Bioresource Technology 131 (2013) 218–225

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Sugar recoveries from wheat straw following treatments with the fungus *Irpex lacteus*

Davinia Salvachúa, Alicia Prieto, María Eugenia Vaquero, Ángel T. Martínez, María Jesús Martínez *

Centro de Investigaciones Biológicas, CSIC, Ramiro de Maeztu 9, E-28040 Madrid, Spain

HIGHLIGHTS

- Wheat thin stillage was a suitable medium for *Irpex lacteus* pre-inoculums production.
- Mn(II) supplementation of wheat straw pretreated with the fungus gave significant improvements in glucose yield.
- The maximum glucose yield reached 68% at 21 days of incubation with Mn(II) addition.
- Xylose digestibility reached 100% under some pretreatment conditions.
- Fungal biomass on wheat straw was a positive indicator of glucose yields.

ARTICLE INFO

Article history:
Received 11 July 2012
Received in revised form 13 November 2012
Accepted 19 November 2012
Available online 20 December 2012

Keywords:
Lignocellulose
Wheat thin stillage
Optimization
Ergosterol
Bioethanol

ABSTRACT

Irpex lacteus is a white-rot fungus capable of increasing sugar recovery from wheat straw; however, in order to incorporate biopretreatment in bioethanol production, some process specifications need to be optimized. With this objective, *I. lacteus* was grown on different liquid culture media for use as inoculums. Additionally, the effect of wheat straw particle size, moisture content, organic and inorganic supplementations, and mild alkali washing during solid-state fermentation (SSF) on sugar yield were investigated. Wheat thin stillage was the best medium for producing inoculums. Supplementation of wheat straw with 0.3 mM Mn(II) during SSF resulted in glucose yields of 68% as compared to yields of 62% and 33% for cultures grown without supplementation or on untreated raw material, respectively after 21 days. Lignin loss, wheat straw digestibility, peroxidase activity, and fungal biomass were also correlated with sugar yields in the search for biopretreatment efficiency indicators.

1. Introduction

Wheat straw, the most abundant agricultural residue in Europe and the second worldwide, presents great potential for ethanol production (Talebnia et al., 2010); however, due to the complexity of its structure, especially the lignin framework, it is a challenge to obtain high sugar release from this substrate.

Steam explosion, one of the most used physico-chemical pretreatment methods to disrupt the lignocellulosic biomass, produces undesirable compounds such as weak acids, furan derivatives, and phenolic and inorganic substances which negatively affect the fermentation step (Hahn-Hägerdal et al., 2006). Biological pretreatments could be an alternative, since some organisms, like white-rot fungi, are able to degrade lignocellulose selectively and produce fewer yeast inhibitors than steam explosion (Salvachúa et al., 2011). One disadvantage of these treatments is the long incubation time necessary to reach yields similar to those obtained with current physico-chemical pretreatments. For this reason, combinations of biopretreatment with mild physical (Yamagishi et al., 2011), alkali (Salvachúa et al., 2011; Saritha et al., 2012; Zhong et al., 2011), organosolv (Canam et al., 2011), or hot water (Wang et al., 2012) treatments have been investigated.

The basidiomycete *Irpex lacteus*, has emerged as a fungus with great biodegradation potential (Novotny et al., 2009). The fungus has an exceptional ability to degrade corn stover (Xu et al., 2010), corn stalks (Du et al., 2011; Zhong et al., 2011), and wheat straw (Pinto et al., 2012; Salvachúa et al., 2011) under SSF conditions and thus to considerably increase sugar yields from these feedstock.

The present study focused on optimizing the production of *I. lacteus* inoculums for use in SSF of wheat straw by studying fungal biomass production. In addition, SSF cultural and nutritional parameters, such as nitrogen and mineral salt supplementation,

Abbreviation: Lip, lignin peroxidase; MnP, manganese peroxidase; SSF, solid-state fermentation.

* Corresponding author. Address: Centro de Investigaciones Biológicas, Department of Environmental Biology, CSIC, Ramiro de Maeztu 9, E-28040 Madrid, Spain. Tel.: +34 918373112; fax: +34 915360432.

E-mail address: mjmartinez@cib.csic.es (M.J. Martínez).

0960-8524/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2012.11.089

wheat straw particle size and moisture were analyzed as well as ergosterol and enzymes secretion during SSF. The efficiency of fungal treatment complemented with mild-alkali washing was determined by sugar yield estimations at 7, 14, and 21 days.

2. Methods

2.1. Microorganism

The white-rot fungus *I. lacteus* (IJFM A792), deposited in the Fungal Culture Collection of the Centro de Investigaciones Biológicas (Madrid, Spain), was maintained on 2% malt extract agar (w/v) at 4 °C and cultured on plates containing the same medium at 28 °C for one week before being used.

2.2. Pre-inoculum production

2.2.1. Culture media screening

Four 1-cm^{-2} agar plugs were cut from actively growing mycelium on agar plates and used to inoculate 250-mL Erlenmeyer flasks with 30 mL of growth medium (CSS) (Salvachúa et al., 2011) that were incubated at 28 °C and 180 rpm for 7 days. These cultures were aseptically homogenized (Omnimixer, Sorvall), and 2.5 mL were used to inoculate 30 mL of different liquid culture media in 250-mL flasks. The screened media were: (i) CSS medium, (ii) K medium pH 5.5 (glucose, 20 g L^{-1}; $MgSO_4 \times 7H_2O$, 0.5 g L^{-1}; KH_2PO_4, 1 g L^{-1}; yeast extract, 2 g L^{-1}; peptone, 5 g L^{-1}), (iii) wheat mush pH 5.5 diluted until the glucose concentration was 40 g L^{-1}, (iv) wheat thin stillage pH 4 (glucose, 4 g L^{-1}), (v) wheat thin stillage pH 4 supplemented with glucose to a final concentration of 40 g L^{-1}, (vi) wheat thin stillage pH 4 supplemented with nitrogen (0.3 g L^{-1}) from ammonium tartrate, and (vii) wheat thin stillage pH 4 with both glucose and nitrogen at the concentrations listed under (v) and (vi). Wheat mush and wheat thin stillage were obtained from first-generation bioethanol production at Bioetanol Galicia S.A. (Abengoa Bioenergy, Spain).

Cultures were collected at 3, 5, 7, and 10 days of incubation and vacuum-filtered through filter paper to separate the solids to measure biomass, and to determine total reducing sugars in the filtrate. The pH influence on biomass values was studied in wheat thin stillage medium, adjusted to pH 4, 4.5, 5, 5.5, and 6 with NaOH. These cultures were analyzed at 24, 48, and 72 h. All cultures were grown in triplicate and incubated at 28 °C and180 rpm.

2.2.2. Inoculums for solid-state fermentation (SSF) experiments

Pre-inoculums were grown in wheat thin stillage medium pH 5 as described in Section 2.2.1 and 2.5 mL of an aseptically homogenized culture was used to inoculate the stillage. The cultures were incubated at 28 °C and 180 rpm, and 2 mL of 1-day-old mycelium was used as inoculums for SSF experiments.

2.3. Wheat straw pretreatment

Wheat straw was harvested from Galician fields (Spain), dried, and chopped (<1 cm). *I. lacteus* basal cultures (ILC) with 2 g of wheat straw and 6 mL of water were prepared and cultured under SSF conditions as previously described (Salvachúa et al., 2011). These basal conditions were modified in other *I. lacteus* cultures to test the effect of: (i) wheat straw particle size by using milled straw (MWS, <0.5 mm), (ii) maintaining moisture content at 75%, either by replacing lost water daily or by increasing the initial moisture content to 86%, and (iii) the addition of 0.3 mM $MnSO_4$, $CuSO_4$ and $FeSO_4$, peptone (20 g L^{-1}), and wheat thin stillage (diluted so as to reach 2 g L^{-1} of glucose). To maintain the original moisture content, the lost weight in control cultures was attributed to water evaporation and this amount was added to all treatments. Additives (salts and others) were dissolved in distilled water (6 mL) before autoclaving. Untreated wheat straw of both particle sizes was incubated under the same conditions as the treatments and used as controls. Assays were performed in triplicate.

Biopretreated and untreated wheat straw collected after 7, 14, and 21 days of incubation were washed with distilled water (15 mL) for 1 h, at 28 °C and 180 rpm, and vacuum-filtered to extract water-soluble compounds. Solid fractions were dried in an aeration oven at 65 °C and weighed. After calculating weight loss and analyzing the main remaining components in the wheat straw (see Section 2.6.), solid fractions were subjected to mild alkali treatments with 0.1% sodium hydroxide (5% w/v), at 50 °C and 165 rpm for 1 h. This alkali mixture was then filtered, washed until neutral with distilled water at 50 °C, dried at 65 °C, and total reducing sugars were analyzed in the alkali filtrates.

2.4. Enzymatic hydrolysis, digestibility and sugar yield estimations

Pretreated fractions were hydrolyzed in duplicate at 5% (w/v) by commercial enzyme cocktails (from Novozymes, Denmark) as 15 FPU g^{-1} of cellulases (Celluclast and NS50010) and 30 U g^{-1} of xylanases (NS50013 and NS50030) in 100 mM sodium citrate buffer (pH 4.8) at 50 °C, and 165 rpm for 60 h and analyzed for fermentable sugar release (Salvachúa et al., 2011). The digestibility of cellulose (D_c) and hemicellulose (D_h) was calculated according to Eq. (1), as the ratio between the percentage of glucose (G_r) or xylose (X_r) released from pretreated fractions and the estimated glucose (G_p) or xylose (X_p) in the fraction prior to enzymatic hydrolysis, respectively. Glucose (G_y) and xylose (X_y) yields were determined by taking into account glucose (G_i) and xylose (X_i) content per gram of dry wheat straw, glucose (G_f) and xylose (X_f) remaining after wheat straw pretreatment, and the digestibility of cellulose (D_c) and hemicellulose (D_h), respectively, as shown in Eq. (2).

$$D_c \text{ or } D_h\ (\%) = (g\ G_r \text{ or } X_{r/g}\ G_p \text{ or } X_p) \times 100 \quad (1)$$

$$G_y \text{ or } X_y\ (\%) = (G_f \text{ or } X_f \times D_c \text{ or } D_h)/G_i \text{ or } X_i \quad (2)$$

2.5. Substrate characterization

Wheat straw weight loss for each fungal pretreatment was calculated as the percentage of total solids lost after water washing. Total hydrolysis of wheat straw was performed to determine Klason lignin (Tappi, 1974) and sugar composition.

2.6. Sugar and protein determination

Sugars from wheat mush and wheat thin stillage were analyzed by gas chromatography (GC) as previously reported (Prieto et al., 2008). Total reducing sugars were estimated by the Somogyi–Nelson method (Somogyi, 1945), using glucose as standard. Glucose, xylose, cellulose, and hemicelluloses estimations were calculated as described elsewhere (Salvachúa et al., 2011). Protein concentrations from wheat mush and wheat thin stillage were determined using the Bradford reagent (Bio-Rad), with bovine serum albumin as standard.

2.7. Enzyme assays

Enzymes were evaluated in the water-soluble fractions from SSF experiments and expressed in international enzyme units (micro moles per minute) per gram of wheat straw. Lignin peroxidase (LiP), laccase, and Mn(II)-oxidizing peroxidase (MnP) were