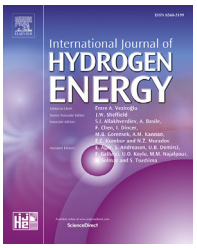
INTERNATIONAL JOURNAL OF HYDROGEN ENERGY XXX (2018) 1–14

Available online at www.sciencedirect.com

ScienceDirect

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



Screening and optimization of pretreatments in the preparation of sugarcane bagasse feedstock for biohydrogen production and process optimization

Ganesh Dattatray Saratale [a], *Rijuta Ganesh Saratale* [b], *Sang Hyoun Kim* [c], *Gopalakrishnan Kumar* [d,*]

[a] *Department of Food Science and Biotechnology, Dongguk University-Seoul, Ilsandong-gu, Goyang-si, Gyeonggi-do, 10326, Republic of Korea*

[b] *Research Institute of Biotechnology and Medical Converged Science, Dongguk University-Seoul, Ilsandong-gu, Goyang-si, Gyeonggi-do, 10326, Republic of Korea*

[c] *School of Civil and Environmental Engineering, Yonsei University, Seoul 03722, Republic of Korea*

[d] *Green Processing, Bioremediation and Alternative Energies Research Group, Faculty of Environment and Labour Safety, Ton Duc Thang University, Ho Chi Minh City, Viet Nam*

ARTICLE INFO

Article history:
Received 3 May 2017
Received in revised form
13 November 2017
Accepted 29 January 2018
Available online xxx

Keywords:
Sugarcane bagasse
NaC + NaS pretreatment
Enzymatic hydrolysis
Clostridium beijerinckii
Biohydrogen production

ABSTRACT

This work evaluated the effects of individual alkaline, sodium carbonate (Na_2CO_3 denoted as; NaC), sodium sulfide (Na_2SO_3 denoted as; NaS) and combination of NaC + NaS pretreatment for the saccharification of sugarcane bagasse (SCB). The effects of different pretreatments on chemical composition and structural complexity of SCB in relation with its saccharification were investigated. For enzymatic hydrolysis of pretreated SCB we have utilized the produced crude enzymes by *Streptomyces* sp. MDS to make the process more cost effective. A enzyme dose of 30 filter paperase (FPU) produced a maximum reducing sugar (RS) 592 mg/g with 80.2% hydrolysis yield from NaC + NaS pretreated SCB under optimized conditions. The resulted enzymatic hydrolysates of each pretreated SCB were applied for hydrogen production using *Clostridium beijerinckii* KCTC1785. NaC + NaS pretreated SCB hydrolysates exhibited maximum H_2 production relative to other pretreatment methods. Effects of temperature, initial pH of culture media and increasing NaC + NaS pretreated SCB enzymatic hydrolysates concentration (2.5–15 g/L) on bioH_2 production were investigated. Under the optimized conditions, the cumulative H_2 production, H_2 production rate, and H_2 yield were 1485 mL/L, 61.87 mL/L/h and 1.24 mmol H_2/mol of RS (0.733 mmol H_2/g of SCB), respectively. The efficient conversion of the SCB hydrolysate to H_2 without detoxification proves the viability of process for cost-effective hydrogen production.

Introduction

The primary energy consumption in the world is documenting rapid increase in last five decades due to the growing world population. Fossil fuel resources are widely used for the development of industrial sector to meet the growing energy demand of the population [1]. However continuous depletion of fossil fuels, their increasing price, environmental and

* *Corresponding author*. Green Processing, Bioremediation and Alternative Energies Research Group, Faculty of Environment and Labour Safety, Ton Duc Thang University, Ho Chi Minh City, Viet Nam.
E-mail address: gopalakrishnankumar@tdt.edu.vn (G. Kumar).

https://doi.org/10.1016/j.ijhydene.2018.01.187
0360-3199/

ecological point of views have stimulated the reawakened interest in developing sustainable and renewable energy sources to lessen the reliance on fossil fuels [2,3]. Hydrogen is viewed as a sustainable energy carrier with high-energy yield (122 kJ g^{-1}) [4,5]. To reduce the production cost, it is preferable to produce biohydrogen from lignocellulosic (LC) biomass, which are the most abundant, less expensive, and renewable resource available in nature that do not compete with food crops. Thus LC biomass signifies a huge potential in terms of renewable energy and has drawn tremendous amount of attention for the conversion of LC biomass to biofuels [1,6].

Sugarcane bagasse (SCB), the fibrous residue acquired after extracting the juice from sugar cane (*Saccharum officinarum*) which is about 280 kg per ton of sugarcane. Worldwide approximately 5.4×10^8 dry tons of sugarcane is processed annually thus generating SCB as a valuable byproduct [7]. Generally, the resulted SCB (~50%) is used in distillery plants for energy production by combustion which causes environmental pollution [8]. There is a great interest to develop biological process for fuel and chemicals production from SCB which can offer economic and environmental advantages [7,8]. However, SCB are usually not readily fermentable by microorganisms thus, pretreatment steps are often required for its conversion into biofuels. The pretreatment is required for removing the lignin, to increase cellulose availability by changing the chemical composition, surface area, and porosity by which it improves the enzymatic susceptibility of biomass [9,10]. An effective and economical pretreatment should use inexpensive chemicals, simple procedures and which must preserve the utility of the hemicelluloses and should evade the formation of fermentation inhibitors [11,12]. Various physical, chemical, physicochemical and biological technologies have been explored for biomass pretreatment taking into account of enhancing the yields of fermentable sugars and subsequently biohydrogen production using dark fermentation strategies [2,6,8,13,14].

The enzymatic hydrolysis represents a more inexpensive and ecofriendly perspective for the release of fermentable sugars from biomass using a multi-component enzyme system. There are many advantages of this process including, low energy requirement, no corrosion issues, less byproduct formation, better yield and process can run under mild environmental conditions [14,15]. Saccharification of LC biomass can be done by directly using pure commercial enzymes which normally result in faster hydrolysis rate and higher sugar yield [16]. However, due to the high cost arising from enzymes production and purification the application of commercial enzymes for hydrolysis limits the entire process. Thus extensive attention for cost-effective and high-efficiency hydrolytic (cellulose and hemicellulose degrading) enzymes production and their utilization for the saccharification of LC biomass is required. This approach will overcome the above limiting issues and could make the lignocellulosic biohydrogen production more cost effective and practically applicable.

In lignocellulosic biohydrogen production mainly two approaches are used. First one is two stage processes and the other known as simultaneous saccharification and fermentation. In case of two stage processes enzymatic hydrolysis and H_2 fermentation were conducted separately. The hydrogen yield was reported to be lower in simultaneous saccharification and fermentation as compared to two stage process under dark fermentation [17,18]. Some investigators utilized two stage process to enhance lignocellulosic biohydrogen production and thus the process becomes more economically feasible, less energy intensive and practically applicable [19–22]. In addition to this, microbial fermentation parameters including type of inoculum, type of substrate and operational and environmental factors (organic loading rate (OLR), initial pH, temperature, etc.) need to be optimized for better H_2 production [23].

This study was aimed to explore the possibility of sugarcane bagasse as a feed stock for biohydrogen production. This paper focuses about the different physical, chemical and physicochemical pretreatment to prepare SCB feedstock. The pretreatment conditions for NaC + NaS including chemical loading and their ratio, incubation time, and solid/liquid ratio were tested to govern the effectiveness of the pretreatment process. Moreover, the enzymatic saccharification of untreated and pretreated SCB was carried out through the produced crude enzymes by *Streptomyces* sp. MDS to make this process more cost effective and practically applicable. The optimization of saccharification parameters for maximum reducing sugar production were also evaluated. Then, the SCB hydrolysate was fermented by *Clostridium beijerinckii* KCTC 1785 to validate its potential as substrate for hydrogen production and also optimized the process parameters to enhance H_2 yield. This work is anticipated to provide useful information for evaluating the feasibility of SCB as efficient feedstock for cellulosic biohydrogen production.

Material and methods

Lignocellulosic substrates used

Wheat straw was used for biomass hydrolyzing enzymes production under solid state fermentation and which was collected from the local farmers. Sugarcane bagasse was used for the pretreatment studies and was collected from local sugar industry, South Korea. The raw substrates were collected, then air dried, milled and sieved through a 0.2 mm and 0.5 mm screen before storing at room temperature.

Hydrolytic enzymes production under solid state fermentation

Pure culture of isolated actinomycetes namely *Streptomyces* sp. MDS was used in this study and the detailed results of 16SrRNA sequencing and phylogentic analysis have been reported elsewhere [24]. Pure culture was maintained on Dubos salt medium (g/L): $NaNO_3$, 0.5; K_2HPO_4, 1.0; $MgSO_4 \cdot 7H_2O$, 0.5; KCl, 0.5; $FeSO_4 \cdot 7H_2O$, 0.01; agar powder, 20; CMC (10 g/L); with pH 6.5, stored at 4 °C and subcultured monthly. Solid state fermentation was carried out in 250 mL Erlenmeyer conical flasks containing 5 g of dry wheat straw moistened with Dubos salt medium by keeping the initial pH (5.0), at 30 °C and moisture content ratio (1:3) for cellulase and hemicellulase production. For inoculation, 1 mL of spore suspension (absorbance ~1.0 at 600 nm) from 7 days old culture of *Streptomyces* sp. was used and prepared in sterile saline and further mixed well. After an appropriate time interval, the fermented substrate were aseptically

Please cite this article in press as: Saratale GD, et al., Screening and optimization of pretreatments in the preparation of sugarcane bagasse feedstock for biohydrogen production and process optimization, International Journal of Hydrogen Energy (2018), https://doi.org/10.1016/j.ijhydene.2018.01.187