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Substrate consumption and hydrogen production via co-fermentation of monomers derived from carbohydrates and proteins in biomass wastes

Ao Xia [a], Jun Cheng [a,*], Lingkan Ding [a], Richen Lin [a], Wenlu Song [b], Huibo Su [c], Junhu Zhou [a], Kefa Cen [a]

[a] State Key Laboratory of Clean Energy Utilization, Zhejiang University, Hangzhou 310027, China
[b] Department of Life Science and Engineering, Jining University, Jining 273155, China
[c] COFCO Nutrition and Health Research Institute, Beijing 100020, China

HIGHLIGHTS

- Monosaccharides and amino acids were mixed for fermentative H_2 production.
- Monosaccharide consumption generally preceded amino acid consumption.
- H_2 was mainly produced via fermentation of monosaccharides but not via amino acids.
- Glutamic acid, serine and alanine were more readily used by fermentative bacteria.
- Energy and carbon conversion efficiencies in fermentation reached 83.3% and 93.3%.

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ABSTRACT

Fermentative hydrogen production from biomass wastes is a promising technology combining waste treatment and clean fuel production. Biomass wastes have various types and components, while the fundamental fermentation reactions involve monosaccharides and amino acids. In this study, typical monosaccharides (glucose and xylose) and amino acids (glutamic acid, aspartic acid, serine, glycine, arginine and alanine) were mixed and fermented by anaerobic fermentative bacteria (AFB) obtained from heat pre-treated anaerobic digestion sludge, to directly examine the substrate consumption and hydrogen production during the co-fermentation of monosaccharides and amino acids. Hydrogen was mainly produced from the fermentation of monosaccharides, but not from the fermentation of amino acids. Monosaccharide consumption generally preceded amino acid consumption. Glucose was more readily utilised by AFB than xylose. The maximum volumetric hydrogen productivity and production rate from the co-fermentation of glucose and mixed amino acids was 2.7 times and 3.1 times higher than those from the co-fermentation of xylose and mixed amino acids. Glutamic acid, serine and alanine were more readily utilised by AFB than aspartic acid, glycine and arginine. The co-fermentation of monosaccharides and amino acids showed efficient energy conversion and carbon conversion, with the maximum efficiencies of 83.3% and 93.3%, respectively.

1. Introduction

Energy crisis and environmental pollution caused by excessive utilisation of fossil fuels (such as coal and petroleum) have been receiving increasing worldwide attention [1–5]. Hydrogen is considered an ideal future fuel on account of its clean combustion product and high energy density by mass [6–11]. In comparison with conventional hydrogen-producing processes (such as water electrolysis and steam reforming), fermentative hydrogen production from biomass wastes has unique advantages, being energy-saving and environment-friendly process [12–14].

The main organic fermentable components in biomass wastes are carbohydrates and proteins [14–16]. For instance, agriculture residues are rich in carbohydrates, while sludge, food wastes and algae are rich in both proteins and carbohydrates. A lot of previous studies have investigated fermentative hydrogen production from biomass wastes, and found that proper protein contents in substrates (e.g., co-fermentation of agriculture residues and sludge) can enhance hydrogen production [15–21]. Prior to

* Corresponding author. Tel.: +86 571 87952889; fax: +86 571 87951616.
E-mail address: juncheng@zju.edu.cn (J. Cheng).

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fermentation, high-molecular-weight carbohydrates and proteins must be generally hydrolysed to low-molecular-weight monosaccharides and amino acids, respectively, for efficient utilisation by anaerobic fermentative bacteria (AFB) via single or combined pre-treatment (such as steam heating, microwave irradiation, enzymatic hydrolysis, acid/alkali hydrolysis and ultrasonication) [22–24]. Biomass wastes have various types and components; however, the fundamental fermentation reactions involve monosaccharides and amino acids. Thus, to reveal the basic characteristics of hydrogen production and substrate consumption from biomass wastes, it is essential to use pure monosaccharides and amino acids as substrates for co-fermentation. However, hydrogen production during the co-fermentation of monosaccharides and amino acids has rarely been reported in the literature.

In the present study, two most abundant monosaccharides (glucose and xylose) and six typical amino acids (glutamic acid, aspartic acid, serine, glycine, arginine and alanine) were utilised as substrates for hydrogen fermentation. Substrate consumption and hydrogen production during the co-fermentation of monosaccharides and amino acids were investigated.

2. Materials and methods

2.1. Inocula

Mixed AFB were isolated from anaerobic digestion sludge collected from a methane plant in Zhejiang Province, China. The original digestion sludge was first heated at 100 °C for 30 min to inactivate methanogenic bacteria, and then enriched three times (3 d each time) to harvest spore-forming AFB [25]. The compositions of the enrichment medium used for AFB are described previously [26]. The enriched and activated AFB, in which the dominant bacterial strain was identified as *Clostridium butyricum* through 16S rDNA sequence analysis [27], were utilised as the inocula for hydrogen fermentation.

2.2. Fermentation processes

The fermentation was performed in 300 mL-scale glass fermenters. A total of 3.0 g of mixed monosaccharides and amino acids (as shown in Table 1) and 230 mL of autoclaved deionised water were added to each fermenter. The initial pH was adjusted to 6.0 ± 0.1 using 6 M HCl and 6 M NaOH. Subsequently, the fermenters were inoculated with 20 mL of activated AFB, sealed with rubber stoppers, purged with nitrogen for 5 min and maintained at 35.0 ± 1.0 °C during the fermentation. The pH was adjusted to 6.0 ± 0.1 every 6 h or 12 h using 6 M HCl and 6 M NaOH, and the fermenters were then purged with nitrogen for 5 min. Gases (mainly hydrogen and carbon dioxide) produced during the fermentation were released from the headspace of the fermenters and collected in graduated containers [28]. The batch tests were conducted in duplicate.

2.3. Analytical methods

The produced gas was sampled using a gas-tight syringe (Hamilton, Switzerland). The concentrations of hydrogen and carbon dioxide in the gas phase were determined using a gas chromatography (GC; Agilent 7820A, USA) equipped with a thermal conductivity detector, as described previously [26]. The volumes of hydrogen and carbon dioxide produced in each time interval were calculated as described previously [25]. The volumetric hydrogen productivity (mL/L) was defined as the ratio of the cumulative hydrogen volume (mL) to the total fermentation liquid volume (L). The specific hydrogen yield (mL/g monosaccharides) was defined as the ratio of the cumulative hydrogen volume (mL) to the consumed weight of monosaccharides. The concentrations of soluble metabolic products (SMPs) in the liquid phase were determined using another GC (Thermo Finigan TRACE 2000, USA) equipped with a flame ionisation detector, as described previously [26]. The carbon conversion efficiency (%) was defined as the ratio of the total weight of carbon in carbon dioxide (g) and SMPs (g) to the total weight of carbon in the consumed substrates (g). The energy conversion efficiency (%) was defined as the ratio of the total heating value in hydrogen (kJ) and SMPs (kJ) to the total heating value of the consumed substrates (kJ). The concentrations of monosaccharides in liquid phase were determined using the 3, 5-dinitrosalicylic acid method [29]. The concentrations of amino acids in liquid phase were determined using a high-performance liquid chromatography (Agilent 1200, USA) equipped with a fluorescence detector and a Zorbax Eclipse AAA column (4.6 mm × 150 mm; Agilent, USA) [30].

3. Results and discussion

3.1. Substrate consumption and hydrogen production during the co-fermentation of glucose and glutamic acid

Glucose (1.5 g) and glutamic acid (1.5 g), corresponding to a glucose concentration of 6.0 g/L and glutamic acid concentration of 6.0 g/L, were used as substrates for hydrogen production during the co-fermentation. The changes in volumetric hydrogen productivity and substrate concentrations are shown in Fig. 1a, and the rates of substrate consumption and hydrogen production are shown in Fig. 1b. Only a small amount of hydrogen was produced in 6 h. When the fermentation time was increased to 12 h, the hydrogen production rate considerably increased to a peak of 97.1 ± 12.3 mL/L/h, and the volumetric hydrogen productivity increased to 583.2 ± 73.7 mL/L. The glucose consumption rate significantly increased to a peak of 0.219 ± 0.114 g/L/h, whereas the glucose concentration remarkably decreased to 3.38 ± 1.37 g/L. The glutamic acid consumption rate slightly increased, whereas the glutamic acid concentration gradually decreased to 5.79 ± 0.15 g/L. After a short adaptation period, AFB started to efficiently use glucose as the main substrate for hydrogen fermentation. When the fermentation time was increased to 36 h, the volumetric hydrogen productivity increased to 1548.6 ± 29.0 mL/L, whereas the hydrogen production rate gradually decreased to 12.8 ± 0.8 mL/L/h. The glucose concentration rapidly decreased to near zero, whereas the glutamic acid concentration gradually decreased to 5.17 ± 0.25 g/L. These findings indicate that AFB prefer glucose over glutamic acid. When the fermentation time was further increased to 96 h, the volumetric hydrogen productivity slightly increased to 1554.9 ± 29.7 mL/L, whereas the hydrogen production rate

Table 1
Substrate compositions during the co-fermentation of monosaccharides and amino acids.

Substrate	Monosaccharide (g)	Amino acid (g)
Glucose + glutamic acid	Glucose (1.5)	Glutamic acid (1.5)
Glucose + mixed amino acids	Glucose (1.5)	Aspartic acid (0.25), glutamic acid (0.25), serine (0.25), glycine (0.25), arginine (0.25) and alanine (0.25)
Xylose + mixed amino acids	Xylose (1.5)	Aspartic acid (0.25), glutamic acid (0.25), serine (0.25), glycine (0.25), arginine (0.25) and alanine (0.25)