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Hydrogen supersaturation in extreme-thermophilic (70 °C) mixed culture fermentation

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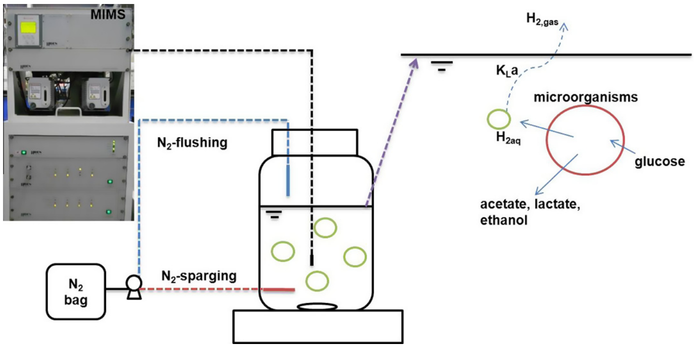
HIGHLIGHTS

- H_2 supersaturation occured in extreme-thermophilic mixed culture fermentation.
- H_2 supersaturation ratio (R_{H2}) increased when reducing H_2 partial pressure (P_{H2}).
- Metabolite distribution changed little under low P_{H2} due to the high R_{H2} value.
- Mass transfer calculation indicated H_2 supersaturation was likely inevitable.
- Suggested gas sparging rate vs H_2 production rate was 2–10 to improve H_2 yield.

GRAPHICAL ABSTRACT



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ABSTRACT

Hydrogen supersaturation in extreme-thermophilic (70 °C) mixed culture fermentation (MCF) was demonstrated for the first time by membrane inlet mass spectrometry. It was found that hydrogen supersaturation ratio (R_{H2}) increased dramatically (from 1.0 to 20.6) when H_2 partial pressure (P_{H2}) was reduced by N_2 flushing or sparging. The distribution change of metabolites was insignificant under low P_{H2} (<0.30 atm) due to the high value of R_{H2}, which indicated that it was more relevant to the concentration of dissolved H_2 (H_{2aq}) rather than P_{H2}. To explain the cause of hydrogen supersaturation, the overall volumetric mass transfer coefficients (K_La) for H_2 were calculated. K_La changed slightly (~7.0/h) with N_2 flushing, while it increased from 7.4 to 10.2/h when N_2 sparging rate increased from 0.3 to 17.9 mL/min/L. However, the required K_La values were orders of magnitude higher than the experimental ones when maintaining low R_{H2} by gas sparging, which indicated that hydrogen supersaturation was likely inevitable in MCF. Moreover, to improve the hydrogen yield of MCF, the gas sparging rate was suggested as 2–10 times of the hydrogen production rate.

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1. Introduction

Conventional fuels such as petroleum and natural gas are amount to 85% of the world energy requirements, which result in the irreversible diminishment of natural reserves in the coming years and the terrible environmental pollutions. Therefore, the alternative technologies to simultaneously produce clean energy and degrade the existing wastes are worldwide needed [1]. Mixed culture fermentation (MCF) is a mature and promising technology to convert organic wastes into the valuable chemicals and biofuels, such as hydrogen, ethanol and polyhydroxyalkanoates (PHAs) [2–5]. Among the above products, hydrogen is an ideal energy carrier for the carbon-free nature and high energy density and so on [6]. H_2 plays a very important role in MCF to control the redox

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balances and metabolic pathways of fermentative microorganisms [7,8]. Most researches use H_2 partial pressure (P_{H2}) as an indicator to understand the role of H_2 in MCF [9–11]. However, H_2 in liquid phase which the organisms are actually exposed to was usually ignored as the complicated and unstable measurement of the concentration of dissolved H_2 in bulk solution (H_{2aq}) [12,13].

Hydrogen supersaturation has been observed in a few MCF processes [13–15]. Kraemer and Bagley have observed this phenomenon in mesophilic MCF regardless of N_2 sparging [13]. Our study of thermophilic MCF showed the hydrogen supersaturation ratio (R_{H2}) increased with the increase of organic loading rate and the decrease of Reynold number [14]. These suggest that H_{2aq} shall be a more proper factor than P_{H2} to study the effect of H_2. Recently, more and more studies were focusing on MCF at extreme-thermophilic conditions (>65 °C) due to better pathogen destruction and thermodynamic conditions [16–18]. Hydrogen supersaturation under these conditions was usually ignored due to higher diffusion coefficient of H_2 at higher temperature [19]. However, H_2 supersaturation was observed in batch experiments of the extreme-thermophilic (70 °C) *Caldicellulosiruptor saccharolyticus* [12], which suggested that it may also exist in extreme-thermophilic MCF. On the other hand, pH has a significant effect on microbial metabolism [5,20–22], while the alkaline condition was seldom investigated in MCF. A high H_2 yield (3.1 mol/mol$_{glucose}$) in fermentation performed under pH 8.0 was reported recently [23]. Under the alkaline condition, more produced CO_2 will be dissolved and the P_{H2} level must be increased, which might result in the elevation of hydrogen supersaturation.

Hydrogen supersaturation in MCF was largely related to different operating conditions, such as stirring and gas sparging [13,14,24]. R_{H2} increased from 3 to 11 when N_2 sparging was used to decrease H_{2aq} in fermentative liquor [13]. It decreased from 2.8 to 1.8 as the stirring rate increased from 120 to 450 rpm [14]. The overall volumetric mass transfer coefficient (K_La) is representative of the rate of gas transfer from liquid to gas, and is clearly specific to a given reactor and mode of operating condition [25,26]. It has been used to explain the relationship between H_2 mass transfer and N_2 sparging rate, and to optimize H_2 production of N_2-sparged bioreactors [25]. Hence, K_La may be a useful parameter to investigate the relationship between hydrogen supersaturation and different operating conditions.

Therefore, the objective of this study was to investigate hydrogen supersaturation in extreme-thermophilic (70 °C) MCF at pH 8.0. To accomplish this objective, N_2 sparging and N_2 flushing were performed to change P_{H2} in a continuous stirred tank reactor (CSTR) under two different stirring rates (150 and 400 rpm). The relationship between hydrogen supersaturation and P_{H2} was investigated. K_La was calculated to describe the possible effects of different operating conditions to hydrogen supersaturation. The variation of metabolites and the effect of different gas sparging rates were also discussed.

2. Materials and methods

2.1. Inoculum, reactor setup and media

The inoculum used in this study was an anaerobic sludge taken from a UASB reactor treating citrate-producing wastewater. As shown in Fig. 1, a glass-made CSTR reactor (1.8 L capacity and 1.35 L working volume) was used. The anaerobic sludge was acclimatized in the reactor before experiments. At the initial period of acclimation, the temperature gradually increased from 30 to 70 °C within 30 days by a water bath, while pH was gradually increased from 7.0 to 8.0 with automatic addition of 2 M NaOH. Hydraulic retention time (HRT) was kept at 10 days for 30 days to ensure the biomass activity. Then, it was gradually decreased to 0.8 day within 30 days. The whole acclimation approximately lasted for 90 days. The equipment was interfaced to computer via an Opto PLC used for data logging and set-point modification. Meanwhile, the system was also equipped with a Hiden HPR-40 DSA Membrane Inlet Mass Spectrometer (MIMS) for online monitoring H_{2aq}.

The synthetic feed consisted of 50% solution A and 50% solution B. Solution A contained 10 g/L of glucose and 1 g/L of yeast extract, autoclaved at 110 °C for 20 min. The composition of solution B (mg/L) was as follows: KH_2PO_4 400; KCl 104; Na_2SO_4 84; NH_4Cl 20; $MgCl_2 \cdot 6H_2O$ 144; $CaCl_2 \cdot 2H_2O$ 20; $MnCl_2 \cdot 4H_2O$ 1.6; $CoCl_2 \cdot 6H_2O$ 2.4; H_3BO_3 0.4; $CuCl_2 \cdot 2H_2O$ 2.2; $NaMO_4 \cdot 2H_2O$ 0.2; $ZnSO_4 \cdot 7H_2O$ 6.4; $FeSO_4 \cdot 7H_2O$ 6.4; $NiCl \cdot 6H_2O$ 1.0; EDTA 0.5.

2.2. Experimental design

N_2 flushing or sparging was used to introduce changes in the headspace gas composition at two different stirring rates (400 and 150 rpm). When the stirring rate was 400 rpm, the N_2 flushing

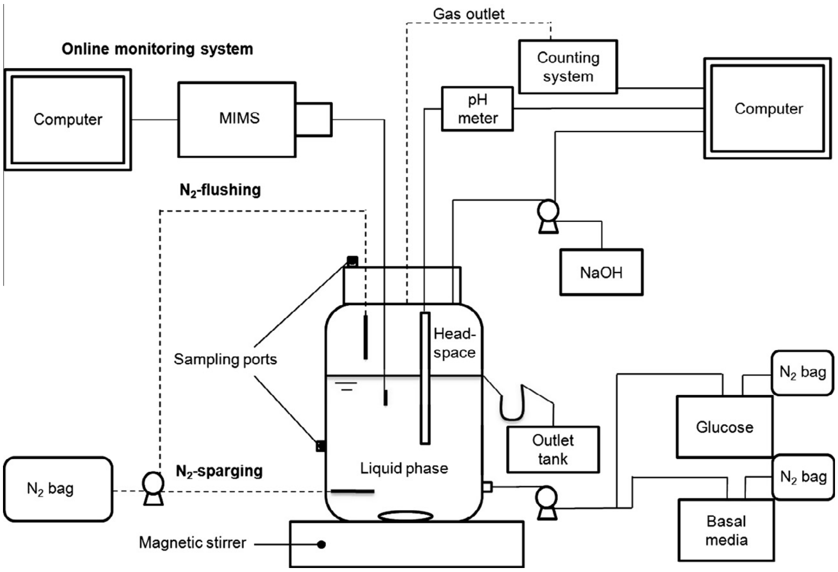

Fig. 1. Schematic diagram of the experimental setup.

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