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Simultaneous valorization and detoxification of the hemicellulose rich liquor from the organosolv fractionation

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ABSTRACT

Fractionation of lignocellulosic biomass with solvents (organosolv process) generates a hemicellulose-rich liquor with a high content of phenolics which is particularly toxic. This work addresses the utilization of this stream as a potential carbon source for the production of ligninolytic enzymes (LE). Among six basidiomycetes species, *Irpex lacteus* and *Ganoderma lucidum* presented the highest activities of manganese peroxidase (646 ± 122 U L^{-1}) and laccase (1497 ± 161 U L^{-1}), respectively, growing on a medium composed mainly of cellulose fibers, lignin and hemicellulose. The influence of each lignocellulosic fraction on the LE production mechanisms was studied in more detail. The high concentration of phenolic compounds in the hemicellulose-rich stream acted as inducer of LE production, with levels even greater than those of xylose. Acute toxicity tests on *Vibrio fischeri* revealed a substantial reduction of the toxicity after the fungal treatment (by ca. 12–21 times). The proposed valorization and detoxification of this currently non-exploited and abundant by-product stream is a promising strategy to enhance the industrial feasibility of the organosolv fractionation process.

1. Introduction

White-rot fungi are the only group of microorganisms currently known that degrade all basic wood polymers, i.e., cellulose, hemicellulose and lignin into low-molecular weight compounds that can be assimilated for growth (Camarero et al., 2014). This is achieved by their ability to produce several hydrolytic enzymes (cellulases and hemicellulases) and their unique network of oxidative (ligninolytic) enzymes. The study of the extracellular ligninolytic enzyme (LE) system of *Phanerochaete chrysosporium* demonstrated that lignin peroxidase (LiP, EC 1.11.1.14), manganese peroxidase (MnP, EC 1.11.1.13) and laccase (EC 1.10.3.2) are the primary enzymes associated with the degradation of lignin (Kuwahara et al., 1984).

The LE system plays a fundamental role in the bioconversion of lignocellulose. Furthermore, LE can also be applied for other purposes, such as the production of second-generation biofuels, organic synthesis (antibiotics, polymers, building blocks), cosmetics (skin-lightening agents), nanobiotechnology (biofuel cells and biosensors for biomedical applications), bioremediation, biopulping and biobleaching in paper industry as well as the food and textile industry (Alcalde, 2015; Yadav and Yadav, 2015).

However, to meet market demands, the large-scale production of these enzymes at low cost is mandatory. Although significant efforts have been devoted to enhance LE production by heterologous protein expression (Alcalde, 2015), the levels of enzymatic production are still rather limited (Eibes et al., 2009) and still have to be obtained from wild strains (Elisashvili and Kachlishvili, 2009).

LE production highly depends on the fungal species, source of lignocellulosic substrate and cultivation method (Elisashvili et al., 2008). The presence of lignocellulose was found to be a prerequisite for LE production by white-rot fungi in submerged cultures (Kapich et al., 2004; Gassara et al., 2012) and the various lignocellulosic fractions have been reported to influence LE production in a different manner. For example, *P. chrysosporium* was found to secrete laccase in the presence of cellulose, but not glucose (Srinivasan et al., 1995). *T. versicolor* produced higher laccase yields in the presence of natural lignocellulose-containing substrates such as wheat straw or wood, rather than with glucose (Schlosser et al., 1997). Toxic aromatic compounds and lignin also have a remarkable influence on LE production with *T. versicolor* or *Phlebia radiata* (Rogalski et al., 1991a, 1991b). Understanding the physiological mechanisms regulating enzyme synthesis by white rot fungi could therefore be useful for improving the efficient production of LE.

The organosolv process, i.e. pulping with ethanol-water, allows the

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fractionation of the lignocellulosic materials on its main constituents, i.e. cellulose fibers, lignin and hemicellulose fraction (Laure et al., 2014). However, only a few works have studied potential applications of the hemicellulose liquor after lignin recovery (Kautto et al., 2013; Hallberg et al., 2011). The presence of degradation products from the carbohydrates, soluble low molecular weight lignin and other possible inhibitors may limit its conversion to biofuel (Kautto et al., 2013). On the other hand, considering this fraction as waste stream, the large costs associated with wastewater treatment would limit the economic viability of the organosolv biorefinery (Laure et al., 2014).

Recently, the interest on the valorization of agro-industrial residues to produce LE at lower cost has increased (Palma et al., 2016). Several residual streams, such as apple pomace sludge (Gassara et al., 2012), fishery residue, brewery waste, pulp and paper industry sludge (Gassara et al., 2010), sugarcane residue (Maza et al., 2015), olive oil wastewater (Mann et al., 2015), oat husks, waste from paper process industry (Winquist et al., 2008) have been evaluated as carbon source. However, to our knowledge, the application of the hemicelluloses fraction from organosolv process for ligninolytic enzyme production has not been reported yet.

This study evaluates the production of LEs in submerged fermentations by different fungal strains using the hemicellulose liquor of an organosolv process as carbon source. With the aim of better analyzing the specific requirements of the fungal strains for LE production, the individual or combined addition of the other fractionation products (cellulose fibers and lignin) was evaluated before using the hemicellulose liquor as the only carbon source. Moreover, the acute toxicity of the treated liquor was assessed with the bioluminescent photobacterium *Vibrio fischeri*.

2. Materials and methods

2.1. Lignocellulosic substrates

The lignocellulosic substrates used as carbon source in this study were cellulose fibers (C_6), organosolv lignin (L) and a concentrated C5-sugars liquid fraction (C_5), all provided by the Fraunhofer Center for Chemical-Biotechnological Processes (CBP, Leuna, Germany). The physico-chemical characterisation of these substrates is given in Table 1. The fractionation of beech wood in the organosolv process was performed at the Fraunhofer-CBP. Debarked beech wood chips were pulped with a 1:1 ethanol/water mixture at 170 °C for 100 min using 0.5% of sulphuric acid (based on dry wood) at a liquor-to-wood ratio of 3.2:1 and the pressure was maintained at 20 bar using nitrogen gas (Laure et al., 2014). The solid fraction obtained after the pulping of the beech wood was washed; disintegrated and dewatered, obtaining the cellulose fibers (C_6). The liquid phase was composed mainly of lignin, C5-sugars from hemicellulose fraction and degradation products. Lignin (L) was precipitated from this stream by the addition of water and filtered for its separation (Schulze et al., 2016). By the recovery of the ethanol from the filtrates of precipitated lignin, a raw hemicellulose fraction was obtained (C_5). This raw hemicellulose stream was subsequently concentrated.

2.2. Microorganisms and inocula preparation

Irpex lacteus (Fr. 238 617/93), *Lentinus tigrinus* (PW94-2), *Stereum hirsutum* (PW93-4) and *Phanerochaete chrysosporium* (BKM-F-1767) were obtained from the culture collection of the Department of Chemical Engineering of the University of Santiago de Compostela (Spain). *Bjerkandera* sp. R1 was isolated from a Chilean forest in Temuco and identified as a new anamorph of *Bjerkandera* sp. (Taboada-Puig et al., 2011). *Ganoderma lucidum* was isolated from mushroom spent substrate, kindly provided by Hifas da Terra S.L. (Pontevedra, Spain). All fungal strains were maintained on MEA agar at 4 °C.

Mycelia were produced in static cultures for fungal inocula preparation. Five plugs of fungal mycelium from fresh agar plates were inoculated with 200 mL of glucose-peptone medium (Kimura et al., 1990) in Fernsbach flasks and incubated at 30 °C. After 7 days, fungal cultures were homogenized in a sterilized blender for 30 s and used as inoculum.

2.3. Culture conditions

Submerged fermentations were performed in 250 mL Erlenmeyer flasks containing 90 mL of culture medium and 10 mL of inoculum. The fungal cultures were incubated on a rotary shaker at 150 rpm, initial pH 4.5 and 27 °C. All culture media were prepared with distilled water and contained 1 g L^{-1} peptone (as nitrogen source), 0.5 mM $MnSO_4$ and 0.15 mM $CuSO_4$ (as inducers for enzyme production). Six different combinations of cellulose fiber (C_6), organosolv lignin (L), concentrated hemicellulose fraction (C_5), and/or xylose (X) were evaluated as carbon source. The concentration of these components in each culture medium is shown in Table 2. Cellulose and lignin concentration was calculated considering the humidity and concentration of each of the components in both streams (Table 1). Hemicellulose concentration was expressed in equivalents of TRS, considering that the concentrated hemicellulose fraction had a TRS concentration of 225 ± 5.6 g L^{-1}.

2.4. Analytical methods

An adapted protocol from NREL (National Renewable Energy Laboratory, Golden, USA) was used for the determination of the biomass composition in glucan, xylan and lignin (Sluiter et al., 2007). Acid hydrolysis allows lignin separation from the sugar fraction, and the amount of lignin recovered is determined by weight. The concentrations of xylan and glucan were calculated from the concentration of the corresponding monomeric sugars using a correction of 0.88 and 0.90

Table 1
Physico-chemical characteristics of the cellulose fibers, organosolv lignin and concentrated hemicellulose fractions obtained from the organosolv process. Mean value ± standard error were calculated using triplicate sets.

Parameters	Cellulose fibers (C_6)	Organosolv lignin (L)	Parameters	Hemicellulose (C_5)
pH	3.4	2.6	pH	5.5
Humidity (%, w/w)	69.8 ± 0.4	12.7 ± 0.6	TN (mg L^{-1})	748 ± 55
Ashes (%, w/w)	0.16 ± 0.02	0.14 ± 0.02	Glucose (g L^{-1})	10.8
Glucan (%, w/w dry basis)	73.5 ± 7.4	0	Xylose (g L^{-1})	144.3
Xylan (%, w/w dry basis)	11.3 ± 3.9	2.4 ± 0.09	Rhamnose (g L^{-1})	44.1
			Xylose oligomer (g L^{-1})	75.7
Acid soluble lignin (%, w/w dry basis)	2.1 ± 0.2	2.0 ± 0.1	Acetic acid (g L^{-1})	17.1
			Total phenols (g L^{-1})	40.8 ± 0.8
Total lignin (%, w/w dry basis)	10.4 ± 1.0	92.7 ± 0.9	Na^+ (g L^{-1})	20.7
			SO_4^{2-} (g L^{-1})	18.8

TN: total nitrogen.

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