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Combining catalytical and biological processes to transform cellulose into high value-added products

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Abstract:
Cellulose, the most abundant polymer of biomass, has an enormous potential as a source of chemicals and energy. However, its nature does not facilitate its exploitation in industry. As an entry point, here, two different strategies to hydrolyse cellulose are proposed. A solid and a liquid acid catalysts are tested. As a solid acid catalyst, zirconia and different zirconia-doped materials are proved, meanwhile liquid acid catalyst is carried out by sulfuric acid. Sulfuric acid proved to hydrolyse 78% of cellulose, while zirconia doped with sulfur converted 22% of cellulose. Both hydrolysates were used for fermentation with different microbial strains depending on the desired product: Citrobacter freundii H3 and Lactobacillus delbrueckii, for H_2 or lactic acid production respectively. A measure of 2 mol H_2/mol of glucose was obtained from the hydrolysate using zirconia with Citrobacter freundii; and Lactobacillus delbrueckii transformed all glucose into optically pure D-lactic acid.

Keywords: cellulose, fermentation, hydrolysis

DOI: 10.1515/psr-2017-0026

1 Introduction

Currently, the source of about 85 % of all the energy used on the planet is fossil fuel [1]. As a result, pollution is a concerning problem all over the world; in addition petroleum reserves are depleting so rapidly that soon its demand will surpass its production. The development of clean and sustainable alternative sources of energy is, therefore, a global priority.

Biomass is an organic material that 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. This makes organic biomass a clear source of renewable energy.

Lignocellulosic biomass is a particular 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 [3]. Lignocellulose is composed of cellulose, hemicellulose and lignin. Cellulose is a linear polymer formed by units of glucose linked to each other through β-(1,4)-glycosidic bonds. These polymers are packed together forming microfibrils; among them intramolecular and intermolecular hydrogen bonds strengthen its structure [4]. Consequently, cellulose is a robust polymer difficult to hydrolyse. The supramolecular structure of cellulose is divided into areas of high order (crystalline) and low order (amorphous) [5]. The presence of one or another is claimed to deeply have an impact on cellulose robustness [6]. While amorphous cellulose is more accessible and simpler to hydrolyse, taking profit of crystalline cellulose is more challenging.

Diverse approaches have been proposed for cellulose hydrolysis [7], the most popular one among them being the enzymatic treatment [8]. However, high enzyme cost and strict control of temperature and pH are a burden when scaling-up these processes [9].

As an alternative, this review presents two different catalytic approaches for cellulose hydrolysis, liquid acid hydrolysis and solid acid hydrolysis. As solid acid catalyst, zirconia and different zirconia-doped materials are tested; meanwhile liquid acid catalyst is carried out by sulfuric acid. Afterwards, the suitability of these methods to produce fermentable sugars is confirmed by two different bacterial strains, *Citrobacter freundii* H3 and *Lactobacillus delbrueckii*, for H_2 or lactic acid production respectively (Figure 1).

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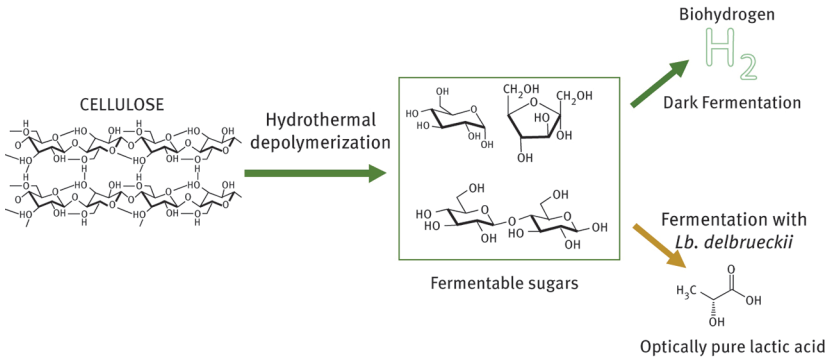

Figure 1. Scheme of a general hydrolysis-fermentation process.

H_2 is a biofuel that is believed to have considerable potential for using 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) [10]. The maximum H_2 yields obtained from these pure carbohydrates vary from 2.40 mol H_2/mol hexose for cellulose [11] to 3.33 mol H_2/mol hexose for starch [12] and glucose [11], indicating that these carbohydrates are indeed suitable as feedstocks for dark fermentation [13].

Lactic acid is a natural carboxylic acid with extended usages in industry. Recently, lactic acid has gained more importance in industry because of its usage as a precursor for polylactic acid (PLA) production. PLA, a thermoplastic aliphatic polyester, is becoming a highly consumed bioplastic. European demand for PLA in 2008 was 25,000 tons per year and could potentially reach 650,000 tons per year in 2025 [14]. Lactic acid is produced either by chemical synthesis or by fermentative processes. Chemical-based synthesis involves the use of petrochemical resources, requires the use of aggressive chemical compounds and leads to a racemic mixture of lactic acid. However, high isomeric purity of the obtained lactic acid is required in order to obtain good physical properties of the further PLA [15]. Rather, using fermentative processes, glucose can be transformed into almost isomerically pure lactic acid [16, 17]. Furthermore, the energy consumption in fermentative process is lower than that in the chemical synthesis process.

2 Materials and methods

2.1 Preparation of the supports and catalysts

Zirconium dioxide (ZrO_2) (commercial sample from Degussa) was prepared for using as a catalyst for cellulose hydrolysis with three different promoters: sulphate, 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 labelled ZrO_2-S, ZrO_2-P and ZrO_2-F.

2.2 X-ray diffraction (XRD)

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 a modified Segal's method (Eq. 1)):

$$CrI = \left[(I_{cel} - I_{am}) \right] / I_{cel} \quad (1)$$

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.

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 2θ = 18° with refinable peak width.

2.3 Cellulose characterization

Before and after the catalytic treatment, the surface morphology of the cellulose was imaged with scanning electron microscopy (SEM-JEOL JSM-35C), operating at 15 kV. The sample was gold-coated to facilitate SEM analysis.

2.4 Cellulose hydrolysis

2.4.1 ZrO_2-catalysed cellulose hydrolysis

The bulk cellulose hydrolysis (molecular biology, ≥99 %) was performed in an autoclave reactor (Parker Autoclave Engineers, UK, 100 mL), using 0.2 g of catalyst at 453 K and 30 bar for 15 h. The solution was continuously stirred at 400 rpm while the reactions were carried out. Prior to the reactions, the autoclave reactor was fed with 50 mL of water, 0.8 g of cellulose and 0.2 g of catalyst and then purged with Argon gas (Ar). The reactor was then heated to 453 K and pressurized to 30 bar with Ar.

2.4.2 H_2SO_4-catalysed cellulose hydrolysis

Cellulose was impregnated with 3 % w/w sulfuric acid and dried overnight at 373 K. The hydrolysis experiments were carried out in a PFTE (polytetrafluoroethylene) reactor loading 1 g of impregnated cellulose and 20 mL of deionized water. The samples were irradiated with a microwave system (Milestone ethos-touch control) at 393 K for 2 h.

2.5 Fermentation

2.5.1 Fermentable substrates

Pure cellulose (molecular biology, ≥99 %) and glucose (D-glucose, anhydrous) were obtained from Sigma Chemical Co. (Madrid, Spain). The water-soluble fractions (WSFs) derived from the hydrolysis reactions were used for fermentation without filtration or any further treatment.

2.5.2 Hydrogen production

Citrobacter freundii H3 was tested for their ability to produce H_2 from the liquid product derived from cellulose hydrolysis. *Citrobacter freundii* H3 was aerobically pre-cultured in a synthetic medium at 310 K in an incubator-shaker overnight at 200 rpm. Per liter, the synthetic medium used contained 7.0 g of K_2HPO_4, 5.5 g of KH_2PO_4, 1.0 g of $(NH_4)_2SO_4$, 0.25 g of $MgSO_4{\cdot}7H_2O$, 0.021 g of $CaCl_2{\cdot}2H_2O$, 0.12 g of $Na_2MoO_4{\cdot}2H_2O$, 2.0 mg of nicotinic acid, 0.172 mg of Na_2SeO_3, 0.02 mg of $NiCl_2$ and 10 mL of trace element solution containing, per liter, 0.5 g of $MnCl_2{\cdot}4H_2O$, 0.1 g of H_3BO_4, 0.01 g of $AlK(SO_4)_2{\cdot}H_2O$, 0.001 g of $CuCl_2{\cdot}2H_2O$ and 0.5 g of Na_2EDTA. A complex medium was prepared by adding 0.5 g/L of yeast extract to the synthetic medium. To study the batch dark fermentation, the reaction medium was prepared by adding different carbon sources, at around 5 g/L. The pH of the synthetic medium was adjusted to 6.8 before autoclaving. Inoculation was performed under strictly anaerobic conditions, using Ar to purge. The cells were harvested at the end of the exponential growth phase and 10 % (v/v) were used as the inoculum for the main batch experiments.

H_2 production by dark fermentation was investigated in a batch system during 120 h, using 100 mL bioreactors sealed with rubber butyl stoppers and aluminium caps with a working volume of 50 mL. They were continuously agitated in a shaker at 200 rpm and a constant temperature of 310 K. Initially, an anaerobic atmosphere was created in each bottle by purging with 30 mL/min of Ar (99.99 %) for 15 min. Before inoculation, all reactors were autoclaved (for 20 min, 393 K and 1.5 Kg/cm^2 of pressure). Each experiment was performed in duplicate. The carbon source used in the experiments was WSF generated from the catalytic hydrolysis of the cellulose.

2.5.3 Lactic acid production

Lactobacillus delbrueckii delbrueckii, CECT 286, was grown on an impoverished MRS broth (5.0 g/L peptone, 5.0 g/L beef extract, 2.5 g/L yeast extract, 2.0 g/L ammonium citrate, 5.0 g/L sodium acetate, 0.2 g/L $MgSO_4 \cdot 7H_2O$, 0.05 g/L $MnSO_4 \cdot H_2O$, 2.0 g/L K_2HPO_4, 1.0 g/L cysteine hydrochloride, 11 g/L HEPES and 10 mg/L resazurin). This medium was selected after different optimization tests and used instead of MRS broth in order to decrease the nutrients, hence reducing media cost. After the pH adjustment at 6.2, the WSF obtained in the hydrolysis experiments was used as a source of carbohydrates for fermentation experiments without any purification step. The fermentative processes were carried out in batch reactors of 50 mL with 25 mL of aqueous media, sealed with rubber cap and aluminum seal and afterwards deoxygenated with 30 mL/min argon flow. Resazurin was used as indicator of oxygen presence. The inoculum consisted of 2.5 mL of previously grown *L. delbrueckii*.

2.6 Analytical methods

The WSFs remaining in the autoclave reactor were filtered and analysed by total organic carbon analyzer (TOC). TOC determined the total concentration of soluble carbon directly related to cellulose solubilization from the hydrolysis. The composition of glucose and other by-products of the liquid phase after the hydrolysis and dark fermentation steps was analysed with a high-performance liquid chromatograph (HPLC) (Agilent Technologies, Spain 1100 series), equipped with an ICSep ICE-COREGEL 87H3 Column, serial no. 12525124, a diode-array detector (DAD) and refractive index detectors (RIDs). A mobile phase of H_2SO_4 (2.2 %) was used at a constant flow of 0.6 mL/min, the temperature of the column was maintained at 323 K and each sample was analysed for 40 min. The total soluble carbon after the hydrolysis of cellulose was then compared to the initial carbon present in the cellulose to determine the extent to which it had dissolved or converted into a soluble chemical. With this information, the cellulose conversion capacity of each catalyst was calculated. Calculations were made analytically from TOC results as follows:

$$\text{Reactedcellulose} = 100 \times \frac{\text{mgC}_{\text{liquid phase}}}{\text{mgC}_{\text{initial cellulose}}} \tag{2}$$

$$\text{Glucose selectivity} = \frac{\text{Carbon moles in glucose}}{\text{Reacted carbon moles from cellulose}} \tag{3}$$

This formula describes the carbon moles of cellulose transformed into carbon moles of glucose.

The composition of the gas was measured using a GC-14B gas chromatograph equipped with a thermal conductivity detector (TCD) and a 80/100 Porapak-Q column. Argon was used as the carrier gas at a flow of 30 mL/min. The hydrogen from the fermentation was calculated by comparison with standard pure gas. Hydrogen was measured using a gas chromatograph GC-14B. The operational temperatures of the GC for the injection port, oven and detector were 423 K, 353 K and 473 K, respectively. The chromatogram was developed and analysed using the Turbochrome Navigator (version 4.1) software from Perkin Elmer, Spain.

A modified Gompertz equation Eq. 4was used to estimate the maximum H_2 production rates.

$$\text{H}_2(t) = H_{\text{max},H2} . \exp\left\{-\exp\left[\frac{R_{\text{max,H2}} .e}{\text{H}_{\text{max,H2}}}(\lambda_{\text{H2}} - t) + 1\right]\right\} \tag{4}$$

where $H_2(t)$ is the cumulative H_2 production (mmol/L), λ the lag-phase time (h), H_{max},H_2 the maximum H_2 production (mmol/L), R_{max},H_2 the maximum H_2 rate (mmol/L h) and t the incubation time (h). This equation was found to be suitable for modelling the experimental data on H_2 production [18].

3 Results and discussion

3.1 Cellulose hydrolysis

Structural characterization of cellulose surface was performed by SEM; pure cellulose (Figure 2a) shows a smooth surface. Figure 2(b)–(e) shows the images of cellulose after catalytic hydrolysis with ZrO_2 materials, while Figure 2(f) shows the cellulose surface after hydrolysis with sulfuric acid.

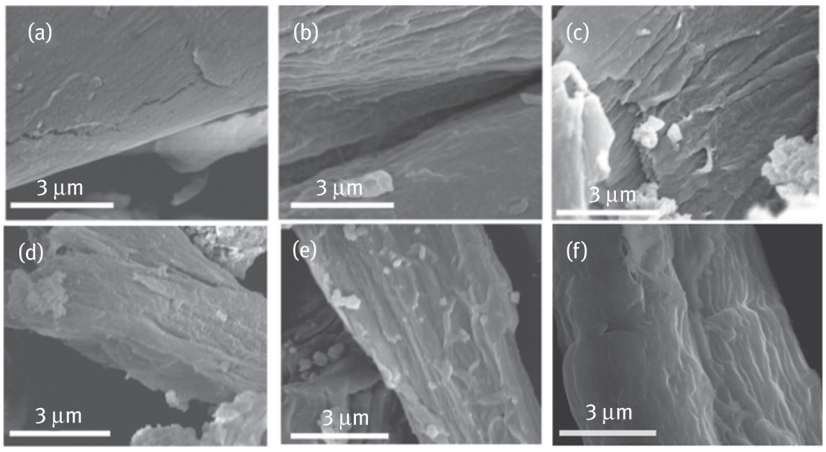

Figure 2. Images of cellulose fibers' wall before (a) and after hydrolysis with ZrO_2 (b), ZrO_2-P (c), ZrO_2-S (d), ZrO_2-F (e) and H_2SO_4- (f)

Cracking of cellulosic fibres occurs alongside with hydrolysis as can be seen in Figure 2(b)–(f). As the images show, the fibres of the cellulose surface have become markedly more exposed. This change in morphology is equally observed independently of the acid catalyst, being patent for all the different promoted zirconia as well as sulfuric acid.

Despite a similar physical aspect, XRD (Table 1) proved that sulfuric acid combined with microwaves is a better method for hydrolysing the crystalline phase of cellulose (Table 1, entry 6). Pure ZrO_2 had a slight effect on depolymerization of crystalline cellulose. Instead, promoted ZrO_2 showed better ability to degrade crystalline cellulose, yet sulfuric acid had greater potential for depolymerizing the crystalline phase.

Table 1: Crystalline index calculated by modified Segal's equation.

Entry	Sample	CI (%)
1	Cellulose	74
2	ZrO_2	73
3	ZrO_2-P	65
4	ZrO_2-S	70
5	ZrO_2-F	72
6	mw-H_2SO_4	57

Figure 3shows the conversion of cellulose after the treatment with different catalysts and the selectivity towards different products. For all promoted ZrO_2 materials, cellulose conversion was greater than for pure ZrO_2. In fact, pure ZrO_2 showed the lowest cellulose conversion (only 9.3 %). Selectivity to glucose (14.6 %) was highest for the ZrO_2-P sample, for which cellulose conversion was 12.8 %. Selectivity to HMF (hydroxymethyl-furfural) (26.9 %) was also highest for the ZrO2-P sample, which may stem from glucose dehydration [19]. On the other hand, cellulose conversion (22.0 %) was highest and selective to glucose and HMF lowest for ZrO_2-S (3.2 % and 8.9 %, respectively). ZrO_2 promotion by sulfate species conferred higher acidity, which may lead to a greater capacity for hydrolyzation and, in turn, other by-products. Thus, the hydrolysis product to be obtained will determine which promoted catalyst is selected.

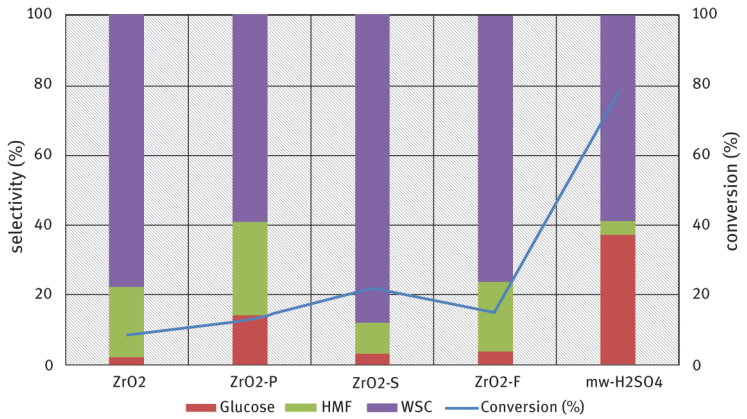

Figure 3: Conversion of cellulose with different catalysts and selectivity towards different products.

However, it is clear that a liquid acid catalyst, sulfuric acid, combined with microwaves is much more efficient for depolymerizing cellulose. Not just reducing the treatment times, a liquid acid catalyst can as well increase cellulose conversion from 22 % (ZrO_2) to 78 % when sulfuric acid was used. It also has to be noted that the conditions of both hydrolysis are not the same: 453 K, 30 bars of Ar and 15 h of treatment time for solid acid catalysis as against 393 K and 2 h for liquid acid catalysis. Even though solid acid catalysts could theoretically be reused, liquid acid catalysis assisted by microwave proved to be a more cost-effective process. In addition, when using H_2SO_4 as catalyst, glucose was the most abundant identified product, hence presenting a more suitable process for production of fermentable sugars.

3.2 Fermentation of the water-soluble fractions (WSFs)

If WSFs are to be used in dark fermentation, monosaccharide yields must be higher, carbohydrate losses minimized and levels of inhibitory substances lower [20]. Glucose is the compound that is most easily fermented by most microorganisms. Such by-products as furfural, HMF, phenols, aromatic substances and some organic acids can also inhibit bacterial growth [21].

Fermentation of the sugars obtained after hydrolysis catalysed by ZrO_2-P and ZrO_2-S by *Citrobacter freundii* H3 yielded 1.19 and 0.99 mol H_2/mol hexose, respectively, as can be seen in Figure 4. The maximum theoretical production is 4 mol of H_2 per mol of glucose consumed [22]. However, thermodynamically, this yield cannot be achieved by mesophilic organisms. The maximum possible yield that can be achieved without such additional adjustments such as lowering the partial pressure by purging inert gases is about 2 mol H_2/mol of glucose [11].

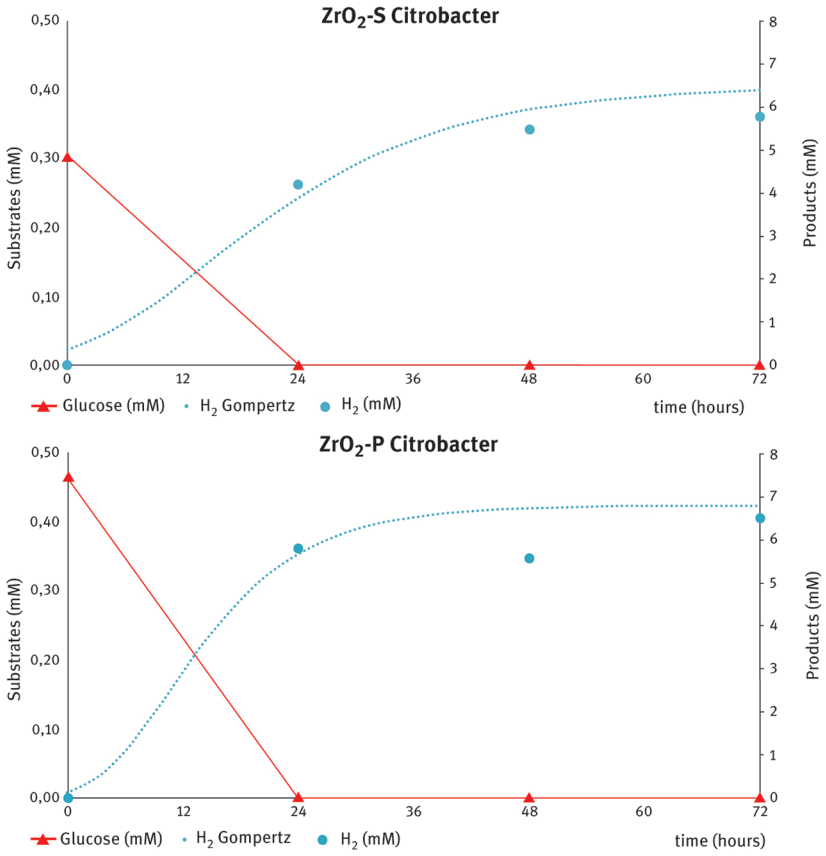

Figure 4 Dark fermentation profile using *Citrobacter freundii* H3 in the WSF resulting from the hydrolysis tests with (a) ZrO_2–P and (b) ZrO_2–S.

As shown in Figure 5, when fermenting the liquid phase obtained by dilute acid treatment of cellulose with 3 % of sulfuric acid for 2 h by *L. delbrueckii*, lactic acid production was directly proportional to the amount of glucose. The rest of monitored compounds (levulinic acid, formic acid and HMF) remained constant during the fermentation. Therefore, there was no interference of the rest of generated compounds during cellulose hydrolysis in the metabolic pathway of *L. delbrueckii*. This fact is important because no separation step is needed. Thus,

after the hydrolysis, the microbial fermentation can be performed directly. Furthermore, highly pure, 99 %, D-Lactic acid was formed by *L. delbrueckii*. TOC measurements revealed no major loss of organic dissolved carbon, meaning that no CO_2 was produced, consequently and only homolactic fermentation occurred. Therefore, the maximum yield of lactic acid per glucose molecule was achieved.

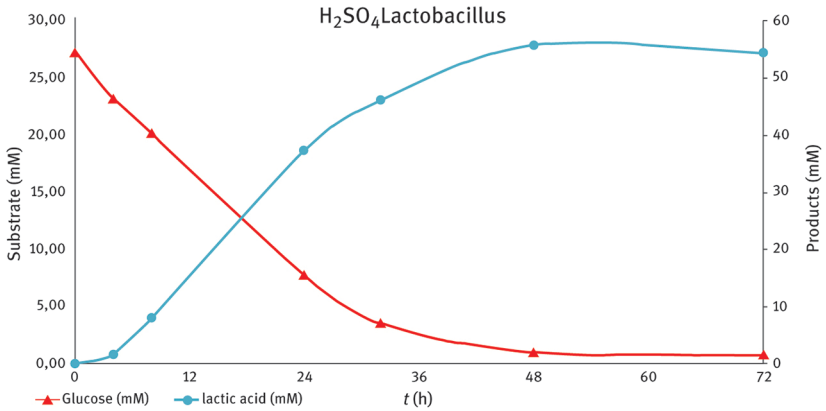

Figure 5. Lactic acid production by *L. delbrueckii* from the WSF obtained after cellulose treatment with 3 % H_2SO_4 at 2 h

Consequently, by electing the appropriate strain, different compounds can be targeted. *Citrobacter freundii* H3 and *L. delbrueckii* were able to grow with the different compounds generated by solid or liquid acid catalysis, respectively, as well as transform glucose in the desired compound.

4 Conclusions

In this chapter, two different strategies for cellulose hydrolysis were presented. In one hand, solid acid catalysis presents the advantage of catalyst reuse and easier downstream processing. While, microwave-assisted liquid acid catalysis has shorter reaction times and greater conversions.

Here as well, two different microbial strains were presented for the production of biohydrogen or optically pure lactic acid. Demonstrating that both strains were appropriate for fermenting the obtained sugars after hydrothermal depolymerization of cellulose. Consequently, a full strategy from biomass to biofuels or green chemicals has proved to be feasible.

Acknowledgements

This study was funded by the Spanish Ministry of Economy and Competitiveness (MINECO), reference CTM2015-69848-R

This article is also available in: Tylkowski, Polymer Engineering. De Gruyter (2017), isbn 978–3–11–046828–1.

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