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Thermophilic bacteria and their thermozymes in composting processes: a review

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Abstract

In this review, the composting process of organic waste is discussed through an in-depth exploring of its thermophilic phase. It starts with the highlight on the thermodynamic evolution, which needs to be assessed when deciding to use reactors for composting, also in the context of energy generation. The composting process is mediated by different types of microorganisms, and the bacteria that play key roles are evaluated. The roles of the genera *Bacillus* and *Thermus* are considered, often described as the main components of the microbiota of compost. Due to their adaptation to the composting processes, they are candidates for technological purposes. Subsequentially, the focus is moved on the thermostable enzymes that can be isolated from them and their succession during the composting processes. Experimental examples of enzyme-related literature are reviewed, for example investigating proteases and ureases, which are found at the beginning of the process. In addition, cellulases, hemicellulases, lignin-modifying enzymes, and esterases have been described for their activities during the thermophilic phase, giving them great potential for biotechnological and industrial applications. Following, the composition of the microbial community is analyzed through the description of approaches of metagenomics. Despite it being a relatively new but fast-growing field within biology, it is intended to be a priority analysis to acquire knowledge on genomes of environmental microorganisms and communities. Finally, a space is dedicated to the description of the composting plant which treats olive oil wastes within the LIFE TIRSAV PLUS project (LIFE05 ENV/IT/00845). Through two plant solutions, being the Dynamic and the Static Composting, it provides a high-quality compost with an effective, flexible and economical process.

Keywords Compost, Thermophilic phase, Thermodynamics, Metagenomics, Thermozyme, Thermophilic bacteria

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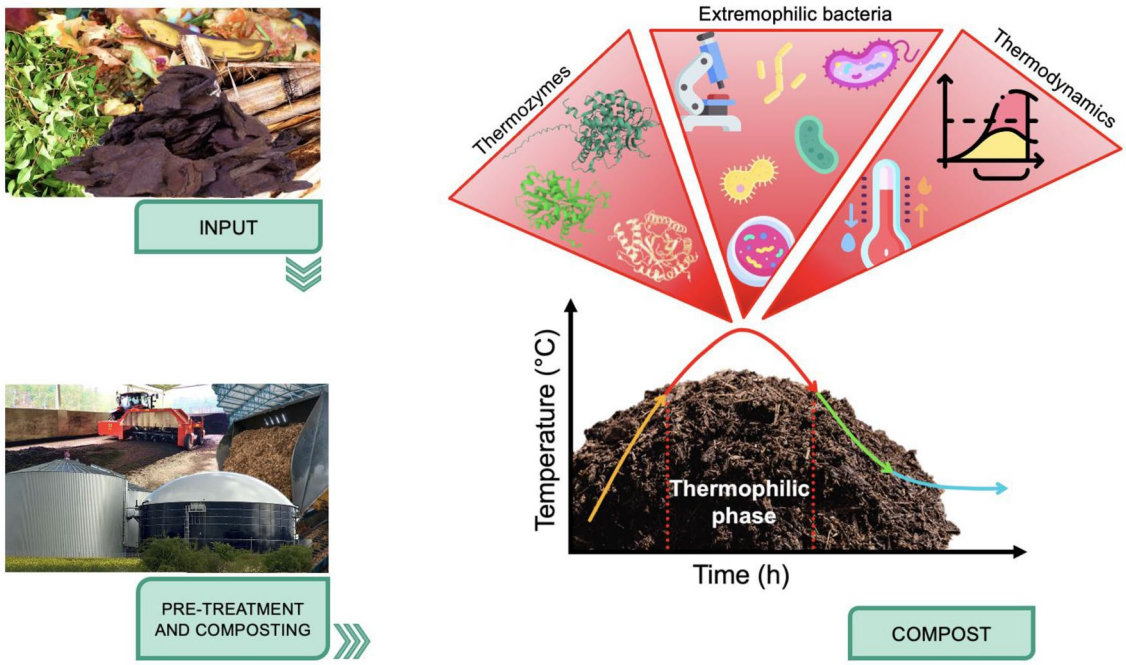
Graphical Abstract



Introduction

Composting represents an efficient process in which organic wastes can be recycled to obtain organic fertilizers utilized in agricultural field [1]. Starting materials, regularly utilized in compost procedure, consist of organic fractions deriving from municipal wastes, animal manures, agro-industrial waste [2], and/or their combinations. The final stable product, named compost, is a sustainable soil improver and fertilizer containing humified fraction comparable to humus which can be used to improve the physico-chemical characteristics of soil, the fertilizer efficiency, and which can promote the growth of crop [3, 4]. Humic substances enrich the soils of nutrients such as nitrogen, potassium, calcium, and phosphorus, necessary for the plant's growth [5, 6]. Furthermore, composting is a smart and sustainable solution for reducing the negative environmental impacts connected to the waste management, and it is based on the circular economy model with the recycling of by-products [7, 8].

A generic composting process is characterized by four main phases, which have been described in different works [8–10]. The first phase is the mesophilic or initial phase, where a rapid increase of temperature in the range 10–42 °C determines the start of the degradation of organic matter, and its duration varies between 24 and 72 h. The second one is the thermophilic phase, characterized by temperatures between 45 and 70 °C in relation to the metabolic activities of endogenous thermophilic microorganisms which degrade the organic compounds, and it can last from several days to several weeks. The temperature can remain fixed for many days according to the properties of the feedstocks, the size of the composting plant and the environment. During the following phase, indicated as the mesophilic II or maturation phase, the temperature decreases between 65 and 50 °C, and it maintains itself for 1–2 months, with the reactivation of mesophilic microorganism and the degradations of the most recalcitrant components. These first three phases can also be referred as the bio-oxidative period of composting. Finally, the last phase is the maturing or curing phase, which can last for 1–4 months with temperature comprised between 50 and 23 °C, and where the organic matter produced stabilizes.

Composting is an aerobic and exothermic process which can occur naturally in the environment, but its efficiency is deeply related to the control of specific physico-chemical parameters which can significantly affect the progress of the bio-oxidative process, such as temperature, pH, aeration rate, moisture content, carbon to nitrogen (C/N) ratio, the type of substrates and their assortment [9, 11, 12]. In addition, the mixing ratios chosen during the preparation phase of composting can

significantly affect the progress of the bio-oxidative process. In detail, a good performance of composting, with an improved efficiency of the bio-oxidative process and a high quality of the outgoing compost, is obtainable when the compost pile reaches a temperature of 55–60 °C during the thermophilic phase, the pH is comprised between 5.0 and 8.0, the humidity in the mixture is almost 40–60%, and there is an initial C/N ratio of 20–40, but the value may vary depending on the substrate [13, 14]. The optimal oxygen concentration is 10%, and while the oxygen content at the beginning of composting is usually adequate, during the process it may be necessary an active aeration to avoid anaerobic conditions, to remove excess moisture from the substrate, and to regulate the temperature [4, 8]. Aeration of the compost pile can be obtained through mixing or through forced aeration, with different studies focusing on continuous and intermittent aeration during composting [8, 15]. The C/N ratio parameter has been recognized as one the most relevant for the final quality of compost [16]. Indeed, the speed of the process results to be affected by this ratio: while low nitrogen values are a limiting factor during composting, making the degradation process slow, an excess of nitrogen is often lost by the system in the form of gases such as ammonium or other nitrogen compounds [17]. Consequently, unsuitable C/N ratios need to be adjusted by addition of further substrates to balance both elements. Other studies considered the role of turning on the process, suggesting its incumbent role in improving the biological heat efficiency and the mass transfer, also in relation to indigenous core bacteria which could be directly activated [18].

The microorganisms required for compost development are furnished by compost feedstocks, keeping an active microbial community during the process [19]. Soil microorganisms (such as bacteria, actinomycetes, fungi and protozoa) are included into the process when the waste materials are mixed with soil. Moreover, mature compost and animal manure are often used as starter in composting processes since they can be a great source of microorganism. Microbial degradation allows the conversion of organic materials into carbon dioxide (CO_2), which is then released into the environment. The composting process is mediated by the intervention of different types of microorganisms. The first degradation processes are carried out by mesophilic organisms that prefer temperatures between 30 and 45 °C [17, 20, 21]. As the temperature rises, due to the intense digestive activity of microorganisms, thermophilic populations settle and continue the conversion of organic compounds into carbon dioxide [22, 23]. This is the "active stage" of composting because the decomposition is considerably rapid, and it persists until organic substrates are available in the piles. Then, the microbial activities decline and with them the pile temperature. During the curing phase, mesophiles populate again the compost and humic substances begin to accumulate generating the mature compost [17, 20, 24].

In composting, different substrates can be added to the starting materials to reduce the negative sides of this process or to improve some characteristics. These compounds can be distinguished between bulking agents, if they influence the structure of the compost in a physical way (e.g., water absorption capacity, air space, and flow between particles), or additives, if they enhance the composting process itself (e.g., reduction of leaching, greenhouse gas emissions, and odor) [25, 26]. Additives are classified as chemical, microbial or physical, and the latter can be further sorted in organic, mineral, and reusable. In addition, the results obtained using a mixture of additives may be better than using a single compound [27].

It has been reviewed by Barthod et al. [25] how the improvement obtained through these compounds can influence different parameters of the composting procedure by way of various mechanisms. For example, the authors described how the thermophilic phase of composting can be extended by using both mineral, organic, and biological additives, thanks to their proficiency in increasing the microbial activity, while biochar, woodchips, or residual straws can increase the aeration and porosity of the composting mass. Moreover, to avoid high humidity that leads to anaerobic conditions during composting, it is required to use fibrous materials, including cornstalk, sawdust or spent mushroom substrate; on the other hand, substrates like clay can reduce large water losses. It has also been described how additives such as food waste or microbial inoculums are employed to increase the pH, which, on the contrary, can be lowered by using bamboo charcoal or zeolite. These compounds can also reduce nitrogen losses, because of their ability to adsorb ammonia. In addition, through the incorporation of rice straw, ash, or several chemical compounds, it is possible to lessen the malodorous gasses full of sulfur and nitrogen, by absorbing them or by expanding the oxygen transfer.

Abdellah et al. [28] described how the incorporation of immobilization additives leads to the diminution of heavy metals or the modification of their oxidation state, in order to decrease the bioavailability and the mobility. Biochar, lime, and graphene oxide are considered effective towards the main heavy metals (Zn, Cu, Ni, Cd, Pb, Cr, As, Hg). In particular, biochar is a carbonaceous material which can be produced by different organic substrates under high temperatures (>400 °C) and a moderate oxygen supply [29], and its role as an organic additive is the main topic analyzed by other researchers.

For example, the shell biochar used by Awasthi et al. [30] in composting improved the efficiency of the process stimulating the microbial growth and the enzymatic activities, providing the optimal physico-chemical conditions for heavy metals resistant bacteria. In another case, bamboo biochar could significantly reduce total carbon and nitrogen losses, with a positive enhancement of enzymatic activities related to their metabolism [29]. The use of wood and wheat-straw biochar done in the work by Awasthi et al. [31] resulted in an interaction with the bacterial culture, determining the adsorption of heavy metals (Cu and Zn) during the process, thus improving the quality of the compost itself.

In this review, the composting process is discussed by exploring in a more detailed way its thermophilic phase, starting with the highlight on the thermodynamic evolution, which needs to be assessed when deciding to use reactors for composting. Subsequently, the bacteria that play key roles are evaluated. The roles of the genera *Bacillus* and *Thermus* are described, being often the main components of the microbiota of compost and being candidates for technological purposes. It follows a focus on the thermozymes which can be isolated from them, and which could have potential biotechnological and industrial applications. Experimental examples of enzyme-related literature are reviewed, investigating proteases, ureases, cellulases, hemicellulases, lignin-modifying enzymes, and esterases. Following, the composition of the microbial community is analyzed through the description of metagenomics approaches, a priority analysis essential to acquire knowledge on genomes of environmental microorganisms and communities. Finally, a space is dedicated to the description of the composting plant which treats olive oil wastes within the LIFE TIRSAV PLUS project (LIFE05 ENV/IT/00845), with the analysis of two plant solutions, that is the dynamic and the static composting.

Thermodynamics of the composting process

As a result of the composting process, organic solid waste (OSW) is decomposed by microorganisms into CO_2, H_2O, NH_3 and a significant amount of energy is released, as made explicit below.

$$\text{Organic matter} + O_2 \rightarrow CO_2 + H_2O + NH_3 + \text{humus} + \text{energy}$$

To sustain its metabolism, the microbial organism spends part of the produced energy, while the remainder is liberated into the surrounding system as heat [32]. When using reactors for composting, it critical to study the heat produced from organic compounds and the balance between the energy needed for the thermophile stage and the one lost through all the dissipation pathways [33].

Various approaches for quantifying the heat generation from composting have been elaborated, i.e., degradation method (DD), oxygen consumption method (OC), heat balance method (HB), CO_2 evolution method (CEM), temperature method (TEM) and heating value method (HVM), which will be discussed hereafter.

Degradation method (DD)

As previously stated, the heat generation is closely linked to the OSW degradation. Thus, a degradation model can be used to describe the composting process and its heat production. The general form can be expressed as shown in Eq. 1:

$$Q_{\text{bio}} = HV_r \times r = HV_r \times \frac{\mathrm{d}m}{\mathrm{d}t}. \quad (1)$$

Qbio is the heat production rate of composting (W); HVr is the heating value of the substrates (MJ kg^{-1}), defined as the amount of heat released per unit of substrate degradation; r is the coefficient of degradation rate (kg s^{-1}); t is the composting time (s); m is the weight of substrates (kg).

The degradation model can be considered as a function of HVr and r. For ease of calculation, HVr is regarded as a constant throughout the composting process, but in practice it gradually decreases. For r, it is difficult to accurately measure the degradation rate. Hence, many mathematical models have been formulated to describe the degradation process, such as first-order models and Monod-type models. In the first-order models, r is related to the concentration of composting substrates. These models are the most employed, because of their simplicity; however, they do not take into account the effects that other factors may have on the value of r. The Monod-type model illustrates the relationship between the microbial growth and the degradation process in composting. Due to the increased complexity and the need to obtain four or more coefficients to describe the process, this model has a more limited use.

O_2 consumption method (OC)

During the composting process, microorganisms require oxygen to start the decomposition of organic matter. The heat production rate during the metabolism is linearly correlated with the O_2 consumption rate, according to Eq. 2:

$$Q_{\text{bio}} = HV_O \times OCR = HV_O \times \frac{\mathrm{d}O_2}{\mathrm{d}t}. \quad (2)$$

HVo is the heating value defined as the amount of heat generated metabolically per mole of O_2 consumption (kJ mol^{-1} O_2), OCR is the O_2 consumption rate (mol kg^{-1}),

defined as the O_2 consumed per unit of time (mol O_2 s^{-1}).

HVo is commonly seen as a constant during the composting process. Most of the time, the O_2 consumption rate is determined by measuring the O_2 concentration difference in the inlet and outlet gas. Furthermore, the O_2 consumption rate can be estimated by first-order models or Monod-type models.

Heat balance method (HB)

Throughout the composting process, the release of energy from decomposition leads to the rise in temperature of the organic matter and water, heat loss via convection and conduction, and water evaporation. The heat basically presents in two forms: sensible heat (energy associated with an increase in temperature) and latent heat (energy associated with phase transformation). The actual heat output can be calculated from the measurement of the components indicated in Eq. 3 [32]:

$$Q_{\text{bio}} = Q_{\text{sensible}} + Q_{\text{latent}} = Q_{\text{gas}} + Q_{\text{sub}} + Q_{\text{loss}} + Q_{\text{vap}} + Q_{\text{rad}}. \quad (3)$$

Qsensible is the amount of sensible heat (W), Qlatent is the amount of latent heat (W). In particular, the components of the thermal balance are convective heat loss of inlet and outlet streams (air, vapor, and water) Qgas (W), sensible heat of composting materials Qsub (W), conductive/convective losses through surface of reactor Qloss (W), latent heat loss of water evaporation Qvap (W), and radiant loss Qrad (W).

It is possible to determinate the total sensible heat, which is the temperature increase of the liquid and solid components in the reactor, by varying the temperature of the matrix per unit time. The heat balance, using accumulated heat as a variable, is calculate in Eq. 4:

$$\frac{\mathrm{d}(mcT)}{\mathrm{d}t} = GH_{\text{in}} - GH_{\text{out}} - UA(T - T_a) + \frac{\mathrm{d}(BVS)}{\mathrm{d}t}H_c. \quad (4)$$

m is the mass of the matrix (kg); c is the specific heat of the matrix (kJ kg^{-1} $°C^{-1}$); T is the temperature of the matrix (°C); t is the time (days); G is the dry air mass flow (kg day^{-1}); Hin and Hout are the air enthalpy of the entrance and exit of dry air, respectively (kJ kg^{-1}); U represents the overall heat transfer coefficient of the matrix (kW m^{-2} $°C^{-1}$); A stands for the total surface area of the reactor (m^2); Ta is the ambient temperature (°C); BVS is the mass of degradable volatile solids (kg); and Hc is the reaction heat value of degradable organics (kJ kg^{-1}). In the equation, mc is not a constant amount and it changes frequently.

Ventilation is the principal cause of heat loss. Air entering the reactor is made up of dry air and water vapor. As this goes through the reactor, these two components are heated up and release the sensible heat. Then, when the water in the treated organic solid waste leaves the reactor as water vapor, heat is carried out in the form of both sensible heat and latent heat for vaporization [34, 35]:

$$Q_{\text{gas}} = F_{\text{gas}} \times (H_{\text{out}} - H_{\text{in}}). \quad (5)$$

Q_{gas} is the ventilation convective heat loss (kJ); F_{gas} is the ventilation air flow rate (kg h^{-1}); Hout is the enthalpy of the air flow leaving the system (kJ kg^{-1} of dry air); Hin is the enthalpy of the air flow entering the system (kJ kg^{-1} of dry air).

Sensible heat of composting materials Q_{sub} can be calculated as in Eq. 6 [19]:

$$Q_{\text{sub}} = m[(1 - MC)C_s + C_w MC]\frac{\mathrm{d}T}{\mathrm{d}t}. \quad (6)$$

m is the mass of composting pile (kg); MC is the moisture content (%); C_s is the specific heat capacity of solid (kJ K^{-1} kg^{-1}); C_w is the specific heat capacity of water (kJ K^{-1} kg^{-1}); T is the temperature of composting pile (K); t is the time (s).

The heat loss due to conductive transfer and radiation heat can be computed by using Eqs. 7 and 8 [33]:

$$Q_{\text{loss}} = UA(T_m - T_a). \quad (7)$$

Q_{loss} is conductive heat loss from the reactor wall (kJ); U is the overall heat transfer coefficient of the matrix (kW m^{-2} $°C^{-1}$); A is the reactor wall surface (m^2); T_m is the matrix temperature (°C); T_a is the ambient temperature (°C):

$$Q_{\text{rad}} = \sigma A_{\text{top}}(T_t^4 - T_a^4)F_a F_e, \quad (8)$$

where Qrad is radiant heat loss from the top surface of the materials (kJ); σ is the Stefan–Boltzmann constant; A_{top} is the surface area of the radiating body (m^2); T_t is the temperature of the top surface (°C); F_a is a configuration factor accounting for the relative position and geometry of the objects (dimensionless); F_e is the emissivity factor accounting for non-black body radiation.

The latent heat loss of water evaporation from composting pile is determined from the water vapor content of exit and inlet gases as after in Eq. 9 [35]:

$$Q_{\text{vap}} = F_{\text{gas}} Q_v (h_{\text{out}} - h_{\text{in}}). \quad (9)$$

F_{gas} is the flow rate of air (kg h^{-1}); Q_v is the enthalpy change of water vaporization (kJ kg^{-1}); h_{out} is the absolute

humidity of exit air (kg kg^{-1}), h_{in} is the absolute humidity of in air (kg kg^{-1}).

In large-scale reactors Qvap takes up the majority of the heat produced, while in small-scale reactors the greatest heat depletion is caused by Qloss. Qrad, instead, is so small that its contribution can be ignored [32].

CO_2 evolution method (CEM)

Variations of CO_2 concentration can manifest the degradation process since microorganisms decompose OSW and produce CO_2. There are several ways to assess the CO_2 concentration; the main ones are the use of CO_2 meters and the estimation via respiratory quotient. The general equation of CO_2 evolution method is Eq. 10:

$$Q_{bio} = HV_c \times CER. \tag{10}$$

HV_c is the heat released per unit of CO_2 evolution (kJ mol^{-1}); CER is the CO_2 evolution rate (mol s^{-1}).

Temperature method (TEM)

The temperature alterations in composting can be attributed to heat production and heat loss processes. TEM is applicable only when the heat loss is a known quantity, or it is equal to zero. For rapid evaluation, the specific heat capacity of the mixed solids and liquids in the system and the pressure at which the process takes place, are assumed constants. The heat production rate can be found by monitoring the rate of temperature change during composting process, for example using a water bath to retrieve the generated heat and measure the temperature difference between the composting and water bath. Owing to these rigorous conditions, TEM is usually used in laboratory bench-scale composting units:

$$Q_{bio} = m_{mix} \times C_{mix} \times \Delta T = m_{water} \times C_{water} \times \Delta T. \tag{11}$$

ΔT is the temperature change of the substrates or water (K); C_{mix} is the specific heat capacity of substrates (kJ kg^{-1} K^{-1}); m_{mix} is the mass weight of OSW (kg); m_{water} is the mass weight of water (kg); C_{water} is the specific heat capacity of water (= 4.2 kJ kg^{-1} K^{-1}).

Heating value method (HVM)

According to this method, it is considered that the difference between the heating value (HV) of composting substrates at the beginning and at the end of the composting is indicative of the measure of energy produced from degradation. The general equation of HVM is Eq. 12. For these measurements, a calorimeter is usually needed [32]:

$$Q_{bio} = HV_{in} - HV_{fin}. \tag{12}$$

HV_{in} is the HV of substrates at composting beginning (kJ kg^{-1}); HV_{fin} is the HV of substrate at the composting end (kJ kg^{-1}).

In conclusion, different methods have been developed to evaluate the effectiveness of the composting process. Moreover, it is fundamental to investigate thoroughly all the energy generation, transfer and loss processes associated to the OSW degradation in order to optimize, control and manage this phenomenon [33]. Finally, the technological and environmental potential of composting heat reuse is a current topic of study, as it can help respond to the energy demand and reduce the pressure of climate change.

Thermophilic microorganisms isolated from compost samples

A wide variety of prokaryotes (bacteria and archaea) tolerates and usually requires high temperatures for their growth and survival; they are known as thermophilic microorganisms. A great diversity of these microorganisms has been isolated and characterized in hot environments within the past few decades. Thermophilic microbial genera have been discovered from natural (geothermal areas, terrestrial hot springs, deep-sea hydrothermal vents, etc.) and man-made environments (waste treatment plants, biological wastes, self-heated compost piles, etc.) throughout the world.

Thermophilic microorganisms are directly involved in the thermogenic phase (50–80 °C) of composting process for treating organic solid waste. The composting processes start immediately, and the temperature of compost rises with time and within a few days reaches the maximum inside the central portion of the compost pile. During the temperature change, the population of thermophiles increases and the one of mesophiles decreases. The growth of the majority of the present microorganisms is inhibited by excessively high temperatures, and the decomposition of organic matter slows down. Thermophilic bacterial strains have been isolated from almost every type and nature of compost, including autotrophs, heterotrophs and mixotrophs, and each research work has highlighted different characteristics (Table 1).

Bacillus, *Thermus* and *Clostridium* show metabolic activity at temperatures above 60 °C [36]. The authors have reported that during the thermogenic phase of compost, only a few species of thermophilic sporogenous bacteria *(Bacillus stearothermophilus* and *Bacillus subtilis*) and the genus *Thermus* Gram-negative, aerobic, nonspore forming bacteria, showed metabolic activity above 70 °C. *Bacillus* spp. often occur as the major components of the microbiota (87%), including *B. licheniformis, B. subtilis, B. coagulans* type B, *B. stearothermophilus,* and

Table 1 Summary of the characteristics highlighted in the respective original works for the thermophilic bacteria

Thermophilic bacteria	Emphasized characteristics	Compost type	Refs.
Bacillus stearothermophilus; B. subtilis; genus *Thermus*	Metabolic activity above 70 °C	n.d.	[36]
Bacillus licheniformis; *B. subtilis*; *B. coagulans* type B; *B. stearothermophilus*; *B. sphaericus*	*Bacillus* spp. found during the thermophilic phase	Table scraps and shredded newspaper	[37]
Hydrogenobacter spp.	Sulfur- and hydrogen-oxidizing, autotrophic thermophilic bacteria	Green waste, wood chips, and kitchen waste, or sewage sludge	[38]
Bacillus stearothermophilus; Thermus thermophilus HB8	Growth between 65–69 °C; growth between 65–82 °C	Kitchen waste and shredded garden waste or methanized sewage sludge, and wood chips	[39]
Thermus thermophilus; Bacillus spp.	Growth above 70 °C	Kitchen waste, garden waste, grass, and shredded wood	[40]
Bacillus pallidus; B. stearothermophillus; B. thermodenitrificans	Isolates identification	Kitchen waste and shredded garden waste or methanized sewage sludge, and wood chips	[41]
Bacillus thermodenitrificans; B. sporothermodurans; B. thermosphaericus	Dominant species between 55–73 °C	General garden and domestic waste	[42]
Aneurinibacillus sp.; *Brevibacillus* sp.	Most abundant isolates	Synthetic food waste	[43]
Geobacillus toebii	Novel species isolated	Hay	[49]
Geobacillus toebii subsp. *decanicus* subsp. nov	Novel subspecies isolated	Organic urban waste and green bush waste	[50]
Geobacillus galactosidasius	Novel species isolated	From the Experimental System of Composting of Teora, Avellino, Italy	[51]
Bacillus composti; B. thermophilus	Novel Fe(III)-reducing species isolated	Sewage sludge and crop straw	[52]
Aeribacillus composti	Novel species isolated	Olive mill pomace	[53]

B. sphaericus [37]. Bacteria belonging to the genus *Thermus* have been isolated mainly from hot-spring ecosystems and they have probably adapted to the conditions in the different composting processes. In contrast to the above-mentioned work, reported during the thermophilic phase of composting the presence of a low diversity of obligatory heterotrophic thermophiles related to the genus *Bacillus stearothermophilus*, Beffa et al. [38] have reported for the first time the isolation of the sulfur- and hydrogen-oxidizing, autotrophic thermophilic bacteria related by high DNA:DNA homology to *Hydrogenobacter* strains. These last strains have been isolated only from geothermal areas and their presence in the thermogenic phase (> 60 °C) of the composting process suggests that they may play a part in inorganic sulfur compound oxidation and mineralization of waste, being candidates for further studies. Organic compost samples of 2–5 weeks with temperatures ranging from 65–69 °C showed only strains related to *B. stearothermophilus,* whereas at temperatures above 70 °C a considerable number of *Thermus* strains related to *Thermus thermophilus HB8* were the dominant active degraders [39].

The study conducted by Blanc et al. [40], from kitchen and garden waste compost, using a molecular approach with restriction enzyme analysis of a library of bacterial 16S rRNA gene clones, showed that among 200 clones investigated, about 70 could be identified as *Thermus thermophilus* and thermophilic *Bacillus* spp. Both taxa were found to be the dominant bacterial populations during the thermophilic phase of composting; Blanc et al. [41] reported through the Amplified Ribosomal DNA Analysis (ARDRA) method *Bacillus pallidus*, *B. stearothermophilus* and *B. thermodenitrificans* as predominant species in hot compost. Zhang et al. [42] from the analysis of 11 compost samples (domestic and garden waste) and using total plate counts of spore in combination with RAPD-based identification (randomly amplified polymorphic DNA), showed the thermophilic *Bacillus thermodenitrificans* as dominant isolate with a prominent role compared to *B. stearothermophilus* within the compost ecology. It was already reported [42, 43] the abundance of *B. thermodenitrificans* in domestic and garden composts, indeed RAPD profiles indicated that the thermophilic strains represented ~ 76.1% of the bacterial community, whereas lower numbers (~ 10%) were represented by *B. sporothermodurans*, *B. thermosphaericus, Aneurinibacillus* sp. and *Brevibacillus* sp., although the latter two strains are not typically associated with garden composts. Moreover, the study of the samples collected from different compost starting materials and managements was based on the use of different molecular phylogenetic techniques such as DGGE, T-RFLP, ARISA, 16S/23S rRNA intergenic spacer amplification, SSCP profiling and FISH hybridization, and the results showed that the thermophilic phase was characterized by the abundance of members of the genus *Bacillus* [44–47] and *Thermophilus* [48].

The combination of both culture-dependent and culture-independent methods (molecular techniques and isolation and identification of bacteria) is complementary, but not overlapping and a traditional microbiological approach is still effectual. New species of thermophilic bacteria have been isolated and characterized by biochemical and phylogenetic techniques, as in the case of *Geobacillus toebii* [49], *Geobacillus toebii* subsp. *decanicus* subsp. nov. [50], *Geobacillus galactosidasius* [51], *Bacillus composti* and *Bacillus thermophilus* [52], *Aeribacillus composti* [53].

To summarize, a considerable heterogeneity of thermophilic bacteria has been isolated from the thermogenic phase of the composting of different substrates, when they are the main responsible for the degradation of organic matter. The main genera represented are *Bacillus* and *Thermus*, often described as the main components of the microbiota of the piles of compost. These bacterial strains can be isolated from natural environments, and probably they adapted to the composting processes, becoming potential candidates for technological and industrial purposes.

Bacterial enzymatic activities in the thermophilic phase of composting

To degrade organic waste through composting, microbial communities produce and secrete a wide spectrum of thermostable enzymes, mainly hydrolytic and oxidoreductase activities [15, 54]: enzymes such as proteases, cellulases, hemicellulases and lignin-modifying enzymes are one of the main factors driving the composting processes and could have many potential industrial applications, thus representing a field of research in industrial enzymology, a sector of biotechnology [23, 54–56].

The joint activities of these extracellular enzymes are strictly dependent on the composition of the microbial communities, so that the readily degradable organic compounds and the more refractory ones are progressively decomposed and mineralized to be used as sources of carbon and nitrogen by the microorganisms, sustaining the microbial growth and ensuring the continuity of the process [57–59]. Additionally, the activities of the enzymes depend on different environmental factors of the composting process [54]. One of the main physico-chemical parameters associated to the efficiency of composting is temperature, and as a prolongation of the thermophilic phase can improve the quality of compost itself, extracellular enzymes secreted by microorganisms have a fundamental role, being associated to the increase of temperature [54, 60].

During the composting process, bacteria are accounted as the leading microorganisms for quantity and diversity, which is related to their small size and their ability to grow in a wide range of pH and temperature [20]. As discussed in the previous paragraph and highlighted in literature [55, 61, 62], most of the extremophile bacterial species which can be found in the thermophilic phase of composting belong to the *Bacillus* and the *Thermus* genera, and with them the correlated enzymatic activities, even considering intrinsic variability depending on the compost itself. For example, Miyatake and Iwabuchi [62] studied the variation of enzymatic activity of thermophilic bacteria in high-temperature dairy cattle manure compost, finding the highest bacterial activity at 54 °C, with a decrease at 60 °C, followed by a new increase at 70 °C but associated with a reduction of the decomposition of organic matter due to the high temperature itself. Increasing attention has been given to these enzymatic activities and to their succession during the composting process since they affect the transformations that happen, thus regulating the ability to decompose organic substances. They vary based on the composition of compost, the physico-chemical parameters, and the dynamics of the microbial community at each stage of the process [63–65].

Proteases and ureases

During the initial phase of composting, due to the presence of oligo- and polypeptides, a high protease activity is usually found. Proteases are related to the nitrogen cycle, being responsible for the hydrolysis of proteins [64, 66]. Urease activity is also related to the nitrogen cycle, especially in the first step of nitrogen mineralization, catalyzing the hydrolysis of urea into ammonium and carbon dioxide [64, 67]. Due to the joint action of urease and protease, together with high pH and temperature, large amounts of ammonia are released, which are then related to the quality of the compost [65].

Table 2 reports the latest research papers with the corresponding protease and urease activities found during the composting process, with the duration of the thermophilic phase, the most prominent bacterial species during that period and the composition of the compost.

It has been described that ammonia inhibits the activity of protease and urease [54], and this is coherent with the higher activity of protease and urease, associated to *Paenibacillus validus* 1VC, that has been found in weeks 4–8, at the end of the thermophilic phase, of the total 24 weeks of composting in the research by Hemati et al. [54]. Ammonia is formed at the beginning of the thermophilic phase because of the degradation of amines [8], but it rapidly decreases in association with high temperature and in relation to the oxygen insufflated through the aeration system [54]. Additionally, an extraordinary high activity of urease has been recorded at the very beginning of composting (week 1), probably because of the presence

Table 2 Proteases and ureases of composting, duration of thermophilic phase, prominent bacterial species, and type of compost

Enzyme	Thermophilic phase	Prominent bacteria	Compost type	Refs.
Protease	4th–8th week/24	*Paenibacillus validus* 1VC	Agricultural residue/sawdust 97:3 and 15:85	[54]
	4th–16th day/25	*Stenotrophomonas*, *Sinibacillus*	Mulberry branches/silkworm excrement 1:9 (w/w)	[23]
	4th–7th day/11	*Ureibacillus*, *Bacillus*, *Pseudomonas*, *Flavobacterium*, *Sporosarcina*	Sludge/corn straw 3:1 (w/w)	[57]
	3rd–16th day/25	*Bacillus* sp.	Chicken manure/rice husk (17.3 C/N)	[58]
	5th–22nd day/39	*Nonomuraea* sp., *Virgibacillus* sp.	Cow manure, mushroom residue, sawdust	[59]
	2nd–7th day/11	*Bacillus* sp.	Sewage sludge/sawdust/ biochar	[68]
Urease	From 6 to 26 days/31	Firmicutes, Proteobacteria	Food waste	[15]
	4th–8th week/24	*Paenibacillus validus* 1VC	Agricultural residue/sawdust 97:3 and 15:85	[54]
	4th–16th day/25	*Stenotrophomonas*, *Sinibacillus*; *Stenotrophomonas*, *Bacillus*	Mulberry branches/silkworm excrement 1:9 (w/w); mulberry branches/cow dung 3:7 (w/w)	[23]
	4th–7th day/11	*Ureibacillus*, *Bacillus*, *Pseudomonas*, *Flavobacterium*, *Sporosarcina*	Sludge/corn straw 3:1 (w/w)	[57]
	3rd–16th day/25	*Bacillus* sp.	Chicken manure/rice husk (17.3 C/N)	[58]
	2nd–7th day/11	*Bacillus* sp., *Candidatus microthrix*	Sewage sludge/sawdust/ biochar	[68]

of urea in the compost itself, that stimulated the expression of the enzyme.

A decrease of urease following a high activity during the first half of the thermophilic phase has been reported in H. Liu et al. [58], where the authors studied the composting of chicken manure and rice straw combined with two different C/N ratio, and in Li et al. [69], where they studied the effects of the addiction of a microbial inoculum to the treatment of pig manure and corn straw (6:1 w/w). They highlighted how there is not a significant association between the urease activity and bacteria during the thermophilic phase. Similarly, the urease activity described by J. Du et al. [68] in the composting of sewage sludge, sawdust, and biochar in different ratios generally resulted in an increase at the beginning of the process, followed by a decrease entering the thermophilic phase. In the same work, the activity of protease increased only later in the composting, at the very end of the thermophilic phase, probably because the involved microorganisms needed to adapt to be active and to start secreting these enzymes. Huang et al. [67] reported about the effects of a hyper-thermophilic pretreatment of pig manure and straw compost, that is a short-term pretreatment at high temperature, which determined a decline in the relative abundance and diversity of the microbial community, especially of the bacteria responsible for the ammonification activities during the thermophilic phase. This contributed to the decreasing activity of the enzymes expressed by these microorganisms with respect to the control pile of composting, such as protease and urease, which has also been one of the causes of a reduced ammonification rate. In turn, the lowering of nitrogen loss could have contributed to the inhibition of these enzymes.

The industrial-scale composting of food waste of 31 days researched by Zhang et al. [15] has been conducted comparing different aeration frequencies. The increase of the aeration rate determined an increasing duration of the thermophilic phase, but the authors excluded a significant correlation with the activity of urease, which gradually decreased starting from the beginning of the process, with a later reduction in the composting due to the accumulation of nitrate. Ma et al. [57] studied the composting of sludge and corn straw, finding even in this case a decrease of the urease activity during the thermophilic phase (day 5–7 over the total 11 days), while the protease activity increased and reached a peak of 3.25 mg g^{-1} from the 7th day, then it maintained this activity until the end of the process. A similar trend in the protease activity has been described in the already mentioned work by H. Liu et al. [58], where, during the composting of chicken manure and rice straw, it showed higher values in the thermophilic phase compared to the initial mesophilic one, from the 7^{th} day to the end on the process (25 days in total), being over 1012 mU g^{-1}.

Among different composting material tested, Q. Liu et al. [23] reported the highest protease activity during the thermophilic phase in mulberry branches and silkworm excrement (1:9 w/w) compost, reaching over 60 μg g^{-1} min^{-1}, explainable with the high crude protein content of silkworm excrements. Also, they reported a high urease activity in both mulberry branches/silkworm excrement and mulberry branches/cow dung (3:7 w/w)

composts, measured over 17 μg g^{-1} min^{-1}. Similarly, Qiao et al. [59] monitored the extracellular enzymatic activities produced during the composting of three starting material, namely cow manure, mushroom residue, and sawdust, with three different C/N ratios (15, 25 and 35). They reported that the protease activity decreased during the composting process, with an activity measured at 91.36 mU g^{-1} during the thermophilic phase, mainly associated to *Nonomuraea* and *Virgibacillus* sp. The decreasing is associated to the initially high availability of degradable organic compounds in the starting material, which is then gradually decomposed.

Cellulases, hemicellulases and lignin-modifying enzymes

Lignocellulose is the major fraction of most organic waste; it consists mostly of cellulose, hemicellulose, and lignin, and it is the most stable and refractory fraction of organic matter in the composting process, so that its degradation is one of the major factors that could limit the composting efficiency [23, 54, 56]. Enzymatic activities can degrade cellulose and hemicellulose into simple sugars, and lignin into polyphenols [56]. However, the enzymatic degradation of lignin is complex due to the phenolic rings that constitute its highly branched and irregular polymer, so that the mineralization could be not completed during the composting process [54, 56]. Fungi are known to degrade lignin in an efficient way, however, during the thermophilic phase of composting most fungi are inactivated. The composting time is mostly dependent on the activity of the enzymes secreted by lignocellulolytic thermophilic bacteria, that can speed up the process in the presence of high percentage of plant residues and green wastes [20, 54, 56, 64]. Additionally, thermophilic microorganisms able to degrade lignocellulose contribute to loosen its structure, facilitating other enzymatic activities in the further phases of composting [70]. The degradation of lignocellulose happens at faster rates during the thermophilic phase due to the abundance of bacteria able to degrade it through the production and secretion of hydrolytic enzymes, whose activity increases with the rise of composting temperature [23]. This has been reported by Zhu et al. [56], who obtained an increment of simple organic matter through a thermal pretreatment of dairy manure, and even an increment of the relative abundance of thermophilic bacteria later involved in the enzymatic decomposition of lignocellulose, thus improving the quality of the compost obtained.

Cellulases and hemicellulases include numerous enzymatic activities which regulate the carbon cycle of composting, and which have an impact on the whole nutrient cycle, increasing soluble nutrients during the process [64, 65]. In particular, cellulases and activities such as endo- and exo-glucanases, cellobiohydrolases, β-glucosidases and β-glycosidases are related to the cellulose cycle, working synergistically to degrade cellulose, while hemicellulases and enzymes such as xylanases are related to the hemicellulose cycle, for the degradation of the polymers of xylan [54, 58, 63]. The lignin-modifying enzymes, formerly named ligninases and lignases, have an activity related to the lignin cycle being able to catalyze the degradation of the polymer of lignin through an oxidative mechanism, with the generation of free radicals [54, 71]. These enzymes are numerous, with some of the main groups being laccase, manganese-dependent peroxidase, and lignin peroxidase [71]. Peroxidases have a key role in the degradation of more refractory organic matter such as lignin and other similar structures [45].

Table 3 reports the latest research papers with the corresponding cellulases, hemicellulases and lignin-modifying enzymes found during the composting process, with the duration of the thermophilic phase, the most prominent bacterial species during that period and the composition of the compost.

Hemati et al. [54] measured the highest cellulase activity within the 4th and the 12th week of the total 24 weeks of composting, between the end of thermophilic phase and the starting of the cooling phase and associated it to *Bacillus nealsonii* 104C. In the high lignin compost (agricultural residue/sawdust 15:85), the increase in temperature during the thermophilic phase promoted the denaturation of polymers, making more substrates available, increasing the microbial community and the enzymatic activities, and lowering the decrease of the cellulase activity with respect to the low lignin composting process (agricultural residue/sawdust 97:3). Additionally, in the same work the authors distinguished the activity of generic cellulases from the one of β-glucosidase, that was higher at the beginning of the composting process, in weeks 2–4 (thermophilic phase), and it has been associated to *Bacillus nealsonii* 104C. β-glucosidases are active on cellulose and other disaccharides, they hydrolyze β-D-glucose chains releasing β-glucose, and their activity is higher in presence of easily metabolizable substrates available to microorganisms, and in presence of high concentration of water-soluble carbon, such as at the beginning of composting [54, 66, 68].

A similar trend in the cellulase activity but on a shorter composting period has been reported by Jiang et al. [65], with a peak on the 4^{th} day on the total 24 days of composting of sewage sludge and saw dust at a ratio of 4:1 (w/w fresh weight). Moreover, their addiction of "garbage enzymes" obtained through the composting of kitchen wastes increased the relative abundance of bacteria of the phylum Firmicutes during the thermophilic phase, with a significant enhancement of cellulase activity. Instead, during the thermophilic phase of the composting of

Table 3 Cellulases, hemicellulases and lignin-modifying enzymes of composting, duration of thermophilic phase, prominent bacterial species, and type of compost

Enzyme	Thermophilic phase	Prominent bacteria	Compost type	Refs.
Cellulase	4th–12th week/24	*Bacillus nealsonii* 104C	Agricultural residue/sawdust 97:3 and 15:85	[54]
	Not specified	*Bacillus subtilis* A-, M- and N-SRETCR	Coffee residues/cow manure 3:1 (v/v)	[61]
	9th–28th week/44	*Devosia*, *Flavobacterium*, *Pseudoxanthomonas*, *Pseudomonas*, and *Achromobacter*	Textile waste	[20]
	4 days/24	Firmicutes	Sewage sludge/saw dust 4:1 (w/w)	[65]
	4th–7th day/11	Mostly *Bacillus* sp.	Sludge/corn straw 3:1 (w/w)	[57]
	3rd–16th day/25	Mostly *Bacillus* sp.	Chicken manure/rice husk (17.3 C/N)	[58]
	5th–22nd day/39	*Nonomuraea* sp., *Virgibacillus* sp.	Cow manure, mushroom residue, sawdust	[59]
	From 6 to 26 days/31	Firmicutes, Proteobacteria	Food waste	[15]
	2nd–7th day/11	*Bacillus* sp.	Sewage sludge/sawdust/ biochar	[68]
β-glucosidase	2nd–4th week/24	*Bacillus nealsonii* 104C	Agricultural residue/sawdust 97:3 and 15:85	[54]
	4th–16th day/25	*Stenotrophomonas*, *Bacillus*	Mulberry branches/cow dung 3:7 (w/w)	[23]
	2nd–7th day/11	*Bacillus* sp.	Sewage sludge/sawdust/ biochar	[68]
Xylanase	4th–12th week/24	*Paenibacillus validus* 1VC	Agricultural residue/sawdust 97:3 and 15:85	[54]
	4th–16th day/25	*Stenotrophomonas*, *Bacillus*; *Stenotrophomonas*, *Sinibacillus*	Mulberry branches/cow dung 3:7 (w/w); mulberry branches/ silkworm excrement 1:9 (w/w)	[23]
	3rd–16th day/25	*Bacillus* sp.	Chicken manure/rice husk (17.3 C/N)	[58]
	9th–28th week/44	*Devosia*, *Flavobacterium*, *Pseudoxanthomonas*, *Pseudomonas*, and *Achromobacter*	Textile waste	[20]
	11 days/180	Firmicutes	Tomato plant waste/pine chips 1:1 (w/w)	[70]
Ligninase	2nd–4th week/24	*Paenibacillus koreensis* 12C; *Paenibacillus validus* 1VC	Agricultural residue/sawdust 97:3 and 15:85	[54]
Peroxidase	4th–7th day/11	*Bacillus* sp.	Sludge/corn straw 3:1 (w/w)	[57]
	2nd–7th day/11	*Bacillus* sp.	Sewage sludge/sawdust/ biochar	[68]

sludge and corn straw done by Ma et al. [57], between the 5th and the 7th day of the total 11 days of the process, the cellulase activity mostly associated to *Bacillus* sp. gradually increased at 250 μg g^{-1} min^{-1}. It did not reach a peak in this phase probably because of the high cellulose content of the substrate, allowing further cellulase production in the following phase: the authors highlighted that since cellulose is a moderately refractory organic matter, its degradation mainly happens in the middle and late phases of composting. A comparable increase in the cellulase activity, even in this case associated to *Bacillus* sp., has been reported by Du et al. [68] in the middle of the thermophilic phase, nevertheless with a peak at the end of the 11 days process, probably because cellulase is a complex polysaccharide, and microorganisms need to gather enough nutrients to decompose it, with a subsequent production of cellulases later in the process. Additionally, the authors differentiated the activity of β-glucosidase, again associated mainly to *Bacillus* sp. which was higher at the beginning of composting until the start of the thermophilic phase.

Siu-Rodas et al. [61] isolated three strains of *Bacillus subtilis* from the thermophilic phase of composting, namely A-, M- and N-SRETCR, that showed endo- and exo-cellulase activity at acid pH (4.8 and 5.8, respectively), with potential applications in animal foods, fruit juice extraction and clarification and paper bleaching industries, and basic pH (9.3), suitable for applications in paper and detergent industries. Additionally, the cellulase from the strain A-SRETCR showed a higher activity at 60 °C. During the 44 weeks composting of textile waste, Biyada et al. [49] detected different bacterial genera, such as *Devosia*, *Flavobacterium*, *Pseudoxanthomonas*, *Pseudomonas*, and *Achromobacter*, with cellulase and xylanase activities associated to the thermophilic phase. The authors related the high concentration of cellulolytic bacteria during this phase to the high temperature itself that could have assisted the degradation of cellulose.

The composting process of 25 days of chicken manure and rice husk in two different C/N ratios, namely 9.61 and 17.3, has been researched in H. Liu et al. [58], and the latter was found more suitable, with an increase in the relative percentage of *Bacillus* sp. also during the thermophilic phase. The authors related this abundance to cellulase and β-glycosidase activities, leading to a prolonged thermophilic phase (from the 3rd to the 16th day) and a fast and broad degradation of cellulose and hemicellulose with respect to the compost with 9.61 C/N

ratio, highlighting a critical role in the progressing of the composting itself. The cellulase activity peaked on the 3rd day, measuring 11 U g^{-1}, while the β-glycosidase reached a high value later in the composting process, being measured 3.80 U g^{-1} during the same day. A similar trend has been highlighted in the already cited study of industrial-scale composting of food waste by Zhang et al. [15]. The authors reported a high cellulase activity at the beginning of the process and then entering the thermophilic phase, followed by a decrease of its activity with the increase of the temperature in the second half of the thermophilic phase. Even in the already mentioned composting process studied by Qiao et al. [59], the authors reported an increase of the cellulase activity during the thermophilic phase, measured at 14.03 U g^{-1}. This resulted in an efficient decrease of cellulose of 78.9%, 64.6% and 61.6% at the end of the overall 39–days process for the three piles of compost considered (cow manure, mushroom residue, and sawdust, respectively). Additionally, they reported that the β-glycosidase activity decreased through the composting, measured at 16.39 U g^{-1} during the thermophilic phase, being related to the presence of substrates that could be readily metabolized.

Depending on the composting materials used, Liu et al. [23] reported different predominant enzymatic activities during the thermophilic phase (from day 4 to day 16 of the total 25 days), when the degradation of organic matter mainly happens. Specifically, the highest β-glucosidase and endo-glucanase activities have been measured for the composting of mulberry branches and cow dung (3:7 w/w), 1.31 and 17.15 μg g^{-1} min^{-1}, respectively, while the activity of exo-glucanase and xylanase were higher in composting of mulberry branches and silkworm excrement (1:9 w/w) and again in the mulberry branches and cow dung compost.

The xylanase activity in the above-mentioned work by Hemati et al. [54] had a similar trend to the one of cellulase in the same work, with the highest activity at the end of the thermophilic phase, when the high temperature determines changes in the polymers, and it has been associated to the *Paenibacillus validus* 1VC isolate. On the contrary, the xylanase activity described in the composting of chicken manure and rice husk researched by H. Liu et al. [48] had a peak at the beginning of the process and another peak in the middle of the thermophilic phase, being $25.4{\cdot}10^3$ U g^{-1} on the 9th day over the total 25 days. The composting of tomato plant waste and pine chips over the course of 6 months in the research done by López et al. [70] resulted in a thermophilic phase that lasted for 11 days, and the authors isolated bacteria with xylanase activity, mostly belonging to the phylum Firmicutes. Given their wide thermostability, the authors suggested as they could have a key role in the degradation of cellulose and hemicellulose during the composting process.

Hemati et al. [54] detected an enzymatic activity generally called ligninase, with the highest activity in the thermophilic phase and they associated it with *Paenibacillus koreensis* 12C and *Paenibacillus validus* 1VC from the 2nd to the 4th week of the total 24 weeks of composting. In the cited work by Ma et al. [57], the authors described a peroxidase related to lignin and to other refractory organic matter degradation, with a high activity during the thermophilic phase and a peak of 192.50 μmol h^{-1} g^{-1} on the 7th day of the total 11 days of composting. A peroxidase activity strongly associated to *Bacillus* sp. has been described also in J. Du et al., [68], with an increase through the composting process, and pointing at a high lignin degradation only at the end of it.

Du et al. [60] studied the addition of exogenous enzymes on the thermophilic composting of wildlife manure. The treatment with 0.1% of the enzymatic complex composed of cellulase 10,000 U g^{-1}, xylanase 20,000 U g^{-1}, β-glucanase 5000 U g^{-1} and pectinase 1000 U g^{-1} resulted in an improvement of the compost maturity, with an increase of duration of temperatures over 60 °C (33 over 38 days of total composting) during the thermophilic phase. The authors suggested that the ligninolytic enzymes improved microbial activity, thus promoting the elimination of organic micro-pollutants and pathogens through a favorable thermophilic phase. Moreover, they reported predominant relative abundance of Firmicutes and of Actinobacteria during the first and the latter half of the thermophilic phase, respectively. A conceptually opposite experiment has been performed in the work by Li et al. [69], where the authors studied the impacts of microbial inoculation screened from a high-temperature compost on the activity of the enzymes during the composting itself. The addiction of 1.0% of liquid inoculum of *Acinetobacter pittii*, *Bacillus subtilis* subsp. *Stercoris*, and *Bacillus altitudinis* in the compost of pig manure and corn straw (6:1 w/w) determined an increase of cellulase activity with respect to the untreated composting process, being 2.40 against 2.02 mg glucose g^{-1} 24 h^{-1}, respectively, on the 24th day of composting of the total 32 days, during the thermophilic phase.

Esterases

Aside from the activities usually considered by most researchers, there are also papers which focus on other enzymes, such as esterases, giving insight on their activity through the composting process and during the thermophilic phase.

Esterases catalyze the hydrolysis of an ester group, with the release of the esterified acid [72]. Phosphatases are related to the phosphorus cycle, being able to catalyze the

Table 4 Esterases involved in composting, duration of thermophilic phase, prominent bacterial species, and type of compost

Enzyme	Thermophilic phase	Prominent bacteria	Compost type	Refs.
Phosphatase	From 6 to 26 days/31	*Firmicutes, Proteobacteria*	Food waste	[15]
	2nd–4th week/24	*Bacillus nealsonii* 104C	Agricultural residue/sawdust 97:3 and 15:85	[54]
	23 days/32	*Rummeliibacillus pycnus*	Pig manure/corn straw 6:1 (w/w)	[69]
	3rd–16th day/25	Actinobacteria, Bacilli	Chicken manure/rice husk (17.3 C/N)	[58]
Esterase	Not specified	*Geobacillus thermodenitrificans* CMB-A1, A2, A3, A7 and A10	Manure compost	[55]

hydrolysis of phospholipids, with the release of free phosphorus, which is assimilated by microorganisms [15, 54].

Table 4 reports works from the literature with esterase activities found during the composting process, with the duration of the thermophilic phase, the most prominent bacterial species during that period and the composition of the compost.

Zhang et al. [15] selected an alkaline phosphatase, compatible with the pH of the industrial food-waste compost considered, ranging from 7.5 to 8.5. In the composting processes, the enzyme showed an increasing activity in the first half of the thermophilic phase, with a decrease in the latter one; however, in the process with the higher aeration frequency, the phosphatase activity was not maintained during the thermophilic phase. Hemati et al. [54] reported about an alkaline phosphomonoesterase, whose activity slightly increased during the first two weeks of the thermophilic phase, and then decreased until the end of this phase. In the high lignin composting, the highest activity has been associated to *Bacillus nealsonii* 104C in the 4th week. The composting experiment performed by Li et al. [69] with pig manure and corn straw showed a continuous decrease of an alkaline phosphatase during the process, however with a positive correlation with *Rummeliibacillus pycnus*, of the phylum Firmicutes. An alkaline and an acid phosphatase have been distinguished in H. Liu et al. [58], both showing a decreasing trend in the activity through the composting process, with an activity in the pile of chicken manure/rice husk (17.3 C/N) measured at 46.0 and 0.97 U g^{-1}, respectively, on the 9th day of composting, in the middle of the thermophilic phase. On a more generic level, bacterial isolates from manure compost in Charbonneau et al. [55] related to *Geobacillus thermodenitrificans*, strains CMB-A1, A2, A3, A7 and A10, showed a thermotolerant esterase activity at 60–65 °C on tributyrin and olive oil. Additionally, in the same work the authors noted a high lipolytic activity for *Aneurinibacillus thermoaerophilus* strain CMB-C1 and for *Bacillus smithii*, from pH 5–9 and pH 5–7, respectively.

In conclusion, different thermostable enzymes can be obtained from thermophilic bacteria isolated from composting processes, mainly hydrolytic and oxidoreductase activities, and they could have biotechnological and industrial applications. Their succession during the composting process regulates the decomposition of organic substances. For example, protease and urease activities are found at the beginning of the process, with a general decrease during the thermophilic phase with the accumulation of ammonia. The progressing of composting mostly related to lignocellulolytic thermophilic bacteria, whose cellulase, hemicellulases and lignin-modifying enzymes work synergistically to degrade lignocellulose, a major fraction in most organic waste. The degradation of cellulose has been reported in different periods of the thermophilic phase. Even lignin-related enzymes and esterases discussed in research have a prominent activity during this phase. The characteristics of resistance to high temperature and high or low pH sometimes reported for these enzymes are of great interest for their technological potential, being suitable for industrial processes where mesophilic enzymes could fail.

Composition and diversity of the microbial community during composting by metagenomics approaches

Metagenomics is the study of genetic material extracted directly from environmental samples, which produces a profile of the microbial communities, opening an ideal window on the microbes present in a habitat and overcoming the "pure-culture" paradigm [73]. This implies that the metagenomics studies focus on a whole microbiota, with reference to all microbial species ranging from the ecological community of commensal to symbiotic and pathogenic strains of a peculiar niche. The term metagenomics, coined in 1998 by Handelsman et al. [74], it has been recently redefined by Chen and Pachter as "the application of modern genomics technique without the need for isolation and laboratory cultivation of individual species" [75]. In fact, these studies revealed that usually less than 1% of microorganisms from natural sources could be cultivated under laboratory conditions [76], and that the uncultured species not only constitute a major part of the microbial communities, but they

could also perform key functions in ecological processes. Therefore, despite that metagenomics is a relatively new but fast-growing field within biology, it is intended to be a priority analysis for the purpose of acquiring knowledge on genomes of environmental microbes, as well as of entire microbial communities. Metagenomics analysis targeting total DNA isolated from the environment or from animal sources could be performed using two types of approaches, being "Sequencing metagenomics or sequencing-based metagenomics" and "Functional metagenomics or function-based metagenomics". The first, through (1) Target oriented method or DNA metabarcoding and (2) Total metagenome method or shotgun metagenomics, allows the characterization of a microbial community in terms of taxonomic composition and the prediction of functional diversity. The second is aimed at the discovery of new genes encoding enzymatic activities of biotechnological interest, and to assess the functional activities and ecological roles of particular microorganisms.

The biological decomposition of organic matter is especially due to the presence of thermophilic and mesophilic microorganisms, and bacterial phyla including *Bacteroidetes, Actinobacteria Proteobacteria* and *Firmicutes* are usually found in the composting process [77]. The succession of microbial communities is the key for an efficient and a profitable process, influencing the quality and the maturity of compost and the rate of biodegradation [78]. In a standard composting process, the aerobic microbial metabolism leads to temperature increases above 50 °C, followed by high temperatures that are maintained until most biodegradable materials are completely digested. Then, the material slowly cools down as well as the microbial activity, and the organic matter stabilizes [79]. The resident microbial community includes mesophilic and thermophilic bacteria and fungi which continuously fit to the changing nutrient supply and which alter the environmental conditions. Moreover, since fungi were not detected in a composting sample with temperature above 65 °C, it was suggested that bacteria are the dominant degraders and the main responsible of the recycling of the organic wastes in thermophilic phase of composting processes, while fungi play a functional role during the cooling and curing phases.

The types of raw materials and the operations of the composting process would give different microbial communities. Overall, the genera *Pseudomonas, Acinetobacter, Steroidobacter, Bacilli* and *Sphingobacterium* were the most abundant genera in rice straw, sugar cane bagasse, and coffee hulls composting processes with addition of cow manure [22]. In sludge and cattle dung composting, *Ureibacillus, Tepidimicrobium, Kribbella* and *Bordetella* were reported to be the dominant ones [80], while in maize straw composting processes, *Sporosarcina, Bacillus, Cellvibrio, Devosia* and *Cellulomonas* were the most abundant [81].

Therefore, to improve the quality of compost and to shorten the duration of the process modulating the functional microbes within the procedure, it is required to investigate the microbial communities, their interaction, and their response to physico-chemical properties of compost, such as temperature, moisture content, C/N ratio, water content, aeration, and other factors. Moreover, because composting is considered an interesting and potential source of novel thermophilic microorganisms and a biotechnologically source of new thermozymes useful for industrial applications, the metagenomics and metatranscriptomics tools are valuable approaches to study these microorganisms and their metabolism. Therefore, these microorganisms and enzymes could be employed in biomass valorization, green processes, and sustainable transformation [78], as well as being helpful in the characterizations of the bioprocess itself [24]. These microorganisms have a crucial role in the global carbon cycle and act as sources of biochemical catalysts for advanced biofuels production [82].

Wang et al. [82] used a metagenomic analysis of the rice straw-adapted (RSA) microbial consortia enriched from compost ecosystems to elucidate the systematic and functional properties of this microbiome. The authors employed high-throughput 16S rRNA gene pyrosequencing and phylogenetic classification, the analysis of the 16S pyrotag library, and 5 Gbp of metagenomic sequence beside the DNA library construction and sequencing. They showed that the phylum Actinobacteria was the predominant group among the Bacteria in the RSA consortia, followed by Proteobacteria, Firmicutes, Chloroflexi, and Bacteroidetes. The Carbohydrate Active EnZyme (CAZyme) profile revealed that CAZyme genes in the RSA consortia were also widely distributed within these bacterial phyla. About 46.1% of CAZyme genes were from actinomycetal communities, which included cellobiohydrolase, β-glucosidase, acetylxylan esterase, arabinofuranosidase, pectinlyase, and ligninase genes. The presence of this distinct repertoire of CAZyme genes in the RSA consortia suggests a synergistic system efficient in the processing and metabolizing of carbohydrates in the compost habitats based on lignocellulosic biomass.

In another study, Tian et al. [21] described the composting experiments conducted using dairy manure and rice chaff as starting materials of the process, in the Lian Ye composting plant (Jiang Yin, China). This study provided an improved understanding of the bacterial composition and dynamics during the composting process since a large number of clones were analyzed and a high

coverage value of each clone library was obtained. The experimental procedure implied the construction of four 16S rRNA gene clone libraries from compost samples taken at fixed days as 0, 12, 42 and 112, and about eight hundred randomly selected clones were sequenced. The results showed that the microbial communities varied significantly during the process. Firmicutes and Proteobacteria were the two most abundant phyla at all stages of sampling. Bacteroidetes and Chloroflexi resulted as ubiquitous, while the phylum Actinobacteria was prevalent only at the thermophilic stage (day 42) in which most of sequences fell into the genus *Arthrobacter*. The authors registered that a short variation in bacterial diversity at the phylum and genus levels was checked as the composting process went on, contrary to what happened at the level of the species that instead, varied greatly. In addition, the candidate phylum BCR1 (Bacterial rice cluster 1) was originally revealed by phylogenetic analysis of 16S rRNA genes amplified from anoxic bulk soil of flooded rice microcosms and found by molecular methods in diverse environments (soils, activated sludge, anaerobic digesters and wastewater treatment reactors, marine and freshwater sediments, geothermal springs), and sequences belonging to it were recorded also in the Lian Ye composting plant. In conclusion, the lower bacterial diversity at the species level was found at the thermophilic stage of process. In contrast, a high level of mesophilic bacterial diversity was observed in the cured compost.

In a recent paper, Zhong et al. [22] studied the dynamic changes in structure and potential function of microbial population during dairy manure composting process. The bacterial community dynamics and diversity were monitored throughout the composting processes by the Illumina MiSeq platform for high-throughput sequencing. Potential function of bacterial community was predicted by advanced bioinformatics tools (e.g., co-occurrence network analysis and PICRUSt). Although the highest alpha diversity was observed in the initial samples, most of the species were not functional microbes for the composting reaction because a self-purification mechanism appears in order to eliminate the undesired microbes and develops the microbial community able to degrade the raw material. The evolution of bacterial community during composting was observed, and a total of 21 phyla were detected throughout the dairy manure composting process, with Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria, Chloroflexi, and Planctomycetes as the dominant. In the initial phases of composting, genera from Firmicutes and Actinobacteria such as *Corynebacterium, Clostridium* sensu stricto, *Clostridium XI, Romboutsia* and *Turicibacter*, were significantly higher. *Corynebacterium, Clostridium, Romboutsia* and *Turicibacter* have been reported to be the dominant species in raw dairy manure. In addition, other genera such as *Bacillus* and *Geobacillus* from the phylum Firmicutes, were found in the thermophilic phase of composting and they are generally considered relevant thermophilic decomposers. In the cooling phase, the relative abundance of the phylum Proteobacteria was significantly predominant and at the genus level, *Chelatococcus, Filomicrobium, Chelativorans, Kofleria, Azomonas, Povalibacter* and *Luteimonas* were identified as indicators for this phase. Moreover, the genera *Thermobifida* and *Thermomonospora* affiliating to Actinobacteria were also found in the cooling phase. Other dominant Actinobacteria, such as *Mycobacterium*, *Nonomuraea* and *Actinomadura* had significantly higher relatively abundance in maturation phase. The phylum Bacteroidetes was dominant in the cooling phase while the phylum Planctomycetes was dominant in the maturation step. Regarding the function profile, PICRUSt indicated that the bacterial metabolism changed as the composting progressed, suggesting that a specific metabolic function was required in different composting steps. These findings will provide significant information for a better understanding of the function and the structure of microbial community during the composting ecosystem.

Even in the case of food processes that have suffered an error during the line of production, the resulted waste can be used in the composting processes for the recovery of organic material. This is the case described by Papale et al. [83], in which waste biomass coming from a local coffee company, which supplied burnt ground coffee after an incorrect roasting process, was employed as a biomass to be used in the composting plan. Genomic and predictive metabolic analysis of the 16S rRNA V3–V4 amplicon and culture-dependent analysis were both used to identify the main microbial factors that characterized the composting process. The abundance of the Bacillales order (phylum Firmicutes) indicated a shift from the mesophilic to the thermophilic phase of the composting processes, confirming the ability to degrade coffee constituents, such as cellulose and hemicellulose, by members of Bacillales. The finding of archaeal component dominated by ammonia-oxidizing Archaea (AOA) within Thaumarchaeota, led to suppose that ammonification (a process in which ammonium can be released from organic nitrogen when present in the fresh substrates) could be a prevalent process in the early thermophilic phase of the ground coffee compost.

Next-generation sequencing and amplification of 16S rRNA and Internal Transcribed Spacer (ITS) gene amplicons genomic regions of bacteria and fungi, respectively,

were employed to analyse the DNA extracted from textile waste compost samples taken both at mesophilic (week 9) and thermophilic phases (week 28) of the process [20]. It was observed that Proteobacteria, Bacteroidetes, and Actinobacteria were the dominant bacterial phyla present in the mesophilic phase, but they did not find them in the thermophilic phase. Composting of textile waste exhibited a sustained thermophilic profile (above 55 °C) that usually precludes fungal activity. Nonetheless, the presence of fungi at the thermophilic phase was observed. In particular, the phyla Rozellomycota, Basidiomycota, and Ascomycota were recorded both in mesophilic and in thermophilic steps.

To summarize, depending on the raw materials and the operations of the composting process, different microbial communities can arise, each characterized by different internal interaction and response to physico-chemical properties. As a direct consequence, the application of metagenomics to study these microorganisms. The functional metagenomics essentially applies two different methodological approaches, being the Sequencing-based approach and the Functional-based screening, and both constitute the most important way to discover novel extremozymes useful for industrial purposes.

Although molecular techniques are of increasing usage in the elucidation of microbial community, the isolation of microorganisms remains an essential task both to relate taxonomic and metabolic diversity of organisms and to recover relevant species as purified microorganism for further use. In fact, composting ecosystem represents a rich source for the isolation of microorganisms that are useful, e.g., inoculants for composting and producers of enzymes that hydrolyze polymers or degrade recalcitrant compounds. Despite the broad research in this field, the potential of this ecosystem for the discovery of novel microorganisms and secondary metabolites is far from being fully exploited, especially considering that each process and raw materials may provide different strains.

Composting example: recovery of olive oil waste in the LIFE TIRSAV PLUS project

In the EU producing countries, it takes place 70–75% of the world's olive oil production, with 1,920,000 tn produced in the 2019/2020 olive-growing season (COI 2020 data). Olive groves are located in nine EU Member States in the Mediterranean region and cover an area slightly lower than 5 million hectares. The environmental impact of the olive sector is partly linked to the production of oil mill waste (OMW): olive mill wastewater (OMWW) and olive pomace waste (OPW). OMW is also a valuable resource of compounds useful through their recovery and enhancement.

As part of the LIFE TIRSAV PLUS project (LIFE05 ENV/IT/00845), it has been developed an industrial composting process able to recover all the waste from the olive sector to produce quality compost. The composting process developed is independent from the extraction systems in use at the mills, such as: the two-phase continuous cycle processes, whose products are olive oil and wet pomace, and the three-phase continuous cycle systems which produce olive oil, wastewater, and olive pomace. In addition, the flexibility of the TIRSAV PLUS composting system allows the recovery of different organic waste from other production chains within the same process. The physico-chemical characteristics of the oil mill waste need the definition of appropriate mixing ratios between the various matrices used, due to their low total nitrogen content (0.96% on average) which is unbalanced compared to the total organic carbon values (60.45%) for the purposes of the controlled bio-oxidation process.

OMW is a relevant organic resource to be returned to agricultural soils, however composting technologies aimed at the recovery of these organic matrices have never become established in the olive oil industry due to the initial investment, the necessary management costs, and the expertise required. The composting process of TIRSAV PLUS is based on the combination of management and process choices able to adapt to the territorial and productive context in which the plant operates. The management solutions adopted in response to the problems highlighted are based on the centralization of composting activities within a single production area, meaning one plant for several mills. This choice allows to reduce management costs and ensures effective process control for a specialized operator.

The composting method developed by LIFE TIRSAV PLUS employs two plant solutions. The first is the Static Composting (SC), a static system which consists of a pre-treatment phase, a slow ripening phase and eventually a refining phase. The second is the dynamic composting (DC), a dynamic system divided into a pre-treatment phase, a comparatively rapid ripening phase, and in the end a refining phase (Fig. 1).

The first phase of the process, common to both SC and DC systems, concerns the preparation of the mixtures to be started at composting. The choice of the composition of the mixtures depends on the composting system to be activated and on the substrate to be produced, i.e., mixed compost soil improver or green compost soil improver. The starting matrices were vegetation waters, virgin olive pomace, wet pomace, Pruning Residues (PR), lignocellulosic materials such as Sawdust, Bark and Shavings (SBS), and raw Wool Waste (WW). Table 5 shows some of the

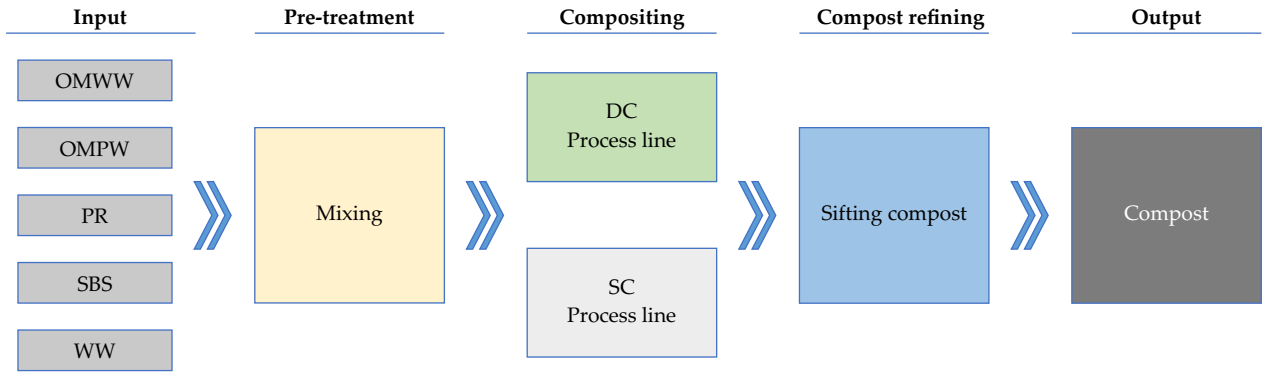


Fig. 1 Scheme of the composting process of the oil waste composting plant realized by the LIFE TIRSAV PLUS project. *DC* dynamic composting, *OMPW* olive mill pomace waste, *OMWW* olive mill wastewater, *PR* pruning residues, *SBS* Sawdust, bark, and shavings, *SC* static composting, *WW* wool waste

Table 5 Mixture composition for the composting processes A, B and C. OMWW: olive mill wastewater; OPW: olive pomace waste; OPWW: olive pomace wastewater

Proceedings		Experimental mixtures		
		A	B	C
		DC–SC	*DC*	*SC*
Additives (%)	Residual pomace (OMWW, OPW, OPWW)	72.00	72.00	72.00
	Pruning residues	11.25	8.5	14.00
	Sawdust, bark, shavings	11.25	8.5	14.00
	Wool waste	5.5	11.00	0.00

experimental mixtures developed during the first phase of the TIRSAV PLUS process, indicated as A, B and C.

The mixing ratios aimed to obtain mixtures with physico-chemical characteristics as close as possible to the standard parameters required by an efficient composting process. The analytical values of the experimented mixtures A, B and C are reported in Table 6.

The pre-treatment phase, from a plant engineering point of view, consists of three stages: (1) the pruning residues shredding; (2) the mixing of olive pomace and vegetation water from three-stage continuous cycle mills (in the case of the two-stage extraction systems this step is not activated); and (3) the second-level mixing and homogenization of all matrices provided in the composition of the recipes (Fig. 2).

The mixtures produced in the pre-treatment phase are sent to the composting lines. The composting process consists of 4 phases, meaning mesophilic I, thermophilic, mesophilic II and maturation. As part of the LIFE TIRSAV PLUS project, two process lines have been created that refer to the DC and SC systems.

Table 6 Analytic characteristics of experimented mixtures

	Experimented mixtures		
	A	B	C
Moisture (%)	68.00	64.30	68.30
pH	5.36	5.39	5.25
CEs [dS(m)$^{-1}$]	2.07	1.87	1.92
Salinity [meq(100 g)$^{-1}$]	80.75	65.63	75.60
Ashes (%)	10.28	10.04	9.86
Total C (%)	44.86	44.98	45.07
Total N (%)	1.44	2.18	0.95
C/N ratio	31.26	20.60	47.69
Fat (%)	6.91	5.58	7.32
K (%)	2.72	1.91	3.06
Ca (%)	0.79	0.29	0.46
Mg (%)	0.18	0.14	0.15
Na (%)	0.36	0.61	0.27
Fe (mg Kg^{-1})	24.43	12.17	15.40
Mn (mg Kg^{-1})	62.12	32.04	55.78
Zn (mg Kg^{-1})	44.29	50.72	28.70
Cu (mg Kg^{-1})	16.76	15.27	85.44
Pb, Co, Ca, Cr (mg Kg^{-1})	< lod	< lod	< lod

The values are related to dry substance

Lod limit of detection

In the case of the DC System, it is structured in three combined phases (Fig. 3):

1. A single system solution in biocontainer for the mesophilic I and thermophilic phases (accelerated bio-oxidation). The duration of this phase varies from 14 to 21 days and the process is entirely controlled by a Programmable Logic Controller (PLC) system that monitors temperature and humidity.

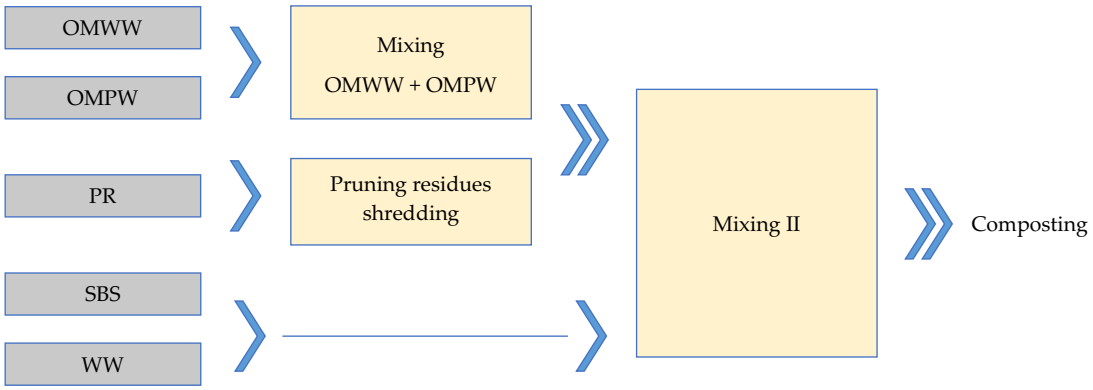


Fig. 2 Functional diagram of the pre-treatment phase. *OMPW* olive mill pomace waste, *OMWW* olive mill wastewater, *PR* pruning residues, *SBS* sawdust, bark, and shavings, *WW* wool waste

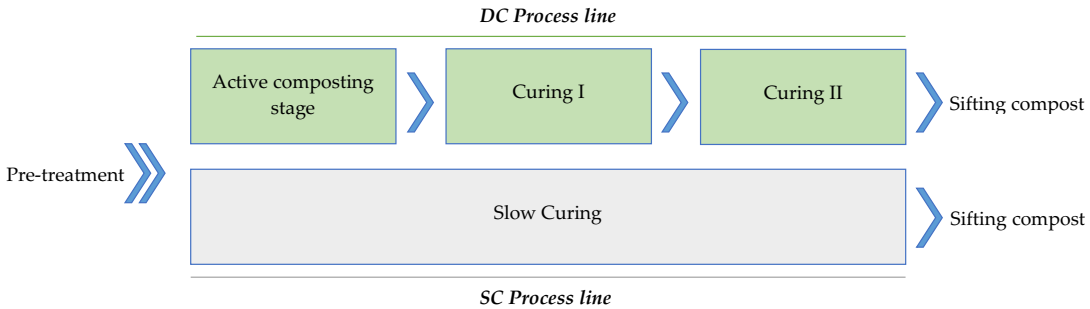


Fig. 3 Functional scheme of the phases of maturation DC and SC. *SC* static composting, *DC* dynamic composting

2. A second phase where the material at the exit of the biocontainer is placed in heaps on panels with forced ventilation (mesophilic phase II) and covered with waterproof and breathable sheets. This phase lasts about 30 days, and the temperature is the controlled process parameter.
3. The third stage is the final ripening. It is managed in static and not ventilated heaps under roofing, and it does not require specific equipment except for a front loader for the periodic turning of the heaps. The duration of the final maturation period is variable, and it depends on the type of mixtures, ranging from 50 to 60 days.

Instead, in the slow ripening line SC (Fig. 3), the process can follow two alternative process lines. In the first, the mixture is loaded into aerated plastic containers (bins or big bags) and stored in a dry place. In the second line, the mixtures are placed in static heaps indoors, about 2.5 m high, which are turned over periodically until complete ripening. For both SC lines there is no automatic process control system but a manual and periodic temperature measurement. The ripening time can be very long, ranging from 3 to 6 months, and the quality of the compost is not always optimal for any market due to the coarse size and the excessive dehydration. The SC process has been designed to significantly reduce the plant and the management costs, and to meet the needs of companies interested in returning a mature compost in their own fields.

At the end of the maturation process, the compost is processed with a dimensional separation to eliminate the coarse elements, such as lignocellulosic residues not completely degraded, lumps of compact compost and impurities. The compost is stored in big bags, or it is given directly to companies for use in the field. The fine fraction (residues of lignocellulosic and lumps of compost) is reused for the preparation of the mixtures to be started at composting. The compost produced by the plant meets high-quality criteria, making it certifiable and usable in organic farming. The main analytical values of some of the soil improvers produced by the LIFE TIRSAV PLUS plants are shown in Table 7. The two composts are classified according to the Italian legislation on fertilizers as mixed compost and green compost.

Table 7 Analytical values of the mixed and green compost soil improvers produced by the LIFE TIRSAV PLUS plants

	Mixed compost soil improver (DC)	Green compost soil improver (SC)
Average analytical values		
Moisture (%)	30.00	31.00
pH	8.0	6.9
Conductivity (ms/cm)	1.18	0.96
Total organic carbon (% s.s.)	36.7	38.3
Total nitrogen (% s.s.)	3.18	1.25
Organic nitrogen (% Tot)	97.80	99.80
C/N ratio	11.54	30.64
Potassium (% s.s.)	1.60	1.11
Phosphorus (% s.s.)	0.14	0.12
Microbiological analysis		
Salmonella spp.	n.p	n.p
Escherichia coli MPN/g (p.f.)	0	0

DC dynamic composting, *SC* static composting

To summarize, the composting method of oil residues developed by the LIFE TISAV PLUS project includes the preparation and the pretreatment of the starting mixtures, followed by the employment of two plant solutions, being the dynamic composting and the static composting. This results in an effective, flexible, and economical process which significantly reduces the environmental impact of the olive sector and contributes to the maintenance of soil fertility, with the production of a high-quality compost.

Abbreviations

AOA	Ammonia-oxidizing archaea
ARDRA	Amplified ribosomal DNA analysis
BCR1	Bacterial rice cluster 1
CAZyme	Carbohydrate active enzyme
CEM	CO_2 evolution method
DC	Dynamic composting
DD	Degradation
HB	Heat balance
HV	Heating value
HVM	Heating value method
ITS	Internal transcribed spacer
OC	Oxygen consumption
OMPW	Olive mill pomace waste
OMW	Oil mill waste
OMWW	Olive mill wastewater
OPW	Olive pomace waste
OPWW	Olive pomace wastewater
OSW	Organic solid waste
PLC	Programmable logic controller.PR: Pruning residues
RAPD	Randomly amplified polymorphic DNA
RSA	Rice straw-adapted
SBS	Sawdust, bark and shavings
SC	Static composting
TEM	Temperature method
WW	Wool waste

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Author contributions
Conceptualization, AP, IR, IF and BN; literature search, AP, AC, LR, IR, IF and PDD; data curation, AC, LR, AF and PDD; writing—original draft preparation, AP, AF, IF and AC; writing—review and editing, AP, IR, IF, AC and LR: supervision, AP, IF and BN; funding acquisition, AP, BN and AF. All authors have read and approved the final manuscript.

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Availability of data and materials
The data presented in this study are available in the article.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
All the authors gave their consent for publication of this article.

Competing interests
The authors declare that they have no competing interests.

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