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Biohydrogen production from mixtures of agro-industrial wastes: Chemometric analysis, optimization and scaling up

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ABSTRACT

Cheese whey (CW) and wheat straw hydrolysate (WSH) were used to produce biohydrogen by anaerobic co-digestion of multiple substrates. In this work, the influence of pH, temperature, substrates concentrations on the biohydrogen production was explored with the application of the principal component analysis (PCA) and the hierarchical clustering analysis (HCA), allowing the identification of the main clusters and the uniqueness of some experiments. Response surface methodology (RSM) was used to evaluate the individual and interactive effects of pH, temperature, CW concentration and WSH concentration in the fermentation. Optimal operational conditions obtained by RMS were 5 g L^{-1} WSH, 25 g L^{-1} CW, 26.6 °C and pH 7.25. With these conditions was expected 5724.5 mL H_2 L^{-1}. When optimal conditions were tested using 0.11-L anaerobic serological bottles, 1-L and 4-L bioreactors the results obtained for biohydrogen production were 4554.5 ± 105, 3685 ± 305 and 4132.3 ± 151 mL H_2 L^{-1}, respectively; on the other hand, the biohydrogen production rate was improved from 66.6 to 89.5 mL H_2 L^{-1} h^{-1}. Results demonstrate that it is possible to use WSH and CW, both individually and in combination, as a substrate for the production of biohydrogen.

1. Introduction

Exhaustion of fossil fuel resources and environmental damages owing to petroleum production and its consumption highlight the importance of a shift to renewable sources for fuels. Bioenergy production from organic wastes is becoming an essential component in the overall development of sustainable energy sources. The biological processes to produce hydrogen are environmentally friendly and can convert a wide variety of abundant organic biomass at low cost. In particular, biological production of hydrogen by dark fermentation can be emphasized for its large use of sustainable substrates, the high hydrogen production rates, and its simplicity of operation [1,2]. The dark fermentation can be defined as the partial oxidation of carbohydrates without external electron acceptor. This process also produces by-products such as fatty acids and solvents, thus there is an opportunity for further combination with other processes that yield more bioenergy. Dark fermentation can be carried out by mixed cultures of bacteria, like *Prevotella*, *Lactobacillus*, *Clostridium*, *Selenomonas*, *Megasphaera,* and *Enterobacter* genera [3–5].

Organic wastes are abundant sources of renewable and low cost substrate that can be efficiently fermented by microorganisms. The main criteria for the selection of waste materials to be used in biohydrogen production are the availability, cost, carbohydrate content and biodegradability. Simple sugars such as glucose, sucrose and lactose are readily biodegradable and preferred substrates for hydrogen production. However, pure carbohydrate sources are expensive raw materials for hydrogen production [6]. The advantages of using organic wastes for biohydrogen production are: reduction of CO_2 and other pollutants emissions, added value agricultural wastes, partial substitution of fossil fuels with sustainable biomass fuels, and reduction of environmental and economic costs for diverging the disposition of municipal solid wastes [2]. The production of renewable energy, a reduction of waste and prevention of environmental pollution promote the industrial application of anaerobic co-digestion for the treatment of agro-industrial organic wastes. Co-digestion is defined as the anaerobic treatment of a mixture of at least two different waste types with the aim of improving the efficiency of the anaerobic digestion process [7]. Due to the ability of dark fermentation to use complex

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substrates as livestock, crop residues, wastes and wastewater, there are several opportunities to develop co-digestion of two or more substrates with supplementary characteristics. Co-digestion can be used to enhance the dark fermentation process due to a better carbon and nutrient balance; in addition, it has other potential benefits such as dilution of toxic compounds, synergistic effect of microorganisms and better biogas yield. Furthermore, in the research about co-utilization of different carbon sources by bacteria is important to reveal the role of each carbon in bacterial physiology and how it enhances biohydrogen production [8–11].

According to FAO, in 2013 there was reported a production of 71.6×10^7 ton of wheat, whose waste contains approximately 8.73×10^6 ton of nutrients, and 2.5×10^6 ton of cheese whey [12]. In Mexico, SIACON-SIAP reported in 2011 a production of 4.4×10^6 ton of wheat straw and 1.9×10^5 ton of milk of which it is estimated that 4.4×10^3 are cheese whey approximately [13]. The wheat straw is rich in cellulose (35–45%), hemicellulose (20–30%) and lignin (18-15%) [14], its pretreatment is necessary to break down the lignocellulose into the three major polymeric constituents [15]. The thermal pretreatment of biomass results in two major streams: the solid fraction mainly consisting of cellulose (hexose: glucose) and liquid phase (hydrolysate) mainly constituted of hemicellulose (pentose: xylose and arabinose) [16]. Meanwhile cheese whey (CW) is a liquid that separates from the milk coagulation during cheese manufacture and corresponds to around 85–90% of the total volume of processed milk. This residue is one of the polluting residues in the dairy industry that can negatively affect the environment and biological processes during wastewater treatment [17]. In a dry basis, bovine whey contains 70–80% of lactose, 9% of proteins, 8–20% of minerals and other minor components, such as some hydrolyzed peptides of *k*-casein and lipids [18]. Therefore, the treatment of the degradable fraction of solid wastes, allows the generation of carbon-neutral bioenergy, nutrients and other resources or valuable products [2].

Since initial pH, temperature, substrate concentration, inoculum type, macronutrients and micronutrients impacts the biohydrogen production; the optimization of the operating conditions of bioreactors stills is a key parameter to improve the production of this energy carrier [19–21]. The optimization of operational conditions can be achieved by using chemometrics approaches through the application of experimental design, response surface methodology and multivariate data analysis. Response surface methodology consists of a group of mathematical and statistical techniques that are based on the fit of empirical models to the experimental data obtained in relation to the experimental design. The procedures are based on the simultaneous variation of numerous factors (independent variables) to a specific number of levels and possible combinations of these levels are used to evaluate the response (dependent variable) in order to determine the effect of individual factors and their interactive influences. The optimization is simple to perform and enables the optimum conditions to be found with a reduced number of experiments. The multivariate data analysis, such as principal component analysis (PCA) and hierarchical clustering analysis (HCA) are used to process a large number of data, assisting the interpretation of the results [22–24].

As mentioned before, co-utilization of different organic material in the fermentation complements the bacterial nutritional requirements, for instance typical wheat straw hydrolysates (WSH) are nitrogen and mineral deficient and they can be obtained from the cheese whey. Therefore, the goal of this work was to study the biohydrogen production by dark fermentation by using two typical agroindustrial wastes as carbon sources with a chemometric approach through the application of a response surface methodology and multivariate data analisys.

2. Material and methods

2.1. Substrates and inoculum

CW was purchased from Land O'Lakes Inc. (Arden Hills, Minnesota) and WSH was obtained from CUCBA (University of Guadalajara, Jalisco, Mex). The lactose content of CW solution was 6.9 g L^{-1}. To obtain WSH, wheat straw was slurred in diluted H_2SO_4 (0.75% v/v) at 4% (w/v) and pre-treated at 121 °C for 1 h in a steam sterilizer with heating and cooling ramps of 30 min each. The liquid fraction was recovered and the samples were taken, it was centrifuged at 10,000 rpm and concentrated by evaporation at 70 °C [25]. WSH contained per litter: total reducing sugars (TRS) 21 g, glucose 1.54 g, xylose 13.96 g, arabinose 1.93 g, furfural 0.12 g, formic acid 1.01 g, and acetic acid 3.6 g. Anaerobic granular sludge was obtained from a wastewater treatment plant in San Luis Potosi, Mexico. The granular sludge was washed with three volumes of tap water and then boiled for 40 min to inactivate methanogenic microflora according to Davila-Vazquez et al. [26] and stored at 4 °C before use.

2.2. Experimental design

A Central Composite experimental design with six central points (Table 1) was used to find the optimal conditions for biohydrogen production using mixtures of CW and WSH as substrate. The independent variables were pH, temperature and concentration of CW and WSH. Three levels for each variable were included and 2 star points. The response variable was biohydrogen production (H_2). The experiments were performed in 120 mL anaerobic serological bottles with a working volume of 110 mL, all bottles containing medium B [26] and 2.75 g L^{-1} yeast extract. The temperature and initial pH, as well as CW and WSH concentrations used in each experiment were determinate by the central composite experimental design. The cultures were shaken at 175 rpm during the period of experiment until no generation of biohydrogen was observed. Consequently, the data was analyzed by the response surface methodology (RSM). Analysis of variance (ANOVA), RSM and the optimum conditions were performed using Design-Expert® Version 7.0 (Stat-Ease, Inc.). The ANOVA *F* test was used to assess the adjusted models. The significance of each coefficient was determined with the *t*-test with a *P* value less than 0.05.

2.3. Batch cultures on bioreactor

Batch fermentations were performed using a mixture of WSH and CW (25 g L^{-1} and 5 g L^{-1}, respectively) in 1-L and 4-L bioreactors (Applikon, Foster City, CA) equipped with two six-blade Rushton turbines, pH was monitored using an autocleavable electrode (Applikon) and controlled at 6.5 by a Bioconsole ADI 1035/ Biocontroller 103 (Applikon). BioXpert 1.3 software (Applikon) was used for data acquisition. The experiments were performed at 26.6 °C with a initial pH of 7.25 and stirred at 175 rpm. Culture samples of 1 mL were taken every 4 h from the bioreactors and centrifuged at 600 rpm. The supernatant was filtered through a 0.22 μm syringe filter (Millipore, Bedford, MA, USA) before analysis of fermentation products.

2.4. Analytical methods

Total reducing sugars (TRS) analysis was performed by the dinitro-salicylic acid (DNS) method, with some modifications as follows: 0.25 mL of WSH with 0.75 mL of DNS reagent (10 g L^{-1} NaOH, 200 g L^{-1} $KNaC_4H_4O_6 \cdot 4H_2O$, 0.5 g L^{-1} $Na_2S_2O_5$, 2 g L^{-1} C_6H_6O, 10 g L^{-1} 3,5-Dinitrosalicylic acid) were heated for 15 min in a