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Continuous bio-hydrogen production from citric acid wastewater via facultative anaerobic bacteria

Haijun Yang[a], Peng Shao[c], Tingmao Lu[b], Jianquan Shen[a,*], Dufu Wang[b], Zhinian Xu[b], Xing Yuan[c]

[a]Institute of Chemistry, The Chinese Academy of Sciences, Zhongguancun, Beijing 100080, PR China
[b]Shandong Environment Protection Industry Co. Ltd, Jinan 250014, PR China
[c]Northeast Normal University, Changchun 130024, PR China

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Abstract

In this study, continuous biological hydrogen production using wastewater from citric acid factory as raw materials was investigated. And enrichment cultures from facultative hydrogen-producing anaerobe together with citric acid wastewater were utilized. For finding out the industrialized feasibility of continuous H_2 bio-production, the ability of H_2-production via facultative anaerobe, optimum hydraulic retention time (HRT) and optimum volume loading rate (VLR) were also studied. The experimental results showed that facultative anaerobic bacteria could yield hydrogen as high as 0.84 mol H_2/mol hexose consumed at the VLR of 38.4 kg COD/m^3 d. Butyric acid was the dominant product among volatile fatty acids (VFAs). An improved upward-flow anaerobic sludge blanket (UASB) reactor with a working volume 50 m^3 was used. Continuous hydrogen yield was 0.72 m^3 H_2/m^3 reactor d under the following conditions: temperature at 35–38 °C, initial pH at 6.8–7.2, HRT = 12 h. The study also indicates that the reactor used has a better operational stability and the facultative anaerobe has an excellent adaptive capacity for organic loading rate. Furthermore, the chemical oxygen demand (COD) removal efficiency exceeded 60% and the total sugar degradation efficiency (TSDE) was close to or over 90%, even up to 96.6% at HRT of 12 h during the experiment.

Keywords: Facultative anaerobe; Bio-hydrogen production; UASB reactor; Citric acid; Industrial wastewater

1. Introduction

At the start of the 21st century, we face significant energy challenges. As a main energy source used, fossil fuel has been overly consumed and is one of the significant causes of global warming and acid rain. The sustainable development urgently calls for a new energy source alternative fossil fuel [1,2]. Hydrogen, a renewable and entirely carbon-free fuel with a high combustion enthalpy (185 kJ/l), is regarded as an alternative to fossil fuel [3]. Its application to fuel cells is a typical example [4,5]. To date, hydrogen is produced in large amounts by the chemical and physical methods, e.g. the steam reforming, partial oxidation of fossil fuels, which make hydrogen production expensive and further environment pollution occurs [2,6]. In contrast, hydrogen generation via biological routes is economical, highly effective, and pollution free. It is expected that it will be the main feasible approach for supplying hydrogen energy in the future [2].

Hydrogen can be produced by four kinds of microbes, including anaerobic fermentative bacteria,

* Corresponding author. Tel.: +86 10 626 20903; fax: +86 10 625 59373.
E-mail address: jqshen@iccas.ac.cn (J. Shen).

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photosynthetic bacteria, cyanobacteria, and algae [6–11]. But most of the relevant researches on them almost used pure cultures as biocatalysts and were carried out on a laboratory scale [7,8]. The mixed anaerobic fermentative bacteria have significant advantages in economical energy production. This bio-energy process not only couples the need for waste treatment on a large scale and hydrogen recovery under mild operating conditions, but also couples high hydrogen production rates and low energy requirement [12,13]. But hydrogen production via acclimated sewage sludge or facultative anaerobe has been analyzed only in a few works, and these researches mainly focus on the mechanism of hydrogen production on batch experiment from single carbohydrates or continuous hydrogen production on a laboratory scale [14–17]. In the present study, facultative anaerobes were a mixed microbial system containing hydrogen production bacteria and other anaerobic microbes. Compared with other hydrogen-producing bacteria such as sludge anaerobic bacteria, hydrogen-producing bacteria used in our research have many merits, e.g. shorter hydraulic retention time (HRT) and easier acclimatization. An improved 50 m^3 upward-flow anaerobic sludge blanket (UASB) reactor was used, whose cubage is by far larger than for other pilot-scale experiments [15–17] and closer to practical production.

It is well known that if the HRT was short, facultative anaerobes would be predominant in this system by virtue of their shorter generation time (8–12 h) than that of methanogenic bacteria (1–2 days). But overshort HRT makes the treatment efficiency low. Therefore, finding the optimum HRT is a key for effective continuous hydrogen production [18–20]. Furthermore, optimized operating parameters and the feasibility of employing UASB reactor were also investigated.

2. Materials and methods

2.1. Wastewater

Citric acid wastewater from a local factory was used in this investigation. The characteristics of the raw wastewater are given in Table 1. This kind of raw wastewater was used as a single carbon source, without any balanced nutrients supplemented, such as nitrogen, phosphorus, and other nutrient microelement. But for the influent pH stability, a buffer tank was prepared by addition of 10 mol/l NaOH solution. In spite of the high cost, a certain amount of sodium carbonate was added into wastewater in order to strengthen buffer capacity.

2.2. Inoculum

Both bacterial culture and growth medium were identical to those used in the previous studies [21,22]. The facultative anaerobic organisms (predominantly *Clostridium pasteurianum*) under anaerobic conditions were inoculated into a 1.5 l completely stirred tank reactor (CSTR), followed by the growth in fed-batch culture for about 1 month. The resultant culture was subsequently acclimated to enrichment cultures in non-sterile 2 m^3 tanks containing the mixture of raw citric acid wastewater and some nitrogen sources in anaerobic conditions. After an additional month's growth, this facultative anaerobic enrichment cultures could be used to treat wastewater, and was finally poured into UASB reactor.

2.3. UASB reactor

The experiment was conducted in an improved UASB reactor made of carbon steel. Its working volume was 50 m^3 with a height of 6 m. In order to ensure complete mixing of the organic wastewater and bacteria, the reactor was equipped with not only distributed inflow but also stirrer. Three liquid sampling ports were evenly installed among the height of the column (1.3, 2.6, and 3.9 m, respectively). The upper part of the reactor was a gas–liquid–solid separator. The whole reactor was wrapped with a thermal insulating layer. A schematic diagram of the experimental setup is shown in Fig. 1. Gas production was measured with a volumetric gas flow meter (LUGB Yutao, China) and pH was controlled by pump (MDP-20R Boshan, China).

2.4. Start-up and continuous operation

The start-up and continuous operation were controlled under 35–38 °C and initial pH of 7.0 ± 0.2. The start-up continued for about 20 days. Influent velocity was increased stepwise from 2 to 8 m^3 till the reactor was full. Once all kinds of concentrations of VFAs maintained steady, the amount of fermentative gas was evolved, apparent hydrogen production occurred, and continuous operation started formally.

Two series of experiments were conducted to investigate both effects of HRT and VLR on hydrogen production. In Series 1, the HRT was gradually decreased from 48 to 8 h while wastewater concentration, initial pH, and temperature were maintained at about 18 kg COD/l, 7.0 ± 0.2, and 35–38 °C, respectively. In Series II, the organic loading rate was increased stepwise from around 10.0 to 75.0 kg COD/m^3 d via adjusting both HRT and substrate concentration under