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Methane production from acid hydrolysates of *Agave tequilana* bagasse: Evaluation of hydrolysis conditions and methane yield

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HIGHLIGHTS

- Acid hydrolysis was evaluated by RSM on two types of *Agave tequilana* bagasse.
- CH_4 production from hydrolysates was evaluated with and without nutrients in AnSBRs.
- AnSBR operated without nutrient addition presented a constant CH_4 yield.
- AnSBR operated with nutrient addition presented a gradual CH_4 suppression.
- Nutrient addition promoted CH_4 suppression by affecting archaeal/bacterial ratio.

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ABSTRACT

Evaluation of diluted acid hydrolysis for sugar extraction from cooked and uncooked *Agave tequilana* bagasse and feasibility of using the hydrolysates as substrate for methane production, with and without nutrient addition, in anaerobic sequencing batch reactors (AnSBR) were studied. Results showed that the hydrolysis over the cooked bagasse was more effective for sugar extraction at the studied conditions. Total sugars concentration in the cooked and uncooked bagasse hydrolysates were 27.9 g/L and 18.7 g/L, respectively. However, 5-hydroxymethylfurfural was detected in the cooked bagasse hydrolysate, and therefore, the uncooked bagasse hydrolysate was selected as substrate for methane production. Interestingly, results showed that the AnSBR operated without nutrient addition obtained a constant methane production (0.26 L CH_4/g COD), whereas the AnSBR operated with nutrient addition presented a gradual methane suppression. Molecular analyses suggested that methane suppression in the experiment with nutrient addition was due to a negative effect over the archaeal/bacterial ratio.

1. Introduction

High dependence on fossil fuels to supply world energy needs has triggered environmental problems and energy crisis. Therefore, research on alternative energy sources has increased in recent years (Parisutham et al., 2014). In this regard, the use of lignocellulosic biomass is especially attractive, since it is abundant and included in the global carbon cycle (Kumar et al., 2009; Menon and Rao, 2012). Moreover, the lignocellulosic biomass can be obtained as a residue from a wide range of industrial processes; such as the *Agave tequilana* bagasse, which is one of the main residues generated by the tequila industry, resulting in an inexpensive feedstock (Caspeta et al., 2014; Saucedo-Luna et al., 2011, 2010).

Agave species are plants considered to have high potential for biofuels and alcoholic beverages production due to their high polysaccharides content and high drought resistance, which make them advantageous over other crops (Davis et al., 2011; Escamilla-Treviño, 2012; Núñez et al., 2011). The *A. tequilana Weber* blue variety is used as feedstock for tequila production in some regions of Mexico. According to the Tequila Regulatory Council, in 2013 the consumption of *A. tequilana* for tequila

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production was estimated at 756.9×10^3 tons, from which, approximately 40% corresponded to bagasse (Saucedo-Luna et al., 2011). Thus, the *A. tequilana* bagasse is a lignocellulosic residue from the tequila industry that has great potential for biofuels production because of its suitable composition and high production volume.

The *A. tequilana* bagasse is produced in either the traditional or the alternative tequila production processes. Regarding the traditional process, it consists of four main steps: cooking of the *A. tequilana* heads, sugar extraction by grinding (where the bagasse is generated), fermentation and distillation. In contrast, the alternative process consists on the following four steps: syrup extraction of the *A. tequilana* heads by grinding (where the bagasse is generated), hydrolysis of the syrup, fermentation and distillation. It is important to mention that in order to favor the production of biofuels from lignocellulosic biomass, such as *A. tequilana* bagasse, hydrolysis of its polysaccharides is required due to its recalcitrant structure (Monlau et al., 2014).

Even though several hydrolytic methods have been developed for sugar recovery from different lignocellulosic biomasses (Kumar et al., 2009; Taherzadeh and Karimi, 2008; Vandenbossche et al., 2014), dilute acid hydrolysis is the most used method due to its effectiveness and low cost (Kumar et al., 2009; Saucedo-Luna et al., 2010). Typically, during dilute acid hydrolysis most of the hemicellulose fraction of the lignocellulosic biomass is hydrolyzed to monosaccharides and oligosaccharides; however, depending on the intensity of the hydrolysis conditions (temperature, reaction time and acid concentration), toxic byproducts formation (furan and phenolic compounds) may also occur (Saucedo-Luna et al., 2010). Thus, in order to increase the sugar yield, while maintaining a low generation of toxic byproducts, evaluation of the acid hydrolysis under different conditions is required prior to biofuel production.

Due to the fact that main sugars from lignocellulosic biomass are hexoses (glucose, mannose and galactose) and pentoses (xylose and arabinose), bioethanol production from hydrolysates of *A. tequilana* bagasse is a limited option, since pentoses are barely consumed by yeast (Hahn-Hägerdal et al., 2007; Young et al., 2010). Conversely, anaerobic digestion is an excellent alternative for energy recovery from lignocellulosic hydrolysates, since microbial consortiums involved in this process present interesting consumption rates for both, hexoses and pentoses (Arreola-Vargas et al., 2014; Gomez-Tovar et al., 2012).

It is worthy to mention that even though vinasses from the tequila industry (a liquid residue from the fermentation step) have been previously evaluated as substrate for methane production (Méndez-Acosta et al., 2010), to the best of our knowledge there is no report in the current literature that evaluates the use of *A. tequilana* bagasse hydrolysates as feedstock for methane production. Therefore, this work aimed to evaluate the feasibility of using acid hydrolysates from *A. tequilana* bagasse for methane production. During the first part, different hydrolysis conditions were evaluated on both types of *A. tequilana* bagasse (obtained from the traditional and alternative tequila production processes) by using the response surface methodology in order to determine the hydrolysis conditions that render the highest sugar yield but without losing sight of maintaining a low generation of toxic byproducts. On the other hand, the second part was devoted to evaluate the feasibility of using the hydrolysates as substrate for methane production in anaerobic sequencing batch reactors (AnSBR) under two conditions, with and without nutrient addition. Finally, molecular analysis were carried out in order to correlate the AnSBR performances with the *Archaea* and *Bacteria* population dynamics by using capillary electrophoresis single-stranded conformation polymorphism (CE-SSCP) and quantitative polymerase chain reaction (Q-PCR).

2. Methods

2.1. Evaluation of the hydrolysis conditions

2.1.1. A. tequilana bagasse and hydrolysis procedure

The *A. tequilana* bagasse was obtained from two different processes of tequila production at Casa Herradura distillery (Amatitan-Jalisco, Mexico), the traditional process in which the *A. tequilana* heads are cooked (cooked bagasse) and an alternative process in which the *A. tequilana* heads are uncooked (uncooked bagasse). Prior to hydrolysis, both types of *A. tequilana* bagasse were dried at room temperature and their fiber sizes were reduced to an average length of 1 cm.

Even though, several studies have reported the use of H_2SO_4 for the acid hydrolysis of different types of lignocellulosic biomass (Kumar et al., 2009), the presence of sulfate in the anaerobic digestion process could promote the growth of sulfate-reducing bacteria, which are electron donor competitors of methanogens (Isa et al., 1986). In fact, the acid hydrolysis of *A. tequilana* bagasse by using H_2SO_4 (1–3% w/w) and high temperatures (100–200 °C) has been previously reported (Saucedo-Luna et al., 2010). Nonetheless, a recent study on the use of lignocellulosic hydrolysates for methane production reported the use of HCl instead of H_2SO_4, as well as lower temperatures (90 °C) for sugar recovery from oat straw (Gomez-Tovar et al., 2012). Therefore, dilute acid hydrolysis was carried out by resuspending the different types of bagasse at 5% (w/v) in a dilute HCl solution. The reaction took place in an oven at controlled temperature and for specific time periods. Different temperatures, HCl concentrations and reaction times were evaluated according to the experimental design presented in Section 2.1.2. At the end of the hydrolysis, the hydrolysate was filtered through a 0.45 μm membrane for its analysis.

2.1.2. Experimental design

A central composite experimental design was proposed in order to evaluate the effect of temperature, HCl concentration (%, w/w) and hydrolysis time over the sugar recovery during the hydrolysis of both types of *A. tequilana* bagasse. Center points for temperature, HCl concentration and hydrolysis time were set at 90 °C, 2 % (w/w) and 2 h, which were based on the values reported by Gomez-Tovar et al. (2012), while the step change values were set at 20 °C, 0.5 % (w/w) and 1 h, respectively. The central composite experimental design was completed with six axial and five central points (Table 1).

The experimental design was performed by using coded values. The relation between coded and actual values is given by the following equation: $x_i = (X_i - X_i^*)/\Delta X$, where x_i is the coded value of the ith test variable, X_i is the uncoded or actual value of the ith test variable, X_i^* is the uncoded value of the ith test variable at the center point, and ΔX_i is the step change value (Davila-Vazquez et al., 2008). Experimental results obtained with the central composite experimental design were analyzed by using the response surface methodology (Davila-Vazquez et al., 2008; Saucedo-Luna et al., 2010). During the analysis of the response surface methodology, the relationship between the independent (temperature, HCl concentration and hydrolysis time) and the response variable (total sugars) was described by the following second degree quadratic polynomial equation: $Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 + \sum \sum \beta_{ij} x_i x_j$, where β_0 is the constant of the model, β_i is the linear coefficient, β_{ii} is the quadratic coefficient, β_{ij} is the interaction coefficient, Y is the response variable and x are the independent variables that have influence on the response variable (Davila-Vazquez et al., 2008).

It is important to remark that all the experiments were run in triplicate and that reliability of the polynomial model was