Bioresource Technology 135 (2013) 246–253

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

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

Coupled production of single cell oil as biodiesel feedstock, xylitol and xylanase from sugarcane bagasse in a biorefinery concept using fungi from the tropical mangrove wetlands

Srijay Kamat [a,b], Mahesh Khot [b], Smita Zinjarde [b], Ameeta RaviKumar [b], Wasudeo Namdeo Gade [a,*]

[a] Department of Biotechnology, University of Pune, Pune 411 007, India
[b] Institute of Bioinformatics and Biotechnology, University of Pune, Pune 411 007, India

ARTICLE INFO

Article history:
Available online 23 November 2012

Keywords:
Biorefinery
Mangrove fungi
Sugarcane bagasse
Xylitol
Biodiesel

ABSTRACT

This work evaluates sugarcane bagasse (SCB) conversion, in a biorefinery approach, to coproduce biodiesel and high value products using two novel mangrove fungi. On acid pre-treatment, sugarcane bagasse hydrolysate (SCBH) resulted in a xylitol yield of 0.51 g/g xylose consumed in 72 h by *Williopsis saturnus*. After SCB pretreatment, sugarcane bagasse residue (SCBR) was utilized using *Aspergillus terreus* for production of xylanase (12.74 U/ml) and cell biomass (9.8 g/L) which was extracted for single cell oil (SCO; 0.19 g/g) and transesterified to biodiesel. The FAME profile exhibited long chain SFAs and PUFAs with predicted biodiesel properties lying within the range specified by international standards. This biorefining approach of SCB utilization for co-production of xylitol, xylanase and SCO gains importance in terms of sustainability and eco-friendliness.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

In the last two decades, interest in biotechnology has increasingly focused on obtaining products of commercial significance from low value residual agro-industrial biomass (Pandey et al., 2000). The effective use of lignocellulosic biomass for production of fuels, power and high value chemicals can only result from the development and implementation of a biorefinery concept. Current strategies for biomass conversion to products are based on individual production chains. However, different bio-based industries can either combine or couple their material flows so that the residue from one bio-industry becomes an input for another industry. This will result in optimal utilization of all biomass components for generation of multiple products in an integrated biorefinery system with economic and environmental perspectives (Cherubini, 2010).

Sugarcane bagasse (SCB), a major agro-residue in tropical countries has been extensively used as a cheap substrate for steam and heat generation in sugar and alcohol industries. The remaining stockpiled bagasse is of low economic value and constitutes an environmental hazard to sugar mills and surrounding areas (Pandey et al., 2000). From a biorefinery perspective, it would be economically and environmentally beneficial to utilize this SCB for production of biofuels, commodity chemicals and industrial enzymes.

Single cell oils (SCOs) accumulated by oleaginous fungi have recently emerged as a potential alternative feedstock for production of biodiesel. Current research efforts are directed towards developing SCO production processes using renewable carbon sources. A variety of agro-industrial residues such as rice hulls (Economou et al., 2011b), wheat straw (Zheng et al., 2012), corn fiber (Xing et al., 2012), sugarcane bagasse (Zhao et al., 2012) are being exploited as substrates for fungal SCO production under submerged fermentation conditions. However, solid and semi-solid state fungal fermentation processes are also being developed e.g. sweet sorghum (Economou et al., 2011a). Other unconventional carbon sources such as glycerol, waste oils and whey have also been shown as suitable substrates for microbial SCO production (Bellou et al., 2012; Katre et al., 2012).

Xylitol has been identified as one of the 12 best chemical building blocks derived from agro-residual biomass (Werpy and Petersen, 2004). Besides this, its proven applications in food and pharma industries make it an attractive candidate for production using a lignocellulosic biorefinery system. It is currently produced by chemical reduction of xylose obtained from wood hydrolysates under alkaline conditions. The drawbacks of commercial chemical preparation are a low initial availability of sugar, non-eco-friendly purification and separation steps. Hemicellulosic fraction of waste materials such as SCB, provide an important source of xylose which can be converted to xylitol by microbial fermentation (Chandel

* Corresponding author. Tel.: +91 20 25694952; fax: +91 20 25692248.
E-mail address: wngade@unipune.ac.in (W.N. Gade).

0960-8524/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2012.11.059

et al., 2011). In this regard, selection of naturally occurring yeasts capable of producing xylitol from lignocellulosic waste is emerging as a promising approach.

Xylanase plays an important role in the bioconversion of agro-residue matter for potential applications such as biobleaching of kraft pulp, production of food and feed, in textile manufacturing and in industrial effluent waste water treatment (Mtui, 2012; Raghukumar et al., 2004). Filamentous fungi are considered suitable sources of xylanases because of their ability to utilize a wide range of inexpensive agro-residues and can be induced to release high enzyme titers on them.

Although SCB represents a suitable fermentation substrate for lipid accumulation due to its high C:N ratio and low ash content, it remains unexploited for production of fungal SCOs as biodiesel feedstock. Also, coproduction of xylitol and xylanase will impart a cost effective and efficient means with minimal SCB waste generation while simultaneously utilizing a part of the produced enzyme for internal processes such as SCB saccharification. Considering the importance of strain selection, two fungal species from mangrove wetlands of Indian west coast, isolated in our laboratory were utilized. A xylose assimilating yeast strain, *Williopsis saturnus*, was used for xylitol production while production of SCO as biodiesel feedstock and extracellular xylanase was demonstrated using *Aspergillus terreus*, earlier shown to accumulate SCO up to 0.54 g/g dry biomass (Khot et al., 2012). Thus, in order to allow for an efficient and sustainable use of biomass and to reduce competition among different uses of a single biomass source, this study focuses on a biorefinery alternative that utilizes SCB hydrolysate for coproduction of xylitol (platform chemical) and its undigested residue for SCO (biodiesel feedstock) and xylanase (enzyme).

2. Methods

2.1. Process description

In brief, the process scheme of the SCB biorefinery concept adopted in this paper is as follows. SCB was subjected to a dilute sulfuric acid hydrolysis treatment to release the monomeric sugars (glucose and xylose) in the liquid fraction i.e. sugarcane bagasse hydrolysate (SCBH) which was used for the production of xylitol while the remaining solid fraction i.e. hydrolysed sugarcane bagasse residue (SCBR) was utilized in the production of SCO as a feedstock for biodiesel as well as a source for production of xylanase.

2.2. Raw material for biorefining and its pretreatment

SCB obtained locally was used as the biorefining material in this study. It was first washed thoroughly with water and heat-dried in an oven at 50 °C for 48 h. The dried material was milled, passed through a 1 mm sieve, packed into sterile plastic bags and stored at 4 °C till further use.

The chemical pretreatment of SCB was carried out by dilute sulfuric acid hydrolysis followed by over-liming with $Ca(OH)_2$ and adsorption using activated charcoal to obtain the detoxified liquid hydrolysate (Alves et al., 1998). The left over residual solid fraction (SCBR) was washed extensively with distilled water to remove water soluble solids until neutral pH was obtained and dried at 80 °C in an oven till constant weight.

2.3. Fungal strains

The present study was carried out using fungal strains previously isolated in our laboratory from mangrove wetlands of the Indian west coast that included yeast strain, *W. saturnus* (GenBank Accession No.: JX242997) and an oleaginous mold, *A. terreus* (GenBank Accession No.: JN639854). Both the fungal isolates were maintained on Czapek Dox agar slants at 4 °C.

2.4. Xylitol production on SCBH

The inoculum of *W. saturnus* was developed in shake flasks (150 mL) containing 30 mL MXYP medium (g/L; xylose 20.0, yeast extract 3.0, malt extract 3.0, peptone 5.0, NaCl 15.0; pH 6.8) and incubated at 28 °C on a rotary shaker (180 rpm) for 24 h. The yeast cells were harvested by centrifugation (10,000*g*, 10 min, at 4 °C),washed twice with sterile distilled water and the seed inoculum added to 100 mL of fermentation medium (1×10^8 cells/mL) containing SCBH and salt solution in a ratio of 1:1. The final composition of the medium was in g/L: 16.55 of sugar (glucose plus xylose), NaCl 15.0, KNO_3 2.0, K_2HPO_4 1.0, $MgSO_4{\cdot}7H_2O$ 0.5 and $FeSO_4{\cdot}7H_2O$ 0.01; pH 6.5. Flasks were kept for incubation on a rotary shaker at 28 °C and 180 rpm, for 144 h. Samples were withdrawn periodically to determine cell biomass, xylose and xylitol concentrations.

2.5. SCO and extracellular xylanase production on SCBR

The production of lipids and xylanase by oleaginous fungal isolate *A. terreus* was studied under submerged fermentation conditions using SCBR (1%, w/v). The cultivation medium was supplemented with yeast extract (1.5 g/L) and minerals consisting of (in g/L) NaCl 15.0, KH_2PO_4 7.0, $Na_2HPO_4{\cdot}2H_2O$ 5.0, NH_4Cl 0.5, $MgSO_4{\cdot}7H_2O$ 1.5, $CaCl_2{\cdot}2H_2O$ 0.1, $FeCl_3{\cdot}6H_2O$ 0.08, $ZnSO_4{\cdot}7H_2O$ 0.01 while $MnSO_4{\cdot}5H_2O$, $CuSO_4{\cdot}5H_2O$ and $Co(NO_3)_2{\cdot}6H_2O$ each with 0.1 mg/L conc. The spore inoculum of *A. terreus* was prepared as described previously (Khot et al., 2012). Shake flasks containing 100 mL medium were inoculated with 1 mL of spore suspension ($1–3 \times 10^8$ spores) and incubated on a rotary shaker (120 rpm) at 30 °C for 120 h. At regular time intervals, a flask was sacrificed, the mycelial mass harvested for biomass estimation and lipid analyses while the culture broth was used for extracellular enzyme (xylanase and cellulase) assays.

2.6. Analytical methods

2.6.1. Chemical composition of bagasse

The partial chemical composition of untreated SCB sample and of sample residue after acid hydrolysis (SCBR) was analyzed to determine the cellulose, hemicellulose and ash contents according to Updegraff (1969), Deschatelets and Yu (1986) and Sluiter et al. (2005), respectively.

2.6.2. Scanning electron microscopy

Bagasse morphology was analyzed by scanning electron microscopy at three different stages, namely – before acid hydrolysis (raw SCB), after acid pretreatment on residue (SCBR) and after fungal growth. Samples from surfaces or transverse sections were dried and coated with gold in a sputter coater. Sample imaging was carried out using scanning electron microscope model – Stereoscan 440 (LEO/Leica, Cambridge, UK).

2.6.3. Biomass estimation

Fungal biomass was harvested by centrifugation and/or vacuum filtration, lyophilized and dried until constant weight and expressed as cell dry weight (CDW).

2.6.4. SCO extraction and preparation of fatty acid methyl esters (FAMEs)

The extraction of total lipids from fungal biomass and the preparation of FAMEs were carried out as reported earlier (Khot et al.,

Download English Version:

https://daneshyari.com/en/article/7083265

Download Persian Version:

https://daneshyari.com/article/7083265

Daneshyari.com