# Evaluation and isolation of natural anaerobic consortia for hydrogen production from wheat straw

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ABSTRACT

Hydrogen production via fermentation has great interest because the possibility to use a wide range of organic substrates such as lignocellulosic substrates derived from the agricultural sector. The anaerobic fermentation of cellulose rich substrates by microbial consortia has the main advantage of using untreated lignocellulosic materials in non-sterile conditions. The objective of this work was to evaluate the hydrogen production from wheat straw utilizing different types of natural anaerobic consortia, as well as to characterize the cultivable members of the consortia. Different reactors were inoculated with cow rumen, garden soil, sludge from an anaerobic digester, and the native microflora present on the substrate. The reactors with the higher hydrogen production were used for isolating of the members of the microbial consortia using selective media. The results showed that the highest hydrogen production was reached with the reactors inoculated with the anaerobic digester (175.6mL$H_2$/L) followed by the reactors with the native microflora (123mL$H_2$/L). The highest diversity of cultivable anaerobic microorganisms was found in the reactors with the native microflora present on the wheat straw isolating a total of 35 strains most of them growing on xylose as sole carbon source, some of the isolates shows a microscopy morphology similar to *Clostridium*. From the fermentations with the sludge from anaerobic digesters as inoculum, a total of 14 cultivable anaerobic microorganisms were obtained growing on cellulose, carboxymethylcellulose, glucose and wheat straw. These microorganisms will be identified and used to enhance the hydrogen yield from wheat straw.

Keywords: *Clostridium*, microbial consortia, wheat straw.

## 1. Introduction

Hydrogen is one of the options for renewable clean energy [1], its theoretical combustion only generates water therefore its use can reduce the green gases emission [2]. The hydrogen production via fermentation has an increasing attention because the high production rates and the possibility to use as raw matter different kind of organic wastes [3]. The agricultural wastes or lignocellulosic materials have a high potential for obtaining biohydrogen since they are abundant and widely distributed. In Mexico, already 150 million tons of dry matter are produced derived from agricultural wastes such as cornstover, sorghum straw, sugarcane baggasse and wheat straw [4]. The chemical composition of the lignocellulosic material shows a high content of fermentable carbohydrates in

form of cellulose and hemicellulose strongly bounded to lignin. The conversion of these sugars typically requires a pre-treatment and hydrolysis of the substrate before the fermentation is performed. The reported pre-treatments include acid or alkali hydrolysis, as well enzymatic hydrolysis [5, 6]. However, the use of these pre-treatments and/or enzymes increases the cost of the process [7]. As alternative, it is possible to utilize mixed cultures or microbial consortia that in natural way have the capacity to metabolize lignocellulosic substrates into hydrogen and soluble metabolites [8].

Diverse types of microbial consortia are present in the ecosystems interacting and performing complex functions that a single microorganism cannot [9]. For example, anaerobic microbial consortia carry on the mineralization of the organic matter to methane and carbon dioxide by the action of 5 different microorganism groups. The main characteristics of a microbial consortium are stability, interdependency and autoregulation [10]. The microbial consortia used as inocula for fermentative hydrogen production include those obtained from sludge from wastewater treatment plants, sludge from anaerobic digesters, compost, soil, cow cattle, silage, hot springs, among others [11-17]. These consortia are manipulated to select only those microorganisms with the capacity of produce hydrogen. For instance, the heat-shock treatment is the most common methods; the inoculum is boiled in a Maria bath already 60 min which kills the vegetative cells (some of them hydrogen consumers) and only survive the sporulating microorganisms as *Clostridium* and *Bacillus*, both hydrogen producers [18]. Other pre-treatment include alkalis, acids, chemical inhibitors, freezing, reactor acidification by substrate overloading, high dilution rates to wash out those hydrogen-consuming microorganisms [10]. However, in a premeditate way these pre-treatments decrease the microbial diversity which from an ecological point of view this fact could decrease the stability of the consortia and therefore the hydrogen production process. In this way, the objective of this work was to evaluate the hydrogen production from a lignocellulosic substrate utilizing different types of natural anaerobic consortia, as well as to characterize the cultivable members of the consortia.

**2. Methodology**

Four types of fermentations were performed utilizing as inocula cow rumen, sludge from an anaerobic digester, garden soil and the native flora present in the lignocellulosic substrate. Untreated wheat straw (*Triticum aestivum* L.) was used as substrate at a particle size of 2mm. The chemical composition was: 419g C/Kg, 4.4 gN/Kg, 86% SV, 8.6% ash, 38.7% crude fiber and 30g/kg protein. The reactors consisted in 250 mL glass flasks with a working volume of 200 mL and with an airtight seal. The reactors were loaded with 20% of each inocula, 20 g/L of unsterilized wheat straw and the volume was adjusted with mineral medium. The reactors were incubated at 37± 1.0 °C and samples for analysis were taken at 0, 7, 14, 21 and 28 days for each type of fermentation by triplicate.

The pH was determined with a potentiometer, microbial growth was determined by means protein with the Bradford method. The biogas accumulation was measured daily by a lubricated syringe placed and the hydrogen content was determined with gas cromatograph Clarus 580 Perkin Elmer with a TCD equipped with an Elite-GC GS Molesieve column. The temperatures of the injector, detector and column were 150, 200 and 50 °C, respectively. Ar was used

as carrier gas. The hydrogen production results were analyzed statistically with Statgraphiscs Centurion 15.2.1, with an ANOVA.

At the end of the incubation, each fermentation was used for the microorganism isolations. Petri dishes were prepared under anaerobic conditions using a sole carbon source: crystalline cellulose, carboxymetylcellulose (CMC), xylose, glucose or wheat straw. The composition of the media was: peptone 1g/L, $KH_2PO_4$ 4.4g/L, $K_2HPO_4$ 0.7g/L, $MgCl_2$ 0.1g/L, NaCl 2.0 g/L, $CaCl_2$ 0.5g/L, KCl 0.1g/L, L-cisteine 0.5g/L and resazurine 0.001g/L). The Petri dishes were spread with the effluents of each fermentation and incubated in an anaerobic glove chamber at 37°C during 96h.

**3. Results and Discussion**

*3.1 Hydrogen production*

The type of inoculum had a significant effect on the hydrogen production during the natural fermentations ($p$<0.05, Figure 1). At 14 days of incubation, the higher hydrogen production were displayed by the reactors inoculated with the anaerobic digesters (145.5mL$H_2$/$L_{reactor}$) followed by the fermentation with the native microflora (116.6mL$H_2$/$L_{reactor}$). It is important to highlight that in the fermentation inoculated with the anaerobic digester, the hydrogen production continued until day 25 unlike the rest of fermentations that stops the accumulation. So, the hydrogen production utilizing sludge from anaerobic digesters as inoculum can reach the highest hydrogen production of 175.6mL$H_2$/$L_{reactor}$ at day 25.

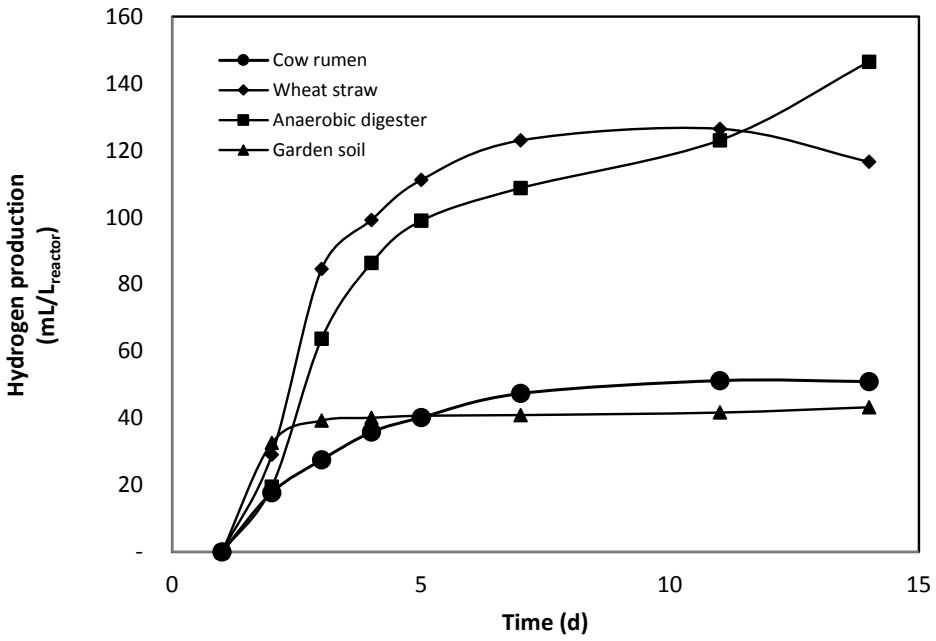


Figure 1. Kinetics of hydrogen production by different types of natural inocula.

Many investigations on hydrogen production have been conducted using refined substrates such as glucose, sucrose, starch and cellulose [19-22]. However, in recent years the use of lignocellulosic materials is increasing due to their high content of sugars and availability. To this respect, microbial consortia have the natural ability to convert directly these materials into hydrogen without a previous pre-treatment, this could reduce dramatically the operation costs at industrial scale. The results obtained in the present work shows that the type of inocula had a significant effect on the hydrogen yield (Table 1). The maximum yield was 9.0 obtained by using sludge from an anaerobic digester as inoculum. This results is close to that reported with heat-shocked anaerobic sludge and higher than those from other studies.

Table 1. Comparison of studies of batch hydrogen production from untreated wheat straw by microbial consortia.

| **Inoculum** | **Pretreatment (inoculum)** | **Temperature (°C)** | **Hydrogen yield (mL $H_2$/g VST)** | **Ref.** |
|---|---|---|---|---|
| Cow dung compost | Infrared | 36 | 1 | [23] |
| CSTR $H_2$ sludge | None | 35 | 6.4 | [24] |
| Anaerobic sludge | Heat-shock | 37 | 10.5 | [25] |
| Sludge from a anaerobic digester | None | 37 | 9.0 | This study |
| Native microflora of wheat straw | None | 37 | 5.9 | This study |
| Cow rumen | None | 37 | 3.5 | This study |
| Garden soil | None | 37 | 2.9 | This study |

*3.2 Isolation of anaerobic microorganisms*

From the natural fermentations, it were selected those with the higher hydrogen accumulation, i.e., those inoculated with the anaerobic digester, native microflora and cow rumen. Table 2 shows the number of anaerobic isolates obtained from each type de natural fermentation.

Table 2. Anaerobic isolates obtained from the natural fermentations.

| | Type of inocula | | |
|---|---|---|---|
| **Carbon source** | **Cow rumen** | **Anaerobic digester** | **Wheat straw** |
| Cellulose | 1 | 3 | 4 |
| CMC | 0 | 5 | 9 |
| Wheat straw | 1 | 3 | 8 |
| Xylose | 1 | 0 | 11 |
| Glucose | 2 | 3 | 3 |
| ***Total*** | ***5*** | ***14*** | ***35*** |

A total of 54 anaerobic strains were obtained. The most strains were isolated from the fermentation with the native microflora on the wheat straw. These microorganisms were naturally presents and could be better adapted for the substrate degradation and conversion into hydrogen. Also, an anaerobic fungus was isolated from the fermentation with the native microflora growing on media with CMC, xylose, wheat straw and cellulose (Figure 2).

Figure 2. Anaerobic fungus isolated from the fermentation with native microflora.

The isolated strains were observed under the optical microscope finding that some of them have spores which are distinctive of the genera Clostridia, microorganisms producers of $H_2$ (Figure 3).

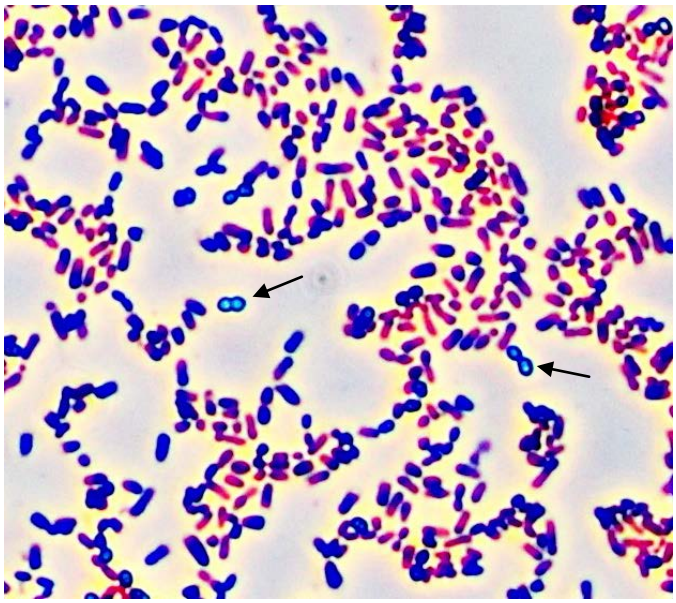

Figure 3. Microscopic morphology of strain isolated from the fermentation with native microflora.

**4. Conclusions**

The type of inoculum has a very significant effect on the hydrogen production and yield from lignocellulosic materials. The higher hydrogen yields were obtained by using sludge from an anaerobic digester and the native microflora present on the substrate. These inocula were better adapted to grow and convert the substrate into hydrogen. The highest diversity of cultivable microorganisms was found in the fermentation with the native microflora, a fungus and several bacterial strains were obtained.

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