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# Enhanced dark fermentative hydrogen production by zero-valent iron activated carbon micro-electrolysis

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## ABSTRACT

This paper investigated the enhancement effect of zero-valent iron activated carbon (ZVI-AC) micro-electrolysis system on fermentative hydrogen production from glucose by a mixed bacterial consortium. The results showed that addition of ZVI obtained 38.2% more hydrogen yield, and ZVI-AC micro-electrolysis could further enhance hydrogen yield (improving rate 50.2%). The ZVI-AC also got the highest hydrogen production potential and maximum hydrogen production rate ( $429 \pm 6.1$  mL and  $11.50 \pm 0.74$  mL/h respectively). The concentration of ferrous ion, final oxidation-reduction potential and pH were measured to understand the mechanism of enhanced hydrogen production by ZVI-AC micro-electrolysis. We concluded that ZVI-AC micro-electrolysis provided a favorable environment which was proposed to be responsible for the improvement of fermentative hydrogen production. In addition, high-throughput sequencing proved that ZVI-AC micro-electrolysis could enhance diversity of microbial community and enrich *Clostridium* spp. for hydrogen production.

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## Introduction

The use and abuse of fossil fuels has caused energy crisis and environmental pollution [1]. Hydrogen gas (H<sub>2</sub>) can be used as an efficient energy vector to power fuel cell systems used in transportation or stationary applications [2,3]. Hence, it is an alternative fuel to reduce the over reliance on fossil fuels and avoid generating harmful gases such as carbon monoxide (CO), nitrogen oxides (NO<sub>x</sub>) and particulates (PM10) [4].

However, more than 96% of global hydrogen production depends on fossil fuels and consumes as much as 2% of the world's energy demand [5]. Therefore, it is necessary to develop other methods for hydrogen production in a cost-effective, sustainable and environmentally friendly way. Among various hydrogen production processes, biological method can provide H<sub>2</sub> from cheap and abundant sources such as wastewater or organic wastes from food industry and agriculture [6]. Meanwhile, biological hydrogen production is less energy intensive compared to other hydrogen processes

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[7]. Biological method mainly includes photosynthetic hydrogen production and fermentative hydrogen production. As compared with photosynthetic hydrogen production, fermentative hydrogen production is technically simpler due to less energy intensity and its independence on the availability of solar irradiation [8].

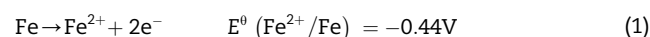
Iron is essential micronutrient for bio-hydrogen production. It serves as the co-factor for hydrogenase and iron-sulphur protein. Hydrogenase presents in a wide variety of prokaryotes and functions in the disposing of excess electrons by forming hydrogen [9]. The iron-sulphur protein in ferredoxin (Fd) acts an electron carrier in pyruvate oxidation into Acetyl CoA and CO<sub>2</sub> [10]. Lee et al. [11] studied the effect of iron concentration on hydrogen production using sucrose solution and mixed microorganism from digester. The maximum hydrogen production rate was found to be 24 mL/g VSS/h at 4000 mg/L FeCl<sub>2</sub> (1760 mg Fe<sup>2+</sup>/L). On the other hand, Dogan Karadag [12] found a much smaller optimal Fe<sup>2+</sup> concentration (50 mg/L) to achieve the maximum hydrogen yield (1.13 mol H<sub>2</sub>/mol glucose). Yang and Shen [13] found a similar optimal Fe<sup>2+</sup> concentration (55 mg/L) corresponding maximum cumulative H<sub>2</sub> volumes (225 mL). Although the optimal Fe<sup>2+</sup> concentration is very different, the authors agree that shortage of iron could limit the hydrogenase activity along with bio-hydrogen production. Addition of an appropriate amount of iron in batch tests enables better production of hydrogen at different temperatures [14]. In addition, the researchers have suggested that when the ambient temperature is relatively lower, bacteria need more ferrous ion to activate the hydrogenase enzyme, so that it can oxidize reduced ferredoxin for better production of molecular hydrogen [15]. Recently, metal oxide (Fe<sub>x</sub>O<sub>y</sub>) nanoparticle has been applied to enhance fermentative hydrogen production due to its affinity for electrons and high reactivity [16,17]. However, it also faces a critical issue that hinders its application. Nanoparticle is prone to aggregation during synthesis and application, and thereafter result in diminished reactivity [18].

Except ferrous compound, zero-valent iron (ZVI) has been applied in bio-hydrogen production because it is readily oxidized to Fe<sup>2+</sup> by chemical and electrochemical reaction. The iron shavings were found to promote the hydrogen production, and the effect was more obvious in low pH buffered medium than in higher buffered medium [19]. Li et al. [20] compared the hydrogen yield from glucose using ZVI and Fe<sup>2+</sup>, respectively. The results showed that ZVI is better to promote bio-hydrogen production than Fe<sup>2+</sup>, and the inhibition of excess ZVI to Hydrogen producing bacteria (HPB) is lower than Fe<sup>2+</sup>. Unfortunately, the corrosion of ZVI is strongly dependent on the pH of solution, and the dissolve ferrous oxides formed on ZVI surface would reduce the activity of iron.

Currently, micro-electrolysis has been widely applied in treatment of various wastewaters for its easy operation, low cost and good treatment efficiency [21]. ZVI is commonly used as sacrificial anode of micro-electrolysis and oxidized to Fe<sup>2+</sup>. Surface area ratio of cathode to anode (S<sub>c</sub>/S<sub>a</sub>) is an important factor affecting micro-electrolytic reaction rate. When S<sub>a</sub> is constant, the larger S<sub>c</sub>, the faster reaction rate. Activated carbon (AC) has exceptionally high surface area (ranges from

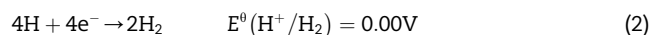
500 to 1500 m<sup>2</sup> g<sup>-1</sup>) and well-developed internal micro-porosity [22], so it is an ideal cathodic material for micro-electrolysis. When ZVI (anode) and AC (cathode) are mixed and contacted with each other, numerous microscopic galvanic cells are formed spontaneously between these two electrodes, and simultaneous occurrences of redox reactions on the surface of a large number of electrodes can result in significant electron flow in micro-electrolysis system. Because the electric energy is converted from chemical energy of ZVI, micro-electrolysis is much less expensive than conventional electrolysis with an external power supply. The half-cell reactions can be represented as [23]:

Anode (oxidation):

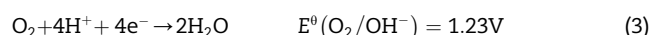


Cathode (reduction):

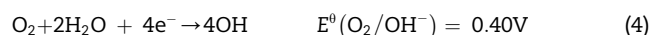
Acidic:



Acid with oxygen:



Neutral:



To our best knowledge, there has been no study that investigated the effects of ZVI-AC micro-electrolysis on dark fermentative hydrogen production till date. Hence, this work was carried out to assess the performance of ZVI-AC micro-electrolysis system in dark fermentative hydrogen production and investigate the mechanism of enhanced hydrogen production in such system. For this purpose, the effect of ZVI dosage on hydrogen production from glucose medium was firstly investigated. Then the enhancement of hydrogen production by ZVI-AC micro-electrolysis were evaluated and compared with adding ZVI only. Finally, the possible mechanism of enhanced hydrogen production by ZVI-AC micro-electrolysis were explored.

## Materials and methods

### Anaerobic sludge and materials

The anaerobic bacteria used in this study was obtained from the secondary sedimentation tank of municipal wastewater treatment plant in Chengdu, China. Then the sludge was heated in boiling water bath for 30 min to inhibit methanogenic microbes and enrich HPB. The pH, total suspended solids (TSS) and volatile suspended solids (VSS) were 7.2, 1.1 g/L and 0.83 g/L, respectively. The sludge was concentrated by setting at 24 h, and storage at 4 °C before use. High-purity ZVI powders (>98.5%) were obtained from Chengdu Chemical Reagent Plant (China). Prior to each experiment, the ZVI powder was pre-treated with dilute hydrochloric acid (pH = 3) for 10 min to remove oxides covering on its surface, and then washed with distilled water, finally dried under N<sub>2</sub> gas. Activated carbon felt was cleaned by soaking 1 mol/L HCl and

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