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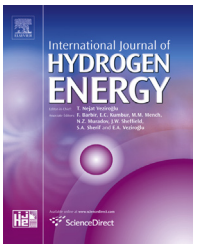

Microscopic analysis of wheat straw cell wall degradation by microbial consortia for hydrogen production

Marisol Pérez-Rangel [a], *Francisco R. Quiroz-Figueroa* [b,**], *Jaquelina González-Castañeda* [c], *Idania Valdez-Vazquez* [c,*]

[a] *Posgrado en BioCiencias, DICIVA, Universidad de Guanajuato CIS, Ex Hacienda El Copal Km. 9, Carretera Irapuato-Silao, Irapuato, Guanajuato, Mexico*

[b] *Instituto Politécnico Nacional, Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional Unidad Sinaloa (CIIDIR-IPN Unidad Sinaloa), Laboratorio de Fitomejoramiento Molecular, Blvd. Juan de Dios Bátiz Paredes no. 250, Col. San Joachin, C.P. 81101 Guasave, Sinaloa, Mexico*

[c] *Depto. de Ciencias Ambientales, DICIVA, Universidad de Guanajuato CIS, Ex Hacienda El Copal Km. 9, Carretera Irapuato-Silao, Irapuato, Guanajuato, Mexico*

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ABSTRACT

In nature, diverse microbial consortia degrade recalcitrant lignocellulosic substrates efficiently through poorly defined mechanisms. Their study can help to design a microbial consortium that performs a consolidated bioprocess for biofuel production. Microbial consortia from anaerobic sludge, epiphytic microorganisms, ruminal fluids, and soil were examined with regard to H_2 production from untreated wheat straw. Cell wall degradation in short cells and the stomata was analyzed by confocal laser scanning microscopy. On day 7, the highest rate and H_2 production were reached by native microflora. For all inocula, sugars from the hemicellulosic matrix were preferably consumed. The microscopic images showed that the cell wall in stomatal areas was degraded more extensively than in short cells. Also, fungal populations were detected in consortia with better H_2 production. Of the consortia that we tested, the epiphytic microorganisms were notable, because they delignified the lignocellulosic substrate and converted the hemicellulosic sugars into H_2 efficiently.

Introduction

The excessive use of fossil fuels has resulted in a global crisis, effecting environmental pollution, global warming, and ocean acidification. Dependence on these nonrenewable energy resources has caused economic and political uncertainty. To mitigate these global problems, the consumption of energy and natural resources must be drastically reduced, and our fossil fuel-driven economy must shift to a low-carbon

* *Corresponding author*. Present address: Unidad Académica Juriquilla, Instituto de Ingeniería, Universidad Nacional Autónoma de México, Blvd. Juriquilla 3001, 76230 Querétaro, Mexico. Tel.: +52 (442) 1926165; fax: +52 (442) 1926185.

** *Corresponding author*. Laboratorio de Fitomejoramiento Molecular, Depto. de Biotecnología agrícola, Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional Unidad Sinaloa, Instituto Politécnico Nacional (CIIDIR-IPN Unidad Sinaloa), Blvd. Juan de Dios Bátiz Paredes no. 250, Col. San Joachin, C.P. 81101 Guasave, Sinaloa, Mexico.

E-mail addresses: labfitomol@hotmail.com (F.R. Quiroz-Figueroa), IValdezV@iingen.unam.mx (I. Valdez-Vazquez).

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economy [1]. Such changes would thrive in a hydrogen-based economy. Hydrogen can be produced from renewable resources, such as wind, water, geothermal energy, solar energy, and biomass. Biomass accounts for 19.8% of renewable energies globally [2], and can be converted biologically into hydrogen when its sugar fraction is dark-fermented. Agricultural and agroindustrial byproducts, most of them lignocellulosic materials, are an important source of biomass, for example, in 2012 the world cereal production was 2300 million tons [3]. In Mexico, wheat straw (WS) is an important agricultural byproduct, it is estimated that ~10.14 million tons of dry WS are produced annually, concentrated in certain regions [4].

The conversion of WS into biohydrogen remains a significant challenge due to its high recalcitrance against biological degradation. Thus, most studies have used chemical and enzymatic pretreatments to release the sugar fraction—for example, hydrothermal pretreatment, ammonia fiber expansion, ozonation, acid pretreatment, enzymatic hydrolysis, and their combination. Then, the recovered sugars undergo dark fermentation. The hydrogen yields from pretreated WS range from 19 to 318 mL H_2 g added sugar^{-1} [5–11]. However, concerns remain with regard to the costs of the implementation of these processes on a large scale: the high cost of the reactors and pressure vessels for hot corrosive conditions, the high cost and consumption of enzymes, the removal of fermentation inhibitors that are formed during pretreatment, the generation of wastewaters that must be treated, and the costs of unconverted substrate [12]. Thus, simple inexpensive pretreatments methods must be developed.

Consolidated bioprocessing (CBP) is a technology in which pretreatment (enzyme production and saccharification) and fermentation of the biomass are carried out in a single unit, requiring minimal equipment and energy consumption and in principle achieving high substrate conversion [13]. A key factor in the development of CBP is the engineered microorganism—cellulase producers with improved fermentative activity constitute category I, and ethanol producers with improved cellulolytic activity are category II microorganisms [14]. Also, a microbial consortium can perform these tasks, the members of which have complementary metabolic activities.

In nature, there are several types of microbial consortia that perform complex functions that a single microorganism can not. In anaerobic environments, microbial consortia mineralize organic matter, and in the ruminant stomach, they convert lignocellulosic materials into organic volatile acids that are absorbed by the ruminant. Microbial consortia contribute to the recycling of fixed carbon in the biomass in forests [15,16]. How microbial consortia work in the wild remains poorly understood, and their study can guide the artificial design of a microbial consortium with the desired capacities.

The objective of this work was to study 4 wild microbial consortia that convert untreated WS into hydrogen by CBP: sludge from an anaerobic digester, the native microflora of WS, forest soil, and ruminal fluids. The metabolic activities were compared and analyzed with regard to yield, production rate, cell growth and cell wall degradation by confocal laser scanning microscopy (CLSM).

Materials and methods

Substrate and inocula

Furrow-irrigated wheat (*Triticum aestivum* L.; cultivar Urbina S2007) was grown in Pueblo Nuevo, Guanajuato, Mexico. The crop was planted in late May 2011, and the plants were harvested after 120 days. The winter WS was harvested mechanically, consisting of the uppermost portion of the straw. The ground WS samples (50 cm) were reground through a 2 ½ mesh (8 mm) on the mill to narrow the range of particle size and thus obtain homogeneous samples. The ground samples were stored indoors in an opaque plastic container in the air-conditioned laboratory (28 °C and 55% RH) prior to use. A sample of 400 g was passed through a set of Endecotts laboratory sieves (Endecotts, London) in an Endecotts sieve shaker for 20 min, and samples of 2 mm were selected for the tests. The chemical composition per kilogram of WS was 956 g total solids, 867 g volatile solids, 387 g cellulose, 190 g hemicellulose, 173 g lignin, 30.6 g extractives, 86 g ash [17,18].

Four wild microbial consortia were used as inoculum to convert untreated sterilize WS into hydrogen. 1) forest soil; 1 kg of soil was collected from an oak forest in the Sierra de Villa Madero, Michoacán, Mexico. The soil sample was stored in a plastic bag at ambient temperature (28 ± 5 °C) prior to use. 2) ruminal fluids; cow ruminal fluids (grass silage diet) were collected from a local municipal slaughterhouse immediately after the slaughter of the animal. The ruminal fluids were stored at 4 ± 1 °C prior to use. 3) anaerobic sludge: it was obtained from an anaerobic digester fed with cow manure: total solid content of 29.8 ± 0.9 mg L^{-1}, solid retention time of 28 d and volume of 1 m^2. The digester was installed in the experimental unit of the Laboratory of Technology for Sustainability, University of Guanajuato. The anaerobic sludge was stored at 4 ± 1 °C. For these treatments, large particles were trapped by the gauze filter and the content of volatile suspended solids was adjusted to the same value prior to use. Each filtrate was used as inoculum separately. 4) native microflora of WS; the reactors were loaded only with the unsterile WS and medium.

Batch tests for hydrogen production

Batch reactors consisted in 250 mL serological bottles with a working volume of 200 mL. The reactors were loaded with 40 mL of each filtrate (for the native microflora inoculum, unsterilized WS was loaded), 4.0 ± 0.1 g of tindalized WS (twice at 85 °C, 2 h with 48-h between the two treatments, [19]), and the final volume was completed with medium (composition per liter: yeast extract 5 g, KH_2PO_4 4.5 g, K_2HPO_4 0.7 g, $MgCl_2 \cdot 6H_2O$ 0.1 g, $MnSO_4 \cdot 6H_2O$ 15 mg, $FeSO_4 \cdot 7H_2O$ 25 mg, $CuSO_4 \cdot 5H_2O$ 5 mg, $CoCl_2 \cdot 5H_2O$ 0.125 mg). The initial pH was adjusted to 6.5. The reactors were sealed with atmospheric gas and incubated statically at 37 ± 0.5 °C. Fifteen identical batch reactors were prepared for each condition, three of them were taken for analysis on days 0, 3, 7, 10, and 14. Seeded treatments with culture medium alone were used as a control, the hydrogen from which was subtracted from those in the inocula treatments.

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