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Selective adaptation of an anaerobic microbial community: Biohydrogen production by co-digestion of cheese whey and vegetables fruit waste

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ABSTRACT

The co-digestion process of crude cheese whey (CCW) with fruit vegetable waste (FVW) for biohydrogen production was investigated in this study. Five different C/N ratios (7, 17, 21, 31, and 46) were tested in 2 L batch systems at a pH of 5.5 and 37 °C. The highest specific biohydrogen production rate of 10.68 mmol H_2/Lh and biohydrogen yield of 449.84 mL H_2/g COD were determined at a C/N ratio of 21. A pyrosequencing analysis showed that the main microbial population at the initial stage of the co-digestion consisted of *Bifidobacterium*, with 85.4% of predominance. Hydrogen producing bacteria such as *Klebsiella* (9.1%), *Lactobacillus* (0.97%), *Citrobacter* (0.21%), *Enterobacter* (0.27%), *and Clostridium* (0.18%) were less abundant at this culture period. The microbial population structure was correlated with the lactate, acetate, and butyrate profiles obtained. Results demonstrated that the co-digestion of CCW with FVW improves biohydrogen production due to a better nutrient balance and improvement of the system's buffering capacity.

Introduction

The problems of global warming and fossil fuel exhaustion caused an urgent need for the development of clean and renewable energies. Hydrogen has been proposed as a clean energy carrier and as potential replacement to fossil fuels, since it has the highest energy content and generates no other products than water when burned [1]. For hydrogen to be considered a sustainable alternative fuel, it should be generated from cheap and readily available feedstocks that are renewable or potentially renewable [2,3].

Biohydrogen production by dark fermentation has received broad attention, since this process can utilize renewable feedstock sources (e.g., complex wastewaters, agro-industrial wastes) [4]. Most of the studies have been conducted utilizing

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mono-digestion processes of food wastes [5], vinasses [6], water hyacinth, soybean oil extraction residues, mushroom wastes [7], and wastewater from cheese processing [8]. Data obtained in these studies shows that the biohydrogen production could be reduced or even inhibit due to some specific characteristics of the wastewater or organic wastes, such as deficient buffer capacity, nutrient imbalance, and presence of microbial populations that may consume hydrogen or produce methane [8]. One strategy to overcome these problems is the use of co-digestion processes due to the positive synergistic effects of the mixed materials with complementary characteristics and the supply of missing nutrients by the co-substrate (adequate carbon/nitrogen (C/N) ratio, and the macro- and micro-nutrients concentration). A balanced C/N ratio allows enhancing the buffer capacity of the system. Additionally, the co-digestion process also reduces the possibility of inhibitory effects, which, in turn, increases biohydrogen production [9,10]. Few novel anaerobic co-digestion processes have been developed for biohydrogen production. In such studies, organic wastes such as municipal foods waste-kitchen wastewater [11], waste glycerol-sludge [12], cow manure-waste milk [13], rice straw-sewage sludge [14], food waste-sewage sludge [15–17], cassava stillage-sludge [10], and pressed mud-sewage [18] have been utilized. However, as far as we know, there is no report in the literature regarding biohydrogen production from co-digestion of crude cheese whey (CCW) with fruit vegetable waste (FVW). CCW is a liquid waste generated after manufacturing cheese with high content of lactose, proteins, and other compounds. It has been reported that around 1 million metric tons are generated annually in Mexico [19,20]. On the other hand, fruits and vegetables waste are massively available and they represent a form of highly degradable feedstock. These residues are largely produced by marketplaces, food industry sectors, and the central food distribution market in Mexico City. In 2008, Mexico produced 20 milliard tons of organic solid wastes, also referred to as the organic fraction of municipal solid waste (OFMSW) [21]. Because of their complementary characteristics, their higher organic composition, easy biodegradable nature, the FVW and CCW can be used as good substrates for the co-digestion process to produce biohydrogen. Additionally, the large amounts of these residues make them an available and low cost feedstock.

The use of non-sterilized organic wastes during fermentation processes will modify the inoculated mixed microflora. Recent studies revealed that, in the co-digestion process, the addition of small amounts of co-substrate altered the bacterial communities of the system [22]. More basic research is needed to determine the effects of co-substrates in co-digestion process to better understand and elucidate the mechanisms to enhance biohydrogen production. The biohydrogen-producing communities' structure identification is a critical step toward microbial communities' optimization and improvement of biohydrogen production [23].

The aim of this study was to investigate the potential of biohydrogen production from CCW with FVW in co-digestion processes by: (1) evaluating the effect of previous acclimation on the microbial distribution of the inoculum, (2) investigating the effect of different C/N ratios obtained by mixing different amounts of CCW and FVW, on hydrogen production, and (3) investigating the effect of co-substrates in the co-digestion process on the metabolic profile evolution and over the buffer capacity of the CCW and FVW mixtures.

Materials and methods

Inoculum and substrates

The inoculum was obtained from a 30 L anaerobic digester regularly fed with FVW [24]. Since the microbial population was mainly adapted to the FVW, an adaptation period for the new substrate (CCW) was necessary. The inoculum was previously adapted to use lactose as only carbon source (up to 30 g/L). The adaptation process was performed during two months. Fresh media with increasing lactose concentrations was sequentially fed to the culture to promote predominance of the microbial population suitable for lactose consumption. The new microbial population was preserved at 10 °C. Pre-inoculums with 24 h of culture, with a ratio of 10% v/v were utilized for the next experiments.

FVW were collected from a marketplace. The FVW composition was the same as previously reported [24]. Pretreatment of FVW consisted in milling the fruits and vegetables in an electrical domestic blender to homogenize the sample. Then, the slurry was sieved through a stainless steel 0.0787-mm sieve (Size No 10).

The CCW used in the experiments was obtained from the Food and Biotechnology Laboratory of the School of Chemistry, UNAM-Mexico. The sample was filtered (filter paper, 8 μm) and refrigerated at 4 °C.

Anaerobic digester setup

The experimental setup of the batch anaerobic digester is shown in Fig. 1. The reactor unit consists of a 2-L Schott Duran bottle with a 1.8 L of working volume. The system was constantly mixed with a magnetic stirrer bar (Cimarec®, Burnstead I Themolyne). The temperature was maintained at 37 °C by using a temperature regulated bath (Poly-Science®, Serial num 109300711), pH was set and maintained at 5.5 by addition of NaOH (10 M) or H_2SO_4 (30% v/v) solutions. Biogas production was measured by a wet gas meter. Gas samples were taken in two ports (head space and wet gas meter) to determine gas composition by chromatography. Liquid samples were taken at regular intervals from a sample port for the analysis of protein, substrate, material degradation, and products concentrations.

Biohydrogen production by a co-digestion process in batch systems

Five CCW:FVW ratios, i.e., 100:0, 75:25, 50:50, 25:75, and 0:100 (%v/v) were tested, corresponding to C/N ratios of 7, 17, 21, 31, and 46, respectively. They were evaluated in terms of their effect over the biohydrogen production. 180 mL of the pre-inoculum was added to 1620 mL of the substrate fresh medium. The initial pH was adjusted to 5.5 with 10 M NaOH. The bottle was sealed and air was displaced by flushing N_2 gas to