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# Hydrogen production using amino acids obtained by protein degradation in waste biomass by combined dark- and photo-fermentation

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## HIGHLIGHTS

- Five amino acids produced limited hydrogen but abundant SMPs in dark-fermentation.
- C conversion efficiencies of alanine and serine were 85.3–94.1% in dark-fermentation.
- Dark-fermentation effluent of alanine gave a high $H_2$ yield in photo-fermentation.
- HVCE of alanine to hydrogen by combined dark- and photo-fermentation was 25.1%.

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## ABSTRACT

The biological hydrogen production from amino acids obtained by protein degradation was comprehensively investigated to increase heating value conversion efficiency. The five amino acids (i.e., alanine, serine, aspartic acid, arginine, and leucine) produced limited hydrogen (0.2–16.2 mL/g) but abundant soluble metabolic products (40.1–84.0 mM) during dark-fermentation. The carbon conversion efficiencies of alanine (85.3%) and serine (94.1%) during dark-fermentation were significantly higher than those of other amino acids. Residual dark-fermentation solutions treated with zeolite for $NH_4^+$ removal were inoculated with photosynthetic bacteria to further produce hydrogen during photo-fermentation. The hydrogen yields of alanine and serine through combined dark- and photo-fermentation were 418.6 and 270.2 mL/g, respectively. The heating value conversion efficiency of alanine to hydrogen was 25.1%, which was higher than that of serine (21.2%).



## 1. Introduction

Hydrogen is an ideal energy carrier with a high calorific value of 242 kJ/mol. The conventional processes for hydrogen production, such as water electrolysis, steam methane reforming, gasification, and water gas shift reaction, are efficient and contribute to most of the produced hydrogen worldwide (Obradović et al., 2013a,b). However, these processes require large energy input from fossil fuels. Biohydrogen production from renewable resources of waste biomass, which has attracted increasing attention, is a promising process that combines waste minimization and energy recovery (Wang and Wan, 2009). Biohydrogen production and upgrading are important parts of current bio-refinery technologies that focus on high value added chemicals for pharmaceutical, cosmetics, nutritional, and biofuel industries (Sostaric et al., 2012; Yang et al., 2011).

Food waste, sewage sludge, manure, agricultural residue, and algae bloom are typical waste biomass utilized as substrates for fermentative hydrogen production (Lee et al., 2010; Yang et al., 2011; Yoshida et al., 2009). The principal fermentative organic components in this process are carbohydrates and proteins. Carbohydrates such as starch and cellulose are better organic substances utilized for fermentative biohydrogen production than proteins (Li et al., 2009). Carbohydrates can be easily hydrolyzed into reducing sugars such as glucose and xylose, which are readily utilized by hydrogen-producing bacteria (HPB) for fermentative hydrogen production (Lin and Cheng, 2006; Su et al., 2009). Proteins are also main components in waste biomass. For instance, the protein content reached up to 72% of the dry weight in *Arthrospira* (*Spirulina*) biomass (Dismukes et al., 2008) and accounted for 40% of the total chemical oxygen demand (COD) in dairy wastewater stream (Barnett et al., 1994). However, the amino acids derived from proteins cannot be easily used by HPB to directly produce hydrogen

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during dark-fermentation because of their unique molecular structures and low C/N ratios (Xia et al., 2013b). The hydrogen-producing potential of carbohydrate-rich wastes (rice and potato) is approximately 20 times larger than that of protein-rich wastes (egg and lean meat) during dark-fermentation (Lay, 2003).

Several investigations have attempted to enhance fermentative hydrogen production from protein-rich biomass. Song et al. (2010) enhanced the hydrogen yield and peak rate from defatted milk powder by adding *Saccharomyces cerevisiae*, which provides a conducive anaerobic condition for HPB and produces several proteinases that facilitates lactoprotein degradation. Xiao et al. (2013) improved the fermentative hydrogen production from protein wastewater by 3.8 times via ultraviolet pretreatment, which effectively disrupts hydrogen bonding networks and unfolds proteins to increase the susceptibility of proteins to proteases. Nagase and Matsuo (1982) reported that amino acids such as alanine can be anaerobically degraded via coupled oxidation–reduction reactions and converted to various short-chain volatile fatty acids (VFAs) by mixed microbial populations, such as *Clostridium* species. Xia et al. (2013b) efficiently converted glutamic acid by using acidogenic bacteria into abundant soluble metabolic products (SMPs), which were reused by photosynthetic bacteria (PSB) to produce 292.9 mL/g (glutamic acid) of hydrogen during photo-fermentation. The low hydrogen production efficiency of typical amino acids derived from proteins in waste biomass (Könst et al., 2010; Rodríguez et al., 1997; Yoshida et al., 2009) is a technical bottleneck that limits the industrial application of biohydrogen production. However, a systematic comparison of fermentative hydrogen production using various pure amino acids has not yet been reported till now. Accordingly, we comprehensively investigated the production of hydrogen from five pure amino acids (i.e., alanine, serine, aspartic acid, arginine and leucine), which are typical degradation products of waste proteins, through combined dark- and photo-fermentation to increase heating value conversion efficiency (HVCE).

## 2. Methods

### 2.1. Feedstock and microflora

Alanine, serine, aspartic acid, arginine and leucine purchased from Sinopharm Chemical Reagent of China were used as feedstock for fermentative hydrogen production. Ethanol, acetic acid, propionic acid, butyric acid, valeric acid, isovaleric acid, and caproic acid purchased from Sinopharm Chemical Reagent of China were used as standard samples for the quantification of SMP components on gas chromatography (GC).

HPB and PSB were separated from anaerobic digestion sludge collected from a methane plant in Huzhou, Zhejiang Province, China. Details on the separation and enrichment of the bacteria were previously described (Su et al., 2009). Both the HPB and PSB inocula were maintained anaerobic for more than 1 week to ensure that no hydrogen was produced before inoculation. The dominant bacterial strains of HPB and PSB were identified to be *Clostridium butyricum* and *Rhodopseudomonas palustris*, respectively, on the basis of 16SrDNA and DGGE sequence analysis (Cheng et al., 2011; Ying et al., 2008).

### 2.2. Dark-fermentation

Hydrogen production through dark-fermentation was conducted in 300 mL glass reactors. Five groups each with one of the five amino acids were established in triplicates. 2.5 g of pure amino acid and 25 mL of HPB were added into each reactor, and the total liquor volume was adjusted to 250 mL with deionized water. The volatile suspended solids (VSS) concentration of the HPB inoculum was 3.5%. Each reactor was stirred until the amino acid was completely dissolved. The initial pH of the dark-fermentation solution was adjusted to 6.0 ± 0.1 with 6 M NaOH and 6 M HCl solutions. The reactors were subsequently sealed with silicone rubber stoppers and purged with $N_2$ gas for 10 min to ensure an initial anaerobic environment. The temperature during dark-fermentation was maintained at 35.0 ± 0.1 °C (Song et al., 2010). The glass reactors were shaded by tinfoil during dark-fermentation.

### 2.3. Photo-fermentation

The residual solutions of dark-fermentation were centrifuged at 6000 r/min for 10 min (Beckman Avanti J-26XP, USA) to separate the liquid supernatant from the solid residue. The zeolites purchased from Zhejiang Shenshi Mining Industry Co., Ltd., China were modified with NaCl to remove $NH_4^+$, which is an inhibitor of the following photo-fermentation process (Cheng et al., 2012b), from the supernatant. Details on the modification and treatment process can be found in our previous study (Cheng et al., 2012b).

The treated supernatant, which mainly contained short-chain VFAs, was diluted to an optimal concentration of 15 mM SMPs and added into an autoclaved enrichment medium mixed with 25 mL of PSB subsequently (Su et al., 2009) in 300 mL-scale glass reactors for photo-fermentation. The total liquor volume in each photo-fermentation reactor was 250 mL, which was identical to that in dark-fermentation. The initial pH was adjusted to 7.0 ± 0.1 with 6 M NaOH and 6 M HCl solutions during photo-fermentation. The reactors were sealed with silicone rubber stoppers, purged with $N_2$ gas for 10 min, and then placed in an illuminated incubator (Shanghai Yiheng LRH-250F, China). The temperature inside the incubator was controlled at 30.0 ± 0.5 °C, and the illumination intensity provided by an incandescent lamp was approximately 6000 lux (Su et al., 2009; Xia et al., 2013b).

### 2.4. Analytical methods

A GC system (Agilent 7820A, USA) equipped with a thermal conductivity detector was used to determine the hydrogen and carbon dioxide concentrations in the biogas. The compositions of the SMPs in the residual solutions of dark-fermentation were determined using another GC (Thermo Finigan TRACE 2000, USA) equipped with a flame ionization detector. Details on this experiment were previously described (Cheng et al., 2012c). The $NH_4^+$ concentration was determined using an ion meter (Mettler Toledo SG8-B, Switzerland) equipped with a DX218–$NH_4^+$ ion electrode (Cheng et al., 2012b).

### 2.5. Calculations

The hydrogen yield was calculated based on the volume and composition of both the headspace gas and the total biogas production in the gas container at each time interval (Fan et al., 2008; Xia et al., 2013b). Dynamic parameters such as the maximum hydrogen yield potential $H_m$ (mL/g), the maximum hydrogen production rate $R_m$ (mL/g/h), the lag-phase time $\lambda$ (h), and the hydrogen production peak time $T_m$ (h) were calculated by best fitting the hydrogen production data for the modified Gompertz equation (Lay et al., 1999) with Origin 9.0.

The carbon conversion efficiency (CCE) of the amino acids during dark-fermentation was calculated according to Eq. (1):

$$\mathrm{CCE} = \frac{\text{Carbon yield of } CO_2 + \text{Carbon yield of the SMPs}}{\text{Total carbon yield of amino acids}} \times 100\% \quad (1)$$