Available online at www.sciencedirect.com

ScienceDirect

http://www.elsevier.com/locate/biombioe

Single-step fermentation of agricultural hemp residues for hydrogen and ethanol production

Valery Agbor [a], Francesco Zurzolo [a], Warren Blunt [a], Christopher Dartiailh [a], Nazim Cicek [a], Richard Sparling [b], David B. Levin [a,*]

[a] Department of Biosystems Engineering, University of Manitoba, E2-376 EITC, Winnipeg, Manitoba, Canada R3T 5V6

[b] Department of Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2

ARTICLE INFO

Article history:
Received 28 October 2011
Received in revised form
26 February 2014
Accepted 14 March 2014
Available online 13 April 2014

Keywords:
Fermentation
Biomass
Cellulose
Consolidated bioprocessing
Ethanol
Hydrogen

ABSTRACT

We have investigated a one-step fermentation process using purified hemp cellulose (PHC), hemp fibres (HF), and hemp hurds (HH), compared to reagent-grade α-cellullose (AC) for ethanol and hydrogen production by *Clostridium thermocellum*. Exponential phase growth and production rates on PHC were comparable to those observed with AC. Net production of ethanol for AC (8.47 mM) was only slightly higher than PHC (6.56 mM), but significantly higher than HF (5.48 mM) and HH (3.52 mM), while the final hydrogen yield was comparable for AC (12.70 mM), PHC (11.01 mM), HF (10.91 mM), and HH (4.72 mM). End-product yields were dependent on the intrinsic cellulosic content as well as the presence of other polymers present in substrate biomass. Rates of ethanol and hydrogen production were similar in early log phase but varied in mid-log, and accessibility to cellulose was shown to determine yield and metabolic flux distribution. Our data suggest that improving the accessibility of the cellulolytic bacterium, *C. thermocellum*, to cellulose fibres has a greater effect on increasing the yield of fermentation products than simply increasing the concentration of cellulose in the substrate.

1. Introduction

Global energy demand, climate change, and environmental pollution caused by fossil fuel combustion have heightened the need for renewable energy sources for both developed and developing nations. In the last decade, approximately 83% of the growing global energy demand (estimated at 467 EJ) was derived from fossil fuels. Biomass energy is by far the most renewable alternative energy source with potential to be optimised for both fuels and co-products [1]. Liquid and gaseous fuels derived from lignocellulosic feedstocks via fermentation will help to curtail our dependence on fossil fuels for transportation and other purposes such as heat and power [2].

Lignocellulosic biomass is the most abundant organic material in nature with 10–50 billion tonnes annual worldwide production and in excess of 180 million tonnes of available cellulosic feedstock per year from agricultural resources [3,4]. Although a variety of fuels currently used can be produced from grains, current renewable fuel standards mandate a 96.4% increase in efficient and 'advanced biofuels', the

* *Corresponding author*. Tel.: +1 204 474 7429; fax: +1 204 474 7512.
E-mail addresses: bisong01@gmail.com (V. Agbor), francesco.zurzolo@gmail.com (F. Zurzolo), wblunt@gmail.com (W. Blunt), umdartia@cc.umanitoba.ca (C. Dartiailh), nazim_cicek@umanitoba.ca (N. Cicek), sparlng@cc.umanitoba.ca (R. Sparling), levindb@cc.umanitoba.ca, levindb@ad.umanitoba.ca, david.levin@umanitoba.ca (D.B. Levin).
http://dx.doi.org/10.1016/j.biombioe.2014.03.027
0961-9534/

majority of which can be derived from cellulosic biomass [5]. Unlike the corn ethanol industry, which faces many industrial challenges including the "food versus fuel debate" [6–8], cellulosic ethanol from biomass has a wide range and variety of feedstocks derived from forestry, agricultural, industrial and municipal sources. Examples of lignocellulosic materials being used as feedstocks for bioethanol production include different types of soft and hardwood, agricultural residues from food crops, such as corn stover and cereal straws, and dedicated lignocellulosic energy crops, i.e. switch grass, miscanthus, and short rotation trees, such as poplar and willow [9]. In this study, we have investigated the potential of using residues of industrial hemp, *Canabis sativa*, as substrate for bioethanol and biohydrogen production.

As an industrial plant, hemp fibre has many applications in the textile industry. Hemp seeds are used for the production of neutraceuticals and food products, while the hurds and fibre have some paper and composite applications [10,11]. Crops that have been cultivated on a large scale like hemp offer great potential as feedstocks for biorefineries. Studies in Sweden have investigated the possibility of using hemp biomass for bioethanol and biogas production [12,13]. In the United States during 1920s, 25% of Standard oils sales in the Midwest were from the ethanol derived from hemp biomass [14]. As an energy crop, hemp biomass has the following advantages: i) Hemp can be grown on marginal land compared to other energy crops grown on agricultural land; ii) it requires little fertiliser, thus minimizing the use of nitrogen-based fertilizers; an essential factor for its Life cycle analysis or LCA as a feedstock; iii) *Canabis sativa* removes smaller amounts of nutrients from the soil compared with other crops, iv) hemp plants are resistant to pest and disease, v) it grows faster than other energy crops, and vi) produces higher energy yield per acre per year than corn, sugarcane, and flax [15–17].

Industrial hemp cultivation in Canada is viewed as a new alternative crop, which compliments prairie crop production rotations by breaking traditional crop cycles and increasing profits [11]. Thus, there is a growing interest for high volume local cultivation of the industrial hemp as a multifunctional industrial crop with a huge supply of residual biomass after cultivation and processing. The residues from hemp cultivation and decortication constitute a mixture of fragments of hemp fibre and hurds or hemp core. We investigated the bioprocessing of this rich source of cellulosic biomass in a one-step fermentation process otherwise known as consolidated bioprocessing (CBP) [18–24].

CBP combines cellulase production and substrate hydrolysis and fermentation of the hydrolysate (both hexose and pentose sugars) in one step, thus saving the cost of investing in a multi-step process [20,23–26]. Of all the reported technological advances to reduce processing cost, a single-step process offers the greatest potential of reducing bioethanol cost by 41% in the processing of biomass to ethanol [27]. A techno-economic evaluation of bioethanol from softwood (spruce), hardwood (salix) and an agricultural residue (corn stover) concluded that the process configuration had greater extent on the outcome compared to the choice of substrate [28].

We have investigated a single-step fermentation of purified hemp cellulose (PHC), hemp fibre (HF), and the hurds or core fraction of the residue (HH), against reagent grade α-cellulose (AC) for bioethanol and biohydrogen production using *C. thermocellum* in batch mode. Previous studies have shown that this gram-positive, thermophilic bacterium has great potential for effective bioconversion of low-value cellulosic feedstocks because of its ability to grow well on amorphous and crystalline cellulose [19,20,29–32]. In this study, the growth and end-product profile from using residues of industrial hemp cultivation and processing have been reported to elucidate the quality of hemp biomass as potential substrate for cellulosic biorefineries.

2. Materials and methods

2.1. Substrates

The hemp (*Cannabis sativa*) used in this study was obtained from the Emerson Hemp Distribution Company, Emerson, Manitoba, Canada. Residue from this industrial hemp is made up of the hemp fibres and hemp hurds. Some of the raw hemp residue was subjected to a thermochemical pretreatment (Organosolv®) process to solubilise hemicellulose and extract lignin. The biomass was pretreated with 50% ethanol, for 55 min, at 195 °C, using 1.5% acid as catalyst, at a 10:1 Liquid: Solid hemp biomass ratio [33,34]. We refer to this water insoluble substrate derived from the Lignols' Organosolv® pretreatment as purified hemp cellulose (PHC). We also separated the fibres in the raw hemp from the hurds to represent two substrate streams from a decortication facility rich in hemp fibres (HF) and hemp hurds (HH) as shown on Table 1. All substrates were added to fermentation reactions at 2 g/L (20 mg). However, the amount of "available cellulose" in each substrate was different, because the amount of cellulose in each substrate was different.

All test substrates (PHC, HF and HH in Table 1) were air dried and milled using a Retsch Rotor Beater mill SR200 equipped with a 34 mesh and 0.5 mm aperture sieve. All the milled samples were transferred into Ziplock bags and stored at room temperature. Alpha (α-) cellulose (C8002) obtained from Sigma Aldrich (Saint Louis, USA) was used as positive control. All test samples were over 93% TS. To test for substrate utilisation, a soluble fraction from raw hemp was obtained by autoclaving Balch tubes (27 mL; Bellco Glass Inc.,) containing 2 g/L (0.2% w/v) substrate concentrate of residual hemp biomass in 1191 and extracting the supernatant. The supernatant was used to test for growth of *C. thermocellum* on the soluble fraction derived from the substrate. The filtered supernatant was transferred into new tubes, sterilised by autoclaving and subjected to CBP with *C. thermocellum*. Negative controls were established using 1191 medium inoculated with *C. thermocellum* but without any substrate.

2.2. Microorganism and media

C. thermocellum strain 1237 was obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ; Braunschweig, Germany) and cultured in 1191 medium [35]. DSMZ 1237 is equivalent to *C. thermocellum* strain 27405 available at the America Type Culture Collection (ATCC). For each experiment, cells used for inoculation were