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Enhancement of biohydrogen production from grass by ferrous ion and variation of microbial community

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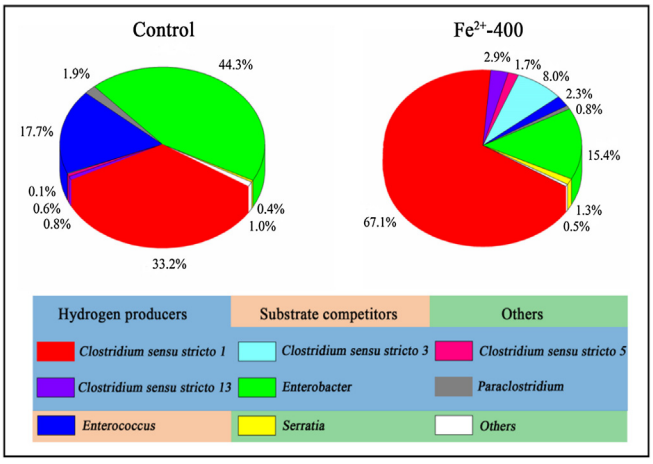
GRAPHICAL ABSTRACT



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ABSTRACT

The enhancement of biohydrogen production from grass was investigated by addition of Fe^{2+}, and the microbial community, microbial activity and kinetic models analysis were used to explore the improving mechanisms of Fe^{2+} addition. The results showed that Fe^{2+} could significantly improve hydrogen fermentation efficiency of grass. The highest hydrogen yield achieved 72.8 mL/g-dry grass at the Fe^{2+} addition of 400 mg/L, which was 49.6% higher in comparison with the control group (48.7 mL/g-dry grass). Organics utilization was also improved from 15.9% to 20.6% at the Fe^{2+} addition of 400 mg/L. Fe^{2+} changed the metabolic pathway towards more efficient hydrogen production and accelerated the hydrolysis of grass, thereby enhancing the conversion process of substrates to hydrogen. Furthermore, microbiological analysis showed that Fe^{2+} improved the microbial activity and enriched more hydrogen-producers, and changed the dominant H_2-producer from *Enterobacter* to *Clostridium*. The addition of Fe^{2+} also significantly decreased the presence of H_2-competitor *Enterococcus*. This study demonstrated that the addition of Fe^{2+} was an effective and a simple strategy to improve fermentative hydrogen production from grass.

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1. Introduction

Hydrogen is an ideal fuel due to the properties of high heating value (122 MJ/kg) and clean combustion product [1]. Recently, renewable hydrogen produced from dark fermentation has gained increasingly attentions. Dark fermentation has some unique advantages, such as low operating cost, low energy demand, fast reaction rate, and simple operation conditions [2]. More attractively, dark fermentation process can utilize various types of organic wastes as feedstock for producing hydrogen [3–6], which simultaneously achieves renewable energy generation and organic wastes utilization.

As a kind of agroforestry waste, grass residue has shown great potential as the feedstock for hydrogen fermentation because of its properties of low cost, stable source, abundance, and high carbohydrate content (50–80% of dry weight) [7]. Some researchers have used grass as the substrate for successful hydrogen production via dark fermentation [8–12]. Despite this potential use, grass is commonly characterized by the lack of trace metals [13,14], which may restrict its efficient hydrogen production. Some specific trace metals (e.g. Fe, Ni and Mg) are crucial for the growth and metabolism of microbes and also the synthesis of essential enzymes in hydrogen evolution pathways [15]. Therefore, to achieve high hydrogen production from grass, it is suggested that specific trace metals should be added into the process.

Fe is the most commonly added trace metal in hydrogen fermentation because of its crucial role in the metabolic machinery of microbes and enzymes active in hydrogen evolution process. Fe is an essential element for the formation of hydrogenases (e.g. Fe-Fe hydrogenase and Ni-Fe hydrogenase) and ferredoxin [16]. Hydrogenases contain Fe clusters and a unique Fe-S center termed the hydrogen cluster (as the site of hydrogen oxidation and hydrogen evolution). The Fe clusters serve to transfer electrons between the external electron carrier (e.g. NADH) and the hydrogen cluster [17]. Some studies have evaluated the impact of Fe^{2+} addition on the performance of hydrogen fermentation [16,18–21]. These studies have demonstrated that the addition of Fe^{2+} can improve hydrogen production and substrate utilization, as well as induce more efficient metabolic pathway in dark fermentation. So far, most of these studies used simple sugars (e.g. sucrose and glucose) as substrate, while the functions of Fe addition on hydrogen fermentation of grass still remain unknown. Actually, hydrogen fermentation of grass is totally different with the fermentation of simple sugars in terms of more complex substrate composition and more complex metabolic pathways (e.g. the hydrolysis of complex carbohydrates) [22]. Meanwhile, previous studies also found that the optimal Fe dosage for various substrates showed a significant difference in dark fermentation. For instance, the optimal dosages of Fe^{2+} were 50, 352.8 and 20 mg/L for glucose [23], sucrose [20] and starch [16] at similar experimental conditions, respectively. Therefore, the optimal Fe dosage for other substrates may provide little reference to grass. However, the optimal demand of Fe for grass hydrogen fermentation is still unclear. Furthermore, most of previous studies mainly focused on the impact of Fe addition on hydrogen fermentation performance (e.g. hydrogen production and substrate utilization) and soluble metabolites formation. Actually, the process performance and the metabolites formation in dark fermentation essentially depend on microbial communities presenting in the system [24]. Thus, understanding the microbial community structure may be particularly important to demonstrate the role of added Fe in dark fermentation, but the knowledge available to date remains limited.

In this study, different concentrations of Fe^{2+} were added into grass hydrogen fermentation system, with the aim to explore an effective and a simple strategy to improve hydrogen production from grass. The optimal dosage of Fe^{2+} for grass fermentation was examined. In addition, the impact of Fe^{2+} addition on substrate utilization, waste reduction as well as soluble metabolites formation was also comprehensively investigated. Furthermore, the analysis of microbial community structure, microbial activity and kinetic models was conducted to reveal the enhancement mechanisms.

2. Materials and methods

2.1. Grass residue and seed sludge

In this work, grass residue (*Lolium perenne* L.) was taken from a lawn of Shanghai (China) in October 2016. Thereafter, the grass residue was dried at 60 °C, and then was milled to about 18-mesh. Before use, the grass residue was conserved at 4 °C. Main characteristics of the grass residue are as follows: moisture content of 74.6%, volatile solids (VS) content of 84.6% of dry weight, total carbohydrate content of 651.6 mg/g-VS, total protein content of 221.3 mg/g-VS, carbon content of 43.9% of dry weight, nitrogen content of 4.2% of dry weight, and hydrogen content of 6.1% of dry weight.

Seed sludge used in this work was collected from the sludge anaerobic digester of a wastewater treatment plant in Beijing, China. The physicochemical properties of the seed sludge were as follows: the pH value of 7.16, total solids of 18868 mg/L, VS of 10693 mg/L, total protein of 5892 mg/L and total carbohydrate of 1475 mg/L. The seed sludge was firstly heat-shocked at 100 °C for 15 min to enrich H_2-producing bacteria [25], and then was directly used as the inoculum for the later fermentation tests.

2.2. Fermentative hydrogen fermentation

Batch hydrogen fermentation tests were carried out in 150 mL glass bottles. 1 g VS of grass and 30 mL of inoculum were added for each bottle [26]. After the mixture of feedstock and inoculum, certain $FeSO_4$ solution with Fe^{2+} of 5 g/L was added into each bottle, respectively. Then, each bottle was filled to the total working volume of 100 mL with deionized water, which made the Fe^{2+} concentrations range from 50, 100, 200, 300, 400 to 600 mg/L in the batch tests. The test without Fe^{2+} addition was served as the control group. No other nutrient solution was added in this work. For each bottle, the initial pH was set as 7.0 with NaOH and HCl solutions. All bottles were flushed with nitrogen for three minutes to remove oxygen. Finally, all bottles were placed in a water bath shaker (100 rpm) at 37 °C for hydrogen fermentation. Batch tests were performed in triplicate for each Fe^{2+} condition.

2.3. Microbial community analysis

2.3.1. DNA extraction and PCR amplification

At the end of hydrogen fermentation, two mixed samples were collected from the control group and the optimal Fe^{2+} addition group for the analysis of microbial community structure, respectively. DNA extraction and qualification were processed based on the methods described in our previous work [22].

For each DNA sample, the V4-V5 region of 16S rRNA gene was amplified using PCR. The primer pairs 515F and 907R were applied for the PCR amplification. The PCR solution (20 μL) contained 10 ng of template DNA, 4 μL of 5× FastPfu Buffer, 2 μL of dNTPs (2.5 mM), 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase and Milli-Q water. The PCR program was performed according to Yu et al. [27]. PCR products were then purified and re-quantified for the later high-throughput sequencing process.

2.3.2. MiSeq sequencing and data analysis

High-throughput sequencing process was carried out with an Illumina MiSeq PE250 platform. Sequences were clustered into operational Units (OTUs) at the 97% similarity level. For the phylogenetic affiliation, 16S rRNA gene sequences were analysed using RDP Classifier against the silva (SSU115)16S rRNA database at a 70% confidence threshold. Alpha diversity in each sample was assessed using the indices of Shannon and Simpson. Spearman correlation analysis was