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High rate hydrogen fermentation of cello-lignin fraction in de-oiled jatropha waste using hybrid immobilized cell system

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ABSTRACT

This study reports high rate hydrogen production from cello-lignin fraction of de-oiled jatropha waste (DJW) using hybrid immobilized cells in continuous operation. A continuous stirred tank reactor (1 L) was operated at the hydraulic retention time (HRT) range of 48–8 h to study the hydrogen production rate (HPR) and yield (HY). The cello-lignin fraction used as the substrate was the non-hydrolyzed residue of acid pretreated DJW. Experimental results showed a peak HPR of 3.65 L H_2/L d and HY of 150 mL H_2/g reducing sugars at the optimum HRT of 12 h and 37 °C. A significant drop in the hydrogen productivity was seen at 8 h HRT. Acetate and butyrate were the major soluble metabolic products at all HRTs, with predominance of butyrate at the optimum HRT. Major hydrogen producers belonged to *Eubacterial* group as evident from the molecular microbial community analysis. The energy production rate and CO_2 emission reduction analyses suggested cello-lignin fraction of DJW as a high energy yielding residue which can produce 2.2 kW electricity/y and an annual CO_2 emission reduction of 4.48 ton/y in a coal based power plant.

1. Introduction

Fossil fuels depletion, energy security and global warming have raised great concern for R&D to promote green and sustainable environment. For developing a green environment, the most important issue which needs attention is the reduction in the use of petroleum based fuels, and encourage biomass based fuels or biofuels. Biofuels such as bioethanol, biomethane, and biohydrogen from organic resources have been extensively studied [1]. Among these, biohydrogen is considered as the most clean and suitable energy carrier due to its high energy content and carbon neutral nature. Dark fermentation for hydrogen production from organic waste is seen as a viable technology to solve the energy related issues in the nearby future [2]. However, it is indicated that there are various technological constraints that need to be tackled to develop an economically feasible hydrogen production such as feedstock cost and availability, hydrogen generation rates, seed inoculum selection and reactor operation [1,3]. Of all these, feedstock selection and its further pretreatment are most crucial for the economical hydrogen production process.

Biohydrogen production in continuous operation has been mostly investigated with wastewaters and organic rich carbohydrates in pure form such as sucrose, glucose and xylose. There are very few reports of utilizing lignocellulosic biomass (LCB) for biohydrogen production in continuous operation, which is poised to be the most feasible and sustainable future technology [3,4]. Although, the direct conversion of LCB to biohydrogen has been demonstrated by various authors [5,6], the hydrogen yield and the production rates were low. The main reasons could be recalcitrant nature of the lignocellulosic feedstocks and lack of cellulolytic enzymes for breaking the complex lignocellulosic heteropolymer matrix [7,8]. Moreover, raw waste addition to the bioreactor in continuous operation also has several limitations, such as incomplete mixing, clogging in pipes and the disposal issues. Nevertheless, pretreatment of LCB remains the major constrain and limiting factor, and is therefore an essential step to improve the LCB-to-hydrogen production process efficiency [3,9].

Biohydrogen production from LCB is mostly done by separate hydrolysis and fermentation (SHP) process, wherein chemical (acid and alkali) or enzymatic mediated pretreatment has been used to obtain the hydrolyzate fraction [10]. Chemically (acid) derived hydrolyzate fraction from birch [11], wheat straw [12], oat straw

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[13] have been used as a substrate for the biohydrogen production. Recently, combined prehydrolysis by acid pretreatment and subsequent enzymatic saccharification is shown to be an efficient method for maximal sugar recovery [14] and further fermentation. In combined method, acid pretreatment increase the porosity and the crystallinity of the LCB making the enzyme saccharification process easier. Only few studies are reported for biohydrogen production from combined pretreated LCB hydrolyzate by mixed culture [15,19,20]. Pan et al. [15] hydrolyzed corn stalk with combined dilute acid (121 °C, autoclave)-enzyme hydrolysis and reported the HY of 209.8 mL/g TVS by anaerobic mixed culture. Arreola-Vargas et al. [16] reported HY of 2.3 mol/mol hexose and hydrogen production rate (HPR) of 0.62 L/L/d in a trickling bed bioreactor from enzyme-treated hydrolysate. In another study using combined pretreated hydrolyzate a maximum HPR of 5.5 L/L d and HY of 1.9 mol/mol sugar is reported [17]. These reports, in general show higher hydrogen production from hydrolyzate prepared by combined method which minimizes the inhibitory compounds formation due to low temperature acid hydrolysis. Besides, the hydrolyzate contains higher fermentable sugar (glucose) owing to enzyme action on the residual cellulosic biomass.

In the present study, de-oiled jatropha waste (DJW), a biodiesel industry residue and lignocellulosic in nature has been used as a feedstock for biohydrogen production. Previous studies on DJW have indicated it as a promising feedstock for methane and hydrogen production [18–20]. DJW predominantly contains three components represented as semi-crystalline cellulose, amorphous hemicellulose and lignin [14]. Direct utilization of DJW to hydrogen production is rate-limiting due to the complexity in the nature of DJW; thus proper hydrolysis method is required to recover the maximal sugars for efficient hydrogen production [14]. Being lignocellulosic in nature, DJW is also amenable to mild acid (HCl) hydrolysis for recovery of the sugars like other lignocellulosic wastes [21,22]. And in our previous study enzymatic saccharification of the acid pre-treated de-oiled jatropha waste produced a hydrolyzate having considerable sugar content [14]. The present study was conducted to study the continuous hydrogen production potential of the cello-lignin fraction in DJW. In most cases continuously-stirred tank reactor (CSTR) operation is considered for continuous hydrogen fermentation due to easy operation and low operation cost. However, the main issue with CSTR operation is the cell washout at very short HRTs. This issue has been overcome using the immobilization technique to achieve a stable high rate hydrogen production [23–25]. The main advantages of the immobilized cells CSTR are complete mixing, intimate contact between the substrate and microflora, and effective pH and temperature control. Thus, considering these merits, hydrogen fermentation of the cello-lignin fraction of DJW was evaluated in immobilized cells CSTR to develop a robust and cost-effective process, with an emphasis on the utilization of the cello-lignin fraction (non-hydrolyzed biomass) of lignocellulosic wastes.

2. Materials and methods

2.1. Inoculum, immobilized cells, and feedstock preparation

The seed sludge and DJW were obtained from a municipal wastewater treatment plant and a jatropha based biodiesel industry, respectively, located in Taichung, central Taiwan. The characteristics of sludge and DJW are mentioned in our previous study [26]. Heat treatment (95 °C for 45 min) was employed to inhibit methanogens and subsequently enrich the hydrogen producers which are spore forming bacteria [21,30].

The hybrid immobilized cells were prepared by adding 5% of the heat-treated inoculum to the prepared suspension of sodium-alginate (2%), following the additions of silicon dioxide (1%) for the mechanical strength, chitosan (1%) for the entrapment of the cells, and activated carbon (1%) for the porous nature of the beads, as described elsewhere [27]. The alginate-cell mixture was then extruded into sterile calcium chloride solution (2%) for cell entrapment and immobilized beads formation. The formed beads (5–6 mm) were further hardened by stirring the beads in a fresh solution of 2% calcium chloride for 2 h. Finally, the beads were washed three times with sterile distilled water and stored for further use.

DJW was initially pre-treated with 2% (v/v) HCl as a hydrolysing agent with the solid–liquid ratio of 1:10 (10 g DJW: 100 mL of 2%

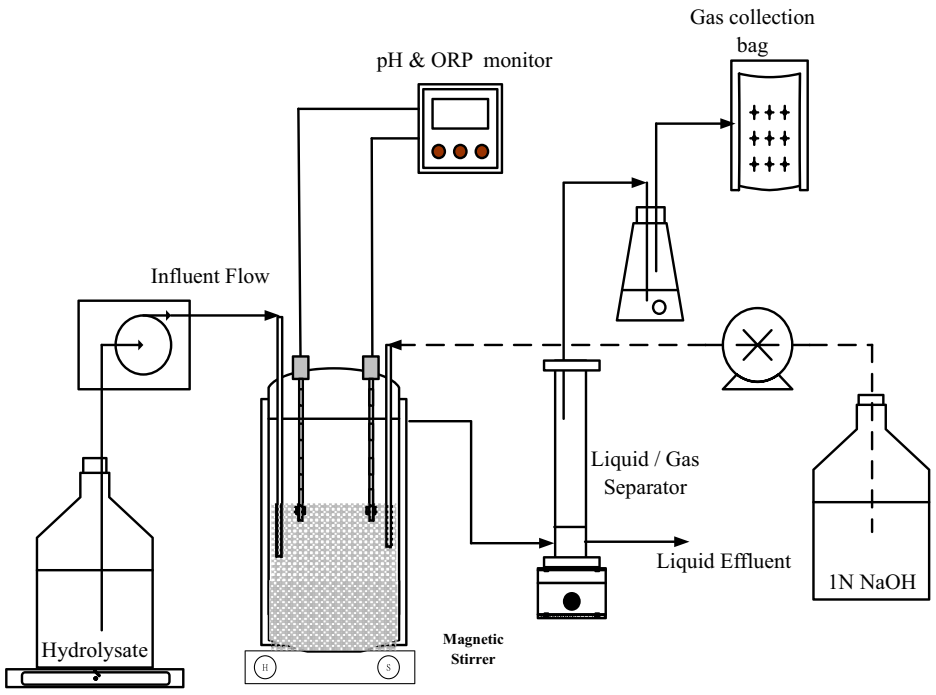

Fig. 1. A schematic diagram of the CSTR for continuous hydrogen production.