ENZYME ACTIVITIES DURING COMPOSTING OF WASTE MICROBIAL BIOMASS FROM PHARMACEUTICAL INDUSTRY

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Abstract

A mixture of wood chips and waste microbial biomass remaining after fermentation and isolation of a pharmaceutical product was treated by composting in a perforated plastic box for five days in several experiments. Maximum temperature of 57 °C was reached, pH varied between 5,6 and 8,3 while humidity was in the range of 56,5% to 51,5%. Different enzyme activities were detected already in the initial mixture for composting, some of them originating from the waste microbial biomass, others from the wooden structure material. Proteases, esterases, cellulases, amylases and two ligninases: laccase and manganese peroxidase were active during the process. In some experiments also some xylanase activity was present. The measured enzymes were extracellular, however, in the case of cellulases, the activity was mostly associated to the cell membrane.

Introduction

During the last decade, composting has gained renewed attention as an alternative technique for treatment of solid organic wastes. It means the decomposition of organic matter by a mixed microbial population in a warm, moist and mostly aerobic environment. More precisely, composting is a breaking down as well as building up process where enzymes play the major role. A big significance for the process of composting represents the cell wall of microorganisms through which mass transfer is possible. Low molecular weight and water-soluble molecules can easily pass through the cell wall where they take part in the cell metabolism, providing energy and being built up into larger polymers, with the help of intracellular enzymes. To attack high molecular weight components, which cannot pass through the cell wall, microorganisms secrete extracellular enzymes. They break molecules down into the fragments that can be assimilated, while the rest is converted into a stable product, humus or compost. [1,2] Reports about the evolution of particular enzyme activities during composting are very rare. Some attention was paid to cellulase,[3] invertase[4] and alkaline phosphatase.[5]

Disposal of microbial biomass remaining after fermentation of antibiotic production is problematic from the environmental point of view. One of the possible solutions can be composting of the waste mycelium to degrade toxic substances and to transform it into a useful product. To enable composting of the wet biomass it must be mixed with some structural material usually of wooden origin. The aim of this research was to study the composting of a mixture of wood chips and waste biomass of *Streptomyces* sp., remaining after the isolation of a pharmaceutical product. The enzyme pattern involved in the process was investigated, the possible effect of enzyme induction was explored and the evolution dynamics of some enzyme activities during composting was followed.

Experimental

Composting system preparation

Several experiments were done in the following manner. Approximately 10 kg of composting mixture was prepared from waste biomass of *Streptomyces* sp.(WMB), remaining after micro filtration of fermentation broth and isolation of pharmaceutical product and chips of waste wood (WWC) as a bulking agent to ensure aerobic conditions (sieved through 2,5cm aperture). Contents of dry matter in waste microbial biomass was about 10% while the humidity of the wood chips and the initial composting mixture were about 40% and 55%, respectively. A perforated plastic box was filled with the material and put under aeration hood for five days. The composting process started at room temperature and after two or three days, when the temperature maximum was reached, the composting material was mixed for better homogenization and aeration.

Analytical methods

Temperature in the middle of the mixture was measured several times a day with a Hg thermometer. Humidity was determined by standard weighing technique after drying the sample at 105^{o}C to constant weight. For chemical analysis a compost extract was prepared as follows. Approximately 100 g of composting mixture was added to double amount of distilled water and mixed for 15 minutes with a magnetic stirrer (Iskra). The suspension was filtered through four layers of gauze and then centrifuged for 20 min at

3200 rpm (Iskra). To obtain the compost extract for extracellular enzyme analysis and pH determination with a digital pH-meter (Iskra), the supernatant was then filtered through a sterile 0,45 μm nitrocellulose filter (Sartorius). Membrane attached cellulases were estimated in the sample after filtration through gauze only.

Some enzymatic activities were determined on solid agar media on Petri dishes, commonly used for screening of microorganisms. However, in our case the technique had to be modified for screening of enzyme solutions as follows. Instead of the whole growth medium, only the specific enzyme substrates were used as listed later and for the application of the enzyme solutions 8 mm holes were drilled into the agar. After inoculation of agar plates, samples were incubated for 3-4 days at 37 ^{o}C. The plates were examined for coloration or clearing zones around the holes. The radius of the zone, indicating the enzyme activity, was determined with a vernier caliper.

The media were prepared by mixing agar (Merck) and the solutions or suspensions of the following specific enzyme substrates: microcrystalline cellulose (Merck) or cellulose azure (Sigma) for cellulases, starch (Merck) for amylases, skim milk powder (Pomurske mlekarne) for proteases, bromcresol purple (Difco) and Tween 80 (Sigma) for fatty acid esterases, tributyrin (Merck) for lipases and xylan (Sigma) for hemicellulases.[6] Ligninase activities were determined spectrophotometrically following the colour change of specific substrates due to enzyme action at room temperature: Laccases, manganese peroxidases and lignin peroxidases were estimated with 2,3-azino-bis(3-ethylbenzthiazoline-6-sulfonate) (ABTS) at 420 nm; ABTS, H_2O_2 and Mn^{2+} at 420 nm; and methylene blue at 677 nm, respectively. [7] Extracellular and membrane attached cellulases were assayed after incubation of the samples at 37^{o}C or at 50 ^{o}C with carboxymethylcellulose (CMC) for 30 min. The activity was determined by a spectrophotometrical determination of reducing sugars released from the CMC. 3,5-dinitrosalicylic acid (DNS) reagent was used to give a yellow colored product. [8]

Results and discussion

The composting mixture of natural materials is heterogeneous and the raw materials vary from charge to charge. Therefore quantitative measurements are only roughly reproducible and many experiments must be performed to enable the evaluation of the

results. To get a representative sample, reliable sampling of the material is of outmost importance.

Typical time courses of temperature and pH value during composting of the waste biomass are shown in Figure 1. It can be seen that after an adaptation period of about 10 hours temperature reached its maximum of 57 °C in one day, and then slowly decreased to room temperature during the next four days. A small effect of mixing after 60 hours can also be seen. A relatively fast increase from the initial pH value = 5,6 to pH = 7,5 during the first day was observed. In the second day it increased further to the value pH = 8,03 while during the next three days the increase slowed down to reach a pH value of 8,34 after five days. The humidity of the composting mixture during the process varied in the range of 56,5% and 51,5 %, respectively.

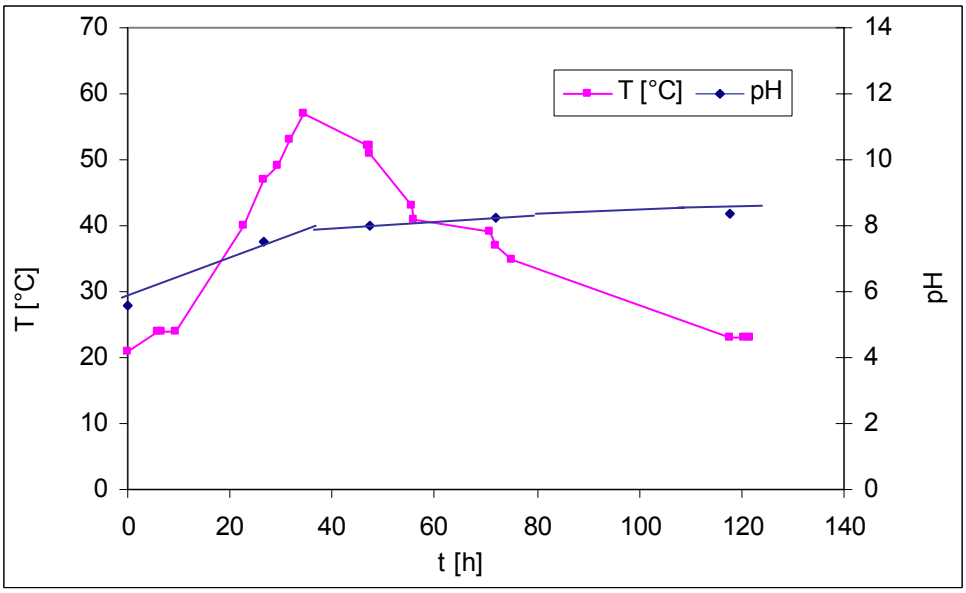

Figure 1. Typical time courses of temperature and pH during composting of waste microbial biomass.

In both starting materials and in the composting mixture different enzyme activities were measured to find out which enzymes participate in the bioconversion of the given organic waste material. Due to the complex composition of the composting mixture, the enzyme activities to be measured were chosen according to the presence of their

possible inducers, including proteins, lipids, polymers of plant origin such as starch, cellulose, hemicellulose, lignin etc.

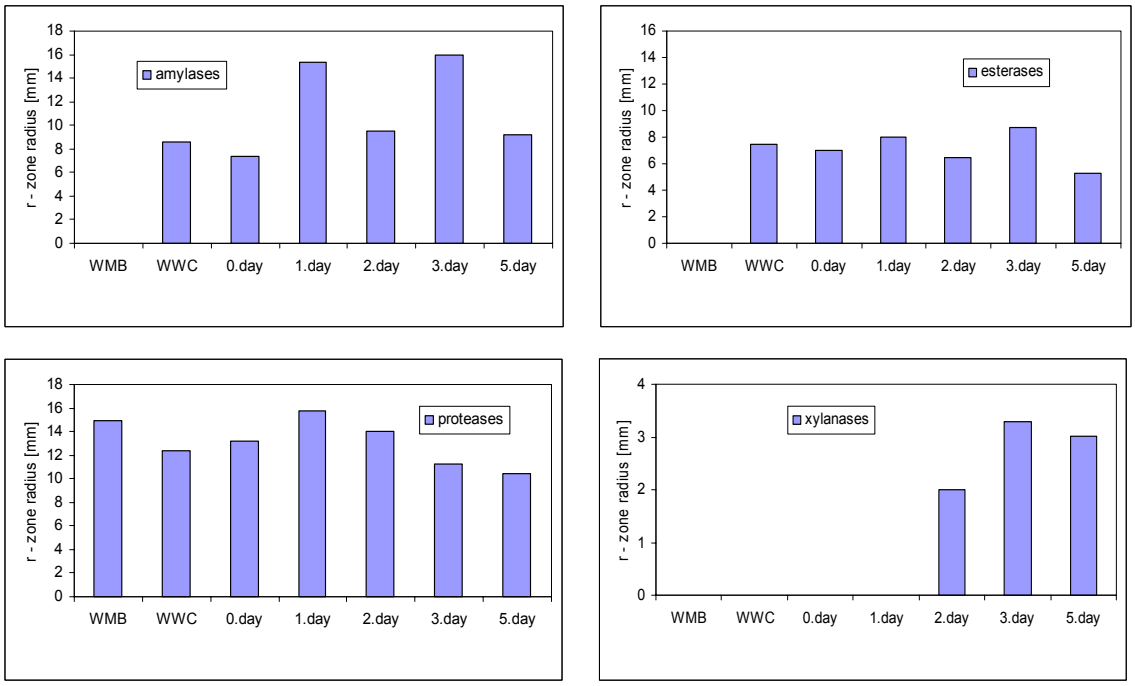

Figure 2. The presence of some hydrolytic enzymes during composting of waste microbial biomass. The activities were detected on agar plates.

Typical evolution of particular enzyme activities is shown in Figures 2 and 3. Amylases and esterases were already present in waste wood chips (WWC), while there was no trace of these activities in the waste microbial biomass (WMB). On the other hand, proteases were initially present in both raw materials. The appearance of these activities is in agreement with the chemical composition of the waste materials. Namely, in waste wood material consisting of branches from forests and orchards as well as river deposits and some green waste, all sorts of microbial population are also present. Therefore, the presence of starch, as well as acetyl esters containing substances of microbial and plant origin induced the respective hydrolytic enzymes. Similarly, proteins are present in plant materials and microbial biomass acting as inducers of proteases. The waste wood contains also hemicellulose if not having been degraded previously. Therefore in such cases xylanases appeared on the second day, probably

induced by the increased temperature. The activities did not vary significantly with time, however, a beneficial effect of mixing of the compost between the second and the third day, leading to the increase of amylase, esterase and xylanase could be detected. Throughout the composting process no lipase activity was observed.

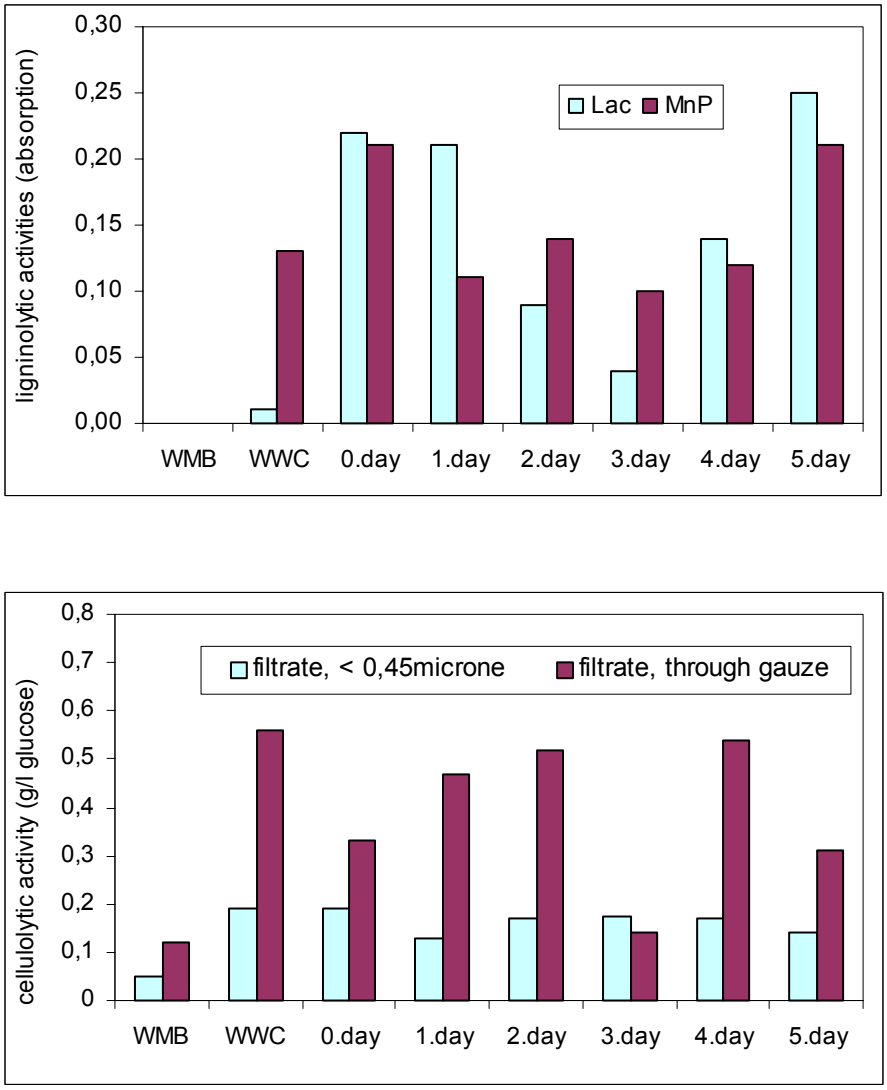

Figure 3. A: Lignolytic activities: laccases (Lac), manganese peroxidases (MnP) and B: cellulase activity after incubation at 37 °C. The activities were determined spectrophotometrically.

Lignin is a wood component and therefore we tested the presence of three groups of ligninases. Two of them, laccases and manganese peroxidases were present at the very beginning of the experiment as a result of microbial activities in the waste wood material before the experiment started, while the presence of lignin peroxidases was not detected.

The results are shown in Figure 3.

We did not prove cellulase activities on agar plates at 37 ^{o}C with substrates such as microcrystalline cellulose and cellulose azure. Since this appeared unusual due to the composition of the raw materials, we repeated the analysis with the quantitative spectrophotometrical method using carboxymethylcellulose as enzyme substrate. The results for lignolytic and cellulolytic activities are shown in Figure 3.

It is seen that both, extracellular and membrane attached cellulases were initially present in waste microbial biomass as well as in wood chips. These enzymes are more expressed in wood chips. Presence of membrane bound cellulases seems to be more pronounced than the extracellular ones. The comparison of two incubation temperatures for cellulase activity measurements showed that there was no notable increase in activity at higher incubation temperature except in the structure material, where the activity was doubled.

With the presented research we tried to reveal a broad spectrum of enzymes which participate in the composting of waste microbial biomass remaining after the isolation of a pharmaceutical product. It has been shown that the enzyme activity of microorganisms during the process was intensive and diverse. Many enzymes were present in the active form: proteases, amylases, esterases, cellulases, ligninases and xylanases. From the measured activities no lipase and lignin peroxidase were detected. For quantitative evaluation of the activities further studies are needed since the material is heterogenic and not well reproducible. The results could be used in further bioremediation studies of various organic compounds in waste microbial biomass from pharmaceutical industry, for example traces of antibiotics, organic solvents and similar.

Acknowledgements

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Povzetek

Za kompostiranje v 40-litrskem perforiranem zabojniku so bile pripravljene mešanice lesnih sekancev in odpadne mikrobne biomase iz proizvodnje farmacevtske učinkovine. Zasledovanje procesa je trajalo pet dni, pri tem je bila pri posameznih poskusih dosežena maksimalna temperatura 57 ^{o}C, pH se je gibal med 5,6 in 8,3, vlažnost pa med 56,5% in 51,5%. Zasledili smo različne encimske aktivnosti, nekatere so bile prisotne že v izhodnih materialih. Določili smo ekstracelularne protease, esterase, celulaze, amilaze in dve ligninazi: lakazo in mangan peroksidazo. V primeru celulaz smo dokazali tudi močno aktivnost encimov, ki so vezani na membrano.