Biomass and Bioenergy 117 (2018) 78–85

Contents lists available at ScienceDirect

Biomass and Bioenergy

journal homepage: www.elsevier.com/locate/biombioe

Research paper

Metagenomic analysis and optimization of hydrogen production from sugarcane bagasse

Laís Américo Soares[a,*], Camila Abreu Borges Silva Rabelo[a], Isabel Kimiko Sakamoto[a], Tiago Paladino Delforno[b], Edson Luis Silva[c], Maria Bernadete Amâncio Varesche[a]

[a] University of São Paulo, João Dagnone Avenue, 1100, 13563-120, São Carlos, SP, Brazil
[b] Research Center for Chemistry, Biology and Agriculture, 13081-970, Campinas, SP, Brazil
[c] Federal University of São Carlos, Rod Washington Luis, Km 235, 13565-905, São Carlos, SP, Brazil

ARTICLE INFO

Keywords:
Hydrogen production
Hydrothermal pretreatment
Lignocellulosic biomass
Metagenomic analysis
Response surface methodology

ABSTRACT

The substrate and yeast extract (YE) concentrations of a batch reactor were changed according to a central composite design in order to optimize the conversion of sugarcane bagasse (SCB) into hydrogen. The optimum hydrogen production (1.50 mmol/L) was obtained using 2.77 and 5.84 g/L of YE and SCB, respectively. Taxonomic analysis using the SEED database indicated that *Clostridium* (33% of total communities) and *Methanothermobacter* (40% of archaeal community) were the most abundant genera in the high hydrogen performance reactor. Key microorganisms and related pathways involved in all steps of the anaerobic digestion were further revealed and may help drive sugarcane bagasse bioconversion.

1. Introduction

Lignocellulosic biomass is abundant, cheap and biodegradable feedstock to generate value-added products by anaerobic digestion process [1]. Sugarcane bagasse (SCB) is one of the major lignocellulosic by-products generated in the Brazilian sugarcane industry (250 kg bagasse per each ton of processed sugarcane). It is estimated that there is sugarcane bagasse surplus of 12.0 and 8.0% in autonomous or annexed sugar mills and ethanol distilleries, respectively [2], justifying its application in biological process for the conversion into value-added chemicals [3].

SCB mainly consists of cellulose, hemicellulose and lignin. Cellulose is a polysaccharide of cellobiose (glucose disaccharide) linked via beta-1,4 glycosidic linkages. Cellulose chains are interlinked by van derWaals forces and hydrogen bonds forming high tensile strength microfibris. It is formed by amorphous (low crystallinity) and crystalline (high crystallinity) regions. Hemicellulose links the cellulose microfibrils and both are covered by lignin [4]. This complex structure renders SCB resistant to biological and chemical attacks. In this sense, pretreatments which break down the lignocellulosic biomass, and make it easily hydrolyzed into fermentable sugars [5] are a key step for SCB bioconversion.

There are many different pretreatments such as physical, chemical, and biological approaches. Hydrothermal treatment, in which the SBC is maintained in pressurized liquid hot water and then quickly depressurized, is an attractive option to improve the breakdown of hemicellulose into free sugars, favoring its conversion into biotechnological products by fermentative bacteria [6]. Besides the pretreatment, there are many other factors that affect the bacterial growth in anaerobic bioreactors, such as substrate and nitrogen availability. Yeast Extract (YE) is a nitrogen source which stimulates the cellular bacterial growth and consequently improves hydrogen production [7]. The type of substrate and concentration also affects the biological hydrogen production [8]. Therefore, the identification of optimal nutrient and substrate concentration for improving hydrogen production is a knowledge gap in lignocellulosic biomass bioconversion.

The taxonomic and functional profiles of the microorganisms responsible for lignocellulosic biomass bioconversion are scarce, although information about the composition and activity of the microbial consortium is critical to optimize reactor performance.

The main molecular approach applied to analyze the microbial community structure in bioreactors include fluorescence in situ hybridization (FISH), denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (TRFLP) and the sequencing of 16 S rRNA gene amplicons [9]. To evaluate the microbial communities related to linear alkylbenzene sulfonate biodegradation.

* Corresponding author.
E-mail addresses: americo.lais@gmail.com (L.A. Soares), camilasilvarabelo@gmail.com (C.A. Borges Silva Rabelo), sakamoto@sc.usp.b (I.K. Sakamoto), tiago.palladino@gmail.com (T.P. Delforno), edsilva@ufscar.br (E.L. Silva), varesche@sc.usp.br (M.B.A. Varesche).

https://doi.org/10.1016/j.biombioe.2018.07.018
Received 22 January 2018; Received in revised form 3 July 2018; Accepted 25 July 2018

However this tools does not provide consistent insights about the functional profile [10] and exhibits many limitations, such as several artifacts related to PCR amplification and sequencing errors [11]. In this sense, the application of the metagenomics approach is imperative to correlate the phylogenetic and metabolic potential of microbial communities.

Therefore, in this paper, response surface methodology (RSM) was carried out to optimize the hydrogen production using hydrothermal SCB as substrate. Finally, the microbial communities from the high hydrogen performance batch reactor was evaluated by the metagenomic approach, providing a clear understanding of the taxonomic and functional profile from the microorganisms and to establish a collection of cellulolytic, fermenter and methanogenic microbial genomes that will simplify future studies regarding the bioconversion of complex biomass into biotechnology products.

2. Material and methods

2.1. Microorganisms and culture conditions

The anaerobic consortium was obtained from a mixed culture from soil and composting residue (4.86 g/L of total solids, 2.55 g/L of volatile solids). To obtain the fermentative and cellulolytic consortium, food-waste composting was collected in University of São Paulo at São Carlos School of Engineering (EESC/USP), Brazil, where this research takes place. Similarly, soil samples were collected close to a sugarcane plantation near EESC/USP. For this, 100 g of soil and composting residue were sieved (mesh of 2 mm) and then added in Erlenmeyer flask containing 900 mL of dissociation solution (0.1% sodium pyrophosphate and 0.1% Tween 80), 50 g of glass beads (5 mm) and maintained under stirring for 20 min at room temperature. The liquid fraction was used as inoculum for batch reactors. Each inoculum (soil and composting residue) were acclimated separately for 48 h in 300 mL of cellulose anaerobe medium [12], supplemented with 1 g/L yeast extract and 1 g/L carboxymethyl cellulose Sigma-Aldrich® (St. Louis, USA). The pH was adjusted to 6.0 and the incubation temperature was 37 °C. The cellulose anaerobe medium consisted of (g/L) $NaHCO_3$, 2.1; NH_4Cl, 6.8; $(NH_4)_2SO_4$, 0.15; $MgSO_4$, 0.12; KH_2PO_4, 0.18; K_2HPO_4, 0.296; $CaCl_2$, 0.061; $Fe_2(SO_4)_3$, 0.021; $C_6H_9NO_6$, 0.015; NaCl, 0.1; $MnSO_4$, 0.005; $CoCl_2$, 0.0001; $ZnSO_4$, 0.0001; $CuSO_4$, 0.0001; AlK(- $SO_4)_2$, 0.0001; H_3BO_3, 0.0001; Na_2MoO_4, 0.0001; and vitamin solutions (10 ml/L). Finally, both inoculums (50-50% v-v) were mixed in a single reactor.

2.2. Sugarcane bagasse pretreatment

The sugarcane bagasse (SCB) was obtained from São Martinho sugar mill (Pradópolis, Brazil). The SCB was pretreated using a hydrothermal system of 100 mL capacity at 200 °C for 10 min at 16 bar. After the hydrothermal pretreatment the solid phase was separated from the liquid phase by vacuum filtration; the SCB fiber was air dried until constant weight, stored at room temperature, and used as substrate in batch reactors.

2.3. Central composite design

CCD was used to optimize the hydrogen production from hydrothermal pretreated SCB. Two independent variables, namely yeast extract concentration and SCB concentration were studied at five levels (± 1; 0; ± 1.42) with three repetitions at the central point and two replicates at axial and factorial points. The variable ranges (center point) of YE concentration and SCB concentration were determined to be 2 and 5 g/L, respectively (Table 1), resulting in total of 11 conditions. The analysis of the experimental data were performed using the Statstica 7.0 software. Test factors of X_i were coded as x_i and appeared in the equation (1).

Table 1
Five-level factorial design.

Batch reactor	Normalization	Yeast Extract	Normalization	SCB
		g/L		g/L
C1	+1	3.00	+1	6.00
C2	−1	1.00	+1	6.00
C3	+1	3.00	−1	4.00
C4	−1	1.00	−1	4.00
C5	0	2.00	0	5.00
C6	0	2.00	0	5.00
C7	0	2.00	0	5.00
C8	+1.42	3.42	0	5.00
C9	−1.42	0.58	0	5.00
C10	0	2.00	+1.42	6.42
C11	0	2.00	−1.42	3.58

$$xi = (X_i - X_0) = \Delta X \quad (1)$$

Where X_i is the value of the independent variable; x_i is the coded value of the variable X_i; X_0 is the value of Xi at the center point, and ΔX is the step change value. A linear model was used to optimize the media compositions (Equation (2)), using the significant coded values.

$$Y = b_0 + b_1X_1 + b_2X_2 \quad (2)$$

Where Y is the predicted response; b_0 is a constant; X_1 and X_2 are the dependent variables (YE and SCB, respectively) b_1 and b_2 are the linear coefficients for the dependent variables (X_1 and X_2, respectively).

2.4. Batch reactors

The experiments were performed in triplicate in the 1 L batch reactor with 500 mL working volume composed of culture medium [12], inoculum 10% v/v and the substrate, and 500 mL of headspace submitted to N_2 (100%) for 10 min. The pH of all experiments was adjusted to 6.0. The reactors were incubated at 37 °C for 400 h, until the hydrogen plateau was reached.

2.5. Analytical methods

The hydrogen and methane were determined by gas chromatography using a GC 2010 Shimadzu system equipped with a thermal conductivity detector (TCD) and a Carboxen 1010 PLOT column (30 m × 0.53 mm) according to [13]. The organic acids and ethanol were analysed by high-performance liquid chromatography (HPLC) equipped with a UV diode array detector (SPD-M10 AVP), a refraction index detector (RID-10 A), a CTO-20 A oven an LC-10 ADVP Pump, an SCL 10 AVP control and column HPX-87H from Aminex, with 300 mm × 7.8 mm (BioRad). The mobile phase consisted of H_2SO_4 (0.01 N) at 0.5 mL/min flow rate [14].

2.6. Kinetic analysis

The experimental data was adjusted to the average values obtained from triplicates using the software package Statistica® 8.0. The data of the accumulated hydrogen production was adjusted using the Gompertz equation, modified by Ref. [15] (Equation (3)).

$$H = P \cdot \exp\left\{-\exp\left[\frac{R_m \cdot e}{P}(\lambda - t) + 1\right]\right\} \quad (3)$$

Where: P – Potential production of H_2 (mmol/L), Rm – Production Rate of H_2 (mmol/L.h), t – time of incubation (h), e – Euler number (2.71828) and λ – time to start the H_2 production (h).