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Lignocellulose-degrading enzymes, free-radical transformations during composting of lignocellulosic waste and biothermal phases in small-scale reactors

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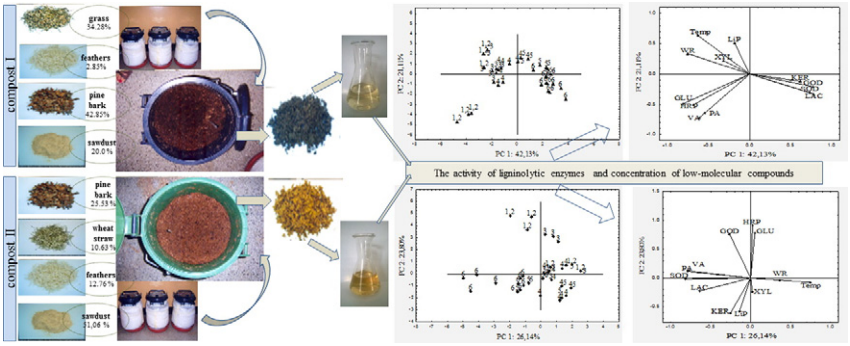
HIGHLIGHTS

- Water extracts of composts have active ligninolytic enzymes.
- Free-radical transformations take place during the composting of lignocellulosic waste.
- Glucose depletion triggers a positive effect similar to the priming effect in soil.
- The priming effect in composts also depends on the amount of keratin component (N).
- Biothermal phases differ by the activity of enzymes and low-molecular weight compounds.

GRAPHICAL ABSTRACT



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ABSTRACT

Environmentally friendly strategies of waste management are both part of legal solutions currently in place and a focus of interest worldwide. Large-scale composting plants are set up across various regions while home composting is becoming increasingly popular. A variety of microbial groups are successively at work during composting and enzymatic activities detected in the composting mass fluctuate accordingly.

Changes in the activities of oxidoreductases and hydrolases, i.e. glucose oxidase, horseradish peroxidase, lignin peroxidase, laccase, xylanase, superoxide dismutase and keratinase, low-molecular weight compounds, i.e. methoxyphenolic and hydroxyphenolic compounds, and the relative level of superoxide radicals and glucose were determined periodically in water extracts of composts to investigate the process of biochemical transformations of ligninocellulose in relation to biothermal phases and to identify a potential priming effect in two composts containing different ratios of lignocellulosic waste and chicken feathers. Composting was conducted for 30 weeks. An important aim of the study was to demonstrate that a positive priming effect was induced during composting of a variety of lignocellulosic waste types using native keratin (chicken feathers) as a source of N. The effect was more evident in compost containing grass, which was related to a more rapid depletion of easily available sources of C and energy (glucose) during composting. Ligninolytic enzymes known to biodegrade recalcitrant organic matter were induced in subsequent biothermal phases of composting. Compost I enriched with grass (pine bark, grass, sawdust and chicken feathers) exhibited a higher enzymatic activity than compost II which did not contain any grass but which had a greater number of hardly-degradable components (pine bark, wheat straw, sawdust, chicken feathers). Similar observations were made for the concentrations of low-molecular weight compounds.

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The enzymes activities and concentration of low-molecular weight compounds listed above can be used to estimate the biodegradation of lignocellulose during composting.

1. Introduction

Composting is a process that is caused by the development of consecutively occurring microbial multi-species populations capable of biodegrading and biotransforming organic matter using a broad range of enzymes. The greater the number of various substrates in the compost, the more complex enzymatic mechanism is triggered (Tuomela et al., 2000; Ball and Jackson, 1995; Singh et al., 2003).

Bacteria, actinomycetes as well as thermophilic and thermotolerant fungi take part in the composting of the lignocellulosic complex (Tuomela et al., 2000). Chamuris et al. (2000) and Kluczek-Turpeinen et al. (2003) report that many potentially lignin-degrading fungi occur in woody compost. White-, brown- and soft-rot fungi are among fungi known to delignify wood (Niladevi, 2009). Brown-rot fungi participate in the biodegradation of cellulose and hemicellulose with limited lignin biodegradation. Soft-rot fungi are important lignin degraders in a mixed population of microorganisms and their efficiency in lignin biodegradation is low (Tuomela et al., 2000). White-rot fungi which secret ligninolytic enzymes are the best known lignin destruents. Pure cultures of these fungi are used to investigate the degradation and removal of toxic compounds (Huang et al., 2008, 2015), to process dyed products in the textile industry (Cheng et al., 2015; Korniłłowicz-Kowalska and Rybczyńska, 2015; Zeng et al., 2015) and as inocula during composting of lignocellulosic waste (Zhang et al., 2013, 2014). Certain mixed cultures of micromycetes degrade organic matter more intensively than pure cultures (Cheng et al., 2015) Fungi degrading the lignocellulosic complex produce three groups of enzymes. The first includes cellulolytic and hemicellulolytic enzymes that attack both carbohydrate components, i.e. cellulose and hemicellulose, and ligninolytic enzymes which degrade lignin and belong to oxidoreductases such as lignin peroxidase (EC1.11.1.14), manganase-dependent peroxidase (EC 1.11.1.13), laccase (EC 1.10.3.2) and horseradish peroxidase (EC 1.11.1.7). The second group of enzymes consists of superoxide dismutase (EC 1.15.1.1) and glyoxylate oxidase (EC 1.2.3.5) which co-operate with the first group but never attack wood on their own. The third group contains enzymes that act as a feedback system as they are crucial in generating hydrogen peroxide, e.g. glucose 1-oxidase (EC 1.1.3.4) and aryl alcohol oxidases (EC 1.1.3.7), and they prevent de-polymerization of quinones and free phenoxy radicals produced during the degradation of the lignin polymer (Leonowicz et al., 1999). Enzymes secreted during lignin biodegradation act synergistically (Tuomela et al., 2000) and their extracellular maturation may be realized by the stimulating activity of proteolytic enzymes (Staszczak et al., 1996). As reported in a study by Tuomela et al. (2000), lignins are first degraded by extracellular oxidizing enzymes to smaller units that are then converted to phenols and quinones during free-radical processes. Low-molecular weight compounds initiate lignin biodegradation processes by permeating between cellulose fibrils (Leonowicz et al., 2001) and preparing the site for the activity of ligninolytic enzymes.

Hemicellulose is the second polysaccharide by number occurring as a natural component in plant cells (Whiterford et al., 2000). Due to the presence of covalent bonds between lignin and hemicellulose, it is more easily degradable than cellulose (Leonowicz et al., 1999; Pérez et al., 2002). Sánchez (2009) reports that the degradation of hemicelluloses is necessary for an efficient induction of lignin degradation. However, it is affected by available N which stimulates the activity of enzymes degrading cellulose and inhibits the activity of xylanase and other enzymes degrading hemicellulose and ligninolytic enzymes (Chen et al., 2014). The role of xylanolytic enzymes in lignocellulose biodegradation is less known than that of the cellulase complex (Rabemanolontsoa and Saka, 2016).

Leonowicz et al. (2001) report that lignin is first depolymerized by demethylation after which ortho-phenols that are produced are oxidized to ortho-quinones by peroxidase or laccase. Laccase is induced by low-molecular weight products of lignin degradation, i.e. methoxyphenolic acids, while ligninase is not induced by the lignin in the substrate although it is an inducing enzyme. Lignin peroxidase is active when the availability of energy compounds is limited or when some low-molecular weight aromatic compounds, e.g. veratryl alcohol, are present.

An important role in this multi-enzymatic system is played by hydrogen peroxide which is produced extracellularly and is required for lignocellulose biodegradation by fungi due to its capacity to produce active oxygen forms. Superoxide dismutase supplies hydrogen peroxide during the dismutation of the superoxide radical ($O_2^{\bullet -}$) as an agent taking part in lignin degradation (Leonowicz et al., 2001). The maximum SOD activity is observed during decreased activity of ligninolytic enzymes (Malarczyk et al., 1995).

Like laccase, horseradish peroxidase can cause the demethylation of methoxyphenols and phenyl-propane polymers, e.g. lignin, in the presence of hydrogen peroxide (Leonowicz et al., 2001). However, the key role is thought to be played by glucose oxidase. It acts as a regulating enzyme which provides H_2O_2 for peroxidase and removes radicals and quinones from laccase's reaction environment.

A humus-rich product is produced as the final effect of composting lignocellulosic waste during which lignin, polysaccharides and nitrogenous compounds of the lignocellulosic complex are biotransformed and biodegraded (Tuomela et al., 2000). Chefetz et al. (1998) report that the synthesis of polyphenols is the main reaction involved in the formation of humic substances from phenols, quinones, carbohydrates and N-sugars during composting and contributes to a relatively rapid humification of organic matter.

The majority of the above studies elaborate on lignocellulose transformation by various microbial strains, especially white-rot fungi in vitro. However, Mondini et al. (2004) and Goyal et al. (2005) notice that the enzymatic activity is an indicator that describes microbiological transformations occurring in situ during composting lignocellulosic waste. On the other hand, Bohacz and Korniłłowicz-Kowalska (2009a) find that enzyme activities may be an indicator of compost maturation. According to Tiquia (2002), the quality and properties of the enzymatic activity during composting reveal the dynamics of the composting process during the decomposition of organic matter and nitrogen transformation. Chen et al. (2014) and Castaldi et al. (2008) report that extracellular enzymes reflect well the function of decomposter communities whose metabolism depends on nutrient availability.

In many investigations into compositing, lignocellulose supplied by waste from the wood- and agricultural industries is a source of carbon and energy. Little is known about the contribution of individual enzymes catalyzing the degradation of natural lignocellulosic residues in specific composting phases. An overall assessment of the biodegradation of lignocellulosic waste in composts based on the dynamics of activity changes of enzymes participating in the degradation of the complex, especially ligninolytic enzymes, and transformations of low-molecular weight compounds is also needed.

This paper addresses the considerations outlined above and proposes a new aspect of research into composting studies. Its aim was to conduct a full and in-depth assessment of the dynamics of the activity of ligninolytic enzymes and low-molecular weight compounds in