INTERNATIONAL JOURNAL OF HYDROGEN ENERGY 39 (2014) 19337–19342

Available online at www.sciencedirect.com

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

Evaluation of hydrogen producing cultures using pretreated food waste

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ARTICLE INFO

Article history:
Available online 12 July 2014

Keywords:
Food waste
Hydrogen yield
Autoclaving
Initial pH
VFAs
Ammonia

ABSTRACT

In order to enhance bio-hydrogen production from food waste, pretreatment methods are widely used. The influence of the initial pH and autoclaving were investigated in batch experiments. Fermentative studies showed that pure cultures like *Clostridium beijerinckii* could directly utilize raw food waste to produce hydrogen, while other cultures (*Clostridium butyricum* and *Clostridium pasteurianum*) could produce hydrogen only after pH adjustment. In this case, the optimal starting pH of the culture was found to be 7. Autoclaving could further enhance hydrogen yields due to increased hydrolysis of food waste. The maximum hydrogen yield was achieved by *C. butyricum* (38.9 mL-H_2/g-VS_{added}) after autoclaving food waste with pH adjustment at 7. In addition, the ratio acetic to butyric acid was decreased by autoclaving pretreatment, because butyrate metabolic pathway was favored in the fermentation process. However, suitable pH for bacteria growth and the low ammonia production could be achieved from autoclaving food waste.

Introduction

Simple carbohydrates such as glucose, sucrose and lactose are ideal substrates for biological hydrogen production. However, pure carbohydrate sources are expensive raw materials. Hydrogen production from mixed organic waste is more realistic as it can meet the goal for waste reduction and energy production. Food waste (FW) has high hydrogen production potential because of its high content of organic matter and carbohydrates, but also of its easily hydrolyzable and biodegradable nature [1,2]. Food waste includes uneaten food and food preparation leftovers from residences, commercial establishments such as restaurants, institutions like school cafeterias, and industrial sources like factory lunch-rooms. Although food waste can be highly diverse, generally it is consisted mainly of starch, protein, and fat, with small amounts of cellulose and hemi-cellulose. The volatile solids to total solids ratio (VS/TS) is more than 80%. These volatile solids are quite biodegradable, of which 80–90% can be converted to biogas [3–5].

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http://dx.doi.org/10.1016/j.ijhydene.2014.06.056
0360-3199/

In recent years, it has been reported that a variety of anaerobic and photosynthetic microbes could produce hydrogen from food waste in different metabolic pathways [6,7]. Hydrolysis of food waste is considered as the rate-limiting step in the overall anaerobic fermentation process. In order to enhance hydrogen production, different pretreatment methods have been proposed such as thermal, alkaline, freeze and thaw, etc. [8–11]. Recently, autoclaving was introduced for municipal solid waste treatment, which involves steam processing under the action of pressure. The high temperature and pressure enhance the hydrolysis of the solid wastes. In Europe, it is considered as an alternative treatment method for diverting solid wastes from landfilling [12].

pH is an important factor that significantly influences the hydrogenase activity and biological metabolic pathways, in order to affect hydrogen production potential from food waste [13]. In batch studies, initial pH is rather important and can not be ignored. A pH range from 4 to 8 has been investigated, which showed that at neutral pH conditions hydrogen production was enhanced. Although higher initial pH heightened hydrogen production rate, however the rate would not last for long time because of the fast pH decrease by acids generation and accumulation. Lower pH values resulted in longer lag periods for bacteria to produce hydrogen due to unfavorable initial condition for fermentative bacteria [14].

To our knowledge, there are limited fermentation studies with pure cultures and food waste. The purpose of this study was to evaluate hydrogen production potential from food waste by four fermentative pure cultures (*Clostridium butyricum*, *Clostridium pasteurianum*, *Clostridium beijerinckii* and *Enterobacter aerogenes*). In addition, the effect of autoclaving and initial pH adjustment as pretreatment methods of food waste were investigated to further enhance hydrogen production potential. The fermentation process was monitored in terms of hydrogen yield, volatile fatty acids, pH and ammonia production.

Materials and methods

Feedstock and bacteria cultures

Food waste (FW) was collected from a canteen at Nanyang Technological University (Singapore). FW was mainly food remaining in plates after lunch consisted of peelings of fruits or vegetables like salad, water melon, cooked potatoes, garden peas, fried potatoes, noodles, rice, cooked meat and bread. After bones and all non-food materials were removed, FW was thoroughly mixed to promote homogeneity of the particulate organics. Then, it was diluted with DI water (the ratio was 100 g FW to 1000 mL DI water) for the subsequent experiments. The characteristics of FW solution are presented in Table 1.

C. butyricum DSM 10702, *C. pasteurianum* DSM 525, *C. beijerinckii* DSM 791 and *E. aerogenes* DSM 30053 were purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) and used in the study. The medium used to cultivate the four species consisted of 3 g/L yeast extract, 10 g/L 'Lab-Lemco' powder (beef extract), 10 g/L peptone, 5 g/L glucose, 1 g/L soluble starch, 5 g/L sodium chloride, 3 g/L sodium acetate, and 5 g/L cysteine hydrochloride. The medium was adjusted at pH 6.8 ± 0.2. Bacteria were cultivated in an incubator at 37 °C and 150 rpm.

Table 1 – Characteristics of raw food waste used in this study.

Parameter	Value	unit	Parameter	Value	unit
pH	4.8 ± 0.2	–	NH_3–N	16.0	mg/L
C	47.2	%	Carbohydrates	24.08	g/L
N	2.7	%	Na	112.6	mg/L
H	7.4	%	K	97.63	mg/L
C/N	17.5	–	Ca	16.32	mg/L
TS	28.05	g/L	Mg	9.61	mg/L
VS	27.22	g/L	Zn	724	μg/L
VS/TS	97	%	Fe	170	μg/L
Acetate	1.22	mM	Mn	32	μg/L
Propionate	0.15	mM	Cu	21	μg/L
Butyrate	0.05	mM	Mo	8	μg/L
TVFAs	1.98	mM	Co	6	μg/L
TCOD	33.55	g/L	Ni	5	μg/L
SCOD	8.95	g/L	Cd	2	μg/L

Experimental design

The dark fermentation batch experiments were performed in 60 mL serum bottles that were purged with argon and then filled with 30 mL food waste solution. The bottles were sealed with both butyl rubber stoppers and aluminum crimp caps. Four different kinds of substrate were used: raw food waste (RFW), pH adjustment food waste (PFW), autoclaving food waste (AFW), and autoclaving and pH adjustment food waste (APFW). For pH adjustment, small amounts of 1 M NaOH were added to ensure minimum increase of the food waste solution. For autoclaving, the sealed bottles were sterilized at 121 °C for 15 min. When cells reached mid-exponential growth phase, the inoculants were injected into serum bottles by 3 mL syringes (cell dry weight was 0.75–0.79 g/L). Afterwards, the bottles were placed in the shaking incubator operating at 200 rpm, at constant temperature 35 °C. The batch experiments were conducted in duplicate and lasted for 120 h. To avoid the influence of the microorganisms in food waste, substrate control experiments were run in order to exclude any hydrogen or other biogas production.

Analytical methods

Samples were analyzed for TS and VS according to the standard methods for the examination of water and wastewater [15]. Carbohydrate concentrations were determined by adapting the colorimetric method of Dubois et al. [16] with UV visible wavelength at 490 nm using glucose as standard (Agilent Cary 50, USA). The C/N ratio was determined by measuring the total carbon and nitrogen using an elemental analyzer (Vario EL cube CHNOS, Germany). Trace metals concentration was measured by an Inductively Coupled Plasma – Optical Emission Spectrometer (Perkin Elmer Optima 8300, USA). COD and NH_4^+ concentrations were measured colorimetrically using a DR 2800 spectrophotometer (Hach, USA). The concentrations of VFAs in supernatant of culture broth were determined using gas chromatography with a