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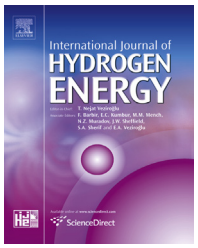

Effect of headspace carbon dioxide sequestration on microbial biohydrogen communities

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ABSTRACT

This study investigated the impact of CO_2 removal from the headspace of a continuous flow biohydrogen production system on the H_2 yield and microbial community structure. A comparative study was conducted in the Integrated Biohydrogen Reactor Clarifier System (IBRCS) with and without potassium hydroxide in the reactor headspace to sequester CO_2 using glucose. Headspace CO_2 sequestration increased the H_2 yield by 22% to 2.96 ± 0.14 mol/mol$_{hexose}$. The impact of headspace CO_2 sequestration was not limited to the improvement in H_2 yield and gas quality, as it also influenced the metabolic pathway increasing acetate concentration, and decreasing butyrate and propionate concentrations. Detailed analyses of the microbial community structure in the IBRCS before and after CO_2 sequestration revealed that removal of CO_2 from the headspace of the bioreactor had a significant impact on the microbial diversity and species distribution which rationalize the observed changes in the metabolic pathways.

Introduction

Hydrogen (H_2) production by dark fermentation is characterized by relatively low yields, with higher yields only possible through thermodynamically unfavourable pathways. In addition, the product gas is a mixture of H_2 and carbon dioxide (CO_2), which creates challenges for the useful application of the H_2 as a fuel [1]. Specifically, CO_2 is a major contaminant in fuel cell technologies that generate electricity from H_2 gas [2], as proton exchange membrane fuel cells (PEMFCs) require high-purity H_2 (greater than 99%) [3].

The two most common dark fermentation pathways for H_2 production from glucose are the acetate and butyrate pathways (reactions 1 and 2) [4], which limit the theoretical H_2 yield to between 2 and 4 moles of H_2 per mole of glucose. Both

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reactions are thermodynamically favourable (i.e. negative ΔG values) and the greater the acetate to butyrate ratio, the higher is the H_2 yield. Therefore, directing the metabolism of the culture towards acetate formation is key to achieving higher H_2 yields [5]. Also, in order to maximize the H_2 yield, metabolism should be directed away from alcohols (ethanol, butanol) and reduced acids (lactate) towards volatile fatty acids (VFAs) production [6]. However, propionate production decreases the H_2 yield, since it is a H_2-consuming pathway (reaction 3) [7].

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad \Delta G_R^\circ = -196\ KJ \tag{1}$$

$$C_6H_{12}O_6 \rightarrow CH_3(CH_2)_2COOH + 2CO_2 + 2H_2 \quad \Delta G_R^\circ = -224\ KJ \tag{2}$$

$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O \quad \Delta G_R^\circ = -279\ KJ \tag{3}$$

Nath and Das [4] stated that removing CO_2 efficiently from the culture medium will shift H_2-synthesizing reactions in the forward direction, increasing H_2 production, and decreasing the consumption of reducing equivalents carried by electron carrier's molecules like Nicotinamide Adenine Dinucleotide (NADH) by competing reactions [4]. Kraemer and Bagley [8] discussed several methods for improving the H_2 yield, one of which was removing dissolved H_2 and CO_2 from the liquid phase of the fermentation process.

In addition, H_2 and CO_2 are the main substrates for both hydrogenotrophic methanogenic bacteria and homoacetogenic bacteria to produce methane (reaction 4) and acetate (reaction 5), respectively [9,10]. Mayumi et al. [11] observed that increasing CO_2 concentrations accelerated the rate of hydrogenotrophic methanogenesis in oil reservoirs. Also, Saady [10] indicated that controlling CO_2 concentrations during dark fermentative H_2 production needs further investigation as a potential approach towards controlling homoacetogenesis. Therefore, dissolved CO_2 removal from the liquid phase may prevent the consumption of H_2 for methane (CH_4) or acetate production.

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad \Delta GR^\circ = -131\ KJ \tag{4}$$

$$4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O \quad \Delta GR^\circ = -104\ KJ \tag{5}$$

One of the common techniques used for dissolved gas removal is gas sparging. Hussy et al. [12] observed an increase in the H_2 yield from 1.0 to 1.9 mol/mol $hexose_{converted}$ using sucrose as the substrate in a continuous stirred-tank reactor (CSTR) operated at a hydraulic retention time (HRT) of 15 h and achieving 95% sucrose conversion after sparging nitrogen (N_2) gas continuously in the reactor. Kim et al. [13] tested the utilization of N_2 as a sparging gas in H_2 production from sucrose in a CSTR operated at an HRT of 12 h and loading of 40 gCOD/L.d and observed a 24% increase in the H_2 yield to 0.93 mol H_2/mol hexose. Tanisho et al. [14] observed a 110% increase in the H_2 yield to 1.09 mol H_2/mol hexose by continuous purging of argon gas in a H_2 producing batch experiment by *Enterobacter aerogenes* using molasses as the carbon source.

Non-sparging techniques to decrease the dissolved gas concentrations include increasing of stirring speed, applying vacuum in the headspace (i.e. decreasing the reactor headspace pressure), using in-reactor ultrasonication, and using an immersed membrane to remove the dissolved gases [8,15,16]. Mandal et al. [17] observed an increase of 105% in the H_2 yield to 3.9 mol H_2/mol hexose of a batch H_2 producing experiment from glucose by *Enterobacter cloacae* by decreasing the headspace total pressure. The increase in H_2 yield was attributed to inhibition of H_2 consumption due to the decrease in total pressure that lead to the production of reduced by-products such as ethanol and organic acids [17]. The aforementioned authors also used a potassium hydroxide (KOH) trap outside the batch reactor headspace to absorb CO_2. Liang et al. [18] used a silicone rubber membrane to separate biogas from the liquid phase in a H_2 fermentation batch reactor using glucose as the substrate, and observed 15% and 10% increases in H_2 yield and H_2 production rate, respectively.

Park et al. [19] were the first to apply headspace CO_2 sequestration using KOH in batch H_2 glucose fermentation, and achieved a H_2 content of 87.4% in the headspace. They recommended assessing CO_2 removal from the headspace of a continuous system instead of batches to measure how effectively CO_2 would be removed, specially under different OLRs [19].

Two H_2-producing pathways from butyrate and propionate that are thermodynamically unfavourable (reactions 6 and 7) [20] can occur if H_2 as a product is decreased to its minimum concentration, converting Gibbs free energy from positive to negative values [20]. Similarly, the propionate to acetate pathway (reaction 6), which is thermodynamically unfavourable, could be shifted forward if CO_2 was removed from the headspace.

$$CH_3CH_2COO^- + 2H_2O \rightarrow CH_3COO^- + CO_2 + 3H_2 \quad \Delta G_R^\circ = +72\ KJ \tag{6}$$

$$CH_3(CH_2)_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + H^+ + 2H_2 \quad \Delta G_R^\circ = +48\ KJ \tag{7}$$

Microbial community composition in a H_2 reactor directly affects the fermentation efficiency [21]. Therefore, it is important to explore the changes in species diversity and population distribution of the predominant H_2 producers due to the removal of CO_2 from the reactor headspace. 16S rDNA-based techniques have been widely used for the qualitative and quantitative analysis of microbial communities [22].

As depicted in this brief introduction, CO_2 presents several challenges to the application of biohydrogen systems, not the least of which is reduced H_2 yield due to hydrogenotrophic methanogens and homoacetogens, and the necessity for biogas cleanup prior to utilization. In addition, the literature is devoid of information on the impact of CO_2 sequestration from continuous flow systems, as most of the few published studies that attempted to sequester CO_2 were done in batch reactors. Moreover, previous studies did not investigate the impact of sequestration on metabolic pathways and microbial community structure, and have only focused on H_2 yield. Therefore, the objective of this study is to evaluate the impact of CO_2 sequestration on H_2 yield, H_2 production rate, chemical buffering requirements, metabolic pathways, and microbial community structure in a novel continuous flow biohydrogen production system.