Biochemical Engineering Journal 101 (2015) 209–219

Contents lists available at ScienceDirect

Biochemical Engineering Journal

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

Combined heterogeneous catalysis and dark fermentation systems for the conversion of cellulose into biohydrogen

E.J. Güell, B.T. Maru, R.J. Chimentão, F. Gispert-Guirado, M. Constantí*, F. Medina

Department of Chemical Engineering, Universitat Rovira i Virgili, P.O. Box 43007 Tarragona, Spain

ARTICLE INFO

Article history:
Received 20 April 2015
Accepted 5 June 2015
Available online 6 June 2015

Keywords:
Bioconversion
Biogas
Cellulose
Fermentation
Bacteria
Zirconium catalyst

ABSTRACT

A two-step combined system consisting of heterogeneous catalysis followed by dark fermentation was investigated for the production of biohydrogen. Cellulose in the aqueous phase was hydrolysed in an autoclave reactor with ZrO_2 catalysts modulated by three different promoters: sulfate, fluoride, and phosphate. The water-soluble fractions (WSFs) resulting from the catalytic cellulose hydrolysis were then submitted to dark fermentation without any additional treatment. The dark fermentation step tested three different microorganisms – *Enterobacter* spH1, *Citrobacter freundii* H3 and *Ruminococcus albus* DMS20455 – for their ability to produce H_2 from cellulose, glucose and the liquid product derived from cellulose hydrolysis. The two enteric bacteria (*C. freundii* H3 *and Enterobacter* spH1) effectively fermented the WSFs to produce H_2 and other organic compounds as metabolites. For the WSFs derived from cellulose hydrolysis with ZrO_2-P and ZrO_2-S catalysts, the values for *Enterobacter* spH1 were 1.40 and 1.09 mol H_2/mol hexose, respectively.

1. Introduction

Fossil fuels are currently the source of about 85% of all the energy used on the planet [1]. As a result, pollution is a problem the world over and the earth's petroleum reserves are depleting so rapidly that there is concern that soon demand will not be met. The development of clean and sustainable alternative sources of energy is, therefore, a global priority.

Biomass is organic material which stores sunlight in the form of chemical energy. The rate of energy capture by photosynthesis around the globe is approximately 100 TW per day [2], about six times the world's energy consumption [3]. This makes organic biomass a clear source of renewable energy, and because its carbon cycle is closed there would be no net increase in atmospheric CO_2 levels. Biomass can also be transformed into compounds that are the same as or similar to those derived from fossil fuels. The energy-carrying solids, liquids, and gases produced from biomass are called biofuels [4]. In order to be used for the production of biofuels, biomass is generally transformed into sugar monomers. Once sugar monomers have been formed, they can be processed by microorganisms [5].

H_2 is a biofuel that is believed to have considerable potential for use with future technologies. Biological H_2 can be produced from a wide spectrum of carbohydrates. Of all known gaseous fuels, molecular hydrogen has the highest calorific value per unit mass (143 GJ/ton) [6]. The maximum H_2 yields obtained from these pure carbohydrates vary from 2.40 mol H_2/mol hexose for cellulose [7,8] to 3.33 mol H_2/mol hexose for starch [9] and glucose [7], indicating that these carbohydrates are indeed suitable as feedstocks for dark fermentation.

Lignocellulosic biomass is a particularly low-impact source of carbohydrates that can be used to produce fuels, chemicals, power and heat, since, unlike other sources such as corn, it does not interfere with the food industry [10]. Lignocellulose is composed of cellulose, hemicellulose and lignin. Cellulose and hemicellulose are two carbohydrate polymers that are tightly bound to lignin to form a recalcitrant matrix, which is difficult to transform [10,11]. Cellulose is the most abundant component in lignocellulosic materials. It is made up of a chain of glucose units bonded by β-1, 4-glycosidic linkages and can form tightly packed strands via hydrogen bonds. This structure makes cellulose highly resistant to chemical attack and deconstruction [12]. Three steps are required to break down lignocellulosic biomass: first, the long-

* Corresponding author. Fax: +34 977 559621.
E-mail address: magda.constanti@urv.cat (M. Constantí).

http://dx.doi.org/10.1016/j.bej.2015.06.004
1369-703X/

chain polysaccharides, cellulose, and hemicellulose need to be separated; second, these polymers need to be hydrolysed into their structural units of five- and six-carbon sugars; and, finally, these sugars need to be converted into biofuels or other value-added compounds. The commercial applications of these steps are still in the early stages of development and need to be improved upon if they are to be efficient enough to become economically viable [13].

The most common method is to use hydrolysis with mineral acids, but this process has the drawbacks that it generates acid wastes and corrodes the equipment, which makes it difficult to manage [14]. A variety of new approaches to biomass transformation have been proposed: for example, hydrothermal liquefaction, catalytic and physical treatments, enzymatic digestion, and bacterial hydrolysis/fermentation [15]. Another approach that deserves attention is enzymatic digestion, but at present it is too expensive and needs to be developed further if the production of enzymes is to be economically viable [12]. Heterogeneous catalysis is one of the most effective methods [13,15–19] and sulfated zirconium dioxide (ZrO_2) has been successfully applied to catalyze hydrolysis reactions on cellulose [20,21]. Onda et al. [20], have shown highly selective hydrolysis of cellulose into glucose under hydrothermal conditions at 423 K in the presence of sulfonated active carbon (AC–SO_3H).

In this study, two methods are combined into an integrated system that uses heterogeneous catalysis and batch dark fermentation. This combined system is designed to provide a new route for converting cellulose into biohydrogen in a light-independent process.

The first step of the combined system is the catalytic hydrolysis of cellulose, which uses an acid catalyst (ZrO_2) to break down the complex structure of the cellulose and produce easily fermentable sugars (the so-called "water-soluble fraction") (WSF). The second step is the dark fermentation of the WSF, without any additional pretreatment. The WSF mainly consists of sugars and other organic compounds derived from the cellulose hydrolysis, including furfural, hydroxymethylfurfural (HMF) and acetic acid. However, these compounds can have inhibitory effects on the growth and metabolic ability of microorganisms [22–26]. Consequently, these compounds need to be removed from the WSF or neutralized before fermentation. This step increases the cost of the process. The present study, therefore, focuses on directly fermenting the WSF produced in the hydrolysis step, without any further treatment, by using microbes that can survive and metabolize under these conditions. Here, two enteric bacteria, *Enterobacter* spH1 and *Citrobacter freundii* H3, were studied. These strains had previously been isolated and shown to effectively convert glucose and glycerol into H_2 and other value-added products such as ethanol and 1,3-propanediol [27]. In addition to these strains, a cellulolytic bacteria, *Ruminococcus albus* DSM20455, was used for cellulose hydrolysis and the fermentation of the remaining WSF. In continuous culture, it can yield 2.4 mol H_2/mol glucose and is known for its ability to degrade cellulose [28].

2. Materials and methods

2.1. Preparation of the supports and catalysts

Zirconium dioxide (ZrO_2) (commercial sample from Degussa) was prepared for use as a catalyst for cellulose hydrolysis with three different promoters: sulfate, phosphate and fluoride. Calcined ZrO_2 at 673 K was impregnated with 5% (w/w) of aqueous solutions of H_2SO_4, H_3PO_4, and HF, respectively. The resulting solids were dried at 373 K for 12 h and calcined at 673 K for 4 h in a muffle. The catalysts obtained were then labeled ZrO_2–S, ZrO_2–P and ZrO_2–F.

2.2. Textural and structural characterization of ZrO_2

Textural properties were obtained by N_2 adsorption–desorption isotherms at 77 K using a Micromeritics ASAP 2000 analyzer. Before analysis, all the samples were degassed in a vacuum chamber at 393 K for 12 h.

X-ray diffraction (XRD) was recorded using a Siemens D5000 diffractometer (Bragg Brentano focusing geometry and vertical θ–θ goniometer) with an angular 2θ – diffraction range from 9.5° to 70°. The samples were placed in a Si (510) support with a cavity that was 0.1 mm deep. The cavity was filled with the same amount of sample to ensure the sample packaging and the same baseline for all analyses. The diffraction data were collected with an angular step of 0.03° at 5 s per step and sample rotation. Cu Kα radiation (λ = 1.54056 Å) was obtained from a Cu X-ray tube operating at 40 kV and 30 mA. The crystalline phases were identified using the ICDD files (International Centre for Diffraction Data, release 2007). The crystallinity index (CrI) of cellulose was calculated using the modified Segal's method [29] (Eq. (1)):

$$\mathrm{CrI} = \frac{[(I_{\mathrm{cel}} - I_{\mathrm{am}})]}{I_{\mathrm{cel}}} \quad (1)$$

where I_{cel} is the sum of intensities of peaks from cellulose that appear in the range 10–27° 2θ and I_{am} is the intensity of the amorphous peak (18° 2θ). It should be noted that this CrI refers only to a ratio between diffracted intensities not to a mass ratio.

All diffractograms were fitted with the TOPAS software (TOPAS, 2009). This software uses the Rietveld method [30] and the fundamental parameters Approach [31], which consists of calculating the instrumental contribution to the peak width by describing the different components of the diffractometer.

The contribution of crystallite size to the peak width (τ) was calculated by fitting a Lorentzian and Gaussian function (double-Voigt approach) and applying the modified Scherrer equation [32]:

$$\tau = \frac{\lambda}{\beta \sin\theta} \quad (2)$$

where β is the mean integral breadth and λ is the wavelength.

The background was considered to be a straight line with constant slope. The amorphous part of the sample was assigned to a pseudo-Voigt peak at $2\theta = 18°$ with refinable peak width. The cell parameters for each phase were refined for each sample. From the same fitting, the relative weight fraction of each crystalline phase, W_i, was calculated from the equation [33].

$$W_i = \frac{S_i \rho_i V_i^2}{\sum_{j=1}^{n} S_j \rho_j V_j^2} \quad (3)$$

where ρ_i is the crystal density, V_i is the unit cell volume and S_i is the refinable scale factor of the crystal structure for phase i. This equation is applicable when all the phases in the sample are crystalline. As this is not true in the present case, we must consider W_i as a relative rather than an absolute weight fraction.

2.3. NH_3-temperature programmed desorption (TPD)

The acid properties of the materials were characterized by NH_3-TPD using a ThermoFinnigan (TPRDO 110) apparatus equipped with a programmable temperature furnace and a TCD detector, and calibrated using NH_3 pulses of differing concentrations. For each sample, 20 mg of catalyst was placed between plugs of quartz wool in a quartz reactor. The sample was first purged using pure He (flowing at 20 cm^3/min) at 393 K for 30 min. After the temperature had been cooled to room temperature, the sample was treated with an