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Effects of pretreatment method of natural bacteria source on microbial community and bio-hydrogen production by dark fermentation

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ABSTRACT

The effects of pretreatment method of cow dung compost, which was employed as natural hydrogen bacteria source, on the microbial community, population distribution of microbes and hydrogen production potential were investigated in the batch tests. The maximum hydrogen yield of 290.8 mL/L-culture appeared in the pretreated method A (infrared drying) by dark fermentation. The pretreated method of compost significantly affected microbial succession, population distribution of microbes. Both *Clostridium* sp. and *Enterobacter* sp. were found to be two species of preponderant hydrogen-producing bacteria, the next best was *Bacteroides* sp. and *Veillonella* sp., the last was *Lactobacillus* sp. and *Streptococcus* sp., which were also essential. The results showed that the mutualism and symbiosis relations of the mixed bacteria played a critical role in hydrogen fermentation process.

1. Introduction

Energy crisis and global environmental impacts from the use of fossil fuels are challenging the scientists to develop nonpolluting and renewable energy source. Bio-hydrogen production is attracting more and more attention based on its cleaning, environmentally friendliness and regeneration [1,2].

As we all know, the microbial community affected directly bio-hydrogen production efficiency during hydrogen fermentation from mixed microbes (such as cow dung compost). Among which, the species and population distribution of microbes of dominant hydrogen-producing microorganisms were also affected importantly by pretreatment method of compost [3]. Consequently, it is of great significance to reveal distribution characteristics of hydrogen-producing bacteria in the different pretreatments of compost. Recently, Ren and his cooperators reported that the pretreatment methods of the seed sludge might lead to the difference in the initial microbial communities, and eventually resulted in the change of fermentation types, such as butyric-acid type fermentation was achieved by the heat-shock and alkaline pretreatments, and ethanol-type fermentation was observed by repeated-aeration pretreatment [4]. Afterward a similar work had also been reported by Liu and co-workers, of which, the acid and heat pretreatments of the seed sludge were found to be effective methods in order to enrich the dominant hydrogen-producing bacteria such as *Clostridium* sp., *Enterococcus* sp. and *Bacillus* sp [5]. In the meantime, the colony counting method has been widely used to investigate microbial succession. e.g., Zdolec et al. studied microbiological succession by Plate Count Agar (PCA) in the ripening of dry fermented sausages, which

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was filled with bacteriocinogenic culture of *Lactobacillus sakei* and semi-purified bacteriocin mesenterocin Y [6]. Torres et al. reported that microbial successions in litter decomposition were strongly influenced by synergic interaction between microbial functional groups (such as ammonifiers, nitrifiers, N-fixing, cellulolytic, and sugar fungi) and litter chemical composition (such as carbon, nitrogen, insoluble fiber, and phenol concentration) [7]. However, the information regarding dynamics and population distribution of bacteria in fermentation hydrogen production is surprisingly lacking to date.

In the present work, we studied the effects of four pretreatment methods of cow dung compost on the microbial community, distribution of microbes and hydrogen production by dark fermentation. The mutualism and symbiosis relations of the mixed bacteria were further observed in the batch tests.

2. Materials and methods

2.1. Seed microflora

The natural microbial consortium, cow dung compost, was obtained from a dairy farm in Zhengzhou City. Prior to use, the compost was composted for two months at ambient temperature. The physical characteristics of compost were as follows: brown, pH = 8.0 and moisture content of 58–60%. Prior to use, the manure was pretreated using the following four different methods to deactivate the hydrogenotrophic methanogens and harvest high yield hydrogen-producing anaerobes.

A (infrared drying): the compost was baked in an infrared oven for 2 h [2];

B (boiling): the mixture of the compost and tap-water with the solid/liquid ratio of 1:4 was boiled for 30 min;

C (forced aeration): the mixture of the compost and tap-water with the solid/liquid ratio of 1:4 was continuously aerated by forced-air pumping for 72 h at ambient temperature;

D (compulsive aeration combined adding sugar): Both compost and tap-water were mixed according to the solid/liquid ratio of 1:4, followed by the addition of sugar (5 g/L), the mixture was compulsively aerated by forced-air pumping for 72 h at ambient temperature.

After that, the pretreated compost was further pre-incubated with sucrose in anaerobic reactors at 36 ± 1 °C and initial pH 7.0 for about 16 h before inoculation [8,9].

2.2. Batch experiments of hydrogen production

The batch experiments were performed with 140-mL serum vials as batch reactors filled to 50 mL with a mixture of the seed microorganism comprising sucrose and 3 mL of nutrient stock solution. Nutrient stock solution was consisted of 80 g/L NH_4HCO_3, 12.4 g/L KH_2PO_4, 0.1 g/L $MgSO_4\cdot 7H_2O$, 0.01 g/L NaCl, 0.01 g/L $Na_2MoO_4\cdot 2H_2O$, 0.01 g/L $CaCl_2\cdot 2H_2O$, 0.015 g/L $MnSO_4\cdot 7H_2O$, and 0.0278 g/L $FeCl_2$, which was slightly modified from the work of Lay et al. [10].

The initial pH values of the medium were adjusted by pH buffer solution. These vials were gassed with nitrogen to remove oxygen and to keep the anaerobic environment, and then capped with rubber stopper. The bottles were incubated at 36 ± 1 °C in an orbital shaker with a rotation speed of 120 rpm to provide better contact among substrates. At each time interval, the total biogas volume was measured by releasing the pressure in the bottles using a gas-collecting vessel of displacement method with saturated brine. All the experiments were carried out independently in triplicates.

2.3. Bacterial enumeration

Samples were taken at 24-h intervals from the hydrogen-producing bioreactor throughout the whole fermentation process and then serially diluted with phosphate buffered saline (PBS). Next, the diluted samples were spread onto the appropriate agar plates as below: Neomycin nagler (NN) medium was used for the enumeration of *Clostridium* sp., and Desoxycholate hydrogen sulfide lactose (DHL) medium was used to enumerate Enterobacteriaceae sp., *Lactobacillus* sp. was enumerated by counting colonies on *Lactobacilli* selective (LBS) Agar. Neomycin brilliant green taurocholate (NBGT) medium was employed as selective medium for *Bacteroides* sp. The *Veillonella* selective (VS) medium was favorable for the growth of *Veillonella* sp. Triphenyl tetrazolium chloride acridine orange thallous sulfate aesculin crystal violet (TATAC) medium was used as *Streptococcus* sp. selective medium, respectively [11,12].

Anaerobic plate counts were performed by manually counting colonies on Plate Count Agar incubated for 72 h at 36 ± 1 °C under anaerobic conditions. To ensure the reliability of results, all the samples were carried out independently in triplicate.

2.4. Analytical methods

Hydrogen concentration was measured by a gas chromatograph (GC, Agilent 4890D) equipped with a thermal conductivity detector (TCD) and 6 feet stainless column packed with Porapak Q (80/100 mesh). The operational temperatures of the injection port, the oven and the detector were 100 °C, 80 °C and 150 °C, respectively. Nitrogen was used as the carrier gas at a flow rate of 20 mL/min. Hydrogen gas was calculated from the headspace measurement of gas composition and the total volume of biogas produced, at each time interval, using the mass balance equation:

$$V = V_o r_i + \sum V_i r_i \quad (1)$$

Where, V is the cumulative hydrogen gas volumes at the current (i); V_0 is the volume of headspace of vials; V_i is the biogas volume discharged from the vials at the time interval (i); r_i is the fraction of hydrogen gas discharged from the vials at the time interval (i) [13].

2.5. Kinetic modeling

The cumulative volume of H_2 production in the batch experiments followed the modified Gompertz equation: