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# Hydration treatments increase the biodegradability of native wheat straw for hydrogen production by a microbial consortium

Anibal R. Lara-Vázquez<sup>a</sup>, Arturo Sánchez<sup>b</sup>, Idania Valdez-Vazquez<sup>a,\*</sup>

<sup>a</sup> Depto. de Ciencias Ambientales, DICIVA, Universidad de Guanajuato, México

<sup>b</sup> Unidad de Ingeniería Avanzada, Centro de Investigación y Estudios Avanzados (CINVESTAV), Av. del Bosque 1145, Zapopan 45019, Jalisco, México

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## ABSTRACT

Enhancing substrate accessibility is one of the prerequisites for efficient pretreatment and bioconversion of lignocellulosic biomass, for which reducing particle size and increasing porosity have been implemented. Biomass porosity, characterized as the water retention capacity (WRC), increases significantly with the use of a culture filtrate of an H<sub>2</sub>-producing microbial consortium, causing native lignocellulosic fibers to hydrate and swell extensively. This study describes the effects of hydration treatment and particle size on the biodegradability of native wheat straw fibers with regard to direct H<sub>2</sub> production by a microbial consortium. A culture filtrate and tap water were used as hydration media, and particle sizes of 0.212 and 3.35 mm were tested. As a result, biodegradability, measured as H<sub>2</sub> production, doubled when the lignocellulosic substrate was hydrated with the culture filtrate. Also, hydration had a significant impact on H<sub>2</sub> yield, increasing it from 6.8 to 86.5 mL H<sub>2</sub>/g total consumed sugars when the 0.212 mm fibers were first hydrated. H<sub>2</sub> production was higher with coarser particles (3.35 > 0.212 mm), but hydrogen yield improved with finer particles (0.212 > 3.35 mm). Based on our results, particle size and hydration improve the biodegradability of native wheat straw for H<sub>2</sub> production, although hydration with a culture filtrate increased the biomass porosity (measured as WRC), which influenced the biodegradability more than a reduction in particle size.

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## Introduction

Hydrogen is an energy carrier that can help global energy requirements be met, due to its high combustion power (H<sub>2</sub> >120 MJ/kg versus >45 MJ/kg for fossil fuels). Further,

hydrogen is considered to be a green fuel, because water is the sole product at the end of its theoretical combustion [1]. Hydrogen can be produced from biological processes, specifically, from the conversion of carbohydrates into hydrogen by fermentative microorganisms under anaerobic environments.

\* Corresponding author. Present address. Unidad Académica Juriquilla, Instituto de Ingeniería, Universidad Nacional Autónoma de México, Blvd. Juriquilla 3001, 76230 Querétaro, México. Tel.: +52 (442) 1926170; fax: +52 (442) 1926185.

E-mail address: [IValdezV@iingen.unam.mx](mailto:IValdezV@iingen.unam.mx) (I. Valdez-Vazquez).

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First generation biofuels are obtained primarily from crops, such as sugarcane and corn, but one of their major drawbacks is that they are part of the human food chain. In contrast, agricultural byproducts can be used as second-generation biofuels, because they are abundant, cheap and a good source of carbohydrates, some of which are plentiful [2]. Annually, nearly 200 billion tons of agricultural byproducts are produced worldwide, energy equivalent to 60–80 billion tons of crude oil [3]. In Mexico, close to 150 million tons of agricultural byproducts are generated from 20 crops, including corn stover and cobs, sorghum straw, the tops and leaves of sugarcane, and wheat straw [4]. In particular, wheat straw is one of the main agricultural products in certain regions; thus, it can be collected with minimal effort and at low cost for use as feedstock in hydrogen production.

Wheat straw is classified as lignocellulosic biomass. In its native form, this biomass is arranged 3-dimensionally as a network, in which the core comprises cellulose microfibrils: large linear chains of anhydroglucose residues that are oriented parallel to hydrogen bonds between successive and adjacent residues, yielding highly ordered and tightly packed crystalline regions that are largely impenetrable by water; and amorphous regions with free hydroxyl groups that interact with the aqueous environment. On the outside of the network, the lignin-hemicellulose matrix is highly branched, with many charged groups rendering it water-swollen [5,6]. The cellulose crystallinity, the association of the lignin-hemicellulose matrix with the cellulose microfibrils, and the accessible surface area contribute to the low biodegradability of lignocellulosic biomass [7]. Thus, the hydrogen yield from direct fermentation of native wheat straw is low ranging from 1.0 to 11 mL H<sub>2</sub> per g of total volatile solids (TVS) [8–11].

Most approaches improve the biodegradability of wheat straw by obtaining hydrolysates with diluted acids [8,11,12], hydrothermal pretreatment [13], and enzymatic hydrolysis [9], which raises the hydrogen yields to 20–68 mL H<sub>2</sub> per g TVS. These pretreatment methods decrease the times that are required for biomass processing and fermentation. However, pretreatment byproducts are released, which strongly inhibit H<sub>2</sub>-producing dark fermentation [14]; thus, fermentation can only be performed with a low hydrolysate percentage or if the inhibitors are removed. These steps can increase the processing costs in a scaled-up plant, compromising the benefit of the pretreatments. Developing economical and simple pretreatment methods that enhance the biodegradability of lignocellulosic biomass remains a significant challenge in the production of lignocellulosic biofuels.

All pretreatment methods aim to increase the accessible surface area [7,15]. Accessibility refers to the substrate that is available for hydrolysis or biodegradation and is governed by biomass porosity (interior surface area) and particle size (exterior surface area) [16]. For years, particle size reduction has been used as an efficient pretreatment method of increasing the exterior surface area to improve biomass biodegradability, the production of biogas and, recently, hydrogen [17–20]. Conversely, cell wall porosity limits the physical, chemical, and enzymatic access to the native lignocellulosic substrate, which impacts the biodegradability of substrate more than the external surface or particle size [21]. In this respect, the biomass porosity can be characterized

by water retention capacity (WRC), which reflects the interactions between the charged surface groups and adsorbed molecules in an aqueous environment [22,23].

Lignocellulosic biomass is considered a cross-linked gel that swells when water molecules are drawn into the pores by osmotic pressure and interact with ionizable groups on the biomass surface. As a result of swelling, the cell wall volume increases and accessibility improves, because the crystallinity is reduced when the bonds between the cross-linked gel are weakened [24]. The hydration medium has significant effects on the WRC: salt ions neutralize the charged groups on the cell wall surface, generally decreasing WRC values and swelling, whereas enzymatic treatment increases them [25,26].

A recent report demonstrated that the WRC and swelling of native wheat straw fibers rose significantly by hydration with a culture filtrate of an H<sub>2</sub>-producing microbial consortium [27]. Based on that result, the objective of this study was to determine the effects of hydration and particle size on the biodegradability of native wheat straw for direct H<sub>2</sub> production by a microbial consortium.

## Material and methods

### Substrate and hydration treatment

Wheat straw (*Triticum aestivum* L.) was used as the substrate and prepared according to [27]; Table 1 shows its chemical composition. Particle size was reduced using a ball mill, and the sample was separated into various fractions using screens; particles that were retained in the 0.212 mm and 3.35 mm-sieves were used.

The wheat straw was subjected to 2 separate hydration treatments: tap water for 10 h and a culture filtrate for 4 h. The substrate:medium ratio was 1:20, and the mixture was homogenized well and kept statically at room temperature (28 ± 5 °C). At the end of the hydration, the soluble fraction of the native wheat straw was released, yielding a reducing sugar concentration of 1.1 ± 0.23 g/L with tap water and 1.9 ± 0.45 g/L with the culture filtrate.

The culture filtrate was obtained from a 3-L New Brunswick fermentor under uncontrolled pH with an initial pH of 5.5 with native wheat straw as substrate (5% w/v), seeded with anaerobic digestate (20% v/v), and incubated for 5 days at 300 rpm and 37 °C. The culture was passed through a gauze filter and used directly in the hydration experiments. The

**Table 1 – Chemical composition of the native wheat straw.**

Component	Content (g/kg)
Total solids	956
Total volatile solids	860
Carbon	419
Fiber content	387
Protein	30.6
Total Kjeldahl nitrogen	4.4
Phosphorus	0.46
Ash	86

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