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# Effect of inoculation with white-rot fungi and fungal consortium on the composting efficiency of municipal solid waste

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ABSTRACT

An investigation was carried out on the effect of inoculation methods on the compost of an organic fraction of municipal solid waste. Three types of white-rot fungi (*Phanerochaete chrysosporium*, *Trametes versicolor* and *Fomes fomentarius*), and a consortium of these fungi, were used. The study assessed their influence on microbial enzymatic activities and the quality of the finished compost. It was found that the addition of white-rot fungi to municipal solid waste (after 37 days of composting) could be a useful strategy for enhancing the properties of the final compost product. In comparison with the control sample (compost without inoculation), it accelerates degradation of solid waste as indicated by changes in C/N, electrical conductivity and pH. However, the effectiveness of waste degradation and compost maturation depends on the type of microorganism used for inoculation. The presence of inoculants, such as *Trametes versicolor* and *Fomes fomentarius*, led to a higher degrading ratio and a better degree of maturity. This resulted in an increase of enzymatic activities (especially dehydrogenase and protease) and a germination index in comparison with inoculation using *Phanerochaete chrysosporium* or a consortium of fungi.


## 1. Introduction

In accordance with framework waste legislation (Tatano et al., 2015), the European Union's approach to solid waste management is based on an integrated, hierarchical system. Waste prevention is the highest priority. In the line with this priority, and to comply with further requirements of the European landfill directive (Tatano et al., 2015), prevention measures and programmes are expected and encouraged in the biowaste category to progressively reduce the amount of municipal biodegradable waste going to landfills. This is followed by recovery options based on separate collection and biological treatment systems for biowaste that cannot be prevented (Tatano et al., 2015). In particular, the European strategy for waste prevention generally calls for actions to be taken at all geographical scales of governance, including regional and local levels (Tatano et al., 2015).

Large quantities of municipal solid waste (MSW) are produced in modern society; its disposal poses serious environmental, social and economic issues. Rapid expansion of cities and towns has caused an increase in per capita generation of MSW. Due to the increasing rate of solid waste generation, limited landfill space, and more stringent environmental regulations for new landfill sites and incinerators, there have increased the waste disposal fees in numerous places. Therefore, municipalities and local governments are under heavy pressure to find sustainable and cost-effective solid waste management practices (Saha et al., 2010; Sharholy et al., 2008).

Biological treatment, such as composting of the organic fraction of MSW (OFMSW), is an environmentally and economically viable solution (Wei et al., 2014). The composting itself is a biotransformation process of organic materials into stable and complex macromolecules under the action of microbe and corresponding enzyme(s) (Zeng et al., 2010). It includes four phases: (i) an initial decomposition phase; (ii) a thermophilic phase of intense microbial decomposition; (iii) a second thermophilic phase; and (iv) a maturation phase. Rapidly multiplying thermophilic bacterial species dominate during the thermophilic phase till the moment when the bulk of the easily decomposable substrate is exhausted. The majority of the remaining material is woody with lignin as the dominating part, which is also stabilised by humic acids and fungi (Belyaeva et al., 2012). The effectiveness of the treatment of MSW

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by bacterial (Adams and Frostick, 2009; Belyaeva et al., 2012), fungal (Belyaeva et al., 2012) and archaeal (Belyaeva et al., 2012; Yamamoto et al., 2011) communities during the composting phases of specific wastes has been investigated by a number of workers using molecular techniques (e.g. polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE)).

The natural composting process is rather time-consuming, but shortage of land and large volumes of OFMSW require these wastes to be treated more quickly. Therefore, inoculation with lignocellulolytic fungi could be a useful strategy for enhancing the properties of final products and shortening the time needed to achieve stability of the compost. Fungal species, such as *Trichoderma viride*, *Aspergillus niger* and white-rot fungi, are able to degrade cellulolytic and lignolytic waste (Awasthi et al., 2015; Huang et al., 2008; Vargas-Garcia et al., 2010). These microorganisms considerably reduce the composting time of OFMSW (Awasthi et al., 2015). Inocula used for composting are generally composed of one or a few strains (Echeverria et al., 2012; Hachicha et al., 2012). Chemical and microbiological changes were studied during OFMSW composting under field conditions in windrows and heaps (Adams and Frostick, 2009), whereas it was found, under laboratory conditions that the keeping the temperature constant is one of the key features for artificial composting (Zeng et al., 2010). Nevertheless, published results are still controversial; therefore further studies are needed for a more detailed explanation of these composting processes.

In this study, white-rot fungi *Phanerochaete chrysosporium*, *Trametes versicolor* and *Fomes fomentarius*, as well as a consortium of these fungi, were employed as inoculants for OFMSW. Investigations were conducted at the OFMSW Composing Plant placed on the landfill body in the Czech Republic. The effects of the fungi tested on rapid biodegradation and the physico-chemical parameters during composting was determined during three months and compared with a consortium of these strains. Differences between control and inoculated piles were evaluated throughout the process.

## 2. Materials and methods

### 2.1. Site description

The Kuchyňky landfill is classified in the S-category for 'other waste', sub-category S-OO3. The area of the landfill is 70,700 $m^2$ in five stages, with a total volume of 907,000 $m^3$, i.e. around $1{,}000{,}000 \cdot 10^3$ kg of waste. The planned service life of the facility is up to 2018. The facility receives waste (in the category of 'other waste') from a catchment area with a population of around 75,000 residents. The annually deposited amount of waste is around $40{,}000 \cdot 10^3$ kg, of which 50% is from the communal sphere.

### 2.2. Composting process

The composting plot is located on the surface of the landfill. Composted materials are biologically degradable wastes that are transported from the surrounding towns and villages. They mainly consist of greenery used in the maintenance of towns and villages, and of biologically degradable communal waste collected from households in the refuse collection area. The estimated capacity of waste received by this composting plant is $2000 \cdot 10^3$ kg/year. The facility is designed for the collection, purchase and exploitation of waste — waste management code R3 pursuant to Annex 3 of the Waste Law as amended.

The composting plant situated within the II b stage plot of the landfill is presented in the Supplemental Information section (Fig. SI-1). On this site, waste disposal was terminated; the plot was subjected to ground shaping, covered with earth and recycled material, and compacted. The size of the composting plant was 20 × 35 m. According to the conditions (amount of compostable waste), 6–8 compost back fills were established of triangular profile sized 30 × 1.5 m and with a height of 0.8 m (Fig. SI-2). Composting piles were divided into five equal parts with dimensions of 6 × 1.5 m and a height of 0.8 m. Aerobic conditions were accomplished by mechanical turning. The turning was accomplished with specialised equipment. Composting piles were turned upside down every 7 days.

### 2.3. Composting materials

Typically, the organic fraction of MSW includes food waste, leaf and yard waste. Food waste represents a significant proportion of organic material; it can originate from residential and commercial kitchens (e.g. restaurants and hospitals) or come from distribution and retail agents. Leaf and yard waste consists of lignocellulosic based materials, such as green grass clippings and thatch, leaves, weeds, brush, and tree prunings, whose production varies widely through the year. In addition, although minor, contribution to the lignocellulosic content of organic MSW is provided by soiled paper. The basic physico-chemical characteristics of the resource materials are presented in Table 1.

### 2.4. Microorganisms and inocula preparation

The white-rot fungi *Trametes versicolor* (TV) and *Fomes fomentarius* (FF) were isolated from the Czech forest near Brno (GPS for TV 50.5395819N, 13.9869047E; GPS for FF 49.2954886N, 16.6351322E). These strains were obtained from the Culture Collection of the Faculty of Forestry and Wood Technology of the Mendel University in Brno (CZ). The lignocellulolytic microorganism *Phanerochaete chrysosporium* (PC) (CCM $8074^T$) was obtained from the culture collection of the Czech Collection of Microorganisms (CCM), at Masaryk University Brno, Faculty of Science (CZ). Tested cultures were stored at 4 °C on potato dextrose agar (PDA).

Prior to inoculum preparation, fungal cultures were cultivated on PDA for 8 days at 22 °C. After incubation, microbial biomass of each strain was collected and suspended in 2 L potato dextrose broth (PDB) at 28 °C for 7 days. The suspension mixture was spread over and injected at different pile locations to ensure the best possible distribution of the inoculum. The control pile received the same amount of sterile distilled water. Inoculation was carried out on the composting piles 37 days after the process had started. This was when the thermophilic phase had finished. The final concentration of each strain or consortium in the piles was 10 g $kg^{-1}$ of wastes.

**Table 1**
Physico-chemical characteristics of the initial resource materials.

| Parameters | Results | Unit |
|---|---|---|
| DM | 42.38 | % |
| pH | 7.0 | |
| C/N | 32 | |
| EC | 180 | μS $cm^{-1}$ |
| Organic carbon | 37 | % DM |
| Total nitrogen | 1.15 | % DM |
| Phosphorus | 2.77 | g/kg DM |
| Potassium | 12.6 | g/kg DM |
| Zinc | 174 | mg/kg DM |
| Cadmium | 0.64 | mg/kg DM |

DM dry matter.

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