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Improving mechanisms of biohydrogen production from grass using zero-valent iron nanoparticles

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ABSTRACT

This paper investigated the improving mechanisms and microbial community dynamics of using zero-valent iron nanoparticles (Fe^0 NPs) in hydrogen fermentation of grass. Results showed that Fe^0 NPs supplement improved microbial activity and changed dominant microbial communities from *Enterobacter* sp. to *Clostridium* sp., which induced a more efficient metabolic pathway towards higher hydrogen production. Meanwhile, it is also proposed that Fe^0 NPs could accelerate electron transfer between ferredoxin and hydrogenase, and promote the activity of key enzymes by the released Fe^{2+}. The maximal hydrogen yield and hydrogen production rate were 64.7 mL/g-dry grass and 12.1 mL/h, respectively at Fe^0 NPs dosage of 400 mg/L, which were 73.1% and 128.3% higher compared with the control group. Fe^0 NPs also shorten the lag time and facilitated the hydrolysis and utilization of grass. This study demonstrated that Fe^0 NPs could effectively improve hydrogen production and accelerate the fermentation process of grass.

1. Introduction

Hydrogen production by dark fermentation using various biomass feedstocks has attracted more and more attentions in recent years, because this process can synchronously achieve clean energy generation and biomass resource recycling (Rajhi et al., 2016; Dennehy et al., 2017; Wang and Yin, 2018). Among biomass feedstock, grass is a promising option for hydrogen fermentation owing to its advantages of abundance, stable source, high carbohydrate content and low cost (Yang and Wang, 2018a). Despite the potential of this process, the main challenge of grass hydrogen fermentation is the unsatisfactory hydrogen yield (4.4–37.8 mL/g-dry grass) (Cui and Shen, 2012; Li et al., 2012; Pakarinen et al., 2008). Currently, the popular means for improving grass hydrogen fermentation typically include mechanical (e.g. microwave and ultrasound), chemical (e.g. acid and alkaline) and biological pretreatments (e.g. enzyme), which aim to disrupt the rigid biomass structure to improve the bioavailability of grass (Sivagurunathan et al., 2017). However, these pretreatments have their drawbacks, such as low efficiency, energy consumption, inhibitory effect and high cost. Therefore, it is necessary to develop other effective, energy-saving and environmentally-friendly strategies to enhance the efficiency of grass hydrogen fermentation.

Zero-valent iron nanoparticles (Fe^0 NPs) have increasingly been used in environmental remediation and industrial wastewater treatment for removing chlorinated contaminants and heavy metals due to its environmental friendliness and high efficiency (Zou et al., 2016). Meanwhile, Fe^0 NPs also have a certain role in improving hydrogen fermentation process (Nath et al., 2015). First, the supplement of Fe^0 NPs to hydrogen fermentation process can remove undesired oxygen in the system, thereby contributing to a higher activity of the oxygen-sensitive hydrogenase. Second, as a reductive material, Fe^0 NPs can decline oxidation–reduction potential (ORP) in the system, providing a more favorable environment for fermentative bacteria. Furthermore, Fe^0 NPs can be easily oxidized to Fe^{2+} in the system, facilitating the synthesis of hydrogenase and iron-sulphur protein in ferredoxin.

Owing to the advantages mentioned above, a few investigations have studied the influence of Fe^0 NPs on hydrogen fermentation performance, and observed that Fe^0 NPs supplement could effectively improve hydrogen production efficiency (Taherdanak et al., 2016; Nath et al., 2015; Taherdanak et al., 2015). At present, almost all studies with Fe^0 NPs supplement used simple sugars as substrate, such as glucose and starch. However, the functions of Fe^0 NPs in hydrogen fermentation of grass are still unclear. Actually, grass fermentation is significantly different with the simple sugars fermentation due to the more complicated substrate composition and more complex fermentation pathway. In addition, previous researches mainly focused on the influence of Fe^0 NPs on hydrogen production. The microbial communities in the system essentially determine the process performance and

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the formation of metabolites (Noblecourt et al., 2018). Thus, the analysis of microbial community structure may be crucial for understanding the essential roles of Fe^0 NPs in hydrogen fermentation, while the information available to date remains limited. Furthermore, although it has been reported that Fe^0 NPs could enhance the efficiency of hydrogen fermentation, the enhancement mechanisms are still ambiguous.

In this study, the potential of using Fe^0 NPs for enhancing hydrogen fermentation of grass was evaluated, with the objective to provide an energy-saving and effective strategy for improving hydrogen recovery from biomass resource. The impact of Fe^0 NPs on the process performance was comprehensively investigated, including hydrogen production, substrate utilization and metabolites distribution. In addition, the impact of Fe^0 NPs on microbial community structure was investigated through high-throughput pyrosequencing. Furthermore, the enhancement mechanisms of Fe^0 NPs in grass hydrogen fermentation were highlighted.

2. Materials and methods

2.1. Feedstock, seeding sludge and Fe^0 NPs

Grass biomass (*Lolium perenne L.*) was collected from a campus lawn. Moisture content, C/N ratio, volatile solids (VS) content and carbohydrate content of the grass were 74.6%, 10.5, 846 mg/g-dry weight and 651.6 mg/g-VS, respectively. Before using as feedstock, the grass was dried and milled to about 18-mesh.

The seeding sludge was sampled from a local sewage treatment plant in Beijing,. Total solids (TS) content, VS content, carbohydrate content and pH of the seed sludge were 18868 mg/L, 10693 mg/L, 1475 mg/L and 7.16, respectively. The seeding sludge was heated to 100 °C and maintained 15 min to kill hydrogen-consuming bacteria (Kim et al., 2006).

Fe^0 NPs (99.9% metal basis, 50 nm) used in this study was purchased from the Shanghai Macklin Biochemical Co., Ltd, China.

2.2. Batch fermentation

Batch fermentation experiments were carried out in 150 mL glass bottles with a working volume of 100 mL. 30 mL of inoculum and 1 g-VS of grass (i.e. substrate concentration of 10 g-VS/L) were mixed in each bottle (Yang and Wang, 2018b). Afterwards, 50, 100, 200, 400 and 600 mg/L of Fe^0 NPs were added into these bottles to explore the impact of Fe^0 NPs on hydrogen fermentation performance. The bottle without Fe^0 NPs supplement was set as the control group. In addition, the bottles with 50, 100, 200, 400 and 600 mg/L of Fe^0 NPs were defined as the Fe-50, Fe-100, Fe-200, Fe-400 and Fe-600 groups in this study, respectively. No other nutrients solution was used in the present work. In all bottles, the initial pH was adjusted to 7.00 ± 0.05 using HCl (1 mol/L) and NaOH (1 mol/L), N_2 gas were purged for 3 min to maintain oxygen-free condition, and then shaken at 37 °C and 100 rpm for hydrogen fermentation. Experiment for each Fe^0 NPs dosage was performed in triplicates

2.3. Microbial community analysis

When hydrogen production process stopped, two samples were collected from the optimal Fe^0 NPs supplement group and the control group to analyze microbial community structure. DNA was extracted using soil DNA extraction kit (Yang and Wang, 2018a). For each DNA sample, the universal primers 515F (5′-barcode- GTGCCAGCMGCCG CGG-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′) were selected for PCR amplification (Wang et al., 2016; Yang and Wang, 2018a), which targeted the V4-V5 region of bacterial 16S rRNA gene. The PCR reactions were carried out in a 20 μL solution containing 2 μL of 2.5 mM dNTPs, 0.4 μL of FastPfu Polymerase, 0.8 μL of each primer (5 μM), 4 μL of 5× FastPfu Buffer and 10 ng of template DNA. PCR conditions were 95 °C for 2 min, 95 °C for 30 s repeated 25 cycles, 55 °C for 30 s, 72 °C for 30 s and a final extension at 72 °C for 5 min (Yin and Wang, 2016a).

High-throughput sequencing was processed using an Illumina MiSeq PE250 platform by Shanghai Majorbio Bio-pharm Technology Co. Ltd. (Shanghai, China). The indices of Shannon and Simpson were calculated for evaluating microbial diversity in each sample (Wang et al., 2016). The indices of Ace and Chao1 were calculated to evaluate microbial richness in each sample (Yin and Wang, 2016a). Correlations between process performance and identified bacteria were assessed by the Pearson correlation analysis.

2.4. Analytical methods

The quantitative analyses of VS and TS were performed using the standard method (APHA, 2005). The value of pH and ORP of the fermentation mixture were measured by a pH meter and an ORP meter. Carbohydrate concentration was analyzed according to DuBois et al. (1956). For Fe^{2+} determination, the fermentation mixture was firstly centrifuged at 10000 rpm for 5 min, and then 5 mL of the supernatant was immediately acidified by adding 1 mL of 1 mol/L HCl solution to prevent the oxidation of Fe^{2+}. After filtering the acidified supernatant through a 0.22 μm membrane, the Fe^{2+} concentration was determined using a UV–vis spectrophotometer (Zhang et al., 2015). Dehydrogenase activity was analyzed based on the process performed in Nikaeen et al. (2015), and expressed as mg of TPF released per gram of VSS. 5 mL of fermentation mixture was thoroughly mixed with 3 mL of 3% 2,3,5-TTC solution and 3 mL of distilled water. After incubation at 37 °C for 24 h in darkness, 10 mL of methanol was added to the reaction mixture and the sample was agitated at 200 rpm for one hour. The suspension was then centrifuged at 10000 rpm for 5 min, and the supernatant was filtered through a 0.22 μm membrane. The amount of TPF in the filtrate was determined using a UV–vis spectrophotometer at 485 nm. A control sample without TTC addition was included for each test.

For each bottle, total biogas volume was determined using displacement of saturated aqueous NaCl in a graduated cylinder. Hydrogen content in biogas was determined using a gas chromatograph (model 112A, Shanghai, China) equipped with a thermal conductivity detector (TCD) and a packed column (model TDX-01, long 3 m, diameter 3 mm). The carrier gas was argon. The pre-column pressure was 0.2 MPa. The temperatures of the injector, column and detector were 180, 160 and 110 °C, respectively (Yang and Wang, 2017b). Ethanol, acetate, propionate, butyrate and valerate were quantified by a high performance liquid chromatography (HPLC) (Shimadzu LC-20AD, Tokyo, Japan) equipped with an Aminex HPX–87H column. The injected volume was 20 μL. The mobile phase was 5 mM H_2SO_4, and the flow rate was 0.5 mL/min. The column temperature was kept at 60 °C. (Yang and Wang, 2018b).

2.5. Kinetic analysis

In the present work, the following models (Eqs. (1) and (2)) were applied to analyze the properties of hydrogen fermentation process.

$$H = P\,exp\{-exp[(\lambda - t)R_m e/P + 1]\} \quad (1)$$

$$H = \frac{P}{1 + (k_{hyd}\,t)^{-n}} \quad (2)$$

where, t is the fermentation time (h); H is the cumulative hydrogen production at fermentation time t (mL); P is the cumulative hydrogen production potential (mL); λ is the lag time (h); R_m is the maximum hydrogen production rate (mL/h); e is 2.718 28; n is the shape factor; k_{hyd} is the hydrolysis rate constant (1/h).