

Enhanced fermentative hydrogen production from cassava stillage by co-digestion with different co-substrates

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Abstract

An efficient strategy to increase the hydrogen yield from cassava stillage (CS) by co-digestion with organic wastes was developed. The effect of different co-substrates on hydrogen yield was evaluated and compared. The maximal hydrogen yield was achieved by CS co-digestion with cassava excess sludge (CES), which resulted in a 46% increase in the hydrogen yield compared with CS alone. Improved hydrolysis and acidification performances, and thus increased hydrogen production, were additional benefits of co-digestion, especially with CES. Also, the presence of a co-substrate promoted butyrate over lactate generation. The influential advantage of co-substrate addition was the enhanced pH buffering capacity and more favourable carbohydrate /protein ratio, which were both correlated linearly with hydrogen yield ($R^2=0.98, 0.86$ respectively). PCR-DGGE analysis indicated that the addition of even small amounts of co-substrate altered the bacterial communities within the reactors, enriching the proportion of hydrogen-producing bacteria.

Keywords

Hydrogen production; co-digestion; cassava stillage; alkalinity

INTRODUCTION

Biohydrogen production by anaerobic digestion is a well-suited energy route allowing simultaneous energy production and waste reduction^[1,2]. The major criteria for the selection of organic residues to be used for biohydrogen production are availability, cost, carbohydrate content, and biodegradability^[3].

Recently, improved hydrogen production was achieved by co-digestion of different organic residues. However, the mechanisms underlying the improved hydrogen production are unclear, although several mechanisms have been proposed^[4,5,6]. Moreover, hydrogen production has only been investigated in co-digestions with two organic residues until now, whereas comparisons among different types of residues as co-substrates with carbohydrate-rich organic residues have not been carried out. Consequently, the reasons for the improved hydrogen production offered by the above authors and in other studies are likely to be neither comprehensive nor sufficient.

To better understand the favorable conditions achieved by the addition of a co-substrate and thus to elucidate the mechanisms underlying the enhanced hydrogen yield, we evaluated a series of co-digestions in which carbohydrate-rich cassava stillage (CS) was mixed with one of four common and easily obtainable wastes: cassava excess sludge (CES), pig manure (PM), cow manure (CM), and waste activated sludge (WAS).

MATERIALS AND METHODS

Feedstock and inoculum

CS and CES were obtained from a cassava ethanol plant in Jiangsu province. PM and CM were collected from a pig farm and a dairy farm in Shanghai. WAS was withdrawn from the secondary sedimentation tank of a municipal wastewater treatment plant in Shanghai. The feedstocks were stored at 4°C before usage. Thermophilically digested CS was used directly as inoculum.

Experimental design and procedure

Five identical 300-ml bottles with a working volume of 200 ml were used as hydrogen-production reactors. Prior to the co-digestion, the feedstocks were concentrated or diluted as needed to achieve a concentration of volatile solids (VS) in all five reactors of 30 g/l, to facilitate comparisons. The $VS_{CS}/VS_{co-substrate}$ ratio was 7:1. The reactors were fed every 24 h with 100 ml of fresh substrate (100 ml CS in the first reactor, 87.5 ml CS and 12.5 ml co-substrate in reactors 2–5) and an equivalent amount of the digestate was removed, corresponding to a hydraulic retention time of 2 d. The initial pH was adjusted to 5.5 with 2 N NaOH or 2 N HCl immediately after each feeding. In the above experiments, the capped reactors with rubber stoppers were placed in a reciprocating water bath shaker at a temperature of 60°C and rotated at 120 rpm.

Analytical methods

TS, VS, TCOD, SCOD, carbohydrate, protein, ethanol, and VFAs, biogas production /composition were determined as described in our previous publication [7].

Lactate was quantified using a high-performance liquid chromatography (HPLC) system (HP 1200; Agilent, USA) equipped with an Aminex HPX-87H column (Bio-Rad, USA) at 54°C and a refractive index detector. The eluting solution was 5 mM H₂SO₄ at a flow rate of 0.6 ml/min.

RESULTS AND DISCUSSION

Comparison of hydrogen production with different co-substrates

The hydrogen reactors were operated for around 35 days, and the performances at steady-states are summarized in Fig 1. Based on the total VS added, the highest hydrogen yield (HY) 57.8 ml/g-total-VS added was obtained by the co-digestion of CS with CES, which was 28% higher than that by digestion of CS alone ($P < 0.05$). The hydrogen yield of carbohydrate-rich wastes was 20 times more than that of fat- or protein-rich wastes [8,9]. Since the carbohydrate contents in CES, PM, CM and WAS used in this study were only 3%~16% of that in the CS (data not shown), hydrogen production was largely dependent on the CS utilization rate. In the four co-digestion reactors, 12.5% of CS was replaced with co-substrates. Our previous work [9] and other authors [10,11] showed that the HYs from the co-substrates themselves were negligible. Thus, HYs were also calculated based on the CS-contributed VS, as shown in Fig. 1. In all four co-digestion reactors, the HYs (ml/g-CS-VS added) were larger than by CS alone, ranging from a 46% increase in the CES co-digested reactor to an 11% increase in the CM co-digestion reactor.

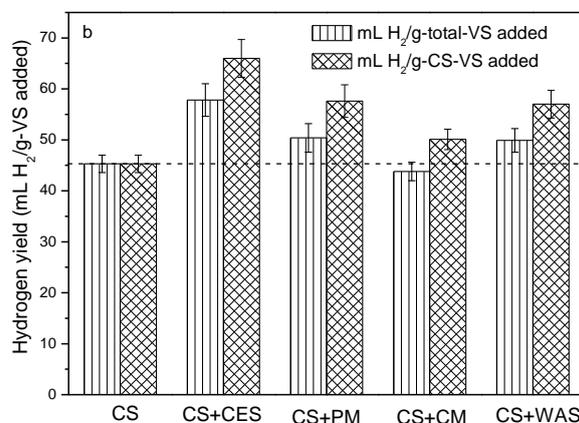


Figure 1. The hydrogen production performances at steady-states.

Despite the addition of only a small amount of co-substrate (the ratio of CS to the co-substrates was 7:1), hydrogen production from CS was significantly enhanced in the four reactors. This result suggests that the co-substrates contribute important components to the fermentation process.

Effect of different co-substrates on the alkalinity of the system

Large amounts of volatile fatty acids are generated during the hydrogen fermentation process when

organic residues with high carbohydrate content are used. However, this causes a drop in pH and consequently the breakdown of the reactor if it is not properly maintained. Since carbohydrate-rich organic residues contain low alkalinity and low nitrogen content [12], chemical reagents are commonly added to these reactions [6, 13]. However, the alkaline cations used for pH regulation in the fermentation process may inhibit anaerobic digestion [14]. Recently, several reports showed that the co-digestion of low-pH buffered organic residues with wastes of suitable alkalinity allowed both effective pH control and economic management [5, 13].

The pH and total alkalinity of CES, PM, CM and WAS are much higher than CS, and the highest total alkalinity was obtained when CS was co-digested with CES (Fig 2a), which also corresponded to the highest hydrogen production. By contrast, the lowest total alkalinity and lowest HY were measured in the CS alone reactor. The use of CM as co-substrate resulted in a comparatively low alkalinity, which corresponded to a lower HY. In agreement with these observations, there was a significant linear correlation ($R^2 = 0.98$) between HY and the total alkalinity of the co-digestion system (Fig. 2a). Thus the improved pH buffering capacity due to the co-substrates addition was an important factor for the enhanced HY.

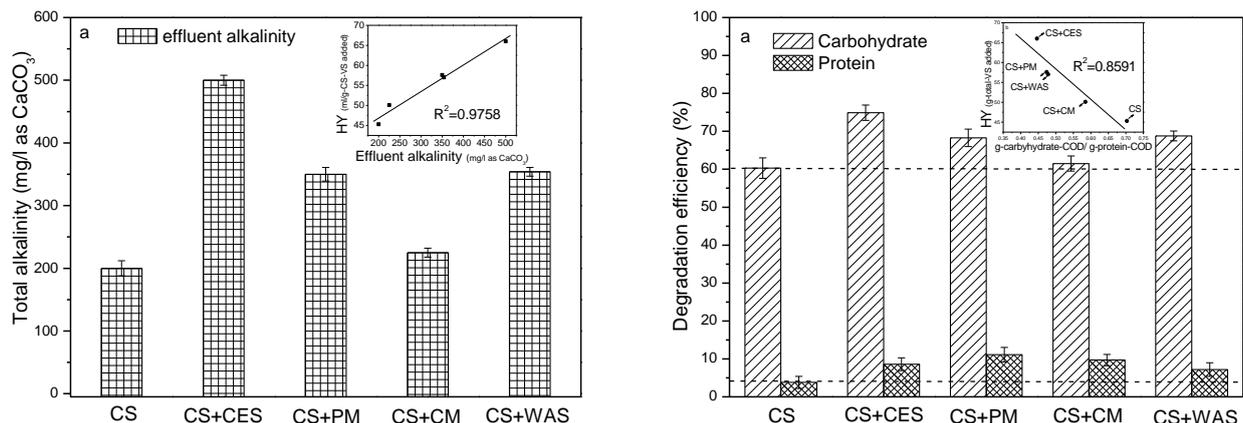


Figure 2. Alkalinity (a) and carbohydrate and protein degradation efficiency (b) of the effluent.

Effect of different co-substrates on hydrolysis and acidification performances

Carbohydrate and protein degradation efficiencies were used to evaluate the degree of hydrolysis and acidification. In all five reactors, carbohydrate degradation efficiencies (>60%) were much higher than those of protein degradation ($\leq 11\%$) (Fig 2b), consistent with the high potential of carbohydrate-rich wastes in intensive biohydrogen production [15]. The differences in the efficiencies of carbohydrate and protein degradation provide further evidence that hydrogen was mainly generated from CS. Moreover, a clear linear correlation ($R^2 = 0.86$) between carbohydrate degradation efficiency and HY was determined. The highest carbohydrate degradation efficiency of 75% was consistent with the highest HY, obtained by the co-digestion of CS with CES, with the lowest efficiency obtained with CS alone (60%). The co-digestion of CS with CES, WAS, PM, and CM allowed a better hydrolysis and acidification performance and CES was an optimal co-substrate.

Kim et al. [4] found a favourable carbohydrate /protein ratio could enhance the hydrogen production. In this study, with different wastes as co-substrates, a linear correlation ($R^2 = 0.86$) between HY and the carbohydrate /protein ratio of the mixture in the reactors was found (Fig 2b), supporting the importance of this ratio in hydrogen production. Co-substrates addition, enhanced the protein portion and decreased the carbohydrate /protein ratio, thus provided balanced nutrient conditions for hydrogen-producing bacteria and enhanced hydrogen production.

Effect of different co-substrates on the composition of acidification metabolites

Butyrate and lactate were the two most abundant metabolites in the five reactors. It is well known that butyrate accumulates with a theoretical production of 2-mol H₂/mol-hexose, while lactate is

involved in a zero-hydrogen-balance pathway. In the co-digestion of CS with CES, butyrate was the dominant metabolite (55%), with slightly lower amounts when WAS (52%) and PM (49%) were the co-substrates, whereas lactate was the major metabolite when CS was either digested alone (49%) or co-digested with PM (48%). In fact, the butyrate concentration was linearly associated with HY ($R^2 = 0.97$). The distribution of acidification metabolites also indicated that the addition of suitable co-substrates, by promoting butyrate production, leads to significantly higher hydrogen production.

Effect of different co-substrates on microbial community structures

As shown in the PCR-DGGE analysis, even a small amount of co-substrate was sufficient to alter the bacterial community structure of the whole system, with the enrichment in hydrogen-producing bacteria markedly improving hydrogen production behaviour. Among the identified bacterial species, hydrogen-producing *Clostridium cellulosi* was present only in the co-digestion reactors, whereas bacteria unrelated to hydrogen production but present in the CS alone reactor were not detectable.

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