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# Sequential generation of hydrogen and methane from glutamic acid through combined photo-fermentation and methanogenesis

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## HIGHLIGHTS

- Glutamic acid was subjected to acidogenic pretreatment to produce metabolites.
- Acidulated solution was treated with zeolite to decrease the $NH_4^+$ concentration.
- Pretreated solution was incubated with photosynthetic bacteria to produce $H_2$.
- Residual solution was reused to generate $CH_4$ in sequential methanogenesis.
- $H_2$ and $CH_4$ cogeneration from glutamic acid enhanced energy conversion efficiency.

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## ABSTRACT

Glutamic acid can hardly produce hydrogen via dark- or photo-fermentation without pretreatment. In this study, a novel process of acidogenic pretreatment with bacteria and zeolite treatment for $NH_4^+$ removal was proposed to use glutamic acid as feedstock in photo-fermentation for efficient hydrogen production. Glutamic acid pretreated with acidogenic bacteria produces soluble metabolite products. After zeolite treatment, the acidulated solution, which mainly contains acetate, butyrate, and $NH_4^+$, shows a decrease in $NH_4^+$ concentration from 36.7 mM to 3.2 mM ($NH_4^+$ removal efficiency of 91.1%). After $NH_4^+$ removal, the treated solution is incubated with photosynthetic bacteria, exhibiting a maximum hydrogen yield of 292.9 mL/g(-glutamic acid) during photo-fermentation. The residual solution from photo-fermentation is reused by methanogenic bacteria to produce a maximum methane yield of 102.7 mL/g. The heating value conversion efficiency from glutamic acid to gas fuel significantly increases from 18.9% during photo-fermentation to 40.9% in the combined photo-fermentation and methanogenesis process.

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## 1. Introduction

The wide utilisation of nonrenewable fossil fuels, such as coal and petroleum, has led to environmental pollution and the energy crisis (Zhao et al., 2011). Fermentative hydrogen production from biomass wastes is attracting increasing attention because of its renewable and environment-friendly characteristics. Compared with other hydrogen-producing methods, such as steam reformation, biohydrogen production through fermentation exhibits unique advantages, such as its energy-saving and carbon-neutralising properties (Argun and Kargi, 2011; Holladay et al., 2009; Kang et al., 2012; Navarro et al., 2009).

The main fermentative components of biomass wastes (e.g. microalgae) are carbohydrates (e.g., starch, glycogen, trehalose, and glucosylglycerol; 11% to 50% dry weight) and proteins (27% to 72% dry weight) (Cheng et al., 2012b; Dismukes et al., 2008; Klaehn and Hagemann, 2011; Song et al., 2010). Most of the carbohydrates in biomass wastes are composed of glucose and xylose, which are easily used by hydrogen-producing bacteria for efficient hydrogen production (Lin and Cheng, 2006; Su et al., 2009). The proteins in biomass wastes are composed of amino acids such as glutamic acid and alanine (Rodriguez et al., 1997). These amino acids cannot easily produce hydrogen during dark- or photo-fermentation without pretreatment. The low C/N ratio of amino acids is one of the bottlenecks in fermentative hydrogen production by hydrogen-producing bacteria. Thus, determination of an effective hydrogen-producing process using amino acids as feedstock is necessary to further improve hydrogen production from biomass. To date, however, fermentative hydrogen production using pure amino acids has yet to be reported.

Heat-treated sludge from pig manure can efficiently ferment organic chemicals, such as glucose and proteins, to low-molecular soluble metabolite products (SMPs), such as acetate, butyrate,

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and ethanol (Song et al., 2010; Xie et al., 2008b). SMPs from the acidogenic treatment solution can be reused by photosynthetic bacteria (PSB) for efficient hydrogen production (theoretically, 4 mol $H_2$/mol acetate and 10 mol $H_2$/mol butyrate) (Christopher and Dimitrios, 2012; Su et al., 2009). However, the $NH_4^+$ from protein (amino acid) anaerobic metabolism can significantly inhibit the activity of nitrogenase, which is the key enzyme responsible for hydrogen production by PSB (Cheng et al., 2012a; Zheng et al., 2010). Previous studies have shown that zeolite, an inexpensive and abundant resource, can be used to extract $NH_4^+$ by selective-ion exchange from a solution (Cheng et al., 2012a; Wang et al., 2011; Wang and Peng, 2010). Acidulated solutions treated with zeolite can potentially be used by PSB for efficient photosynthetic hydrogen production. To further improve heating value conversion efficiencies from feedstock to biofuels and reduce SMP emissions, the residual solution from photo-fermentation, which mainly contains SMPs and the PSB biomass, could be reused by methanogenic bacteria for methane production. However, the combined photo-fermentation and sequential methanogenesis process has not been reported in the literature.

In the present study, glutamic acid, a typical amino acid degraded from protein compositions in biomass wastes (Rodriguez et al., 1997; Romero Garcia et al., 2012; Wu and Pond, 1981), is used as a feedstock to efficiently produce photosynthetic hydrogen. Glutamic acid is pretreated with acidogenic bacteria to produce SMPs. The acidulated solution is treated with zeolite to reduce the $NH_4^+$ concentration. The $NH_4^+$ adsorbed by zeolite is reused as nitrogen source to grow PSB. The treated solution after $NH_4^+$ removal is incubated with mixed PSB for efficient hydrogen production in photo-fermentation. The photo-fermented residual solution is reused by methanogenic bacteria to produce methane, consequently increasing the heating value conversion efficiency dramatically.

## 2. Methods

### 2.1. Bacteria

#### 2.1.1. Acidogenic bacteria

Mixed acidogenic bacteria were isolated from anaerobic digestion sludge (pig manure) sampled from the Huzhou marsh gas plant in Zhejiang Province, China. The original digestion sludge was first heated at 100 °C for 30 min to inactivate the methanogenic bacteria and then enriched three times (72 h each time) to harvest the spore-forming acidogenic bacteria (Cheng et al., 2011a). The compositions of the enrichment medium used for the acidogenic bacteria are described in our previous study (Cheng et al., 2011a). The mixed acidogenic bacteria, the dominating bacterial strain of which was identified to be *Clostridium butyricum* by 16S rDNA sequence analysis (Cheng et al., 2011b), were used as the inoculum during acidogenic pretreatment.

#### 2.1.2. Photosynthetic bacteria

Mixed PSB were also isolated from the anaerobic digestion sludge. The isolation method and enrichment medium applied are described in our previous study (Cheng et al., 2012a). The diversity of the PSB, which was identified as by 16S rDNA sequence analysis, was described in details in our previous study (Ying et al., 2008). *Rhodopseudomonas palustris* was the dominant strain of PSB. Some other bacteria, such as *Pseudomonas putida* and *Pseudomonas pseudoalcaligenes* were also detected in PSB (Ying et al., 2008). The mixed PSB were used as the inoculum for hydrogen production during photo-fermentation.

#### 2.1.3. Methanogenic bacteria

Mixed methanogenic bacteria, including *Methanosarcina* and *Methanothrix* enriched from the anaerobic digestion sludge (Cheng et al., 2010), were used as the inoculum for methanogenesis. The enrichment method and medium applied are described in our previous study (Xie et al., 2008b).

### 2.2. Zeolite

Zeolite was purchased from the Zhejiang Shenshi Mining Industry Co., Ltd., China. The chemical properties and modification procedures for natural zeolite are described in our previous study (Cheng et al., 2012a). The modified zeolite was stored for use in the $NH_4^+$ removal experiments.

### 2.3. Pretreatment and fermentation methods

The experimental processes for pretreatment, hydrogen production, and methane production are shown in Fig. 1. Batch tests for acidogenic pretreatment were performed in 417 mL glass bottles. 2.5 g of pure glutamic acid (Sinopharm Chemical Reagent, China), 25 mL of mixed acidogenic bacteria, and 225 mL of autoclaved deionised water were added into each bottle to mix with 0.25 g of yeast extracts. The initial pH was adjusted to 6.5 ± 0.1 with 6 M NaOH solution. The bottles were sealed using rubber stoppers, purged with $N_2$ gas for 20 min to ensure anaerobic conditions, and then kept at 35 °C for 144 h.

The acidulated solution, which mainly contained acetate and butyrate, was collected by centrifugation at 5000*g* for 10 min (Beckman Avanti J-26XP, USA). 100 mL of the pretreated liquid ($NH_4^+$,>30 mM) and 20 g of modified zeolite were added into a 250 mL conical flask. The initial pH was adjusted to 7.0 ± 0.1 with 6 M NaOH and 6 M HCl solution. The conical flask was sealed with a glass stopper and kept at 30 °C for 6 h in a shaker at 120 rpm. The treated solution collected by centrifugation was diluted to an optimal concentration of 20 mM acetate and then added into the autoclaved enrichment medium with 25 mL of mixed PSB for photosynthetic hydrogen production (Cheng et al., 2012a).

Batch tests for photosynthetic hydrogen production were performed in 350 mL bottles. The fermentation liquor volume of each bottle was 250 mL. The initial pH was adjusted to 7.0 ± 0.1 using 6 M HCl solution. The bottles that had been sealed and purged with $N_2$ gas were placed in an illuminated incubator (Shanghai Yiheng LRH-250F, China) at 30 °C ± 0.5 °C and then subjected to an illumination intensity of approximately 6000 lx (incandescent lamp) (Cheng et al., 2011a).

250 mL of the residual hydrogen-producing solution was inoculated with 10 mL of mixed methanogenic bacteria to produce methane in 417 mL bottles at pH 8 ± 0.1. The fermentation liquor volume of each bottle was 260 mL. The bottles were sealed, purged with $N_2$ gas for 20 min to ensure anaerobic conditions, and then kept at 35 °C for methanogenesis (Song et al., 2010).

### 2.4. Utilisation of the $NH_4^+$ adsorbed by zeolite

The used zeolite was collected by centrifugation at 5000*g* for 10 min, washed with autoclaved deionised water, and then used in growth tests for mixed PSB. About 25 mL of mixed PSB and 225 mL of growth medium mixed with used zeolite (10 g initial dry weight; 1.7 mmol adsorbed $NH_4^+$-N equivalent) were added into a 350 mL bottle. The growth medium used was identical to the enrichment medium used for the mixed PSB without additional nitrogen. Mixed PSB incubated in the complete enrichment medium was used as the control.

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