

Microbiological activities in the composting process – A review

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Abstract: Composting is a technological process of waste management that is, with the help of microbiological activities in aerobic conditions, organic material is decomposed and stabilized into a biodegradable mixture and transformed into compost. This process of decomposition of organic matter has recently attracted a lot of attention due to its environmentally friendly methods in which additional environmental pollution is avoided. The composting process follows four phases (first mesophilic phase, thermophilic phase, second mesophilic phase, and maturation phase). The most important factors influencing the decomposition success are C/N ratio, humidity, temperature, substrate particle size, pH, oxygen content and microorganisms. Microorganisms such as bacteria, fungi, and actinomycetes act as chemical decomposers in the process of decomposition of organic matter into carbon dioxide, heat, water, hummus, and a relatively stable final organic product - compost. In the process of composting, microorganisms decompose the complex molecules of lignin, cellulose, and hemicellulose. The presence of different types of microorganisms is influenced by the composition of composite mixtures and changes in temperature through the phases of the composting process. At the beginning of compression, the microbial activity increases significantly, which causes a temperature rise. The initial dominance of bacteria is replaced by fungi that are most active in the process of compost maturation. This scientific paper aims to present an overview of the composting process and the role of beneficial microorganisms in the process of decomposition of organic matter of the compost mixture.

Keywords: Microorganisms, compost, biodegradable material, microbiological degradation

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Introduction

The composting process has been known since ancient times. The earliest evidence of the first composting dates back to the ancient Akkadian Empire, which used it to improve plant breeding (Vidović and Luttenberger, 2019). Waste today is one of the leading environmental problems of the modern way of life. An increasing amount of waste is generated due to the increase in human activities. Composting technology can be solution for reducing biodegradable waste, stabilization and environmentally friendly use and by-product recycling (Holes et al., 2014.). Today, there are many alternative ways to dispose of organic waste, and one of the ecological ways of disposing of biowaste is

composting, which involves the controlled oxidative microbiological decomposition of organic matter (Vargas-García et al., 2010). The resulting composts are organic fertilizers that are produced by the controlled microbiological decomposition of mixtures of fresh, dry, or processed plant residues, manure and organic waste from the processing industry, animal residues, and mineral additives (Lončarić et al., 2019).

Factors affecting the composting process include C/N ratio, humidity, oxygen, pH, temperature, and composition of all raw materials of the compost mixture (Kumar et al., 2010). The C/N ratio significantly affects the composting process because too wide ratio prolongs the composting process, and carbon deficiency causes nitrogen desta-

bilization and results in significant losses in ammoniac form (Wichuk and McCartney, 2010). Humidity is necessary for the chemical and microbiological processes in composting, and ideally the humidity during composting is 50-60%. The optimal oxygen concentration is 10%, while the ideal temperature depends on the phase in which the compost is (Bernal et al., 2009). The composting process consists of four phases; the first mesophilic phase, the thermophilic phase, and the second mesophilic phase, and the maturation phase of the compost (Sánchez et al., 2017).

The composting process as a biological process involves many microorganisms. It is the microorganisms that break down organic substances and organic compounds by their action and enzymes and turn them into a rich humic product (Albrecht et al., 2008; Aslam et al., 2008; Said-Pullicino et al., 2007). The microbiological population is affected by temperature, oxygen, humidity, nutrients, and pH reactions (Krstić et al., 2018). The most influential parameter on the presence of microorganisms in compost is the temperature (Steger et al., 2007). The starting components in compost also greatly affect the development of different microbial communities. The characterization of microbial communities during the composting process can provide important information on the development of the compost biodegradation process and on the maturity of the final product. Microbial activity is achieved by the action of enzymes responsible for the hydrolysis of complex macromolecules that make up the organic waste. The action of this type of enzymatic activity indicates the rate of decomposition of organic matter and the stability of the product (Mondini et al., 2004). Dehydrogenase activity indicates the index of biological activity due to its role in the oxidative process of phosphorylation (Vargas-García et al., 2010).

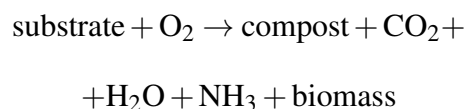
This scientific paper aims to present the com-

posting process, the role of beneficial microorganisms in compost conditioning, and the decomposition of organic matter in the compost mixture.

Composting process

Field trail

Composting is an aerobic microbiological process in which the decomposition of organic matter of materials found in initial mixtures occurs (Insam and de Bertoldi, 2007). The composting products produced by compost are gases (ammonia and carbon dioxide), water, and heat (Diaz et al., 2007). The composting process is also shown by the following equation (Haug, 2018):



The composting process is considered to be an environmentally friendly way of disposing of biological waste from the aspect of environmental protection with landfilling. Compost obtained only from biological waste can be used in organic agricultural production (Haug, 2018). Substrates used in composting are most commonly of plant, animal, or microbiological origin (Kuhad and Singh, 2007). The largest share is occupied by plant substrates, and animal and microbial components are occupied by smaller fractions in the compost mixture (Diaz et al., 2007).

The decomposition of complex structural aggregates is influenced by microorganisms, and their activity is present in certain phases of composting. The decomposition process begins with the oxidation of more easily degradable organic compounds, and this process is called putrefaction, followed by stabilization or decomposition of complex organic molecules and humification of lignocellulosic materials (Diaz et al., 2007).

Composting phases

The composting process consists of four basic phases: the first mesophilic phase (25–45 °C), the thermophilic phase (45–65 °C), the second mesophilic phase (also called the cooling phase), and the maturation phase (Wichuk and McCartney, 2010).

The first mesophilic phase lasts on average several days then the temperature of the compost mixture increases above 40 °C after the 3rd day and thus the thermophilic phase begins. In this phase, soluble sugars and starches are broken down (Lončarić et al., 2015) under the influence of bacteria, fungi, and actinomycetes, which are called primary degraders by one name (Diaz et al., 2007). The number of mesophilic organisms in the compost mixture is three times higher than the number of thermophilic organisms, and the increase in temperature is due to their metabolic activity (Epstein, 1997).

The onset of the thermophilic phase depends on aeration, C/N ratio, and humidity (Vukobratović et al., 2008). This phase can last 10-30 days and is extended by mixing and additional wetting of the compost mixture (Lončarić et al., 2015). At this phase, decomposition acceleration occurs which increases until the temperature of the compost pile reaches a temperature of 62 °C (Tuomela et al., 2000). In the thermophilic phase, mixed populations of thermophilic bacteria and actinomycetes, and fungi that are tolerant to high temperatures develop. Under the influence of microbiological activities, the breakdown of proteins, fats, cellulose, and hemicellulose occurs (Sole-Mauri et al., 2007). Temperatures above 50 °C destroy the germination of weed seeds and pathogenic microorganisms. However, temperatures above 65 °C also destroy beneficial microorganisms, and then aeration of the compost pile can be useful or necessary (Lončarić et al., 2015). In the compost mixture, the temperatures in all parts are not the same, so for this reason it is important to reg-

ularly mix the compost mixture, which ensures that all parts of the compost are brought to the central part where the temperature is highest. The microbiological aspect indicates four zones of the compost mixture. The outer zone is well supplied with oxygen but has the lowest temperature, the inner zone is highly compacted and poorly supplied with oxygen, the lower zone has a high temperature and good oxygen supply, and the upper zone is the warmest and in most cases well supplied with oxygen (Figure 1) (Diaz et al., 2007).

The second mesophilic phase or cooling phase lasts variably which and depends on the initial mixture of the compost pile (Vukobratović et al., 2008). At this phase, mesophilic organisms are reactivated and long-term and slow degradation of lignin and other resistant components occurs (Huang et al., 2010).

In the compost maturation phase, the number of bacteria decreases, and there is an increase in fungi that break down agar and residual lignin (Diaz et al., 2007). This phase lasts from several days to several months, ie until the decomposition of carbon compounds takes place, and ends when stable and mature compost is obtained with a lower C/N ratio and a mild alkaline pH value (Abd El Kader et al., 2007).

Composting process factors

The successful composting process depends on various physical, chemical, and biological factors, namely: substrate, particle size, humidity, temperature, pH, oxygen content, C/N ratio, and the number and type of microorganisms (Abd El Kader et al., 2007). It is important to have good starting materials for the composting process, and this usually includes fruit and vegetable residues, garden residues, kitchen waste, straw, manure, etc. In addition to plant residues, animal residues can also be mixed into the compost mixture. When making a compost mixture, it is important to choose good starting components that

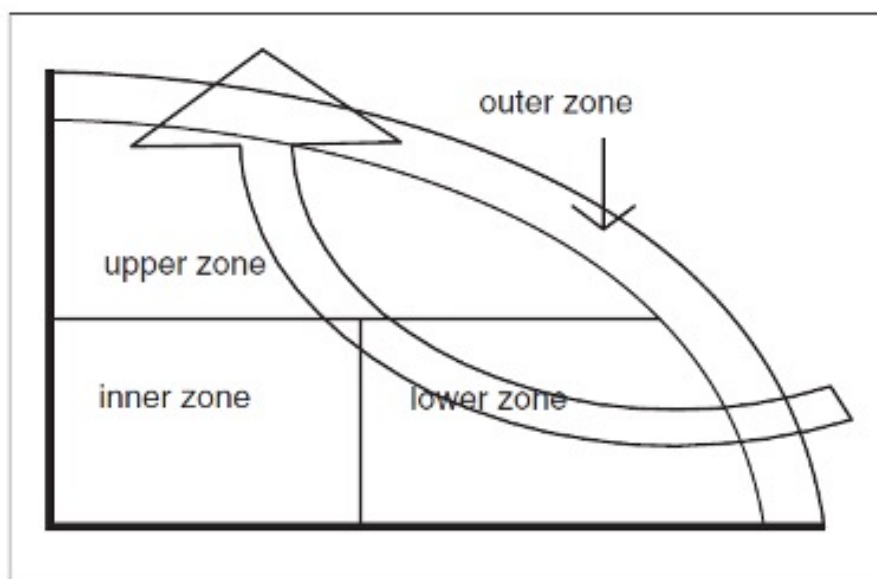


Figure 1: Compost piles zones. Source: (Diaz et al., 2007.)

contain enough nitrogen (green grass, green parts of plants, etc.), but also carbon (straw, sawdust, hay, cardboard, etc.) for the C/N ratio to be optimal (Malakahmad et al., 2017).

The optimal particle size of the initial components is 4–5 cm (Malakahmad et al., 2017). Tongneti et al. (2007) state that the initial components should be chopped to the size of an inch to increase the area available to microorganisms and speed up the composting process.

The ideal humidity at the beginning of the composting process is 50–60% (Castaldi et al., 2009). Excessive humidity above 70% affects the aeration of the compost mixture, i.e. reduces the air space and makes it difficult for oxygen to pass, which creates anaerobic conditions. The water content below 40% causes dehydration of the compost mixture and interruption of biological processes. If the moisture content of the compost mixture drops below 8% then all microbial activities cease (Abd El Kader et al., 2007).

The temperature of the compost mass rises rapidly above the ambient due to microbial activities. Composting is an exothermic process in which a large amount of en-

ergy is produced, but only about 45% of microorganisms are used to synthesize ATP, while the remaining energy is lost as heat in the compost mass (Diaz et al., 2007). The most active microbial activity is in the first mesophilic phase at a temperature of 30–45 °C (Majbar et al., 2018). Temperatures above 60 °C for at least 3 days are significant for sanitizing the compost pile and reducing pathogens. Lowering the temperature of the compost pile to the environment occurs at the end of the second mesophilic phase in which microbial activity decreases (Wang et al., 2013).

The optimal pH range for most actinomycetes and bacteria is 6.5–8.0, and this value corresponds to the final pH of mature compost. In the first mesophilic phase, the pH of the reaction is reduced because the decomposition of easily degradable organic materials from which organic acids are formed also occurs among the products (Kuhad and Singh, 2007). In the thermophilic phase, ammonia is formed due to the decomposition of the amine, which causes an increase in the pH of the compost mixture. In the second mesophilic phase, a

reduced pH occurs due to the reduced activity of microorganisms. In the maturation phase, the pH reaction stabilizes to a neutral value, which is closely related to the buffer capacity of humus (Yu and Huang, 2009).

It is important to provide a sufficient amount of oxygen to the microorganisms that carry out the decomposition of organic matter. In most cases, the oxygen content in the compost mixture at the beginning of the process is sufficient, however, to avoid anaerobic conditions during the composting process, an oxygen supply is required. Aeration of the compost pile is ensured by mixing or forced aeration by blowing air, and the optimal oxygen content for the activity of microorganisms must be greater than 10% (Guo et al., 2012). Oxygen content is highest during the thermophilic phase and decreases during the maturation phase because it slows down microbiological activity and releases carbon dioxide (Awasthi et al., 2016).

Carbon and nitrogen content is important in the composting process because microorganisms use carbon as a source of energy and nitrogen for cell construction and protein synthesis (Iqbal et al., 2015). The optimal C/N ratio in the composting process is considered to be 25/1 to 35/1 (Guo et al., 2012). If the C/N ratio is higher than optimal, the composting process is slowed down because the activity of microorganisms is reduced, and at a lower C/N ratio, ammonia and unpleasant odors are generated (Neugebauer et al., 2017). There is a possibility of a successful composting process even at values of C/N ratios that are lower than optimal, and the research of some authors is shown in table 1. Reduction of C/N ratios can be achieved by adding nitrogen-rich materials such as fruit and vegetable residues. Increasing the C/N ratio is achieved by adding raw materials with a higher carbon content such as cardboard and paper (Makan and Mountadar, 2012).

Microbiological processes in composting

The biological circulation of nutrients is necessary for life, and the main mediators in that process are microorganisms. Biotransformation is a biological modification that changes the chemical structure of matter (Insam and de Bertoldi, 2007). Biotransformation can synthesize atoms or convert simple molecules into more complex compounds (biosynthesis) or vice versa (biodegradation and mineralization) (Michel et al., 2002).

Microorganisms involved

Microorganisms found in compost are mostly beneficial, however, some are potentially harmful to humans, animals, plants, and the environment (Fuchs, 2010). Harmful microorganisms are introduced mostly through the initial substrates of compost mixtures. Input substrates, compost pile size, rotation frequency, particle size, aeration, and wetting directly affect microbial processes (Fuchs, 2010).

Bacteria participate in biodegradation by producing carbon dioxide and heat to produce energy (Insam and de Bertoldi, 2007). The importance of non-mycelial bacteria during the composting process has long been neglected, probably due to better visibility of fungi and actinomycetes. Bacteria provide the fastest and most efficient composting by excreting nutrients, nitrogen, phosphorus, and magnesium (Abu-Bakar and Ibrahim, 2013). There are various types of bacteria in compost piles, where psychrophiles, mesophiles, and thermophiles predominate (Lee, 2016). Compared to other bacteria, psychrophilic bacteria secrete a small amount of energy and are most active at a temperature of 13 °C. Mesophilic bacteria are most active at a temperature of 21–32 °C and their role is similar to psychrophilic bacteria. When the temperature of the compost pile rises above 45 °C, the main role is taken over by thermophilic bacteria that con-

Table 1: Successful composting processes at lower C/N ratios

C/N ratio	Compost mix	References
15	Pig manure with wood sawdust	Huang et al., 2004
19,6	Waste from green areas and waste from the food industry	Kumar et al., 2010
20	Pig manure with rice straw	Zhu, 2007
20	Chicken manure with wood sawdust	Ogunwande et al., 2008

tinue to biodegrade in the composting process (Lee, 2016). For the genus *Bacillus*, the most optimal temperature is in the range of 50–65 °C, and at temperatures above 65 °C *Stearothermophilus* dominates, which is found in almost pure culture under such conditions (Insam and de Bertoldi, 2007).

Actinomycetes prefer a neutral or slightly alkaline pH of compost mixtures and participate in the degradation of more difficult to degrade substrates. Most actinomycetes thrive best when the compost pile is sufficiently moist and sufficiently supplied with oxygen (Insam and de Bertoldi, 2007). Actinomycetes can break down resistant materials such as starch and proteins while releasing carbon, nitrogen, and ammonia causing the earthy smell of compost (Shukla et al., 2014). Despite the lack of a nucleus, actinomycetes can grow multicellular threads such as spider nets and have enzymes that help break down cellulose, lignin, chitin, protein, woody stems, and tree bark.

Representatives of the *Thermus/Deinococcus* group grow on organic materials at temperatures of 40–80 °C, while their optimal temperature for growth is between 65 and 75 °C. *Thermus* species were once present only in geothermal sites and probably adapted to the hot compost system and play a major role at the peak heating phase of the compost mixture (Beffa et al., 1996). Many autotrophic bacteria have also been isolated from compost such as the *Hydrogenobacter* strain that was once also known only in geothermal sites (Insam and de Bertoldi, 2007).

Fungi get nutrients from dead plant mat-

ter, which is why they break down residues in compost, allowing bacteria to decompose even without cellulose (Lee, 2016). They form hyphae with which they penetrate the materials in the compost and thus decompose harder degradable substances such as lignin, hemicellulose, and cellulose (Nutongkaew et al., 2014). Fungi can break down dry and acidic residues and residues with low nitrogen content that are resistant to bacterial action. Complex polymers such as polyaromatic compounds or plastics are also degraded by fungi (Lee, 2016).

Microorganisms at the beginning of the composting process

At the beginning of the composting process, bacteria are most numerous, and fungi and actinomycetes are also important microbial community members. The composition of the microbial community in the compost pile is influenced by the starting materials (Klammer et al., 2008). All microorganisms present in the compost are found in a normal natural environment (Fuchs, 2010). The input components of the compost pile are often heterogeneous, and so are the initial microbial communities. Food waste containing plant residues has a low initial pH, which is why fungal and yeast proliferators are present and bacterial growth is slowed (Ryckeboer et al., 2003). Gram-negative, α -, β -, and γ -proteobacteria were found primarily on compost samples containing leaves and grass on the first day of composting (Michel et al. 2002). Few mesophilic fungi and a large number of thermophilic bacteria and fungi

were found in the household waste (Ryckeboer et al., 2003). The presence of harmful organisms primarily depends on the input components of the compost mixtures. Animal waste from manure and food contains significant amounts of potential human and animal pathogens such as *Salmonella* sp., *Escherichia coli*, and *Listeria* sp. (Heinonen-Tanski et al. 2006, Wichuk and McCartney 2007; Grewal et al., 2007). Plant residues can also contain various plant pathogens. Soon after the onset of the composting process, the microbial population changes drastically and soon it no longer resembles the initial population (Fuchs, 2010).

The succession of microorganisms during the composting process

Shortly after the start of the composting process, microbiological biomass grows drastically (Fuchs, 2010). During the first day of composting, Klamer and Baath (1998) recorded a sixfold increase in microbiological mass in the composting of shredded straw with the addition of slurry. The physical and chemical properties of the compost mass change over time, and the microorganisms that are first active degrade the original substrate and produce metabolites and create a new physicochemical environment (Ryckeboer et al., 2003). One of the main factors influencing the microbial population in the composting process is the concentration and composition of dissolved organic matter (Fuchs, 2010). The main components of the organic matter of compost mixtures are proteins, carbohydrates, lipids, and lignin, and microorganisms produce different enzymes required for the degradation of different feedstocks (Ryckeboer et al., 2003). In the process of decomposition, bacteria dominate microbial communities, and during this phase, large amounts of organic carbohydrates are usually available in the compost mixture (Fuchs, 2010). The amount of nitrogen depends on the input raw materials,

and the optimal C/N ratio for the activation of microbial communities is 25–40. The activity of microbial communities causes an increase in the temperature of the compost mixture and a thermophilic phase occurs. It is at this phase that the greatest number of microbes and enzymatic activities occur (Cunha-Queda et al., 2007). As the temperature of the compost mixture increases, significant changes occur in the microbial community, which is important for the self-sterilization of compost, i.e. the destruction of harmful microorganisms (Fuchs, 2010). Different microbial communities were found in different places, which is related to different compost pile temperatures (Guo et al., 2007). The population of Gram-negative bacteria and fungi increased up to 50 °C but decreased at higher temperatures. After cooling, these two groups of microorganisms increased again (Ryckeboer et al., 2003). During the compost ripening phase, the number of bacteria decreases, but their diversity increases. At the same time, the fungi population is increasing in quantity and diversity (Ryckeboer et al., 2003). Fungal activity is expressed to be important in the compost ripening phase (Fuchs, 2010).

Decomposition of organic matter in the composting process and the role of microorganism

Lignocellulosic material consists of 38–50% cellulose, 23–32% hemicellulose and 15–25% lignin. In plants, it is also possible to identify structural polymers (waxes and proteins) that are represented by 5–13% (Deobald and Crawford, 1987). Lignocellulosic materials are by-products of various agroindustries and represent major problems in the environment. Various studies have examined the properties and methods of disposing of lignocellulosic materials such as sugarcane residues (García-Gómez et al., 2005), paper (Sung and Ritter, 2008), olive pomace (Komilis and Ham, 2003), tobacco waste,

(Pérez et al., 2002), leaves (Ekinci et al., 2000) and sawdust (Atkinson et al., 1996).

Lignin is the basic structural component of plants, and its degradation is the slowest compared to other components such as hemicellulose, starch, pectin and cellulose (Kuhad and Singh, 2007). The cause of slow degradation is due to the exceptional diversity of binding between monomer units (Ekinci et al., 2000). The biodegradation of lignin is mainly of the cometabolic type and therefore the amount of energy released is insignificant. Degradation of this polymer is most commonly achieved by white-rot fungi (*Stereum hirsutum*, *Phanerochaete chrysosporium*, *Trametes versicolor*) (Zeng et al., 2010). Some fungi, such as *Pleurotus ostreatus*, degrade both lignin and cellulose at the same time (Insam and de Bertoldi, 2007).

Cellulose is a polymer of glucose, which is made up of long rows of interconnected molecules of cellobiose disaccharides from which glucose is formed by complete hydrolysis (Diaz et al. 2007). Cellulose is rich in carbon and does not contain nitrogen, and fungi of mycelial structure (*Fusarium* sp., *Aspergillus* sp. and *Chaetomium* sp.) have a better ability to decompose (Insam and de Bertoldi, 2007). *Pseudomonas* and related genera are also known to degrade cellulose, and only a few actinomycetes are involved in this process (Sánchez et al., 2017).

Cellulolytic bacteria are ubiquitous and successfully degrade cellulose, while their ability to mineralize lignin is limited (Insam and de Bertoldi, 2007). *Cytophages* and *Sporocytophages* are the dominant cellulolytic microorganisms present in all phases of the composting process (Singh and Nain, 2014). Mesophilic aerobic and anaerobic forms of bacteria *Bacillus subtilis*, *B. polymyxa*, *B. licheniformis*, *B. pumilus*, *B. brevis*, *B. firmus* and *B. circulans* degrade hemicellulose (Singh and Nain, 2014).

Three types of fungi living on dead wood

found in compost mixtures, and these are soft rot fungi, brown rot fungi, and white-rot fungi (Singh and Nain, 2014). Soft rot fungi (Ascomycetes and Fungi imperfecti) effectively degrade cellulose, but only degrade slowly and incompletely the lignin content. Brown rot fungi (Basidiomycetes) show a tendency to break down carbohydrates and demethylate lignin. White rot fungi degrade both lignin and cellulose (Singh and Nain, 2014).

The composting process relies heavily on the function of decomposing organic matter by actinomycetes. Actinomycetes *Thermoactinomyces* and *Streptomyces* successfully decompose cellulose in the thermophilic phase of composting. The ability of actinomycetes to completely break down lignin is limited, but they extensively modify the structure of lignin with their enzymes (Singh and Nain, 2014).

Hemicellulose is a branched polymer of arabinose, xylose, glucose, and mannose. Together with lignin forms cross-linked structures that provide structural strength, but thus complicate the process of decomposition by microorganisms (Ladisch et al., 1983). Xylan is the most important among hemicellulose and is found in straw and the rest of sugar cane processing (up to 30%) and wood (2–25%). Xylan consists of pentose (xylose and arabinose) or hexose (glucose, mannose, and galactose) (Insam and de Bertoldi, 2007). The major enzymes for degrading, xylanase, are produced by many bacteria and fungi (Diaz et al., 2007). Pectin consists of unbranched polygalacturonic acid chains and is degraded by pectinase, which is common among fungi and bacteria (Insam and de Bertoldi, 2007).

Starch consists of amylose and amylopectin. Amyloses are unbranched chains of D-glucose, due to the position of the β -glycosidic bond, amylose is spiral, unlike cellulose. Amylopectin contains phosphate residues and magnesium and calcium ions

(Diaz et al., 2007).

Chitin is less important than cellulose, and the chemical composition of chitin is very similar to cellulose. The cellulose monomer is glucose and the chitin monomer is N-acetylglucosamine. The main difference for microorganisms that degrade chitin is that it contains a high concentration of nitrogen (about 7%), and the C/N ratio of chitin is approximately 5 (Park et al., 2005). Various fungi (e.g., *Aspergillus*) and bacteria (e.g., *Flavobacterium*, *Cytophaga*, *Pseudomonas*) use chitin as the source of nitrogen and carbon they need in degradation processes (Poulsen et al., 2008). Chitin is broken down through exoenzymes to N-acetylglucosamine, which is resorbed and broken down to fructose -6-P and is thus involved in carbohydrate metabolism (Insam and de Bertoldi, 2007).

Conclusion

The composting process has been known since ancient times, but only in the last decades has more attention been paid to its importance in waste disposal. Transformation into compost of the biodegradable organic fraction of solid waste is one of the most validated methods of recycling. It is a process with low energy consumption and permits the disposal of the organic fraction of the solid waste which represent the greatest portion of refuse. Composting is the economically and ecologically most appropriate method of disposing of biological types of waste. Knowledge of the microbiological as-

pects of composting has permitted the optimization of all the factors which have a direct influence on the process. Optimal compost use in agriculture needs an appropriate determination of compost stability in relation to microbial activity. Stability prevents nutrients from becoming tied up in rapid microbial growth, allowing them to be available for plant needs. Microorganisms play an important role in the composting process because their enzymes break down the organic matter of compost mixtures. There is a complex interaction between different types of microorganisms, and their presence depends on the initial mixtures of the compost pile and the stage of the composting process. Although the outcome of a successful composting process is mostly known the interaction of all mechanisms and processes is still not sufficiently researched. Studying different types of microorganisms can help to discover more efficient and faster-composting models. It is for this reason that further research on microorganisms is important to achieve a better understanding of the composting and biowaste disposal process.

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