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Effect of humic acid on photofermentative hydrogen production of volatile fatty acids derived from wastewater fermentation

Naidong Xiao [a, b], Yinguang Chen [b, *], Wenbing Zhou [a]

[a] Laboratory of Eco-Environmental Engineering, Microelement Research Center of Huazhong Agricultural University, Wuhan 430070, China
[b] State Key Laboratory of Pollution Control and Resource Reuse, School of Environmental Science and Engineering, Tongji University, Shanghai 200092, China

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ABSTRACT

Humic acid (HA) containing in the inoculated waste activated sludge (WAS) would be released into the fermentation liquid during dark fermentative hydrogen production. Nevertheless, the influence of HA on the photofermentative hydrogen production from volatile fatty acids (VFAs) left in the dark fermentation liquid by photosynthetic bacteria (PSB) had not been investigated. This study measured the effects of sludge humic acid (SHA) and AQS (anthraquinone-2-sulfonic acid, model humic acid) on the photofermentative hydrogen production from VFAs. Results showed that the photofermentative hydrogen production was reduced by 12.0% and 35.4% in synthetic wastewater with 100 mg/L of the added SHA or AQS, respectively. Mechanistic studies showed that high concentrations of SHA and AQS inhibited the activity of nitrogenase and development of PSB biomass, as well as damaging the cell membranes and causing significant death of PSB, leading ultimately to a significant decrease of photofermentative hydrogen production. Moreover, fluorescence spectra showed that SHA had a small molecular weight and a low degree of humification compared to that of AQS, which caused more negatively influence of AQS on photo hydrogen generation from VFAs. Finally, the feasibility of removing SHA from anaerobic dark fermentation liquid of wastewater to improve photofermentative hydrogen production was testified.

1. Introduction

With rapid economic development and improvements to the overall standard of living globally, there has been a corresponding increase in organic wastes [1]. Anaerobic dark fermentative hydrogen production from organic wastes, such as food waste [2], activated sludge [3], and high-concentration organic wastewater [4], has been widely studied. There were a large amount of volatile fatty acids (VFAs) accumulated in the liquid fermentation medium during anaerobic dark fermentation of organic wastes. Further converting the residual VFAs to hydrogen by photosynthetic bacteria (PSB) was an important strategy for the whole energy recovery in the dark fermentation [5–7]. Generally, waste activated sludge (WAS) was used as the inoculum for microorganisms for hydrogen production in dark, anaerobic fermentation reactors [4,8–10]. In these cases, maximal hydrogen and VFAs were usually produced under alkaline fermentation conditions [3,8,11,12], and in addition to carbohydrates and proteins, significant amount of humic acid (HA) has also been detected in WAS [13,14]. Other studies have shown under alkaline conditions that humic acid from inoculated WAS is also released into the fermentation medium [15,16]. In light of this co-occurrence, it is noteworthy that the influence of HA on the photofermentative hydrogen production from VFAs has never been investigated.

It is well known that humic acid is formed by the decay and transformation of organic matter and contains many functional groups, which could result in HA reacting physically and chemically with many organic or inorganic substances [1,17]. According to literatures reported, humic acid can significantly affect the biodegradability and bioavailability of organic wastes via changing the hydrolysis of substances and the activities of enzymes and microorganisms. For instance, HA may substantially enhance sludge-derived VFA production by improving the solubilization and acidification of sludge while inhibiting the activity of acetoclastic methanogens [14]. Studies have also shown that HA can significantly decrease biological methane production in biomass

* Corresponding author.
E-mail address: yinguangchen@tongji.edu.cn (Y. Chen).

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fermentation by inhibiting the activity of hydrolytic enzymes involved in cellulose degradation [18]. Anthraquinone-2-sulfonate (AQS), as humic analog, also has been used to increase PSB biomass production from fermentation products of VFAs from kitchen waste in a dark photo-fermentation reactor through preventing sulfate-reducing bacteria (SRB) overgrowth [19]. In addition to potential interactions with enzymes, HA can also significantly influence the electron transmission between bacteria and extracellular electron acceptors or between syntrophic bacteria [20]. It was found that HA enhanced denitrification of biological wastewater by increasing the ratio of intracellular NADH/NAD+ and thus stimulating metabolic capability of denitrifying bacteria [21]. Our previous study showed that the methane production from acetate via acetoclastic methanogens could be inhibited by HA for its inhibition on the biotransformation of acetyl-CoA to 5-methyl-THMPT and competition for electrons via blocking the way of ferredoxin regeneration [14].

To investigate the influence of humic acid derived from inoculated WAS on the photofermentative hydrogen generation from VFAs left in the anaerobic dark fermentation liquid, the fluorescence characteristics of sludge humic acid (SHA) and AQS (a humic analog) were firstly measured. Then the effects of SHA and AQS on hydrogen generation from VFAs in synthetic wastewater were compared. Subsequently, changes in nitrogenase activity, relative cell viability, lactate dehydrogenase (LDH) release, surface morphology, and LIVE/DEAD quantification of photosynthetic bacteria were examined for to determine the mechanisms for SHA and AQS inhibition of hydrogen production during photofermentation. Finally, the feasibility of removing SHA to increase hydrogen production during this fermentation process was examined.

2. Materials and methods

2.1. Preparation of anaerobic dark fermentation liquid of protein wastewater and photosynthetic bacterium

The anaerobic dark fermentation liquid of protein wastewater was collected from a hydrogen-producing bioreactor containing synthetic wastewater fermentation medium supplemented with 2.0 g/L bovine serum albumin (BSA). The BSA wastewater was pretreated with sodium hydroxide (NaOH) (pH 12) before fermentation at pH 10 for 84 h to produce hydrogen. The pretreatment and fermentation was conducted in the dark, as described in our previous publication [4]. The liquid phase was collected by centrifugation of the fermented sludge mixture at 4500 rpm for 15 min. To remove ammonia from the medium, $MgCl_2$ and KH_2PO_4 were added to form struvite, as per our previously reported optimal conditions [22]. To precipitate the sludge humic acid from the fermentation liquid during ammonia removal, the pH was adjusted to 2.6 ± 0.1 by adding 6 M HCl [23]. The fermentation liquid with ammonia and SHA removed was obtained by centrifugation at 4500 rpm for 30 min, and the characteristics of this liquid phase before and after ammonia and SHA removal are shown in Table 1. Supernatants of the liquid phase were then stored at −20 °C until used for further experiments. The photosynthetic bacteria used in this study were isolated from activated sludge in a previous study [5], and identified as *Rhodopseudomonas palustris* by 16S rDNA sequence. The cell morphology of PSB was ovoid without buds or capsule. The isolated PSB were cultured at 30 ± 1 °C with phototrophic medium for 7 d before used for the photofermentative hydrogen production experiments.

2.2. Effects of humic acid on hydrogen production through photofermentation of synthetic VFAs wastewater

Since VFAs are the primary source of organic compounds in the fermentation liquid phase for conversion to hydrogen by PSB, we investigated the effects of SHA and the model humic acid, AQS, on photofermentative hydrogen generation using VFAs in synthetic wastewater. SHA was extracted from activated sludge obtained from the secondary sedimentation tank of a municipal wastewater treatment plant in Shanghai, China. The extraction and purification of SHA was conducted according to the methods for soil humic acid extraction used by the International Humic Substance Society (IHSS) [24]. The AQS ($C_{14}H_7NaO_5S$) was purchased from Sigma-Aldrich (USA). Batch experiments on the effects of humic acid on fermentative hydrogen production were carried out in 8 identical serum bottles, each with 600 mL working volume of synthetic wastewater. The composition of synthetic VFAs wastewater is similar as that of fermentation liquid (see Table 1). The 8 bottles were divided equally into two groups to study the effects of SHA and AQS, respectively. For each group, 300 mL of synthetic wastewater had an added dosage of 0, 10, 30 or 100 mg/L humic acid. After addition of 0.45 g K_2HPO_4 and 0.45 g KH_2PO_4 as buffer salt, the pH in each bottle was adjusted to 7.0 ± 0.1 by adding 4 M HCl or 4 M NaOH. PSB were inoculated to achieve a biomass in the bottle of approximately 500 mg/L. Air in the bottles was removed by argon gas flushing upward from the liquid surface for 60 s under the gauge pressure of gas cylinder, gas flow rate, and nozzle area of 0.025 MPa, 90 L/h and 5 mm^2, respectively. After that all bottles were capped with rubber stoppers, sealed and placed in an air-bath shaker (TS-211GZ) with the rate of 160 ± 5 rpm. The photofermentation by PSB was conducted at 30 ± 1 °C using fluorescent lamps as a light source with a light intensity of 7000–7200 lux. The total gas volume was measured by releasing the pressure in the bottle using a glass syringe of 60 mL to equilibrate with the room pressure according to previously published protocols [8]. Gas was sampled with a 5 mL syringe for the composition assay. All tests were performed in triplicate, and the results were expressed as mean ± standard deviation.

2.3. Experiments of ammonia and sludge humic acid affecting photofermentative hydrogen generation from anaerobic dark fermentation liquid of protein wastewater

During fermentative hydrogen production from protein wastewater, ammonia is generated through deamination of amino acids. At the same time, SHA in the inoculated sludge is released into the

Table 1
Composition of synthetic VFAs wastewater and dark fermentation liquid of protein wastewater.

Wastewater	VFAs (mg COD/L)							NH_4^+-N (mg/L)
	acetic	propionic	isobutyric	n-butyric	isovaleric	n-valeric	TCOD	
Synthetic VFAs wastewater	972.3 ± 24.6	283.6 ± 8.8	220.0 ± 7.3	526.3 ± 13.2	532.4 ± 12.4	0.0 ± 0.0	2534.7 ± 66.3	0.0 ± 0.0
Dark fermentation liquid	903.6 ± 18.8	299.7 ± 9.1	211.3 ± 6.9	477.6 ± 12.5	556.2 ± 13.1	0.0 ± 0.0	2448.4 ± 60.4	281.3 ± 7.5
Liquid with ammonia removal	894.5 ± 17.9	295.4 ± 9.0	206.2 ± 6.8	471.3 ± 12.7	550.7 ± 13.7	0.0 ± 0.0	2418.1 ± 60.1	25.6 ± 3.3
Liquid with ammonia and SHA removal	885.3 ± 17.0	291.6 ± 8.7	202.8 ± 6.5	467.2 ± 12.2	546.4 ± 13.1	0.0 ± 0.0	2339.3 ± 57.5	24.2 ± 3.0