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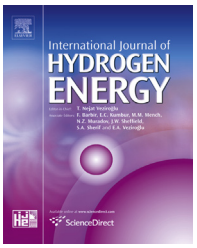

Stability of *Clostridium butyricum* in biohydrogen production from non-sterile food waste

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ABSTRACT

The stability of *Clostridium butyricum TISTR 1032* during biohydrogen fermentation of non-sterile food waste in 5-L semi-batch operation was investigated under controlled and uncontrolled pH conditions at the initial pH 6, and 37 °C. Profiles of carbon mass balance, microbial community and metabolite dynamics were used to evaluate the process efficiency. The results showed that under the uncontrolled pH condition, the maximum hydrogen yield, production rate and specific production rate were 362 mL H_2 g^{-1} VS, 695 mL $h^{-1}$and 174 mL h^{-1} L^{-1}, respectively while under the controlled pH condition, those were 350 mL H_2 g^{-1} VS, 1092 mL h^{-1} and 273 mL h^{-1} L^{-1}, respectively. Regarding the carbon distribution, food waste was still remained in the solid fraction more than 30% at which the maximum hydrogen production was achieved for all cases. The main factor, which controlled the route of fermentative process under uncontrolled pH condition was acidic condition while acetogenesis was the major effect for the production stability in the controlled pH condition. DGGE profiles showed that *C. butyricum TISTR 1032* was still the dominant group in the reactor in both conditions. However, *Klebsiella oxytoca*, *Staphylococcus* spp., *Enterobacter* spp., *Lactococcus* spp. and *Acinetobacter* sp. were observed for all cases. The metabolite analysis revealed the correlation of *K. oxytoca* and solventoganesis process under the uncontrolled pH condition while the presence of *Lactococcus* spp. was related to lower yield of the hydrogen production under the controlled pH condition.

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Introduction

Hydrogen (H_2) is regarded as one of the alternative sources for clean and renewable energy and is considered as an alternative non-polluting fuel for the future. This topic has attracted many researchers interested in improving and developing H_2 production for use on an industrial scale.

Hydrogen can be generated by various methods including chemical and biological processes [1]. Among the biological hydrogen production processes, the dark fermentative process using anaerobic bacteria is often easier than others in practice. Dark fermentation has the advantage over alternative methods that it uses an inexpensive photo-bioreactor for the direct biophotolysis and photofermentation processes. Other attractive points for dark fermentation are a lower energy requirement, various types of feedstock, higher production yields and higher supported rates in the process [2–4].

Various studies have revealed that many different types of organic feedstock such as glucose, starch, municipal waste, livestock manure, crop residues, food waste, and wastewater can be utilized as substrates in the dark fermentation process [5–8]. Food waste is regarded as a major fraction of municipal solid wastes that contains high carbohydrate content and that can be considered as a potential feedstock for biohydrogen production. Over the last decade, dark H_2 fermentation from food waste has been extensively studied in lab-scale bioreactors under mesophilic and thermophilic conditions [9–11]. However, up-scaling production of H_2 by pure cultures from food waste has still not been widely mentioned in the literature. Extensive studies using pure cultures for H_2 production have mainly been set up in a batch mode and have used pure simple carbon sources such as glucose and sucrose as substrates [11–13]. Nevertheless, biohydrogen production using pure cultures in a semi-batch operation from organic waste is a desirable goal for waste minimization and alternative energy generation. Clostridia are well known species for fermentative hydrogen production especially *Clostridium butyricum* [11,13,14] and *Clostridium acetobutyricum* [15]. Previous reports have revealed that approximately 64.6% of mesophilic hydrogen producing sludge is Clostridia [16]. Clostridia groups are spore-forming anaerobic bacteria and are able to utilize glucose for dark H_2 production with a higher yield (2 mol H_2/mol glucose) compared with other fermentative anaerobic bacterial groups such as Enterobacter sp (1mol H_2/mol hexose)

In the dark fermentation process, several types of anaerobic fermentative bacteria can be used for high yields of hydrogen during both day and night as they do not depend on energy provided by an external source [17]. The microbial community is one of the key factors which controls the overall success of dark fermentation processes. In the hydrogen production experiment [18], the competitive relationship of many microorganism groups in the reactor resulted in the loss of some pure strain cultures due to their difficulty in environmental adaptation. Previous studies have reported that optimal H_2 fermentation occurred with an initial pH of 5–6 for food waste [19–21]. However, an extensive study on the stability of pH during the fermentation process has not been reported. These previous studies have revealed the interesting result that the mixed indigenous microorganisms in natural substrates can use some products produced during hydrogen production as metabolism in their own cell growth. For example volatile fatty acids (VFAs) and free hydrogen can act as electron donors to produce many byproducts such as methane, organic solvents and other reduced substances. Therefore, the microbial community and culture conditions during the fermentation process are directly related to the end metabolites and result in different hydrogen production yield under different fermentation conditions.

The purpose of the present study was to investigate the stability of *C. butyricum TISTR 1032* added into non-sterile food waste for hydrogen fermentation under both controlled and uncontrolled pH conditions. Analysis of microbial profiles was used to examine the relationships between an added pure stain of *C. butyricum TISTR 1032* and indigenous microorganisms in non-sterile food waste during the fermentation. Kinetic data and the patterns of metabolites that resulted in different types and directions of fermentative processes, and the carbon mass balance and microbial communities in the reactor were also examined.

Materials and methods

Microorganisms

C. butyricum (TISTR 1032) was purchased from Thailand Institute of Scientific and Technological Research (TISTR) and then a culture was prepared as follows. Prior to cultivation, *C. butyricum* was reactivated by transferring 2 mL of the stock culture into 20 mL of Reinforced Clostridial Medium (RCM). The cultural serum bottle was flushed with nitrogen gas for 2 min to create anaerobic condition and incubated at 37 °C for 10 h with constant shaking (150 rpm). The culture was further enriched by inoculating 10% (v/v) of the culture into 60 mL of Tryptone Sucrose Yeast Extract (TSY:1 L of culture contains 5.0 g of tryptone; 3.0 g of sucrose; 5.0 g of yeast extract; 1.0 g of K_2HPO_4 [14]) and then incubated at 37 °C for 10 h with constant shaking. The enrichment process was then repeated three times. The optical density (600 nm) of the inoculum was then measured to be 0.8. The prepared culture was then used as the starter culture for the fermentation process.

Food waste

The synthetic food waste used throughout the experiment was prepared with the following composition; 65% (w/w) carbohydrate (rice), 17% (w/w) vegetable and 18% (w/w) meat. All materials were ground in a blender to particles of diameter approx. 0.5 mm and used as the feedstock with no prior sterilization. The ground food waste contained total solid (TS) and total volatile solid (VS) of 45,520 and 27,578 mg L^{-1}. Minimal amounts (<1 mM) of volatile fatty acids (VFAs) and ethanol were detected in the ground food waste. Prior to use, the food waste was adjusted to the desired concentration with distilled water approximately 2.5% (w/w). This desired concentration was selected from a preliminary study of optimal condition for hydrogen production. In the controlled pH experiment, the pH of synthetic food waste was adjusted to 6.0 with 1 M NaOH and concentrated orthophosphoric acid.

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