ARTICLE IN PRESS

INTERNATIONAL JOURNAL OF HYDROGEN ENERGY XXX (2018) 1–10

Available online at www.sciencedirect.com

ScienceDirect

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

Two-stage thermophilic bio-hydrogen and methane production from lime-pretreated oil palm trunk by simultaneous saccharification and fermentation

Saruda Sitthikitpanya [a], Alissara Reungsang [a,b,*], Poonsuk Prasertsan [c]

[a] Department of Biotechnology, Faculty of Technology, Khon Kaen University, Khon Kaen 40002, Thailand

[b] Research Group for Development of Microbial Hydrogen Production Process from Biomass, Khon Kaen University, Khon Kaen 40002, Thailand

[c] Department of Industrial Biotechnology, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai 90112, Thailand

ARTICLE INFO

Article history:
Received 14 November 2017
Received in revised form
4 January 2018
Accepted 14 January 2018
Available online xxx

Keywords:
Lignocellulose
Optimization
Anaerobic digestion
Biogas
Energy analysis
Thermophilic microorganisms

ABSTRACT

A simultaneous saccharification and fermentation (SSF) process was applied for thermophilic bio-hydrogen production from lime-pretreated oil palm trunk (OPT) by *Thermoanaerobacterium thermosaccharolyticum* KKU19. The SSF hydrogen fermentation conditions were optimized to maximize hydrogen yield (HY). Based on Plackett-Burman design, substrate loading and initial pH had significant effects on HY. The substrate loading and initial pH were further optimized using response surface methodology with a central composite design. The optimum conditions were a substrate loading, enzyme loading, inoculum concentration, initial pH and temperature of 4.6%, 10 filter paper unit (FPU)/g-OPT, 10% (v/v), 6.3 and 50 °C, respectively, which yielded the highest HY of 60.22 mL H_2/g-OPT. Structural analysis showed that lime pretreatment and SSF decreased the crystallinity of OPT. Methane production was carried out following the hydrogen production to improve the energy yield from OPT. The results showed that methane production increased total energy yield from 0.65 to 11.79 kJ/g-OPT under the optimal conditions.

Introduction

Biological hydrogen production via dark fermentation is an alternative technology to promote sustainable energy production and to solve environmental problems. This is possible due to its low operation cost, lack of carbon dioxide emissions, high hydrogen production, and a great potential to utilize renewable biomass as the feedstocks [1]. Lignocellulosic biomass obtained from agricultural or agro-industrial residues, such as sugarcane bagasse, rice straw, and corn stover, is considered cost-effective feedstocks for bio-hydrogen production because of their abundance, availability, low cost or no cost and non-competition with food crops [2,3]. Oil palm trunk (OPT) is a waste material of the palm oil industry. Oil palms are replanted every 20–25 years, leaving a large amount

* *Corresponding author*. Department of Biotechnology, Faculty of Technology, Khon Kaen University, Khon Kaen 40002, Thailand.
E-mail address: alissara@kku.ac.th (A. Reungsang).

https://doi.org/10.1016/j.ijhydene.2018.01.063
0360-3199/

of OPT [4]. Since the oil palm is systematically planted on a large scale, steady and stable supplies of OPT can be estimated. Therefore, the utilization of OPT for biofuel is an interesting approach to sustainable energy.

Lignocellulosic biomass is mainly composed of cellulose, hemicellulose, and lignin. Cellulose and hemicellulose are polysaccharides that have potential to be converted into biofuels [5]. Lignin is a complex aromatic polymer that is recalcitrant to biodegradation. Thus, pretreatment of lignocellulosic biomass is necessary to remove lignin and make cellulose more accessible. Lime pretreatment is considered as a promising pretreatment method due to its proven effectiveness, low cost, good carbohydrate preservation and easy recovery [6–8]. After pretreatment, cellulose is solubilized to glucose by enzymatic hydrolysis. The glucose is then used to produce hydrogen by microbial fermentation. These two steps are commonly applied to convert lignocellulosic biomass to biofuel [9–11]. Together, they are known as separate hydrolysis and fermentation (SHF). The advantage of the SHF process is that hydrolysis and fermentation can be carried out at their optimum conditions [12]. However, the major drawback of SHF is that hydrolysis step can be inhibited by an accumulation of sugars in the hydrolysate [13]. To overcome this challenge, simultaneous saccharification and fermentation (SSF) was developed. In the SSF process, the sugars produced during hydrolysis are immediately consumed by fermentative microorganisms to produce biofuel such as hydrogen [14–16] or ethanol [17–19]. As a result, the inhibitory effect of the end products of cellulase activity can be eliminated and the productivity increased [20,21]. Moreover, SSF shortens operation time and reduces capital costs due to the requirement of only one reactor [13,22]. Previous research reported higher production of hydrogen by SSF than SHF. Ibrahim et al. [23] reported a significantly higher cumulative hydrogen of 282.42 mL was achieved from pretreated oil palm empty fruit bunches by SSF compared to 222.02 mL hydrogen by SHF. Nasirian et al. [24] studied a bio-hydrogen production by fermentation of wheat straw, pretreated wheat straw, supernatants derived from acid hydrolyzation, SHF of pretreated wheat straw, and SSF of pretreated wheat straw. The results indicated that SSF had the shortest lag phase for gas production and proved to be the effective and economical way to convert wheat straw to bio-hydrogen. However, a major bottleneck of SSF is its different optimal operating conditions, i.e., temperature and pH, for the cellulolytic enzymes and fermenting microorganism [12,13]. The optimum initial pH and temperature values for enzymatic hydrolysis by cellulase are 4.8–5.0 and 50–55 °C, respectively [25], while the optimal initial pH and temperature values for hydrogen production are 4.5–6.5 and 30–35 °C, respectively [26]. Therefore, optimization of the operating conditions for enzymatic hydrolysis and hydrogen fermentation is necessary for the SSF process.

Another approach to decrease the difference in optimal temperatures is to operate the SSF hydrogen production under thermophilic conditions using thermophilic hydrogen producing bacteria. *Thermoanaerobacterium thermosaccharolyticum* is often used for thermophilic hydrogen production. *T. thermosaccharolyticum* strains such as PSU-2 [27], W16 [28] and KKU19 [29] efficiently use various kinds of sugars, e.g., glucose, xylose, arabinose, mannose, galactose, cellobiose and others that are present in the lignocellulosic hydrolysate. Additionally, thermophilic hydrogen production has a high hydrogen production rate (HPR) with less variety of the fermentation end products [30]. Thermophilic conditions also suppress other microorganisms in the fermentation broth [31]. Thus, thermophilic conditions were chosen for SSF hydrogen production as a compromise regarding the optimum temperatures for enzymatic hydrolysis and hydrogen fermentation.

In addition to bioenergy from hydrogen production, the energy gain from the biomass can be improved by methane production [32,33]. The hydrogen fermentation process has low substrate conversion efficiency [34]. After this process, over 65% of the energy contained in the substrate still remains in the effluent as volatile fatty acids (VFAs), i.e., acetic, butyric, lactic and propionic acids [35]. These VFAs are easily converted to methane by methanogens via the methanogenesis step in anaerobic digestion [36,37]. The coupling of a methanogenesis step with a hydrogen production process increased the energetic potential and energetic flux by 46 and 31 fold, respectively [38]. Massanet-Nicolau et al. [39] found that two-stage processes increased the energy yield by 13.4% compared to single stage methane production using grass as a substrate.

To the best of our knowledge, there is a very limited information on two-stage thermophilic bio-hydrogen and methane production from lime-pretreated OPT by SSF. Therefore, the goal of this research was to optimize the thermophilic SSF conditions for maximizing bio-hydrogen production from lime-pretreated OPT by *T. thermosaccharolyticum* KKU19. Methane production from the acidic effluent of hydrogen production was performed to increase the energy yield from the raw material.

Materials and methods

Lime pretreatment of OPT

OPT was collected from the demonstration farm of the Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand. OPT was chopped using a knife and passed through a 10 mesh sieve. The chopped OPT was further pretreated using lime under the optimal conditions determined in a previous study [32]. Briefly, the lime pretreatment was carried out in a 250 mL laboratory glass bottle containing 10% (w/v) of OPT, 0.2 g $Ca(OH)_2$/g-OPT and 100 mL of distilled water. The contents were heated to 121 °C for 60 min in an autoclave. After pretreatment, the solid fraction was separated by filtration through a muslin cloth. The solid fraction was washed with tap water to remove lime residues until the pH was approximately 8 and then air-dried at 60 °C in an oven for 24 h [9]. The solid recovery was calculated by dividing the dry weight of lime-pretreated OPT by the dry weight of untreated OPT and then multiply by 100. The compositions of untreated and lime-pretreated OPT are shown in Table 1.

Inoculum preparation

T. thermosaccharolyticum KKU19 (GenBank Accession No. JN020648), previously isolated in our lab [29], was used as a