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ORIGINAL SCIENTIFIC PAPER

Solid-state fermentation of agro-food waste: applicability comparison of two bioreactor configurations

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Abstract

The applicability of two laboratory-scale bioreactors for solid-state cultivation of white-rot fungus *Trametes versicolor* TV6 was investigated. Sugar beet pulp (SBP) was used as a non-inert carrier. Two bioreactor configurations were investigated: horizontal bioreactor (HB) and tray bioreactor (TB). SBP biodegradation was observed in both investigated bioreactors, whereas the obtained material mass loss was 21.33% and 46.50% for HB and TB, respectively. The decrease of cellulose (64.65% HB and 42.34% TB) and pentosane content (42.39% HB and 62.61% TB), as well as the ash content increase (175.5% HB and 78.9% TB), indicated good biodegradation capability of the fungus. Furthermore, the results indicate that both bioreactors could be used for biological degradation/pre-treatment of lignocellulosic materials for different biotechnological purposes using white-rot fungus *T. versicolor* TV6.

Key words: solid-state fermentation, *T. versicolor*, sugar beet pulp, horizontal bioreactor, tray bioreactor

Introduction

By definition, solid-state fermentation (SSF) is a method of growth of microorganisms on a solid support and occurring in near-absence of free water. The solid support or carrier can be inert (e.g. plastic foams), serving only as an attachment place for the microorganism, or non-inert (e.g. lignocellulosic waste), serving as both attachment place and substrate (Pandey et al., 1992.). Although still underrepresented in the everyday biotechnological production (compared to submerged fermentations, SmF), SSFs are gaining more and more attention as they have proved to be suitable for the enzymes production using filamentous fungi. Furthermore, since bacteria are less suitable for SSF due to the fact they require high water activity (a_w), the chances of bacterial contamination are reduced in SSF (O' Toole 2006.). Bioreactors operating under solid-state conditions encounter problems in control of process parameters, namely pH, temperature, aeration, oxygen transfer and agitation. Two operating principles SSF bioreactors usually employ are static (tray, packed-bed or column bioreactors) and agitated (vertical or horizontal with mechanical agitation, and rotating drum bioreactors) (Figueroa-Montero et al. 2011.). However, still, only a few designs of SSF bioreactors are available. Most industrial-scale SSF processes involve fungi producing extracellular enzymes, while growing on moist agricultural waste, as a non-inert carrier.

A large portion of agro-food wastes is comprised of lignocellulosic materials in which hemicellulose and lignin form a matrix surrounding cellulose, thus providing the high degradation resistance of these polymers to become fermentable sugars (Wyman et al., 2005.). Lignin, after cellulose the second most abundant natural polymer, is an aromatic polymer that provides the strength of plant cell wall and is particularly resistant to biodegradation. There are but few organisms capable of degrading lignin, the most efficient

of which are fungi. White-rot fungi are capable of degrading and mineralizing all major wood polymers and can extensively degrade lignin to CO_2 and H_2O. This ability is due to the fact that these fungi produce a variety of hydrolytic and oxidative enzymes. Extracellular enzymes essential for lignin degradation include lignin peroxidase, Mn-dependent peroxidase, and laccase (Elisashavili, 2009.). SSFs using white-rot fungi are a comprehensive tool for utilization of lignocellulosic waste materials in biotechnological production.

The aim of this study was to investigate the applicability of two bioreactor systems operating under solid-state conditions for the cultivation of white-rot fungus *Trametes versicolor* TV6 using the lignocellulosic agro-food waste material as a substrate and support.

Materials and methods

Substrate/carrier

Dried sugar beet pulp (SBP, donated by "Sladorana Županja d.d." Županja, Croatia), was used as a substrate and carrier for SSF. SBP of identical initial mass and moisture content was used in all runs.

Microorganism and inoculum preparation

The white-rot fungus *Trametes versicolor* TV 6 (Microbial Culture Collection of the National Institute of Chemistry, Ljubljana, Slovenia) was maintained at 4 °C on potato dextrose agar (Biolife Italiana Fr. L. Viale Monza, Italy). Mycelial discs (6 mm diameter) of seven days old culture grown on PDA at 27 °C were used as inoculum for further experiments.

Laboratory-scale bespoke bioreactors configuration and operating conditions

The solid-state cultivation of *T. versicolor* TV6 on sugar beet pulp was carried out in two different laboratory-scale homemade bioreactor systems equipped with appropriate sensors for temperature and relative humidity monitoring, as well as aeration systems: a) horizontal -cylindrical bioreactor (HB); b) tray bioreactor (TB). HB (Figure 1a), with the overall volume of 4250 mL, was filled with SBP and water in a ratio 1:2.2 (500.55 g of wet substrate) and sterilized (121 °C / 20 min). After inoculation, the experiment was conducted at ambient temperature for 30 days. The content of the bioreactor vessel was manually stirred every 24 h for 5 min. TR (Figure 1b) consisted of a metal base holder for four trays (dimensions: 275 x 190 x 15 mm) positioned one above the other (the distance between the trays of 45 mm) and placed in the glass chamber with a lid. Sterilised SBP (1024 g of wet substrate equally spread onto 4 trays) was placed on chemically sterilised trays forming a layer of 12 mm thickness. After inoculation (20 mycelial plugs per tray), the experiment was conducted under static conditions (no stirring) at ambient temperature for 30 days. Aeration was carried out continuously (airflow rate was set at 30 L h^{-1}) in both bioreactor systems using the sterile air filter and air compressor. The inoculation was carried out directly in the bioreactor vessels: 40 (80 TB) mycelial discs (diameter 6 mm), excised from a 7 days old colony growing on PDA, were used as inoculum. The change of process parameters (temperature in different layer substrate and biomass, ambient temperature and relative humidity in the bioreactor vessels), as well as mass loss and changes in the chemical composition of the substrate (dry matter, cellulose, pentosans, and ash contents), were monitored during the experiment. The samples were taken on 15^{th} and 30^{th} day of cultivation.

Process parameters monitoring during the experiment

The temperature was monitored on-line at different positions in the bioreactor vessels (headspace, inlet, outlet and bioreactor surroundings) and in the fermentation beds, using stainless steel thermocouple penetration probes (type T). Thermocouples were connected to the 8-channels Pico A/D converter and a PC application PicoLog for the data acquisition (Pico Technology Limited, England). Testo 635 and 350 devices (Testo Inc., Sparta, New Jersey, USA) were used for additional temperature and relative humidity monitoring.

Analytical determinations

Dry matter content was determined gravimetrically using Halogen Moisture Analyzer HR73 (Mettler Toledo, Switzerland). Mass loss was calculated and expressed as a rough estimation since the fungal biomass present in the sample was not considered in the calculation. Mass loss is given on a percent dry weight basis and wet weight basis. Ash content was determined by high-temperature incineration in an electric muffle furnace for 3 hours. The applied temperature was 575 ± 10 °C. The residue was cooled to room temperature, weighed, and the ash content was calculated. Cellulose content was determined according to the modified protocol by Rivers et al. (1983.) in samples previously extracted (ethanol/benzene 1:1) using Soxtec system 1040 Extraction Unit (Foss Tecator). Pentosane content was determined according to the protocol TAPPI T 223 cm-01 (2001).

All analytical results were expressed as means of three replicates.

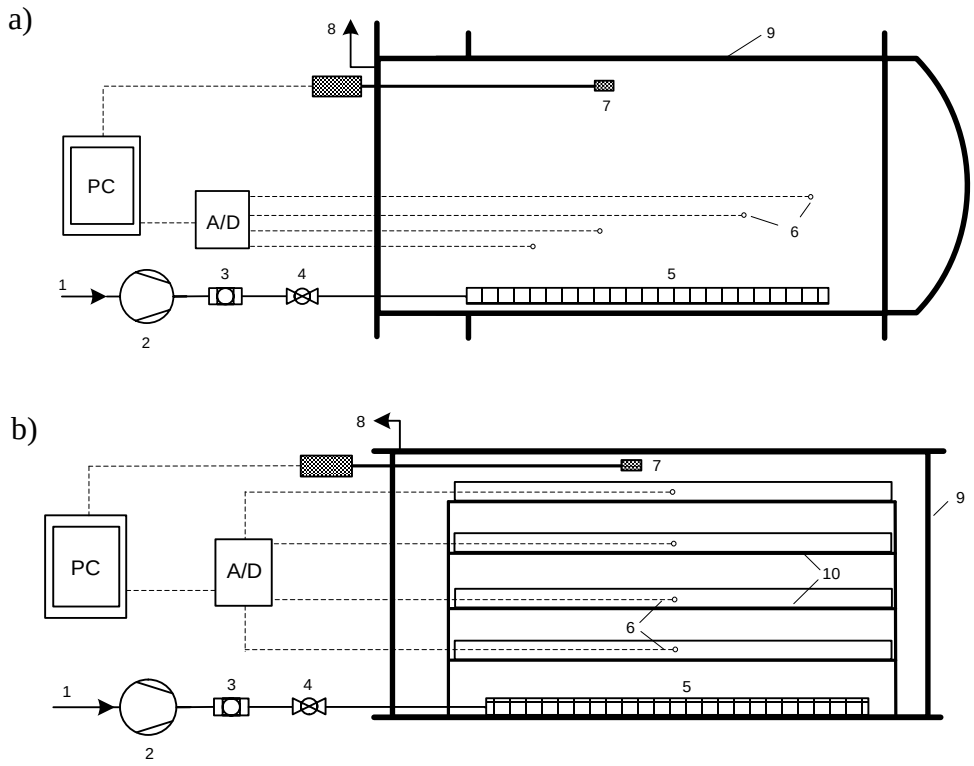

Figure 1. Laboratory-scale bioreactor systems equipped with appropriate sensors for temperature and relative humidity monitoring, as well as aeration systems: a) horizontal - cylindrical bioreactor (HB), b) tray bioreactor (TB). 1- air inlet, 2 - air compressor, 3 - filter, 4 - valve, 5 - air sparger/diffuser, 6 - temperature probes (T-type), 7 - %RH and temperature probe, 8 - gas exit, 9 - reactor chamber, 10 - trays (TB)

Results and Discussion

Visual confirmation of fungal colonization of substrate/carrier indicated that white-rot fungus *T. versicolor* TV6 grew readily on SBP as the non-inert carrier under solid-state conditions in both tested bioreactor systems. Furthermore, the increase of substrate bed temperature in both bioreactors (Fig 2), compared to ambient temperature as a result of intensive microbial metabolic activity, also indicated actively growing fungus. The temperature increase was higher in TB than HB, probably due to static growth conditions, which resulted in poorer heat exchange compared to HR where mixing was employed. Relative humidity was not significantly changed during the cultivation in both bioreactors (data not shown). The results given in Table 1 show the changes of substrate mass loss and chemical composition of samples, namely ash content, pentosans content and cellulose content, during fermentation in both bioreactors. The mass loss, which can be contributed to the utilization of substrate due to fungal growth, after 30 days of fermentation was higher in TB (46.50%) than in HB (21.33%). The continuous increase of ash content during fermentation in all samples can also be contributed to fungal growth, as spent substrates after SSF using fungi often show increased mineral content (ash content) (Peksen et al., 2011.). The decrease of pentosans content in all samples indicate the ability of *T. versicolor* TV6 to degrade hemicellulose. The total decrease of pentosan content after 30 days of fermentation was 42.39% and 62.61% for HB and TB, respectively. This is consistent with the results of Bénes et al. (2013.) that reported remarkable pentosans degradation capacity of *T. versicolor* when grown on SBP under SSF conditions. The decrease of cellulose content in both HB (64.65%) and TB (42.34%) indicate strong cellulolytic activity of *T. versicolor* TV6 grown on SBP under solid-state conditions.

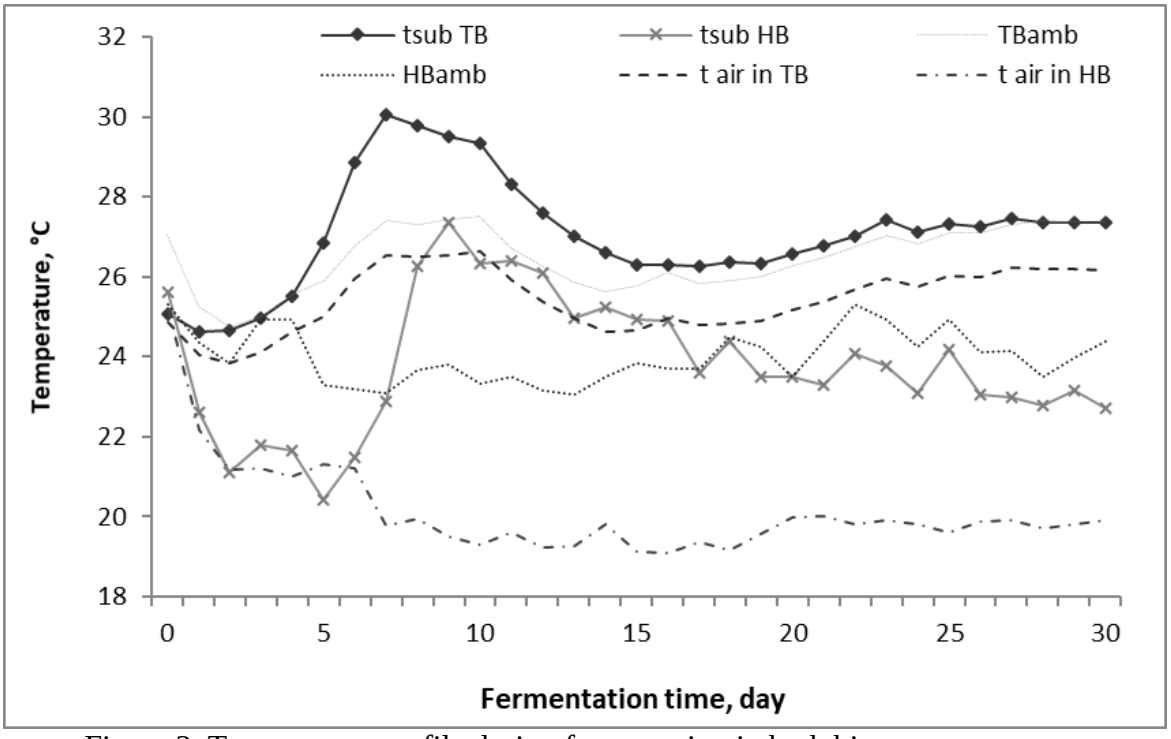

Figure 2. Temperature profile during fermentation in both bioreactors systems

Table 1. The changes of substrate mass loss and chemical composition of SBP samples during fermentation in TB and HB.

	Substrate mass [g]		**Ash content** [%]		**Pentosans content** [%]		**Cellulose content** [%]	
Fermentation days	**TB**	**HB**	**TB**	**HB**	**TB**	**HB**	**TB**	**HB**
0	547.80	500.55	3.79	3.79	29.58	29.58	23.76	23.76
15	398.21	455.38	5.54	6.09	14.91	13.90	16.96	12.73
30	139.93	393.77	6.79	10.44	11.06	17.04	13.70	8.40

Conclusion

All the results presented in this study indicate that both tested bioreactor systems could be successfully employed for biological degradation of the lignocellulosic material, such as SBP. However, because of the higher substrate mass loss and higher pentosans conversion, as well as simplicity of design and construction, the employment of TB for biological SBP degradation using *T. versicolor* TV6 grown under solid-state conditions, seems more appropriate.

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