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Free ammonia enhances dark fermentative hydrogen production from waste activated sludge

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ABSTRACT

Ammonium and/or free ammonia (the unionized form of ammonium) are generally thought to inhibit the activities of microbes involved in anaerobic digestion of waste activated sludge. It was found in this work, however, that the presence of ammonium (NH_4^+-N) largely enhanced dark fermentative hydrogen production from alkaline pretreated-sludge. With the increase of initial NH_4^+-N level from 36 to 266 mg/L, the maximal hydrogen production from alkaline (pH 9.5) pretreated-sludge increased from 7.3 to 15.6 mL per gram volatile suspended solids (VSS) under the standard condition. Further increase of NH_4^+-N to 308 mg/L caused a slight decrease of hydrogen yield (15.0 mL/g VSS). Experimental results demonstrated that free ammonia instead of NH_4^+-N was the true contributor to the enhancement of hydrogen production. It was found that the presence of free ammonia facilitated the releases of both extracellular and intracellular constituents, which thereby provided more substrates for subsequent hydrogen production. The free ammonia at the tested levels (i.e., 0–444 mg/L) did not affect acetogenesis significantly. Although free ammonia inhibited all other bio-processes, its inhibition to the hydrogen consumption processes (i.e., homoacetogenesis, methanogenesis, and sulfate-reducing process) was much severer than that to the hydrolysis and acidogenesis processes. Further investigations with enzyme analyses showed that free ammonia posed slight impacts on protease, butyrate kinase, acetate kinase, CoA-transferase, and [FeFe] hydrogenase activities but largely suppressed the activities of coenzyme F420, carbon monoxide dehydrogenase, and adenylyl sulfate reductase, which were consistent with the chemical analyses performed above.

1. Introduction

Hydrogen is widely considered the most promising alternative to fossil fuels, as it has a high energy yield (142.35 kJ/g) with at least 2.75 times that of any hydrocarbons and produces water instead of greenhouse gases when it is combusted (Cai et al., 2004). As the major byproduct of biological wastewater treatment, waste activated sludge is produced in huge quantities (Feng et al., 2015; Li et al., 2016; Wang et al., 2017a, 2017b). Treatment and disposal of the sludge are costly, accounting for up to 60% of the total operation costs of wastewater treatment plants (WWTPs) (Zhao et al., 2017; Wang et al., 2017c). On the other hand, sludge contains high levels of organic constituents such as protein and carbohydrate (Zhao et al., 2016; Xu et al., 2017; Xie et al., 2016; Chen et al., 2018; Wang et al., 2018), which can be used as substrates for hydrogen production. Therefore, biological production of hydrogen from waste activated sludge has recently attracted much attention (Zhao et al., 2010; Gioannis et al., 2013; Li et al., 2009), by which sludge is reduced and reused, fossil fuels are saved, and greenhouse gas productions are reduced.

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The hydrogen yield of dark fermentation of waste activated sludge is usually low due to rapid hydrogen consumption in comparison to sludge disintegration. Most previous investigations to enhance hydrogen production, therefore, focused on optimizing sludge-pretreatment methods (Yang et al., 2012; Assawamongkholsiri et al., 2013; Kim et al., 2013), operational conditions (Zhao et al., 2010; Zhou et al., 2013; Jung et al., 2011), or sludge composition (Kim et al., 2012; Chen et al., 2012a; Wang et al., 2015). For instance, Cai et al. (2004) found that sludge pretreated by alkaline (pH 11) for 24 h could increase hydrogen yield from 9.1 to 16.6 mL of H_2/g of dry solids. The bioconversion of sludge proteins and hydrogen production were found to be largely enhanced by co-fermentation of sewage sludge and carbohydrate-rich substrates, such as food wastes and agricultural wastes (Kim et al., 2012; Chen et al., 2012b; Liu et al., 2013). By elevating the content of sludge polyhydroxyalkanoates from 25 to 178 mg/g volatile suspended solids (VSS), hydrogen production from alkaline anaerobic fermentation of sludge increased from 26.5 to 58.7 mL/g VSS (Wang et al., 2015). Apart from these parameters, byproducts that are *in situ* generated in the anaerobic fermentation process may also affect hydrogen yield. To date, however, little information is available to this field.

NH_4^+-N, which is produced from the disintegration of nitrogen-rich compounds such as proteins, urea, and nucleic acids, could be accumulated at high concentrations in the sludge digestion or fermentation process (Yan et al., 2010; González-Fernández et al., 2009). The NH_4^+-N concentration in the sludge fermentation liquid is usually at ~300 mg/L (Chen et al., 2007; Zhao et al., 2015), and this value could be up to 1500 mg/L in the sludge digestion liquid (Wang et al., 2014). Almost all previous studies demonstrated that the presence of NH_4^+-N especially at high levels showed inhibitory effects on bio-gas production from anaerobic digestion. For example, Sung and Liu (2003) found that compared with the control (0.4 g/L), 5.0 and 5.8 g/L of NH_4^+-N resulted in 39% and 64% decreases in methane production, respectively. Nakakubo et al. (2008) reported that a 50% reduction in methane yield under thermophilic anaerobic digestion was observed at NH_4^+-N concentration of 11.0 g/L, as compared with 4.6 g/L NH_4^+-N. These findings suggested that it was essential to remove or reduce NH_4^+-N levels in the anaerobic digestion process to ensure bio-gas production.

Nevertheless, it was found in the current work that when 63–272 mg/L NH_4^+-N was added into the sludge mixture, hydrogen yield from alkaline pretreated-sludge was enhanced rather than reduced, as compared to the case without NH_4^+-N addition. There were two varying parameters in these fermentation systems. One is the NH_4^+-N added. As pH variation is similar among these fermentation systems, thus the other varying parameter is free ammonia (FA), the unionized form of ammonium, due to the high concentrations of ammonium added and alkaline condition maintained. FA can diffuse through cell membrane, shuttle protons between the two sides, and result in cell inactivation (Kayhanian, 1999). This could cause variations in the activities of function microbes involved in sludge anaerobic fermentation, which thereby may affect hydrogen production. However, the role of FA on hydrogen production from dark fermentation of sludge has not been clarified so far. It is unknown which one (NH_4^+-N or FA) is the main contributor to the enhanced hydrogen production.

The aim of this study was to identify whether and how FA enhances dark fermentative hydrogen production. First, hydrogen production from sludge with alkaline pretreatment (pH 9.5) in the presence of NH_4^+-N at different levels (36–308 mg/L) was compared. To identify the potential contribution of NH_4^+-N to hydrogen production, hydrogen yield from acidic fermentation of sludge (pH 5.5) was also compared at the same NH_4^+-N levels. It should be emphasized that the variations of FA level among these acidic fermenters were negligible, thus the results obtained could be used to indicate the impact of NH_4^+-N on hydrogen production. Finally, the mechanisms for FA enhancing hydrogen production were explored. The findings reported in this work reveal the details of how FA enhances hydrogen production for the first time, erase the concern regarding the inhibitory effect of NH_4^+-N or FA produced in the fermentation process on dark fermentation, and may guide engineers to develop more economic strategies for hydrogen production from sludge fermentation.

2. Materials and methods

2.1. *The sources and characteristics of waste activated sludge*

The sludge employed in this work was withdrawn from the secondary sedimentation tank of a municipal WWTP in Changsha, China. The raw sludge was filtrated by a stainless steel mesh (2.0 mm) and concentrated by setting at 4 °C for 24 h before use. The major characteristics of the concentrated sludge are as follows: pH 6.8 ± 0.1, total suspended soils (TSS) 14920 ± 260 mg/L, VSS 12140 ± 170 mg/L, total chemical oxygen demand (COD) 14780 ± 290 mg/L, total carbohydrate 1650 ± 230 mg COD/L, total protein 7780 ± 310 mg COD/L, lipid and oil 170 ± 20 mg COD/L, and NH_4^+-N 36 ± 4 mg/L. It can be seen that protein and carbohydrate are the top two organics in the sludge, accounting for about 64% of total sludge COD.

2.2. *Hydrogen production from sludge with alkaline pretreatment in the presence of NH_4^+-N at different levels*

This batch test was conducted in eight serum bottles with a working volume of 1 L each. Each serum bottle was first fed with 500 mL concentrated sludge, as mentioned above. Then different volumes of NH_4Cl stock solution (4.0 M) were added at the beginning of the test, which resulted in the initial NH_4^+-N concentration of 36, 36, 99, 141, 182, 224, 266, or 308 mg/L. It should be noted that NH_4^+-N was not added to the first two bottles, and 36 mg/L NH_4^+-N was the background ammonium concentration. Except one serum bottle with NH_4^+-N concentration at 36 mg/L (set as the blank), the tested sludge in all other serum bottles was pretreated under alkaline condition (pH 9.5) at 35 °C for 24 h before fermentation, as alkaline pretreatment was demonstrated to be an effective method for enhancing biohydrogen production from waste activated sludge (Cai et al., 2004).

The NH_4^+-N concentration, temperature, and pH applied gave rise to initial FA concentrations of 0.3 (the blank), 34, 95, 135, 174, 214, 254, or 294 mg/L. FA concentration was determined by the formula $S_{(NH3\text{-}\overset{+}{N}+NH4\text{-}N)} \times 10^{pH}/(K_b/K_w+10^{pH})$, where $S_{(NH3\text{-}\overset{+}{N}+NH4\text{-}N)}$ represents the concentration of NH_3-N + NH_4^+-N, K_b represents the ionization constant of the ammonia equilibrium equation, and K_w represents the ionization constant of water (Anthonisen et al., 1976). The value of K_b/K_w was calculated via the formula of $K_b/K_w = e^{6,344/(273+T)}$ (Anthonisen et al., 1976). After pretreatment, the serum bottles were sparged with nitrogen gas for 5 min to ensure anaerobic condition. Finally, all serum bottles were capped with rubber stoppers, sealed, and placed in an air-bath shaker (150 rpm). No extra inoculum was added into these reactors, and hence the sludge was used as both fermentation substrate and inoculum. In the fermentation process, the pH value in all bottles was not controlled but was recorded periodically. The total gas volume was measured by releasing the pressure in the bottle using a glass syringe (300 mL) to equilibrate with the atmospheric pressure (Oh et al., 2003). The cumulative volume of hydrogen gas was calculated as: