Co-composting as a management strategy to reuse the white-rot fungus *Trametes versicolor* after its use in a biotechnological process

E. Marco, X. Font, A. Sánchez*, T. Gea and X. Gabarrell

Departament d'Enginyeria Química,
Escola Técnica Superior d'Enginyeria,
Universitat Autónoma de Barcelona (UAB),
08193 Bellaterra, Spain
Fax: 34 93 581 2013
E-mail: Ernest.Marco@uab.cat
E-mail: Xavier.Font@uab.cat
E-mail: antoni.sanchez@uab.cat
E-mail: Teresa.Gea@uab.cat
E-mail: Xavier.Gabarrell@uab.cat
*Corresponding author

G. Caminal

Unitat de Biocatàlisis aplicada Associada al IIQAB (CSIC-UAB),
Unitat Associada de Biocatàlisi Aplicada, IQAC-CSIC,
Departament d'Enginyeria Química,
Escola Técnica Superior d'Enginyeria,
Universitat Autónoma de Barcelona (UAB),
08193 Bellaterra, Spain
Fax: 34 93 581 2013
E-mail: Gloria.caminal@uab.cat

Abstract: White-rot fungi are extensively used in biotechnological processes but little is known about the disposal of fungal biomass after its use. Final products stability parameters (self-heating test and respiration index) indicate that co-composting of the white-rot fungus *Trametes versicolor* with Organic Fraction of Municipal Solid Wastes (OFMSW) ensure a higher stable final product than that obtained in OFMSW composting. Results suggested that the absence of fungus in the final product is probable owing to the thermophilic temperatures achieved during the composting process. These results indicate that composting may be extended to other residual biomass produced in biotechnological processes with white-rot fungi, considering spent biomass as a useful resource and minimising its risks for soil application.

Keywords: *Trametes versicolor*; composting; white-rot fungi; viability; municipal solid waste.

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Biographical notes: E. Marco is currently working as a Lecturer Professor at the Universitat Autònoma de Barcelona (Spain). He received his PhD from the Universitat Autònoma de Barcelona; and he hold a Marie Curie grant for postdoctoral studies at the Helmholtz Centre for Environmental Research (UFZ), Leipzig (Germany). His research interests focus on the application of white-rot fungi for biodegradation of emerging pollutants and more recently the use of proteomic tools to shed light on the metabolism of *Dehalococcoides* species.

X. Font Professor in the Chemical Engineering Department of Universitat Autònoma de Barcelona. His main research activities have been focused on biological waste treatment especially toxic waste waters and organic solid waste treatment through composting and anaerobic digestion. He is also focusing his research on the environmental impact evaluation of solid waste treatment facilities. Nowadays he is also researching on the use of nanoparticles in wastewater remediation and its ecotoxocity.

Antoni Sánchez is Professor in the Chemical Engineering Department at the Universitat Autònoma de Barcelona. He is a Chemical Engineer and obtained his PhD in Biotechnology in 1998. His main research activities have been focused on biological waste treatment, especially on composting processes. He is now the Coordinator of the Composting Research Group of Universitat Autònoma de Barcelona.

Teresa Gea is Professor in the Chemical Engineering Department at the Universitat Autònoma de Barcelona. She is a Chemical Engineer and finished her PhD thesis in 2005 which dealt with the characterisation and monitoring of the composting process of industrial organic wastes and focused on the utilisation of biological activity measurements such as respiratory techniques and enzymatic activities. Her post-doctoral research has also considered the composting process advanced control and optimisation and the study of the influence of physical properties on process evolution.

X. Gabarrell His research work in the Chemical Engineering Department and ICTA at the Universitat Autònoma de Barcelona (UAB) has been focusing on the field of Environmental Engineering and Biochemical Environmental Engineering during the last 15 years. Expert in industrial ecology, Life Cycle Analysis, waste management, and industrial wastewater treatment by fungi. His research has been funded by the European Union; the Spanish CICYT, the Catalan DURSI and different companies. He is the coordinator of the research group Sostenipra.

G. Caminal is researcher from Consejo Superior de Investigaciones Científicas (CSIC) in Spain. She is working at the Associate Unit of Applied Biocatalysis placed in the Universitat Autònoma de Barcelona (UAB). She is PhD from UAB since 1983. Her research interest area is biochemical engineering. At this moment her work is focused on the production of recombinant proteins and the application of white-rot fungi for biodegradation of emerging pollutants.

1 Introduction

Fungal biotechnological applications, including bioremediation and the production of medicines, food and fibres, generate large amounts of residual biomass. These processes can use both genetically modified and wild organisms, and the spent biomass should be treated prior to disposal. The spread of these exogenous microorganisms can generate an ecological impact, as it demonstrates the viability of industrial relevant fungi for several seasons after its release into the environment (Providenti et al., 2004).

Recently, our laboratory has developed a long-term continuous decolourisation treatment of the textile dye Grey Lanaset G in an air-pulsed bed bioreactor with retained pellets of the white-rot fungus *Trametes versicolor* (Blánquez et al., 2004, 2006). Work is in progress to consider near-industrial-scale operational conditions, and one of the main concerns is the post-treatment of the residual fungal biomass obtained from the bioreactor.

Composting has appeared as a fast, safe and relatively simple treatment that may offer a cost-effective method for disposal of biowastes (Singh et al., 2006). Composting is a biotechnological process characterised by an initial phase in which different microbial communities degrade organic matter into simpler nutrients and thermophilic temperatures are developed owing to the heat generation. The high temperatures achieved in this phase appear to be the most important factor in killing heat-sensitive microorganisms. In a second stage, complex organic macromolecules such as humic acids are formed and temperature decreases to ambient levels, leading to a product that may contribute to soil conditioning and fertility (Hsu and Lo, 1999).

In this work, a co-composting process using Organic Fraction of Municipal Solid Wastes (OFMSW) as co-substrate for fungal biomass used for the decolourisation treatment described previously was studied. Subsequently, the survival of *T. versicolor* in the compost intended for soil application was examined.

2 Materials and methods

2.1 Materials

T. versicolor (strain ATCC # 42530) was maintained on 2% (w/v) malt agar slants at 25°C until use. Subcultures were routinely made.

2.2 Fungus composting

Composting materials

For *T. versicolor* and OFMSW co-composting evaluation, three experiments were carried out: two replicates containing a mixture of OFMSW and fungus (7.5% wet weight), and a control experiment containing only OFMSW.

Fungal biomass (88.2% – weight basis – water content, 96.3% – dry basis – total organic matter content, OM) used for composting was obtained from a bioreactor inoculated with pellets of *T. versicolor*. This bioreactor was used to decolourise Grey Lanaset G, which consists of a mixture of metal complexed dye (Blánquez et al., 2004, 2006).

OFMSW was obtained directly from the composting plant of Jorba (Barcelona, Spain), one day after its arrival and mixed with the bulking agent (shredded pruning wastes). The material was sieved (Filtra Vibración, FT-400) for a better homogenisation. Only the small fraction with a particle size of <80 mm was used for the experiments. OFMSW-*T. versicolor* mixtures for composting experiments were handmade.

Equipment and operating conditions

Laboratory-scale experiments were undertaken using 4.5-l Dewar® vessels conditioned for static composting providing a stopper and placing a rigid wire net near the bottom to separate the material from possible leachate. The stopper was perforated in three points for temperature and oxygen content monitoring and air supply. Air was supplied to the vessels in aeration cycles where the total time of the cycle, aeration time and air flow were programmed and changed on the basis of the measured oxygen concentration, to ensure an oxygen concentration over 10% (v/v). Pt-100 sensors were used for temperature monitoring connected to a data acquisition system (DAS-8000, Desin, Spain), which was connected to a standard PC. The system allowed, by means of the proper software (Proasis® Das-Win 2.1, Desin, Spain), the continuous online visualisation and registration of temperature. Pt-100 sensors were placed in the material to measure temperature at half of the height of the material in the Dewar vessel. During all composting processes, water content was always maintained within 40–60%, range recommended for composting processes (Haug, 1993).

2.3 Isolation of T. versicolor from compost

The resultant compost for each replicate was homogenised manually and 10 subsamples were assembled, mixed and sieved through a 4 mm mesh screen.

The isolation of *T. versicolor* from these samples was carried out following the soil dilution plate method (Parkinson, 1994). 10 g of compost was added to a 90 ml 0.2% Triton X100 solution and stirred at 250 rpm for 15 min. The resulting solution was diluted with different volumes of 0.2% Triton X100 solution. Several 0.1 ml-aliquots were then spread on Petri dishes containing malt agar and incubated at 25°C. Fungal colonies in these Petri dishes were isolated and the determination of *T. versicolor* survival was conducted by laccase activity test and further visual hyphae identification by microscopical observations. Laccase activity was analysed as described previously (Blánquez et al., 2004).

2.4 Effect of temperature on viability of T. versicolor

To investigate the viability of fungus at thermophilic temperatures, the effect of short exposition to 50°C and 60°C was studied observing the growth of *T. versicolor* pure strain in malt agar slants. For each temperature, three replicates were cultured for 60 h and then reinoculated and cultured at optimal temperature.

2.5 Analytical methods

Analytical parameters (water content and OM) and final product stability parameters (Dewar Self-Heating Test and Respiration Index) were determined according to the standard procedures (US Department of Agriculture and US Composting Council, 2001).

The Dewar Self-Heating Test is a standardised procedure used to measure self-heating as an indicator of biological activity. With this test, compost is classified from class I (Raw Feedstock; fresh compost, mixed ingredients) to class V (Finished Compost; stable to very stable compost). Two replicates were carried out and the highest temperature difference is considered. Respiration index was measured in a static respirometer, as described previously (Barrena et al., 2005). Results of the static respiration index referred to total organic matter content are presented as average of three replicates.

2.6 Data analysis

Statistical significance of values of different radial growth rates obtained in cultures that were previously grown at different conditions was carried out by means of *F* test (variance analysis) and *t*-Student (mean analysis) both at 95% of interval of confidence.

3 Results and discussion

3.1 T. versicolor co-composting with OFMSW

OFMSW was selected as a standard co-substrate for *T. versicolor* co-composting evaluation. Figure 1 shows the results obtained for the three co-composting experiments: Replicates A and B for OFMSW mixtures with 7.5% fungus (Figure 1(A) and 1(B)) and control experiment (Figure 1(C)). Typical composting temperature profiles were obtained in all cases, with a rapid initial increase to values above 60°C during the first 24 h. Air requirements were similar for the replicates containing *T. versicolor* and higher than those of control experiment, showing more oxygen consumption and hence a higher biological activity.

As shown in Table 1, maximum temperatures rose to around 70°C for all three processes. However, noticeable differences were observed for the length of the thermophilic phase (temperatures maintained above 45°C). Thermophilic phase for the control experiment lasted for almost four days, while 2.5 and 5.7 for replicates containing fungal organic matter. Although international sanitation requirements were not achieved with these laboratory-scale experiments (temperature over 55°C for 2 weeks, US Environmental Protection Agency, 1995), thermophilic phase was longer than two days in any case, which indicates that the sanitation requirements can be reached considering composting of these wastes at industrial scale (Barrena et al., 2006b; Gea et al., 2007).

To evaluate the stability of the final product obtained after composting, self-heating test and respiration index were determined (Table 1). Stable compost is considered when class V is achieved for self-heating test. Different stability limits between 0.5 mg and 1.5 mg O_2 g OM^{-1} h^{-1} have been suggested in the literature for respiration indices (Barrena et al., 2006a). As is shown in Table 1, a high level of stability was observed in duplicates containing fungal biomass. However, control experiment reached a lower stability level (Class III, corresponding to active compost, material decomposing and unstable) and a high biological activity was still present according to respiration index.

The stability results together with the differences observed in air requirements seem to indicate that the presence of *T. versicolor* has a positive effect in the composting

process. Other authors (Baheri and Meysami, 2002; Bolta et al., 2003; Xi et al., 2005) have reported a higher biological decomposition of organic matter when inoculating the organic wastes with white-rot fungi and an improvement of the composting process, since these microbes play an important role in lignin degradation and generate various desired enzymes.

Figure 1 Temperature profiles during co-composting experiments: (A) OFMSW + 7.5% fungus (duplicate A), (B) OFMSW + 7.5% fungus (duplicate B) and (C) OFMSW control. Temperature (continuous line), Room Temperature (dotted line) and Air Flow (semicontinuous line)

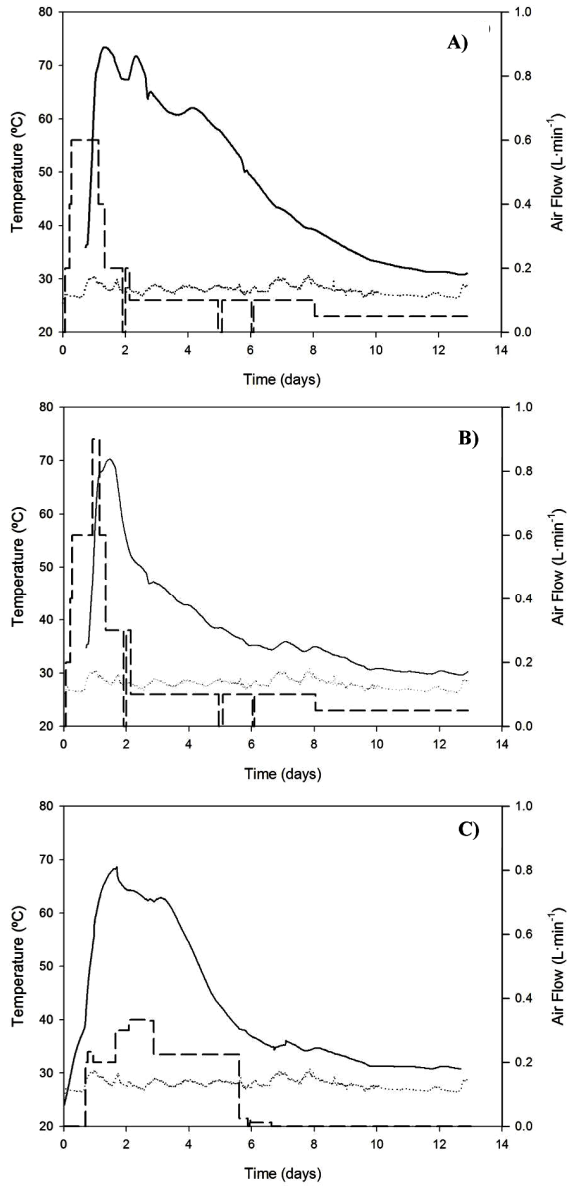

Table 1 Main data of the composting experiments

Parameter	*OFMSW*[1] *+ fungus 7.5% duplicate A*	*OFMSW + fungus 7.5% duplicate B*	*OFMSW control experiment*
Maximum temperature (°C)	73.4	70.3	68.6
Thermophilic phase length ($T > 45$ºC) (days)	5.7	2.5	4.0
Final product self-heating test[2]	Class V	Class V	Class III
Final product respirometric index [mg O_2 g $OM^{-1}h^{-1}$]	1.30	1.58	4.33
Final product dry matter content (%, w/wet weight)	41.2	39.15	35.8
Final product organic matter content (%, w/dry weight)	45.5	50.9	50.7

[1]Culture conditions were described in materials and methods. OFMSW means organic fraction of municipal solid wastes.

[2]COMPOST is classified from class I (raw feedstock; fresh compost, mixed ingredients) to class V (finished compost; stable to very stable compost) for the final product self-heating test.

3.2 Post-composting T. versicolor viability

The survival of *T. versicolor* in the compost was examined by the dilution plate method, microscopical observation and laccase analysis to determine the effectiveness of the treatment in sanitising biowaste. Polymerase Chain Reaction (PCR) analysis has been used in previous reports (Becker et al., 1999; Providenti et al., 2004) to quantify levels of the strain in soil but this method does not provide information on the viability of the microorganism.

Several samples were cultured in Petri dishes after extraction from final compost and plating dilution, but filamentous fungi were observed only in 10% of the dishes. However, no laccase activity was determined in any case and therefore it was concluded that *T. versicolor* was not among the viable species. Same results were obtained by microscopic observations.

3.3 T. versicolor viability at thermophilic temperatures

To confirm that the absence of *T. versicolor* could be due to the thermophilic temperatures achieved during the composting process, the fungus was exposed at 50°C and 60°C as is detailed in materials and methods. After the exposition, no fungus survival was observed, and hence it was concluded that *T. versicolor* can be killed during the thermophilic phase of the process, avoiding any further fungus propagation and allowing the use of the compost obtained as organic amendment.

4 Conclusions

Our laboratory results showed that co-composting is a feasible management strategy to reuse the white-rot fungus *T. versicolor* after its use in a biotechnological process. Stability parameters (self-heating test and respiration index) together with the observed air requirements indicated a high level of compost stability and the positive effect of the addition of the white-rot fungus in the composting process. Furthermore, the possible disappearance of fungus owing to the thermophilic temperatures achieved during co-composting suggests that this technology is suitable to minimise the risk for soil application. This work opens new research horizons in the treatment of biowastes containing white-rot fungi.

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