Energy Conversion and Management 163 (2018) 260–267

Contents lists available at ScienceDirect

Energy Conversion and Management

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

Enhancement effect of amino acids on hydrogen production from organic fraction of municipal solid waste using co-culture of *Escherichia coli* and *Enterobacter aerogenes*

Preeti Sharma*, Uma Melkania

Department of Environmental Science, GB Pant University of Agriculture and Technology, Pantnagar 263145, Uttarakhand, India

ARTICLE INFO

Keywords:
Hydrogen production
Alanine
Cysteine
Lysine
Methionine
Histidine

ABSTRACT

In the present study, the effect of amino acids (viz. methionine, alanine, histidine, cysteine, and lysine) on hydrogen production from organic fraction of municipal solid waste was evaluated using co-culture of *E. coli* and *E. aerogenes*. The amino acids were applied in the concentration range of 1.0, 2.5, 5.0, 7.5, and 10.0 g/L. Modified Gompertz model was used to analyze cumulative hydrogen production (P), maximum hydrogen production rate (Rmax) and lag phases (λ). The results exhibited that the hydrogen production was positively affected by each amino acid at every concentration applied. Application of alanine resulted in the highest cumulative hydrogen production and volumetric yield of 685.4 ± 10.1 mL and 1.9561 $L_{H2}/L_{substrate}$, respectively. Amino acid supplementation resulted in the increase of hydrogen yield by 87.7%, 126.6%, 75.5%, 108.7% and 101.7%, respectively by addition of methionine, alanine, histidine, cysteine, and lysine as compared to control. COD removal and VFA generation were positively affected by amino acid addition.

1. Introduction

Hydrogen is emerging as a clean and sustainable alternative fuel that can be an effective way to control carbon emission. Biological hydrogen is a carbon-free renewable energy carrier, with high energy density [1]. Although electrolysis of water, auto-thermal processes, coal gasification and reforming of natural gas, are well-known technologies for hydrogen production, these processes are cost-intensive due to high energy consumption [1]. In contrast, biological hydrogen production methods are less energy intensive, environmental-friendly and sustainable compared to the current energy production methods [2]. Hydrogen production by dark fermentation can simultaneously achieve dual benefits i.e. waste treatment as well as biofuel production. Several types of substrate has been utilized for biological hydrogen production through anaerobic digestion such as food waste [3], sugarcane straw [4], kitchen waste [5], fruits and vegetables waste [6,7], brown algae [8], organic waste [9] and organic fraction of municipal solid waste [10]. The organic fraction of municipal solid waste (OFMSW) is highly degradable [11,12], thus anaerobic fermentation of OFMSW can be one of the promising methods to generate hydrogen as it is abundant and free of cost [13]. It provides an eco-friendly solution to organic waste management by converting waste to biofuel [14].

Co-culture of facultative anaerobes *E. coli* and *E. aerogenes* was used in the present study based on the results of our previous investigations [15]. These microorganisms have been reported to generate molecular hydrogen by their metabolic activities [16]. Both are facultative anaerobes and can grow under aerobic as well as strict anaerobic conditions. Both *E. coli* and *E. aerogenes* are very useful hydrogen producer with higher hydrogen evolution rate [17,18].

Kumar et al. [19] investigated hydrogen production from industrial wastewater using cultures of *E. coli* XL1-Blue/*E. cloacae* DSM 16657 and found a remarkable increase in hydrogen production by using bacterial co-culture. Maru et al. [20] used co-culture of *Enterobacter* sp and *E. coli* and found a 3.1-fold higher hydrogen productivity from pure glycerol. Application of co-culture system offers advantage of improved hydrogen production and yield as compared to mono-culture systems [21]. In co-culture system, different microbial strains are mixed which improves the individual characteristic that other strain lacks. Thus, co-culture system eliminates the need of pretreatment steps and use of expensive reducing agents. Therefore, co-culture system is cost-effective as compared to mono-cultures. It offers various advantages such as resistance to environmental fluctuation, reduction in lag phase and provides eight times more stability in hydrogen production rate as compared to mono-culture systems [21].

Several approaches such as application of pretreatment methods for substrates, mesophilic and thermophilic modes of anaerobic digestion

* Corresponding author.
E-mail address: preetisharma.doc@gmail.com (P. Sharma).

https://doi.org/10.1016/j.enconman.2018.02.072
Received 5 November 2017; Received in revised form 17 February 2018; Accepted 19 February 2018

etc have been applied to increase biological hydrogen production and yield [4,3]. To enhance hydrogen production, optimization of fermentation conditions is very important. Microorganisms are sensitive to their culture conditions in the anaerobic digesters. Nutrients in the culture medium are vital to their growth. Among nutritional requirement, nitrogen is an important factor essential for microbial growth. Nitrogen is an important process-limiting factor for fermentation [22]. In anaerobic digestion, nitrogen is added mainly in the form of urea, protein, and amino acids [23]. A variety of microorganisms ferment the amino acids obtained from degradation of proteins via purine and pyrimidine-bases and extracellular proteases [24]. Some amino acids can be used as sole nitrogen or carbon sources, however, often NH_3/NH_4^+ is the end-product of the decomposition process [25]. These by-products are used by the microbes directly as a source of nitrogen which additionally acts as a buffering system for the surrounding media [26]. A lot of previous investigations have shown that proper protein content in the substrate can improve hydrogen production [27–29]. However, the protein-derived amino acids cannot be easily used by hydrogen-producing bacteria due to low C/N ratios and their unique molecular structure [30]. Many investigations have been done to improve the hydrogen production from the protein-rich substrate. Song et al., [31] improved the hydrogen peak rate and yield by adding *Saccharomyces cerevisiae* to defatted milk powder, which produced several proteinases to facilitate degradation of lactoprotein. Xiao et al. [30] produced hydrogen from ultraviolet pretreated protein waste-water and reported 3.8 times greater hydrogen production as compared to control. The increase in the hydrogen production was due to effective disruption of hydrogen bonding networks which unfolds protein and increases their susceptibility to proteases [32]. Amino acids such as alanine can be degraded anaerobically and converted to short-chain volatile fatty acids via coupled oxidation-reduction reaction [33]. The amino acids derived from proteins of the waste substrate have low efficiency of hydrogen production [34,35], which is a technical limitation of their industrial application for hydrogen production. In several studies, it has been shown that prior to anaerobic digestion, high molecular weight proteins have to be hydrolyzed to low-molecular-weight amino acids for efficient utilization by fermentative bacteria [36,37]. The studies investigating the effect of pure amino acids on hydrogen production are limited.

There are very few reports on the effect of amino acids on the hydrogen production via anaerobic digestion. To the best of author's knowledge, the influence of amino acids on hydrogen production from OFMSW using co-culture of *E. aerogenes* and *E. coli* has not been investigated so far. Hence, in the present study pure amino acids (viz. alanine, cysteine, lysine, histidine, and methionine) are applied in various concentration ranges to improve hydrogen production and yield.

2. Methods and material

2.1. Microorganisms and media

Selective and differential media EMB (eosin methylene blue) agar was used to isolate the facultative anaerobes viz. *E. coli* and *E. aerogenes* from sewage sludge. EMB is a selective stain for gram-negative bacteria. EMB contains dyes that are toxic to gram-positive bacteria. EMB is the selective and differential medium for coliforms. It is a blend of two stains, eosin and methylene blue in the ratio of 6:1. A common application of this stain is in the preparation of EMB agar, a differential microbiological medium, which slightly inhibits the growth of Gram-positive bacteria and provides a color indicator distinguishing between organisms that ferment lactose and those that do not. EMB Agar is recommended for the isolation and differentiation of gram negative enteric bacteria from clinical and nonclinical specimens. Lysogeny broth medium (5 g yeast extract, 10 g peptone and 10 gNaCl per liter) was used for further growth of isolated strains. The isolated bacteria were incubated at 37 °C. The isolated bacteria grown for five generations were used in the experiments.

Table 1
Characteristics and composition of OFMSW.

Parameters	OFMSW
Density (kg/m^3)	1132.5 ± 20.1
Total solids (TS)%, (w/w)	29.3 ± 1.09
Volatile solids (VS)%, (w/w)	22.0 ± 2.45
Particulate chemical oxygen demand (pCOD) (g/L)	46.0 ± 3.22
Soluble chemical oxygen demand (sCOD) (g/L)	12.2 ± 1.67
Total chemical oxygen demand (tCOD) (g/L)	60.2 ± 4.89
Total kjeldahl nitrogen (TKN) (g/L)	3.89 ± 0.15
Ammonia (g/L)	0.523 ± 4.02
Carbohydrates (g/L)	48.6 ± 5.21
Lipids (g/L)	2.9 ± 0.7
Proteins (g/L)	23.8 ± 2.72
C/N	45.55 ± 3.67
Acetate (HAc) (g/L)	0.589 ± 0.12
Butyrate (HBu) (g/L)	0.496 ± 0.11
Propionate (HPr) (g/L)	0.083 ± 0.04
Lactate (HLa) (g/L)	0.127 ± 0.15
Total volatile fatty acids (TVFAs) (g/L)	1.295 ± 0.42

2.2. Feedstock preparation

The OFMSW was collected from a municipal landfill site located in Rudrapur, Uttarakhand, India. The OFMSW mainly composed of household wastes such as bread, beans, rice, fruits, vegetables, paper, and meat. OFMSW was ground using an electrical grinder resulting in the particle size of less than 2 mm. To avoid its degradation; the feedstock (OFMSW) was stored at low temperature (4 °C). Characteristics of the feedstock are presented in Table 1.

2.3. Amino acids

Amino acids viz. alanine, cysteine, lysine, histidine, and methionine were purchased from Sigma-Aldrich, India. Their characteristics of the feedstock were estimated using methods given in Section 2.5 and are presented in Table 2.

2.4. Experimental set-up

The experiments were conducted using Duran reagent bottles (500 mL) with screw caps having butyl rubber fitted in it for gas sample extraction. A 350 mL of working volume was used in the bottles. In each bottle, 300 mL of OFMSW (20 g/L OFMSW) was added. A slurry containing 20 g/L of OFMSW was prepared by adding 6 g OFMSW in 300 mL of distilled water. Nitrogen sparging of the medium was done for 5 min. The bottles were capped tightly and sealed with aluminum caps. The media was sterilized by an autoclave (Hitech equipment,

Table 2
Properties of amino acids.

Inhibitor	Alanine	Cysteine	Lysine	Histidine	Methionine
Chemical formula	$C_3H_7NO_2$	$C_3H_7NO_2S$	$C_6H_{14}N_2O_2$	$C_6H_9N_3O_2$	$C_5H_{11}NO_2S$
Molar mass (g mol^{-1})	89.09	121.15	146.19	155.16	149.21
Color	White	White	Colorless	White	White crystalline
Density (g/cm^3)	1.424	1.68	1.125	1.423	1.340
Melting point (°C)	258	240	215	282	281
Boiling point (° C)	212.9	254	311.5	458.9	306.9