₄⁺; WSN-NO₃⁻) during MSW composting. (For each parameter data with same letter do not differ significantly at the 5% level according to the Fischer's Least Significant Difference test)*. * Note: Elaborated from: Melis and Castaldi 2004; Castaldi *et al.* 2005, 2008.

rix temperatures below 40 °C and under favourable aeration conditions (Tognetti *et al.* 2007; Said-Pullicino *et al.* 2007; Bustamante *et al.* 2008). The final N-NH₄⁺ values reached in MSW composts should be in general below 400 mg kg⁻¹ dm, that is the maximum recommended value for a mature compost (Zuconi and de Bertoldi 1987; Bustamante *et al.* 2008).

Low concentrations of nitrates are common at the beginning of the MSW composting process. Nitrates concentration then increases at the end of the active (thermophilic) phase and during the following mesophilic phase, suggesting active nitrification (Tognetti *et al.* 2007; Bustamante *et al.* 2008). However, the increase of nitrates during these phases is not always directly proportional to the decrease of N-NH₄⁺ (see for example Fig. 2). This could be due to an inhibition of the activity of nitrifying bacteria as a consequence of not optimal temperatures, partial volatilization of N-NH₄⁺ or excessive amount of ammonia, leaching of N-NO₃⁻ with the watering of the maturation piles, and denitrification processes (Huang *et al.* 2004; Tognetti *et al.* 2007; Bustamante *et al.* 2008).

At the end of the MSW composting, but only if the process has been carried out under proper aeration conditions, the N-NO₃⁻ content should be always higher than that of N-NH₄⁺ (Bernal *et al.* 1998). The NH₄⁺/NO₃⁻ ratio has been used to evaluate compost stability and a limit value of 0.16 has been proposed for stable composts (Bernal *et al.* 1998). However, since the mineral N forms can change irregularly during MSW composting (see for example Said-Pullicino *et al.* 2007), their concentration in the final compost can be globally considered as an unreliable indicator of compost stability (Gómez-Brandon *et al.* 2008) and should be carefully interpreted.

Minimising nitrogen losses during the composting pro-

cess is always desirable since this determines an increase of the N concentration in the final product. This also means a higher agronomic and economic value of the compost and a limited odour generation during the process (Tognetti *et al.* 2007; Bustamante *et al.* 2008).

Carbon/nitrogen ratio (C/N ratio)

It is widely agreed that the general optimum C/N ratio in the starting material to be composted should be between 25–35 (de Bertoldi *et al.* 1983). If this ratio is greater than 40, the bio-oxidation activity within the composting pile is usually slowed, whereas excessive nitrogen in the starting material (low C/N values) allow a rapid decomposition and significant nitrogen losses due to ammonia volatilization, especially at high pH and temperature values (Sanchez-Monedero *et al.* 2001; Goyal *et al.* 2005; Bustamante *et al.* 2008).

During MSW composting the C/N ratio generally decreases, especially at the beginning of the process, due to the carbon losses as CO₂. This ratio then stabilizes as the process proceeds ending up the final compost in the range of 10–15 (Margesin *et al.* 2006; Bustamante *et al.* 2008). Values of the C/N ratio <20 usually indicate a suitable maturation degree of the compost (Bustamante *et al.* 2008). However, the use of the C/N ratio as an index to evaluate compost maturity has been also questioned (e.g. Goyal *et al.* 2005; Raut *et al.* 2008), since the values for well-composted materials present a great maturity variability, due to the characteristics of the waste used and to the methods of N measurement (Hue and Liu 1995). Perhaps to obtain a valid C/N ratio, this has to be based on the biodegradable carbon really available for the microorganisms (Sánchez 2007). Moreover, it has been pointed out that the C/N ratio can

also level off before the compost stabilizes (Namkoong *et al.* 1999). For example, when MSW are mixed with wastes rich in nitrogen, such as sewage sludge or manure, the C/N ratio can be within the above mentioned optimal values (that define a stable compost) even though it may still be unstable (Namkoong *et al.* 1999; Gómez-Brandon *et al.* 2008). On the other hand, the evolution of the C/N ratio can be more useful as a monitoring parameter along the evolution of the composting process and not as a concrete value of stability or maturity.

Water-soluble fractions

Most of the biochemical transformations of the organic compounds during the MSW composting take place in the water-soluble fraction (Gonzales *et al.* 1999; Castaldi *et al.* 2008). Indeed, the analysis and the characterization of the water-soluble fraction could be a rapid and useful tool for improving our understanding of the entire process and evaluating the evolution and stability of the OM during composting. The degradation of the different organic components (lignin, cellulose, hemicellulose, proteins and lipids), leads to the formation during composting of a number of simple water-soluble organic compounds such as carbohydrates, amino acids, peptides and phenolic compounds of lower structural complexity with respect to the starting components. All these simple organic compounds can be utilized by microorganisms as a source of carbon and energy or used as precursors for the synthesis of new macromolecules with properties similar to those of the humic substances in soils (Sánchez-Monedero *et al.* 1999; Fig. 1). Here we review a number of water soluble fractions that have been used to monitor and characterize the MSW composting process as well as the end product.

Water soluble carbon (WSC)

WSC is the most readily biologically active carbon fraction which maintains a high level of microbial activity in the composting pile. Sugars, hemicellulose, phenolic substances, amino acids, peptides, and other easily biodegradable compounds, account for the main organic carbon in water extracts of immature MSW compost (Hsu and Lo 1999; Castaldi *et al.* 2008). The study of the chemical and biological transformations occurring in this fraction can be helpful for a better understanding of the dynamics of MSW composting and assists in the prediction of the stability and maturity of the end product.

A slow increase of the WSC concentration is typical during the first two weeks of MSW composting (Fig. 2). This increase is likely due to a relatively fast solubilization of simple organic compounds that is not accompanied by a proportional degradation or utilization by microorganisms (Castaldi *et al.* 2008). On the other hand, since composting is also a process of synthesis this increase may be due to new WSC originated by the microbial activity (Hernández *et al.* 2006). After the second week of composting the WSC consistently decreases indicating that microbial populations, whose growth was largely supported by growth on easily degradable substrates, are acclimating to the degradation of more recalcitrant substrates (Zmora-Nahum *et al.* 2005; Hernández *et al.* 2006; Castaldi *et al.* 2008), eventually leading to compost stabilization (Bernal *et al.* 1998; Chefetz *et al.* 1998a; Said-Pullicino *et al.* 2007). Indeed, the reduction of WSC after the first two weeks of composting can be attributed to the consumption of easily degradable organic compounds as to the synthesis of complex organic molecules that precipitate or flocculate. These latter molecules arise from the continuous mineralization of soluble organic compounds and repolymerization and/or condensation pathways that lead to the formation of complex organic substrates with low solubility in water and which tend to flocculate out the liquid phase (Hernández *et al.* 2006; Said-Pullicino and Gigliotti 2007). During the curing phase the concentration of water-soluble organic carbon in the hydro-

phobic fraction increases until the concentration of carbon in this fraction is actually equal or even greater than that in the hydrophilic fraction. This increase has been attributed to (i) the contribution of hydrophobic constituents from the degradation of lignocellulosic materials in the bulk OM occurring during the curing phase, (ii) a faster mineralization of hydrophilic water-soluble organic moieties with respect to the more refractory hydrophobic constituents, and (iii) the transformation of hydrophilic components into hydrophobic moieties by abiotic polymerization and condensation reactions that occur in solution (Said-Pullicino *et al.* 2007).

The study of WSC evolution during composting satisfies a large number of requirements, it follows a consistent trend, its determination is neither time-consuming or expensive and is easy to interpret (Gómez-Brandon *et al.* 2008). Threshold values for this parameter have been proposed by different authors in order to evaluate the degree of compost stability. For instance, Eggen and Vethe (2001) and García *et al.* (1992a) defined a stable compost as that with WSC<0.5% d.m., while Bernal *et al.* (1998) (WSC<1.7% d.m.), Hue and Liu (1995) (WSC<1% d.m.) and Zmora-Nahum *et al.* (2005) (WSC<0.4% d.m.) proposed different values. It is clearly apparent from these latter studies that this parameter can not be used univocally to evaluate compost stability. Moreover, several authors argued on the possibility to define a threshold value for WSC in the final compost in order to judge its stability. WSC at the end of process is in fact largely dependent on the raw material used for composting (Goyal *et al.* 2005).

Water-soluble nitrogen (WSN), water-soluble ammonium (WSN-NH₄⁺) and water-soluble nitrate (WSN-NO₃⁻)

WSN and WSN-NH₄⁺ show a similar trend during MSW composting (Fig. 2). As generally recognized, soluble organic-N is subjected to ammonification and the increase of WSN-NH₄⁺ during composting reflects the ammonification of easily degradable compounds (Said-Pullicino *et al.* 2007). In particular, both WSN and WSN-NH₄⁺ have been shown to increase during the first 7-10 days of the process whilst decreasing afterwards (Fig. 2) (Castaldi *et al.* 2008). The increase of WSN and WSN-NH₄⁺ during the first days is probably due to a solid-liquid transfer of N fractions, while their subsequently depletion has been explained by several authors with the degradation of soluble amino acids, oligopeptides and proteins by microorganisms. Furthermore, certain organic compounds produced during composting can bind N-containing compounds removing them from solution and thus contributing to decrease the content of WSN. In particular, it is generally agreed that organic molecules arising from lignin degradation such as phenols, quinones and more complex compounds, are the main precursors in the formation of stabilized OM in compost. This latter process is mediated by their polymerization and condensation with N-compounds such as proteins, amino acids and nucleic acids (Sánchez-Monedero *et al.* 1999; Said-Pullicino *et al.* 2007).

Differently from WSN and WSN-NH₄⁺, the WSN-NO₃⁻ has been shown to gradually increase starting from the end of the active (thermophilic) phase of the MSW composting, reaching at the end of the process its highest value (Fig. 2). Nitrifying bacteria, which oxidize reduced N compounds for energy and fix carbon dioxide for their carbon source, are likely responsible for nitrate accumulation in compost during the curing phase (Castaldi *et al.* 2008). The contribution of autotrophic nitrifiers during MSW composting is basically absent during the first phase since the nitrification process is very sensitive to temperature (nitrification proceeds when temperature decreases under 50°C), as well as to excessive ammonia concentrations that inhibit the process. Moreover, the active degradation of easily degradable OM during the first phase of MSW composting decreases the availability of oxygen to these microorganisms (Tiqua and Tam 2000).

Water soluble carbohydrates (WS-Carb)

The WS-Carb fraction is mainly constituted by hexose and pentose monomers. Intuitively, the amount of these simple sugars in the water soluble fraction is rather indicative of the biochemical transformations of the matrix during composting.

The variation of WS-Carb during the MSW composting process is rather debated with some authors showing an increase of WS-Carb during the first 1-2 weeks of composting (Fig. 2) (e.g. Said-Pullicino and Gigliotti 2007; Castaldi *et al.* 2008) whereas others reported a sharp decrease during the same period (e.g. García *et al.* 1991; Sanchez-Monedero *et al.* 1999; Zmora-Nahum *et al.* 2005). As mentioned on top, WS-Carb are easily available carbon and energy sources for microorganisms and a sharp decrease, during the first part of the composting, is indeed expected. This could be also due to a delayed biodegradation of cellulose during composting which can be related to the sheathing of cellulose by lignin rather than chemical inhibition (Tuomela *et al.* 2000; Said-Pullicino and Gigliotti 2007). On the other hand, the microbially-mediated degradation of polymers deriving from vegetable waste, present in the matrix, such cellulose and hemicellulose and the production of microbial polysaccharides can be responsible for the opposite trend leading to an increase of WS-Carb.

After the first two weeks WS-Carb generally fell sharply and progressively until the end of the curing phase (Sánchez-Monedero *et al.* 1999; Castaldi *et al.* 2008; Fig. 2). Most of the WS-Carb are used as carbon and energy sources by microorganisms suggesting that their mineralization is most probably responsible for the decrease of these hydrophilic fractions during the composting (Said-Pullicino *et al.* 2007).

It can be concluded that there is compensation between the release of soluble carbohydrates, as a result of the degradation of the cellulose and hemicellulose fractions, particularly during the thermophilic phase, and the processes of mineralization carried out by the microbial populations.

Water soluble phenols (WS-Phenols)

The phenolic fraction generated during the partial degradation of lignin is generally insoluble and substantially more resistant to microbial degradation, because of its aromaticity. Moreover, the fraction of WS-Phenols, that is usually characterized by molecules with small molecular size/weight and a simple structure, is more prone to changes and transformations during composting with respect to phenols with high molecular weight. For this reason WS-Phenols have been proposed as indicators of the process evolution and MSW compost stability and maturity (Sánchez-Monedero *et al.* 1999).

In general, the presence of high levels of water-soluble phenolic compounds in the final compost may have a temporary adverse environmental impact especially when compost is added to agricultural soil. Seed germination can be severely inhibited by the residual presence of polyphenols and soil nitrogen immobilisation can also occur due to reaction with polyphenols (Bustamante *et al.* 2008).

The amount of WS-Phenols generally increases during the first weeks of composting, then a progressive decrease occurs (Fig. 2). This suggests that after a first period of approximately 20-30 days, WS-Phenols are slowly utilized by microorganisms either as an energy source or as precursors for the synthesis of new molecules (Bustamante *et al.* 2008). However, different trend have been also reported. Said-Pullicino *et al.* (2007) referred that the concentration of phenolic compounds in water-extractable OM remained constant during most of the composting and tended to increase towards the end of the curing phase. This increase of WS-Phenols is difficult to explain, even mentioning a concentration effect due to their recalcitrance. On the other hand, a significant activity of white-rot fungi, which was reported during the curing phase, can partly explain a

constant presence of WS-Phenols. The activity of white-rot fungi can be responsible for lignin degradation thus contributing to the build up of phenols in water-extractable OM (Said-Pullicino *et al.* 2007).

Humic substances

Compost stability implies the formation of humic-like substances. For this reason humification processes have been widely investigated in order to find out helpful parameters or indexes able to assist in the evaluation of compost stability (Chen and Inbar 1993; Chefetz *et al.* 1998a; Lhadi *et al.* 2006; Gómez-Brandon *et al.* 2008). However, it should be mentioned here that humification indexes can vary greatly, in terms of absolute values, among composts produced from different starting materials (Inbar *et al.* 1992; Gómez-Brandon *et al.* 2008; Jouraiphy *et al.* 2008). This also implies that a generalized utilization of such an index should be avoided or alternatively carefully evaluated case by case.

In general, humic substances recovered and identified from different composts are characterized by a great heterogeneity which is basically reflecting the complex and still unknown processes involved in their formation, transformation and/or partial degradation (Hayes *et al.* 1989; Jouraiphy *et al.* 2008). Humic substances are characterized by the presence of different aromatic rings (phenolic and quinonic) which bear acidic functional groups and peripheral aliphatic, polysaccharidic and peptidic chains. These macromolecules are assembled into a variety of ways that determine the final structure of humic substances (Swift 1989; MacCarthy *et al.* 1990; Stevenson 1994; Senesi *et al.* 1996; Piccolo and Conte 1999; MacCarthy 2001; Piccolo 2002). Humic substances can be divided into humic and fulvic fractions (MacCarthy 1990; Stevenson 1994) and their specific determination and/or quantification requires a suitable separation of the two fractions (Stevenson 1994). Depending on the methodology applied the humified C content could be overestimated. This overestimation is more common at the beginning of the MSW composting process, due to the presence of a large amount of carbon compounds that can be co-extracted (proteins, lipids and intermediate microbial metabolites) (Ciavatta *et al.* 1990; Grigatti *et al.* 2004). This is likely the reason why there is not a general agreement on the trend of humic and fulvic fractions throughout the process of composting (Benito *et al.* 2003; Bustamante *et al.* 2008).

The content of HA generally decreases during the first stage of MSW composting likely because of the depletion of easily degradable compounds bound to humic substances (Adani *et al.* 1999; Genevini *et al.* 2002). An increase of HA concentration has been reported by different authors, particularly at the beginning of the "curing phase" (Roletto *et al.* 1985; Senesi 1989; Tomati *et al.* 2000; Castaldi *et al.* 2005; Bustamante *et al.* 2008). However, these increases are rather small compared with the pre-existing humified fraction, and could be due to the concentration of the recalcitrant fraction (Adani *et al.* 1999; Lhadi *et al.* 2006) and/or to the synthesis of new humic substances (Castaldi *et al.* 2005). The general trend of HA during the MSW composting process indicates a certain HA degradation during the high rate phase (Adani *et al.* 1999) followed by a simultaneous formation and degradation of an *ex novo* HA fraction. Finally, Lhadi *et al.* (2006) suggested that the humification process during composting is closely connected to, and depends on the degradation processes of the more complex recalcitrant material (alkali insoluble fraction: humin).

Enzymatic activities

The enzymes released by the microorganisms during MSW composting play a key role in the biological and biochemical transformations of the matrix. Microbial enzymes within the composting pile are responsible for the breakdown of several organic compounds characterised by a complex structure (i.e. cellulose and proteins) finally leading to the

solubilization of more simple water-soluble compounds (i.e. glucose, amino acids and urea) (Castaldi *et al.* 2008). These latter organic compounds became available as a source of carbon and energy for microorganisms, enabling them to multiply and actively persist within the composting matrix. The presence of a high content of degradable organic compounds in the initial mixture stimulates microbial growth and enzyme synthesis whereas as complex available substrates decrease, a reduced enzyme synthesis also occurs. Monitoring the activity of specific intracellular and/or extracellular enzymes during MSW composting may provide further insights into the waste biodegradation processes (Castaldi *et al.* 2008) and might be useful to evaluate compost stability (Mondini *et al.* 2004). Moreover, enzymatic activity measurements are reasonably easy, fast and not too expensive (Gómez-Brandon *et al.* 2008; Raut *et al.* 2008).

Enzymes with a significant role in the MSW composting process include dehydrogenases, cellulases and β -glucosidases (involved in C cycle), proteases and ureases (involved in N mineralization), and phosphatases (that remove phosphate groups from organic compounds). The activity of dehydrogenase, cellulase, β -glucosidase and protease typically increase during the first 15-20 days of MSW composting, usually in accordance with the dynamics of water-soluble fractions (i.e. water-soluble carbon and water-soluble carbohydrates). This indicates that the indigenous microflora that colonize the composting pile is metabolically active and that the synthesis of a variety of enzymes is occurring.

The dehydrogenase activity is commonly used as a measure of the overall microbial activity since this group of enzymes are involved in the production of energy in the form of ATP through the oxidation of organic compounds (Ros *et al.* 2006). During the first 15-20 days of composting a high dehydrogenase activity is generally recorded while afterwards a progressive decline occurs. The dehydrogenase activity usually does not vary significantly during the last part (approximately during the last month) of composting indicating that a residual metabolic activity is still present (Castaldi *et al.* 2008).

Protease activity is closely related to the N cycle. This group of enzymes catalyze the hydrolysis of proteins and, understandably, protease activity increases sharply at the beginning of the process, when a high availability of oligo- and polypeptides is present in the initial mixture. As for dehydrogenase, protease activity decreases quickly after a

first peak that is typical during the first 15-20 days, then it drops down and remains stable during the following phases of composting (Castaldi *et al.* 2008). Urease is another key enzyme closely related to the N cycle. It is involved in the hydrolysis of urea to ammonium and carbon dioxide. Urease activity usually increases during the first 2-3 weeks of composting when a large availability of WSN is present (García *et al.* 1992a), while a considerable reduction is generally observed afterwards. The increasing concentration of WSN-NO_3^- after the first month of MSW composting can be responsible for a decline of urease activity, that seems strongly repressed by WSN-NO_3^- (Nannipieri *et al.* 1980). The trend of urease activity during MSW composting can be quite different from the other enzyme activities discussed. In particular, after urease activity reaches a minimum, it is not uncommon to detect a second peak likely due to urease resynthesis (Nannipieri *et al.* 1980; Castaldi *et al.* 2008). It has been proposed that this resynthesis may be due to an increased level of proteins in the composting pile consequent to the lysis of microbial cells. This is accompanied by an increase of protease (Raut *et al.* 2008) followed by a peak in urease activity (Castaldi *et al.* 2008). During the last part of MSW composting, generally urease activity drops down and stabilizes.

Cellulases are a class of enzymes that catalyze the hydrolysis of cellulose. The cellulases within the composting mixture are related to the types of cellulolytic microorganisms (e.g. bacteria and fungi) present in the composting mixture (Goyal *et al.* 2005). The rate and extent of cellulose decomposition is one of the most important parameters influencing the time of the composting. It can be assessed that cellulose content influences the process more than any other substrates (Raut *et al.* 2008). Cellulase activity gradually increases during MSW composting reaching a peak around 20-30 days and then declines (Goyal *et al.* 2005; Raut *et al.* 2008).

β -Glucosidase is another key enzyme governing the carbon cycle. It hydrolyzes the reducing terminations of β -D-glucose chains releasing β -glucose monomers (Nannipieri *et al.* 2002). Indeed, β -glucosidase activity is indicative of the presence of β -D-glucose chains arising from the labile OM. Generally, β -glucosidase activity increases during the first 15-20 days of MSW composting, then sharply decreases reaching a minimum after about 3 months of composting (Castaldi *et al.* 2008), and finally remaining stable in the last part of the process. As recently reported by Cunha-Queda *et al.* (2007) the decline in the activity of both cel-

Table 1 Values of some enzymatic activities at the end of composting process

Authors	Dehydrogenase	Protease	Urease	Cellulase	β -glucosidase	Alkaline Phosphatase	Acid Phosphatase
Ayuso <i>et al.</i> 2006	–	5 $\mu\text{mol N-NH}_4^+ \text{g}^{-1} \text{h}^{-1}$	20 $\mu\text{mol N-NH}_4^+ \text{g}^{-1} \text{h}^{-1}$	–	20 $\mu\text{mol PNP g}^{-1} \text{h}^{-1}$	300 $\mu\text{mol PNP g}^{-1} \text{h}^{-1}$	–
Castaldi <i>et al.</i> 2008	26.17 $\mu\text{g TPF g dw}^{-1} \text{h}^{-1}$	209.81 $\mu\text{g tyr g dw}^{-1} \text{h}^{-1}$	217 $\mu\text{g N-NH}_4^+ \text{g}^{-1} \text{dwt h}^{-1}$	8.6 $\mu\text{g gluc g}^{-1} \text{dwt h}^{-1}$	187.21 $\mu\text{g PNP g}^{-1} \text{dwt h}^{-1}$	–	–
Cunha-Queda <i>et al.</i> 2007	–	0.3-9.0 $\text{mmol tyr g}^{-1} \text{dm}^{-1} \text{h}^{-1}$	3-65 $\mu\text{mol N-NH}_4^+ \text{g}^{-1} \text{dw}^{-1} \text{h}^{-1}$	8-40 $\mu\text{mol gluc g}^{-1} \text{dw}^{-1} \text{h}^{-1}$	0.1-3.2 $\mu\text{mol PNP g}^{-1} \text{dw}^{-1} \text{h}^{-1}$	0.7-5.0 $\mu\text{mol PNP g}^{-1} \text{h}^{-1}$	1.5-4.5 $\mu\text{mol PNP g}^{-1} \text{h}^{-1}$
Gómez Brandon <i>et al.</i> 2008	–	800 $\text{mg tyr kg}^{-1} \text{dm}^{-1}$	–	4000 $\text{mg eq gluc kg}^{-1} \text{dw}^{-1}$	370 $\text{mg PNP kg}^{-1} \text{dw}^{-1}$	–	–
Goyal <i>et al.</i> 2005	–	811-1183 $\text{mg tyr kg}^{-1} \text{dm}^{-1}$	–	63-36 $\text{mg red. sugar kg}^{-1} \text{d.m.h}^{-1}$	–	–	–
Mondini <i>et al.</i> 2004	–	–	–	–	1000-2500 $\mu\text{g PNP g}^{-1} \text{h}^{-1}$	5000-13000 $\mu\text{g PNP g}^{-1} \text{h}^{-1}$	3000-6000 $\mu\text{g PNP g}^{-1} \text{h}^{-1}$
Raut <i>et al.</i> 2008	–	6 Activity $\text{mg gm}^{-1} \text{hr}^{-1}$	–	0.8 mg/gm/hr	–	2 mg/g/hr	–
Ros <i>et al.</i> 2006	700-1000 g INTF/g	9.31-25.32 $\mu\text{mol N-NH}_4^+ \text{g}^{-1} \text{h}^{-1}$	–	–	4.55-8.49 $\mu\text{mol PNP g}^{-1} \text{h}^{-1}$	–	–

lulases and β -glucosidase in the second part of the process could be reasonably attributed to a substantial decrease in available organic substrates.

Phosphatases are enzymes with significant agronomic implications since they hydrolyze organic compounds containing phosphate groups and release inorganic phosphorus assimilable by plants (Ayuso *et al.* 1996). Phosphatases are exclusively produced by microorganisms and hence their presence in compost can be directly related to the microbial abundance and activity (Raut *et al.* 2008). The evolution of phosphatase activity during MSW composting is not straightforward. Raut *et al.* (2008) recorded an increase of alkaline phosphomonoesterase activity up to the first 9 days of composting, followed by a sharp decrease during the active phase. This was likely attributed to a feedback inhibition of this enzyme by inorganic phosphate (Ayuso *et al.* 1996). Finally, Raut *et al.* (2008) and Ros *et al.* (2006) recorded a maximum activity of phosphatases near the end of the composting process.

Generally, hydrolytic activities are able to reveal the dynamics of the OM degradation during the composting process of the MSW. With few exceptions, the trend of the different enzyme activities (mentioned in this paper) during composting are similar, even if absolute values can vary greatly depending of the composition of the starting composting materials (Table 1) (Garcia *et al.* 1992a; Castaldi *et al.* 2008; Gómez-Brandon *et al.* 2008). This also implies that the use of threshold values for specific enzyme activities does not seem suitable to univocally define compost maturity. On the other hand, the evolution of the kinetics and/or dynamics of selected hydrolases can be certainly helpful for process monitoring and, ultimately, to judge compost maturity (Castaldi *et al.* 2008; Gómez-Brandon *et al.* 2008).

Thermal analyses

Thermal degradation techniques, such as thermogravimetry and differential thermogravimetry (TG/DTG), differential scanning calorimetry (DSC) and differential thermal analysis (DTA), have been used for many years for the characterization of the OM during different biological stabilization processes (Otero *et al.* 2002; Marhuenda-Egea *et al.* 2007; Carballo *et al.* 2008). Thermogravimetry in particular has been used in the past and in recent years to evaluate compost stability and maturity (Dell'Abate *et al.* 2000; Melis and Castaldi 2004; Baffi *et al.* 2007; Carballo *et al.* 2008). In general, all of these techniques are based on a programmed heating of the sample (i.e. compost, soil, etc) in a controlled atmosphere which ultimately provides detailed quali-quantitative information on the stability of the OM analyzed. Thermal analyses revealed particularly useful because of the rapidity of determination, simplicity and reproducibility. Additionally, thermal degradation techniques are relatively inexpensive yet reliable methods, and are able to provide precious information on the chemical characteristics of the sample without any preliminary extraction step (Melis and Castaldi 2004). These methods appear promising for compost characterization and could be used to integrate the information provided from standard chemical or biochemical analyses (Blanco and Almendros 1994; Atanasov *et al.* 1985).

TG and DTG analyses imply the measurement of changes of the sample mass with temperature. A graph (thermogram) in which the sample mass is varying as a function of the temperature allows the evaluation of thermal stability, reaction speed, reaction processes and sample composition. Based on TG and DTG analyses, different authors reported that the first weight loss of the OM from MSW compost occurs in the 50–150°C range, and is indicative of dehydration reactions (Fig. 3) (Dell'Abate *et al.* 2000; Melis and Castaldi 2004; Smidt and Lechner 2005; Marhuenda-Egea *et al.* 2007; Carballo *et al.* 2008). Between 200 and 760°C, the thermal degradation process of the sample occurs mainly in two steps (Dell'Abate *et al.* 2000; Melis and Castaldi 2004;

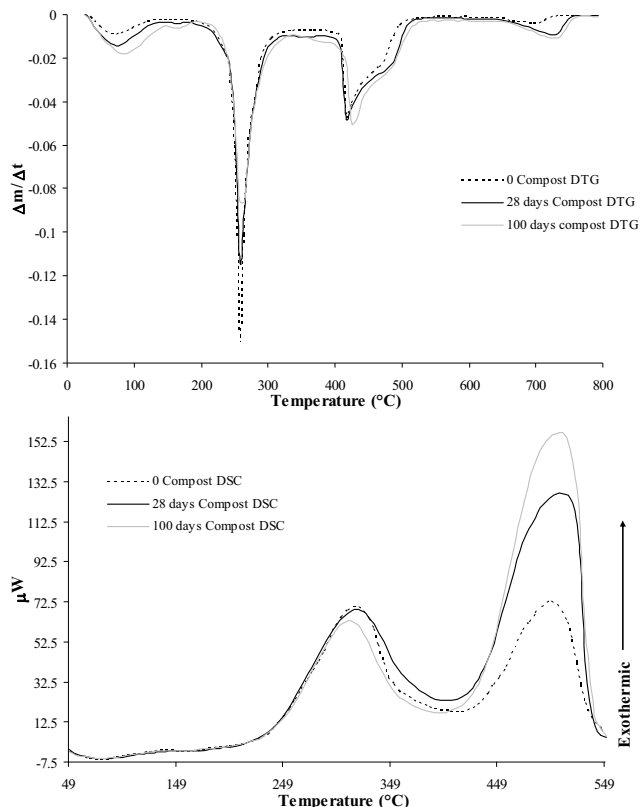


Fig. 3 DTG and DSC curves of compost samples at different times of the MSW composting process. Reprinted from Melis P, Castaldi P (2004) Thermal analysis for the evaluation of the organic matter during municipal solid waste aerobic composting process. *Thermochimica Acta* 413, 209-214, with kind permission from Elsevier, Ltd.

Marhuenda-Egea *et al.* 2007). The first peak, recorded in the 200–400°C range, is indicative of carbohydrates combustion (Fig. 3) (Marhuenda-Egea *et al.* 2007), whereas the second peak, usually recorded between 400–520°C, is indicative of thermal degradation of aromatic structures (Geyer *et al.* 2000; Melis and Castaldi 2004). It has been shown by several authors that the weight loss in the 200–400°C range can reach approximately 40% d.m. for immature compost while is approximately one half (~20% d.m.) for stabilized compost (Geyer *et al.* 2000; Melis and Castaldi 2004). During composting, as simple carbohydrates are metabolized by the microorganisms, and aromatic compounds are concentrated or synthesized, the weight loss recorded for the second peak (400–520°C range) increases (Fig. 3). These trends suggest a progressive transformation of the biomass in the polyelectrolyte macromolecules known as humified matter (Buurman *et al.* 2002; Melis and Castaldi 2004). Usually, this is also accompanied by an increase in stability and aromatization degree of the chemical constituents of the OM (Dell'Abate *et al.* 2000; Marhuenda-Egea *et al.* 2007). Further, some authors observed a shift of all DTG peaks during composting toward higher temperature ranges indicating that the presence of more stabilized OM increases during the process (Fig. 3) (Melis and Castaldi 2004; Marhuenda-Egea *et al.* 2007). Thermograms with a modest weight loss in the 695–750°C range, were also reported by some authors. These weight losses were mainly attributed to thermal degradation of carbonates present as “accessory” compounds (Atanasov *et al.* 1985). These weight losses generally increase with the composting time (Fig. 3) as carbonates concentrate during the process (Melis and Castaldi 2004).

DSC thermoanalysis provides characteristic DSC curves for each sample analyzed in which heat flux is expressed as a function of temperature. Peaks in DSC curves reveal the occurrence of exothermic or endothermic reactions in the sample in correspondence to certain temperatures. DSC

curves of MSW composting materials are generally characterized by an endothermic peak near 100°C, representative of dehydration reactions, and two distinct exothermic peaks (around 250–350 and 440–540°C) which indicate the reactions of organic components characterized by different thermal stability (**Fig. 3**) (Dell'Abate *et al.* 2000; Melis and Castaldi 2004; Marhuenda-Egea *et al.* 2007). In particular, these exothermic peaks were assigned respectively to dehydration, loss of peripheral polysaccharide chains, loss of peptidic chains, and oxidation and polycondensation of aromatic nuclei (Dell'Abate *et al.* 2000; Senesi *et al.* 2007; Carballo *et al.* 2008). As the composting proceed, the intensity of the second exothermic peak usually increase, while the first one slightly decrease until disappearing in the end product (Smidt and Lechner 2005; Smidt and Tintner 2007; Carballo *et al.* 2008). The small shift towards higher temperatures of the second exothermic peak is also obvious as the composting material increases its stability with time (Provenzano *et al.* 1998; Ouattmane *et al.* 2000; Provenzano *et al.* 2000; Smidt and Tintner 2007).

The exothermic reactions in DSC curves of MSW compost are commonly associated with two distinct weight losses on the TG and DTG curves (200–400 and 400–520°C range) (Provenzano *et al.* 1998; Ouattmane *et al.* 2000; Provenzano *et al.* 2000; Marhuenda-Egea *et al.* 2007). The presence in DSC curves, relative to stabilized composts, of a more intense peak in the 440–540°C range could be an indication of the substantial increase of humified OM during composting (**Fig. 3**) (Melis and Castaldi 2004; Smidt and Tintner 2007). Such an intense peak in correspondence to stable compost could be related to an increase of the stability and aromatization degree of the OM during the process. This can be due either by a concentration effect through the loss of the less stable molecules during the process or/and by the *ex-novo* synthesis of more stable molecules (Marhuenda-Egea *et al.* 2007). It is important to mention that DSC curves of stabilized compost can be very similar to those of the native soil HA (Senesi *et al.* 2007). Therefore, as recognized by several authors (Otero *et al.* 2002; Melis and Castaldi 2004), DSC analysis is a useful tool for the evaluation of the humification/stability achieved by the composting material (Senesi *et al.* 2007).

Fourier transform-infrared (FTIR) spectroscopy

It is becoming increasingly clear that suitable and reliable criteria to evaluate either the stability and/or the maturity of organic amendments should be mainly based on chemical and physicochemical features of humic-like components, i.e., HA-like and fulvic acid (FA)-like fractions (Senesi *et al.* 2007).

Each step in the decomposition of OM during MSW composting is characterized by the production of metabolites that can be investigated using infrared (IR) spectroscopy. This approach can provide useful information on the functional groups of these metabolites and on their physicochemical features (Smidt and Meissl 2007; Carballo *et al.* 2008). Infrared spectra illustrate the plot of infrared radiation absorbed by the matter or sample investigated (Meissl *et al.* 2007; Carballo *et al.* 2008). The absorption bands in the infrared spectrum are reflecting the energy absorbed from infrared light while causing the vibration of molecule bonds of functional groups present in the sample analyzed (Smidt and Meissl 2007).

FTIR spectroscopy has been widely applied to investigate the composting process, to characterize humic substances and to assess compost stability and maturity (Inbar *et al.* 1991; Mikki *et al.*, 1997; Provenzano *et al.* 2001; Ouattmane *et al.* 2002; Sánchez-Monedero *et al.* 2002; Baddi *et al.* 2004; Castaldi *et al.* 2005; Carballo *et al.* 2008; El Hajjoui *et al.* 2008). FTIR spectroscopy has been also used for rapid qualitative assessments of composts during the process (Ouattmane *et al.* 2000; Smidt and Meissl 2007).

Generally, FTIR as well as ¹³C NMR methodologies imply HA extraction. Commonly, methods for the extrac-

tion of humic substances from organic wastes are derived from the analytical procedures used to extract HA from soils. It should be noted that considerable differences exist between soil and materials with a high content of OM such as composts, muds, etc. The presence in the compost of high amount of carbon compounds such as lipids, proteins and carbohydrates could interfere with the determination of humic substances, since the solvents employed in the extraction are not completely selective (Ciavatta *et al.* 1990; Adani 1995; Grigatti *et al.* 2004). Therefore the estimation of the amount of humic substances in compost needs more suitable extraction procedures such as those described by Chefetz *et al.* (1998a) and Adani *et al.* (1999). These methodologies are able to extract "true humic acid" fraction which is purified from the interfering substances.

The characteristics of the main bands present in the FTIR spectra of HA extracted from MSW compost, the assignment to functional groups and their behaviour during the composting process are reported in **Table 2**.

The FTIR spectra of HA extracted during composting typically show a characteristic broad band at 3400–3300 cm⁻¹ due to the stretching of the H–O bonds (Chefetz *et al.* 1998a; Provenzano *et al.* 2001; Castaldi *et al.* 2005). Its width can be used as an index of alcohol and phenol association. Moreover, two distinct peaks are usually present at about 2930 and 2850 cm⁻¹ (stretching of aliphatic C–H bonds) (García *et al.* 1992b; Stevenson 1994; Castaldi *et al.* 2005; Spaccini and Piccolo 2007; Carballo *et al.* 2008). These latter signals have been assigned to the symmetrical and asymmetrical stretching and bending vibrations of CH₂ groups in long-chain aliphatic molecules respectively (Spaccini and Piccolo 2007). A strong adsorption in this range is indicative of a high content of lipids in the HA extracted and hence is suggesting that the compost is not fully mature and/or it could depend on the amount of fat in the initial mixtures. The 2000–400 cm⁻¹ zone can provide the following information on the OM in compost: a shoulder around 1711 cm⁻¹ usually indicates the presence of not conjugated carbonylic (C=O) groups, a wide peak at 1650 cm⁻¹ is reflecting the presence of C=O conjugated groups and C=C groups in aromatic structure, small peaks or shoulders at 1550 and 1520 cm⁻¹ are indicating the presence of amide and carboxylate C=O and an evident peak at 1430–1460 cm⁻¹ is indicative of the presence of C–H deformation of CH₂ or CH₃ groups; of the CO₂ stretch of carboxylates and also of the C–O stretch vibration of carbonates (Inbar *et al.* 1989; García and Hernández 1992; García *et al.* 1992b; Senesi *et al.* 1996; Mikki *et al.* 1997; Ouattmane *et al.* 2000; Provenzano *et al.* 2001; Chen 2003; Castaldi *et al.* 2005; Carballo *et al.* 2008).

A peak at 1628 cm⁻¹ can be related to the ring vibration of aromatic compounds, whereas the inclusion of peptidic molecules in a poorly mature compost can be revealed by an intense band around 1550 cm⁻¹ (Amide II band) (Spaccini and Piccolo 2007). A peak around 1323 cm⁻¹ is also recognizable in spectra of HA extracted from not mature MSW compost. This peak has been attributed to aromatic primary and secondary amines (Inbar *et al.* 1989; Senesi *et al.* 1996; Carballo *et al.* 2008). At 1200 cm⁻¹ a peak is also generally present (stretching of single C–O bond in carboxylic acids, esters and ethers) (Castaldi *et al.* 2005). Some authors found a very broad peak at 1160 and 1030 cm⁻¹ that was attributed to C–O bonds in both polyalcoholic and ether functional groups (Spaccini and Piccolo 2007), and polysaccharides (García and Hernández 1992; Carballo *et al.* 2008). Finally, a sharp band at 875 cm⁻¹ may be assigned to the C–O out-of-plane bend of carbonates salts formed during the composting process (Spaccini and Piccolo 2007; Carballo *et al.* 2008; Spaccini and Piccolo 2008).

The FTIR spectra reported in several studies indicate a decrease of the relative intensity of polysaccharide and aliphatic peaks during the MSW composting. This reflects different reactions of degradation and condensation of organic molecules occurring in the composting matrix as the process proceeds (Chefetz *et al.* 1998a; Castaldi *et al.* 2005;

Table 2 Major bands present in FTIR spectra of HA extracted from MSW compost at different stages of the process, the assignment to functional groups, and their behaviour during the MSW composting.

Location wavenumber (cm ⁻¹)	Vibration	Functional groups or components	Behaviour of peak relative intensity (I= increase; D= decrease; ↔= stable)
3400-3300	O-H stretch	Bonded and non-bonded hydroxyl groups and water	D
3180-3090	NH ₂ stretch (2 bands)	Primary amides	D
2930	C-H stretch	Symmetrical and asymmetrical stretching of CH ₂ groups	D
2850	C-H bend	Bending vibrations of CH ₂ groups	D
1711	C=O	Not conjugated carbonylic C=O	↔
1650	C=O; C=C	C=O conjugated, C=C in aromatic structure	I
1628	C=C	Ring vibration of aromatic compounds	I
1570-1540	N-H in plane	Secondary Amides	D
1520	N-H; C=O	Amide and carboxylates	↔
1430-1460	C-H deformation; CO ₂ stretch; C-O stretch	Alkanes; carboxylates and carbonates	I
1323	N-H	Aromatic primary and secondary amines	D
1200	C-O	Stretching of single C-O bond in carboxylic acids, esters and ethers	I
1160	C-O	Polyalcoholic and ether functional groups	D
1030	C-O	Polysaccharides	D
875	C-O stretch	Carbonate	I

Senesi *et al.* 2007). Spaccini and Piccolo (2008) observed a large decrease of alkyl signals in the 2926–2853 cm⁻¹ region after 90 days of stabilization period, suggesting the depletion of the degradable lipid component. Other main variations during composting are represented by the rapid disappearance near the end of the process of the peak at 1550 cm⁻¹ and the progressive decrease of the intensity of the band relative to alcoholic and ether functional groups (1030–1160 cm⁻¹) as the process proceeds (Spaccini and Piccolo 2008). Therefore FTIR spectroscopy indicates that coating materials such as polysaccharides, peptides and lipids bound to the HA core structure are degraded during composting, leading to more aromatic humic structures (Chefetz 1998a; Baddi *et al.* 2003, 2004; Castaldi *et al.* 2005; Senesi *et al.* 2007).

¹³C nuclear magnetic resonance (¹³C NMR) spectroscopy

Solid-state ¹³C NMR is one of the most powerful tools for examining the carbon composition of OM (Hafidi *et al.* 2005; El Hajjouji *et al.* 2008). ¹³C NMR spectroscopy has been used for the monitoring of the stabilization process of OM during composting. In particular, ¹³C NMR spectroscopy has been used to investigate either the compost sample or the HA extracted (Inbar *et al.* 1991; Chefetz *et al.* 1998a; Almendros *et al.* 2000; Baddi *et al.* 2003; Amir *et al.* 2004; Castaldi *et al.* 2005; Hafidi *et al.* 2005; El Hajjouji *et al.* 2008; Jouraiphy *et al.* 2008).

The distribution of the different carbon types in the ¹³C NMR spectra of HA extracted from MSW compost is reported in **Table 3** along with their trends during the process.

The ¹³C NMR spectra of HA extracted from MSW composts show a characteristic peak at 215 ppm (carbonyl C) (Almendros *et al.* 2000; Castaldi *et al.* 2005), which progressively increases during the process. The region between 210 and 161 ppm (carboxylic, ketone and amide C) is typically dominated by a peak at 175 ppm, attributed to the carboxylic C (Spaccini and Piccolo 2007, 2008); even in this case the peak intensity increases in mature composts. In the aromatic region (160–116 ppm) major peaks are recognizable at 148 ppm (phenolic C) (Chefetz *et al.* 1998a) and 152 ppm (O-substituted aromatic C): both peaks usually show a greater intensity in HA extracted at the beginning of the process (Castaldi *et al.* 2005); an intense peak within the above mentioned region is also visible at 130 ppm (aromatic C). The intensity of this latter peak increases during composting (Castaldi *et al.* 2005). On the contrary, the intensity of the aliphatic C region (115–0 ppm) peaks decreases in the HA of mature compost; in particular, the greatest reduction is in the 100–47 ppm region. In this region the

Table 3 Distribution of the different carbon types in the ¹³C NMR spectra of HA extracted from MSW compost at different stages of the process, the assignment to functional groups, and their behaviour during the MSW composting

Chemical shift ppm	Functional groups or components	Behaviour of peak relative intensity as the process proceeds (I= increase; D= decrease; ↔= stable)
215	Carbonyl C	I
210–161	Carboxylic, ketone and amide C	
175	carboxylic C	I
160–116	Aromatic region	
152	O-substituted aromatic C	D
148	phenolic C	D
130	aromatic C	I
115–47	Aliphatic region	
106 and 115	anomeric C	D
70–90 and 102	carbohydrates	D
75	aliphatic alcohols	D
56	methoxyl C	D
46–10	Alkyl region	
33	polyethylene C	↔

main peaks are detected at 56 ppm (methoxyl C) and 75 ppm (carbohydrates or aliphatic alcohols) (Inbar *et al.* 1991; El Hajjouji *et al.* 2008). In addition, carbohydrates often appear around 70–90 and at 102 ppm. The peaks at 106 and 115 ppm, corresponding to anomeric C (El Hajjouji *et al.* 2008; Spaccini and Piccolo 2008) typically decrease during composting. In the alkyl region (46–10 ppm) a peak with a constant intensity at 33 ppm (polyethylene C) is commonly present in ¹³C NMR spectra of HA extracted from compost (Lorenz *et al.* 2000; Ouattmane *et al.* 2000; El Hajjouji *et al.* 2008). This signal is characteristic of (CH₂)_n present in fatty acid chains and ramified aliphatic structures and/or long chains of HA.

The ¹³C NMR spectroscopy of HA extracted during MSW composting indicates that degradable organic compounds, such as aliphatic chains, are modified during the composting process to generate condensed aromatic structures containing large proportions of hydroxyl, methoxyl, carboxyl and carbonyl groups (see the increase of peak intensities at 215, 175 and 130 ppm; **Table 3**). These resistant structures are preserved from degradation and contribute to the neoformation of HA (El Hajjouji *et al.* 2008). An increase of the molecular bonds and inter- and intra-molecular associations has been highlighted by Schnitzer *et al.* (2000) in the OM during MSW composting. This indicates the for-

mation of large molecules characterized by a reduced chemical and biological reactivity with respect of the original compounds. Jouraiphy *et al.* (2008) considers the aliphatic polyesters/ethers as new structures incorporated into the humic fraction during the composting process.

A common trait that characterizes the MSW composting matrix during the process is the decrease of carbohydrate compounds, and the progressive increase in aromaticity and carboxyl content (Chen *et al.* 1989; Inbar *et al.* 1991; Amir *et al.* 2004; Castaldi *et al.* 2005; Senesi *et al.* 2007; Jouraiphy *et al.* 2008). By contrast, Spaccini and Piccolo (2007) showed a significant decrease of alkyl carbons in samples of MSW compost at the beginning of the stabilization period. The authors suggested that an overall mineralization of the most degradable lipid compounds occurred, with no equivalent changes found for carbohydrate carbons.

The analyses of ^{13}C NMR spectra of HA extracted from MSW compost globally indicate that simple carbohydrates and polysaccharides and/or the most degradable lipid compounds are degraded during the process. At the same time, humic structures incorporated aliphatic polyesters and more aromatic structures that are more stable and less reactive than the starting material (Senesi *et al.* 1996; Castaldi *et al.* 2005; Jouraiphy *et al.* 2008).

CONCLUSIONS

Mineralization and humification of fresh OM are the main processes characterizing the composting of MSW.

Water soluble OM is subject to significant changes during composting process which directly reflect the overall OM transformation. Monitoring the chemical and biological changes occurring in water-extractable fraction during the process (particularly the WSC, WSN, WSN-NH_4^+ , WSN-NO_3^- and WS-Carb) can certainly improve our knowledge on the evolution, stability and maturity of the OM during composting. High concentrations of soluble and labile organic compounds such as those present in the hydrophilic fraction of water-extractable OM, are primarily responsible for the lack of compost stability and maturity. The attainment of stable and mature compost can be traced through to their disappearance from the water-extract fraction during the process.

Enzyme activities provide precious indications on the extent of the microbial activity during the composting as well as the relative content of labile (and often toxic) organic compounds. Globally, enzymatic activities and water soluble fractions could be used as indicators of the state and evolution of the OM during MSW composting. The highest enzyme activities and concentrations of water soluble fractions are usually recorded between 0 and 30/60 days, during the active phase of decomposition. Subsequently, an invariance (versus time) of these parameters occurs until the end of the process likely indicating a suitable stability and maturity of the end product. However, composting can be performed starting from very different organic substrates and involves a large number of biochemical and chemical transformations. Since the level of these parameters measured on stable composts largely depends on the raw material used, it is extremely difficult to establish generic threshold values for each enzymatic activity or water soluble fraction. This also means that absolute values relative to these parameters can not be promptly used as an index of compost stability and maturity. To the contrary the monitoring of kinetics and dynamics of selected hydrolases and/or water soluble fractions during the process, that incidentally would be expensive and laborious, can be helpful in order to judge compost stability and maturity.

On the other hand, other physicochemical parameters including C/N and $\text{NH}_4^+/\text{NO}_3^-$ ratios, as well as WS-Phenols, do not appear as reliable indexes able to provide a clear understanding of the degradation processes of various organic substrates.

During MSW composting the breakdown of decomposable OM involves a decrease of the polysaccharide content,

degradable lipid compounds and peptidic molecules and the production of hydrophobic structures incorporating more aromatic compounds and aliphatic polyesters/ethers. Thus, the OM in the final product presents both aromatic and aliphatic structures that are chemically and biologically more stable with respect to its original state.

However, the study of changes of humic substances concentrations during MSW composting should not be used alone to estimate the degree of OM evolution, maturation and stability during the process. Indeed, the concentration of humic substances generally increases during composting but this increase is very limited. On the contrary, the study of the evolution of humic fraction during the process, carried out by thermal and spectroscopy analyses, can provide useful details on the humification degree, stability and maturity of the OM in compost. TG/DTG thermograms, and DSC curves of compost can reveal the state of OM evolution during MSW composting and in particular its progressive thermal stabilization as the process proceeds optimally. Stabilization of the OM during MSW composting is clearly indicated by the shift of TG/DTG peaks towards higher temperatures and by the presence of an intense peak corresponding to the second exothermic reaction in DSC curves.

Both non-destructive FTIR and solid-state C^{13} NMR spectroscopy are analytic techniques capable of revealing a variation in HA composition during the composting process. FTIR analyses indicate that materials such as polysaccharides, peptides and lipids bound to the HA core structure are degraded during composting, leading to more aromatic humic structures. Furthermore, C^{13} NMR spectroscopy of HA extracted from MSW composts clearly showed a decrease of the intensity of the aliphatic C region during the process, with the greatest reduction involving carbohydrates. C^{13} NMR spectroscopy also indicates that this trend is accompanied by an increase in aromaticity and carboxyl content.

In conclusion thermal analysis and FTIR and C^{13} NMR spectroscopy appear reliable and suitable indicators for the assessment of the evolution, maturation and stabilization of the OM during MSW composting.

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