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Bio-hydrogen production by SSF of paper industry wastes using anaerobic biofilms: A comparison of the use of wastes with/without pretreatment

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ABSTRACT

In this research, we carried out the process of simultaneous saccharification and fermentation (SSF) of paper industry wastes with/without chemical pretreatment, using reactors in batch with anaerobic biofilms developed in spheres covered with ixtle fiber cord. Biofilms were previously developed a UASB reactor using anaerobic sludge with acid/thermal pretreatment to remove hydrogen consuming bacteria. Subsequently, the process of saccharification and simultaneous fermentation (SSF) of paper industry waste without chemical pretreatment was evaluated using batch reactors with developed anaerobic biofilms. The key process parameters were: pH (4, 5 and 6) and enzyme loading of Celluclast enzyme (10, 40 and 70 FPU) at a temperature of 45° C The results for hydrogen production with paper industry wastes without pretreatment showed optimal working conditions to maximize hydrogen production by SSF process at: pH (5) and an enzyme load of 70 FPU, so the maximum hydrogen yield obtained was 31.188 mmol/h × gSV. The results obtained from the evaluation of the process of SSF performed with paper industry wastes subjected to chemical pretreatment with H_2SO_4 2.5% showed the optimum working conditions to maximize hydrogen production: pH (4), an enzyme load of 70 FPU, the maximum value of hydrogen yield obtained was 55 844 mmol/h *gSV. The key process parameters were optimized by the response surface methodology (RSM) based on a two factor-three level central composite design (CCD), using as variables: pH (4.5 and 6), enzyme loading of Celluclast® (10, 40 and 70 UPF) and temperature (45 °C), for paper industry wastes with/without acid pretreatment. The results showed the analysis of variance was performed to test the importance of the polynomial equation of second order, so equations obtained for both residues (with/without pretreatment) describe the hydrogen yield in this study.

Introduction

Worldwide, the extensive use of fossil fuels for power plants, automobiles, and rapid industrialization is increasing day by day, resulting in not only environmental pollution, but also economic and diplomatic problems owing to their limited reserves and uneven distributions [1].It is an urgent issue to find alternative energy sources that are environmentally

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benign, cost-competitive, and renewable [2,3]. Hydrogen is a viable alternative source to replace conventional fossil fuels owing to its clean, renewable and high energy yielding (122 kJ/g) nature. As one of the various hydrogen production methods, biological hydrogen production becomes increasingly attractive, because it can be carried out at ambient temperature and pressure with low energy input and high efficiency. Biological method mainly includes photo-fermentative hydrogen production and dark fermentative hydrogen production [4], and can use several types of substrates, such as sucrose, xylose, residues, food waste, and animal waste. Ligno-cellulosic biomass is a second-generation raw material which may also be used for production of high value products via fermentation (e.g. ethanol, butanol, biogas) [5]. Ligno-cellulosic biomass such as paper, cardboard, wood, agricultural residues and other fibrous plant material is the most abundant raw material used for production of bio-fuels, these materials are composed of carbohydrate polymers (cellulose, hemicellulose), and an aromatic polymer (lignin), and contain different sugar monomers (six and five carbon sugars) and they are tightly bound to lignin. Waste paper is identified as particularly attractive feedstock for bio-fuels production due to high cellulose content [5]. Several pretreatment methods such as physical, chemical and biological have been used for hydrolysis of ligno-cellulosic materials [6]. Acid hydrolysis is one of the most effective methods used for hydrolysis of ligno-cellulosic biomass despite formation of undesirable products such as furfurals. When using biomass like waste paper as substrate, the hydrogen production process usually has two steps: enzymatic hydrolysis, and fermentative bio-hydrogen production [7,8]. However, these two steps can also be conducted simultaneously, which is referred to as simultaneous saccharification fermentation (SSF). Compared with separate hydrolysis and fermentation, SSF has several advantages, including weakened feedback inhibition on cellulase by glucose and cellobiose, short processing time, reduced number of reactors, low experimentation cost, and high productivity [4]. Anaerobic sludge is usually used for biohydrogen production. In this study, acid and thermal (heat) methods were chosen to pretreat the anaerobic sludge due to its simplicity, no special equipment required, and less time consuming. The heating and acid process provides the selection of spore forming anaerobic bacteria and removal of methanogens that consume hydrogen gas. Biofilms are cellular organizations that represent the mode of growth that allows cells to survive in harsh environments, disperse to form new niches and give them significant advantages of protection against fluctuations in the environment such as humidity, temperature and pH, as well as concentration of nutrients and the disposal of waste. Due to the above, it was proposed in this research the use of previously developed biofilms developed in ixtle fiber cord in an upflow reactor using anaerobic sludge with acid-thermal treatment and later used in batch reactors for the production of biohydrogen from paper industry wastes with/without acid hydrolysis by SSF. Initial pH (4, 5 and 6) and cellulase enzyme loadings (10, 40 and 70) were analyzed to find their effects on the hydrogen production process. Response surface methodology with central composite design was adopted to determine optimal levels of selected factors for simultaneous hydrogen production [4].

Materials and methods

Raw material

The paper industry solid wastes were collected from a paper processing industry located in the state of Coahuila, Mexico. The residue was crushed into powder, and screened with 25-mesh sieve. The composition of the processed sample is as follows in Table 1.

Pretreatment of paper industry wastes was conducted by placing 5 g with 50 mL of 2.5% sulfuric acid, autoclaving for 30 min at 120° C, then filtering the supernatant and drying the residue at 100° C.

Preparation and conditioning support

Solid plastic spheres with a diameter of approximately 25.5 ± 0.04 mm were used and were coated with 2.20 mt ± 0.02 of ixtle fiber cord. Two spheres per reactor were used and each sphere had 2.23 g ± 0.02 of adhered biofilm.

Microorganisms and enzyme

Mixed anaerobic microbial consortium was obtained from a UASB reactor, from brewing industry located in Zacatecas, Mexico. 0.5 L of granular sludge were macerated and subsequently were pretreated by combining two pretreatments described by Chen et al. [9] and modified by Moreno-Davila et al. [10]. For heat pretreatment, the anaerobic sludge was placed in a water bath at least 90° C temperature for a minimum period of 30 min with vigorous stirring during this time and finally subjecting it to thermal shock in ice water. The heating process provides the selection of spore forming anaerobic bacteria and removal of methanogens that consume hydrogen gas. Subsequently, the consortium at room temperature, was subjected to acid pretreatment, in which the pH was lowered to 3.0 with 3 N HCl solution for a period of 24 h and was finally readjusted pH to 7.0 with 3 N NaOH solution.

The enzyme employed was cellulase (*Trichoderma reesei* ATCC 26921), with an cellulase activity of 74 FPU/mL and β-glucosidase activity was of 1200 U/g.

Table 1 – Proximate analysis of paper industry wastes.

Composition	Wt%
Protein	0
Extractives	0.836 ± 0.02
Moisture	2.00 ± 0.03
Ash (600° C)	50.98 ± 0.12
Cellulose,	16.16 ± 0.25
Hemicellulose	19.40 ± 0.34
Lignine	10.44 ± 0.29
Total	99.81

Values are mean ± SD (n = 3).

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