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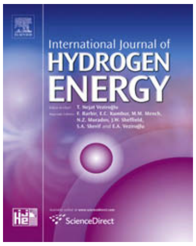

# Sodium inhibition of fermentative hydrogen production

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ABSTRACT

A continuous-stirred-tank reactor (CSTR) was fed with low-sodium influent containing 0.27 g of $Na^+$/L for 70 days (Phase I), and then subjected to higher concentrations of $Na^+$/L, i.e. 2.41 (Phase II), 5.36 (Phase III), and 10.14 g (Phase IV-1). At the quasi-steady state of each phase, biomass was sampled for an acute sodium toxicity assay. Unlike the control biomass, which exhibited a monotonic decrease of specific $H_2$ production activity (SHPA) with increasing sodium concentration from 0.27 to 21.00 g $Na^+$/L, the acclimated biomass maintained their activity up to 6.00 g $Na^+$/L. Soluble microbial product analysis revealed that a sudden increase of the exterior sodium concentration changed the metabolic pathway such that it became favorable to lactate production while depressing butyrate production. Meanwhile, when the biomass was allowed for sufficient time to adapt to the chronic toxicity condition, the volumetric $H_2$ production rate (VHPR) was maintained above 4.05 L $H_2$/L/d at up to Phase III. However, an irrecoverable $H_2$ production drop was observed at Phase IV-1 with a significant increase of lactate and propionate production. Although the sodium concentration decreased to 8.12 (Phase IV-2), 6.61 (Phase IV-3), and 5.36 g $Na^+$/L (Phase V) at further operation, the performance was never recovered. A PCR-DGGE analysis revealed that lactic acid bacteria (LAB) and propionic acid bacteria (PAB) were only detected at Phases IV and V, which are not capable of producing $H_2$.

## 1. Introduction

The current energy system based on fossil fuels is now facing two fundamental problems in sustainability: the depletion of fossil fuel and environmental pollution. This has led to an extensive search for new alternative energy sources and carriers [1]. Among various candidates, hydrogen is regarded as the most promising energy carrier, since it produces only water when combusted and has a 2.75 times higher energy yield (122 kJ/g) than hydrocarbon fuels. In addition, as an automotive fuel, $H_2$ can be easily applied in proton exchange membrane fuel cell vehicles as well as conventional internal combustion engines [2].

$H_2$ can be made via several ways, including electrolysis of water, thermocatalytic reformation of hydrogen-rich organic compounds, and biological processes. Currently, $H_2$ is exclusively made by steam reforming of gas, requiring electricity derived from fossil fuel combustion, a process that is energy intensive and expensive. Feedstock and energy for $H_2$ production must be renewable if its purpose is to decrease the

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current dependency on fossil fuel. Sustainable generation of $H_2$ may be achieved by a range of technologies, including biological processes [3].

Biological $H_2$ production can be achieved by phototrophic and non-phototrophic methods. With the help of light, autotrophs such as algae and heterotrophs such as *Rhodobacter* sp. produce $H_2$ from water and organic wastewater, respectively. However, phototrophic $H_2$ production confronts several obstacles: (1) low solar energy conversion efficiency (2) slow reaction rate, and (3) low light penetration due to biofilm formation on the reactor wall. On the other hand, non-phototrophic production, often called fermentative $H_2$ production (FHP), offers many advantages such as fast reaction rate, degradation of solid organic wastes, technical simplicity, and no need of light. As a result, it can serve to address two critical global issues simultaneously, energy supply and environmental protection [4].

FHP is one of the ways releasing excess electrons derived from organics with the help of the ‘hydrogenase’ function in bacteria. The following equations, Eqs. (1) and (2), are the main $H_2$ production reactions involved in FHP from glucose. Comprehensive studies have been conducted dealing with operating parameters such as pH, hydraulic retention time (HRT), carbon source, and $H_2$ partial pressure [5]. However, little information is available about the effect of cations on FHP.

$$\text{Glucose} + 2\ H_2O \rightarrow 2\ \text{Acetate} + 2\ CO_2 + 4\ H_2 + 4\ \text{ATP} \tag{1}$$

$$\text{Glucose} \rightarrow \text{Butyrate} + 2\ CO_2 + 2\ H_2 + 2\ \text{ATP} \tag{2}$$

Cations play essential roles in adenosine (ATP) synthesis, nicotinamide adenine dinucleotide (NAD) oxidation/reduction, and enzyme activity, thereby accelerating microbial metabolism if maintained at proper concentrations. However, excessive cations can cause plasmolysis and loss of cell activity by creating high osmotic pressure and improper enzyme linkages. In particular, sodium, the main cation in biomass and seawater, leads to many problems in biological treatment systems of wastewater from seafood processing, the dairy industry, and chemical production. Besides, sodium concentration can be increased by buffer addition such as NaOH, $Na_2CO_3$, and $NaHCO_3$. Therefore, research has been carried out on anaerobic digestion and biological nutrient removal processes to identify the inhibition mechanisms and alleviation of its inhibition [6,7].

For methane fermentation, it has generally been reported that sodium concentration over 2 g $Na^+$/L will cause a performance drop [8,9]. However, some studies have reported that continuous exposure of microorganisms to higher sodium levels increased the sodium tolerance. Seafood processing wastewater containing 5–12 g/L of sodium was successfully treated with an anaerobic filter process [10]. According to Feijoo et al. [11], sodium concentration causing 50% inhibition exceeded 10 g $Na^+$/L for the sludge obtained from digesters treating high saline wastewater, but it was lower than 5 g $Na^+$/L when unacclimated sludge was used. Also, a stepwise increase of sodium level exhibited higher tolerance than a shock increase [12]. These findings suggest that acclimation could mitigate sodium inhibition.

There are two known bacterial strategies to adapt to and cope with high sodium concentration; salt-in and compatible-solute strategies [13]. The main mechanism in the salt-in strategy is the extrusion of sodium ions outside cells concurrent with accumulation of potassium ions inside cells via a proton electrochemical gradient and at ATP expense. As this strategy does not reduce the osmotic pressure inside the cell, all intracellular systems should be tolerant at high osmotic pressure. On the other hand, in the compatible-solutes strategy, compatible solutes such as glycerol, ectoine, and glycine betaine are created for the proper functions of intracellular systems at high osmotic pressure. Debate continues over which strategy is more energy efficient, but it is clear that life in a salty environment is costly.

Although salty organic substances, such as waste from the foodstuff industry, could be suitable sources for FHP and sodium is employed as the cation in general buffers and nutrients for FHP, research on this subject is scarce. Therefore, the present study aimed at evaluating the sodium inhibition of FHP. Chronic and acute sodium toxicity was investigated by continuous-stirred-tank reactor (CSTR) operation and batch tests at various sodium concentrations, respectively.

## 2. Materials and methods

### 2.1. *Seed sludge and substrate*

The seed sludge was taken from an anaerobic digester in a local wastewater treatment plant. The pH, alkalinity, and volatile suspended solids (VSS) concentration of the sludge were 7.5, 2.83 g $CaCO_3$/L, and 5.3 g/L, respectively. The sludge was heat-treated at 90 °C for 15 min to inactivate hydrogen consumers and to harvest spore-forming anaerobic bacteria such as *Clostridium* sp. [14]. Sucrose of 25 g COD/L was used as a substrate. Concentrations of $NH_4Cl$, $KH_2PO_4$, and $FeCl_2 \cdot 4H_2O$ were added to yield a COD:N:P:Fe ratio of 100:5:1:0.33. Feed also contained the following nutrients (in mg/L): $NaHCO_3$ 1000; $MgCl_2 \cdot 6H_2O$ 100; $CaCl_2 \cdot 2H_2O$ 75; $Na_2MoO_4 \cdot 4H_2O$ 0.01; $H_3BO_3$ 0.05; $MnCl_2 \cdot 4H_2O$ 0.5; $ZnCl_2$ 0.05; $CuCl_2$ 0.03; $NiCl_2 \cdot 6H_2O$ 0.05; $CoCl_2 \cdot 2H_2O$ 0.5; $Na_2SeO_3$ 0.05 [14].

### 2.2. *Continuous operation*

In this study, a CSTR with a working volume of 5.0 L (325 mm high by 140 mm ID) was used. The chemical oxygen demand (COD) loading rate was maintained at 50 g/L d during the entire period of operation, which corresponded to 12 h of hydraulic retention time (HRT). The reactor was mixed by mechanical stirring at 100 rpm. The pH was maintained at $5.3 \pm 0.1$ using a pH sensor, pH controller, and 3 N KOH. Biogas production was monitored by the water displacement method and then corrected to standard temperature (0 °C) and pressure (760 mmHg) (STP). All experiments were conducted in a constant temperature room at $35 \pm 1$ °C. After being seeded with heat-treated sludge equivalent to 30% of the total effective volume, the reactor was purged with $N_2$ gas for 5 min to provide an anaerobic condition, and then operated in batch mode. When the $H_2$ yield reached 0.2 mol $H_2$/mol $\text{hexose}_{\text{added}}$ in the batch operation, continuous operation started [15].