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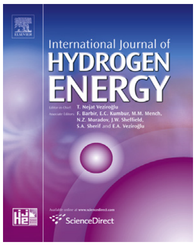

Fermentative hydrogen production under moderate halophilic conditions

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ABSTRACT

Dark fermentation is an intermediate microbial process occurring along the anaerobic biodegradation of organic matter. Saline effluents are rarely treated anaerobically since they are strongly inhibited by high salt concentrations. This study deals with the characterization of microbial communities producing hydrogen under moderate halophilic conditions. A series of batch experiments was performed under anaerobic conditions, with glucose as substrate (5 g L^{-1}) and under increasing NaCl concentrations ranging from 9 to 75 g_{NaCl} L^{-1}. A saline sediment of a lagoon collecting salt factory wastewaters was used as inoculum. Interestingly, a gradual increase of the biohydrogen production yield according to NaCl concentration was observed with the highest value $(0.9 \pm 0.02\ mol_{H_2} \cdot mol_{Glucose}{}^{-1})$ obtained for the highest NaCl concentration, i.e. 75 g_{NaCl} L^{-1}, suggesting a natural adaptation of the sediment inoculum to salt. This work reports for the first time the ability of mixed culture to produce hydrogen in moderate halophilic environment. In addition, maximum hydrogen consumption rates decreased while NaCl concentration increased. A gradual shift of the bacterial community structure, concomitant to metabolic changes, was observed with increasing NaCl concentrations, with the emergence of bacteria belonging to *Vibrionaceae* as dominant bacteria for the highest salinities.

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1. Introduction

The actual increasing interest in hydrogen (H_2) as a promising clean and sustainable energy carrier is mainly due to its high energy density (122 kJ g^{-1}) as well as the high efficiency of fuel cells to convert H_2 to electricity for transportation purposes [1–3]. Nowadays, most of the hydrogen produced worldwide is generated by natural gas reforming. However, producing hydrogen from fossil fuel produce more than twice carbon oxide equivalent compared to biohydrogen and contribute to resource depletion [4,5]. Developing alternatives technologies to produce hydrogen from renewable energy sources and minimizing their environmental impact are therefore of high priority. Producing hydrogen in biological processes is considered more environmentally friendly and sustainable than from conventional techniques. Particularly, dark fermentation is a process that aims at producing biohydrogen and treating organic waste at the same time [1–3,6–9]. During anaerobic digestion of those compounds, several microbial metabolism pathways outcompete for hydrogen which is a key intermediate in the trophic chain, as electron carrier (Fig. 1). When applying specific operating conditions (low pH, high substrate/inoculum ratio), consumption and concurrent routes for hydrogen production can be avoided [3].

Moreover, saline wastewaters, that can be generated by fish, seafood, petroleum and leather industries, may contain large amounts of organic matter that have to be treated [10,11]. Wastewaters discharged from drinking water treatment plants

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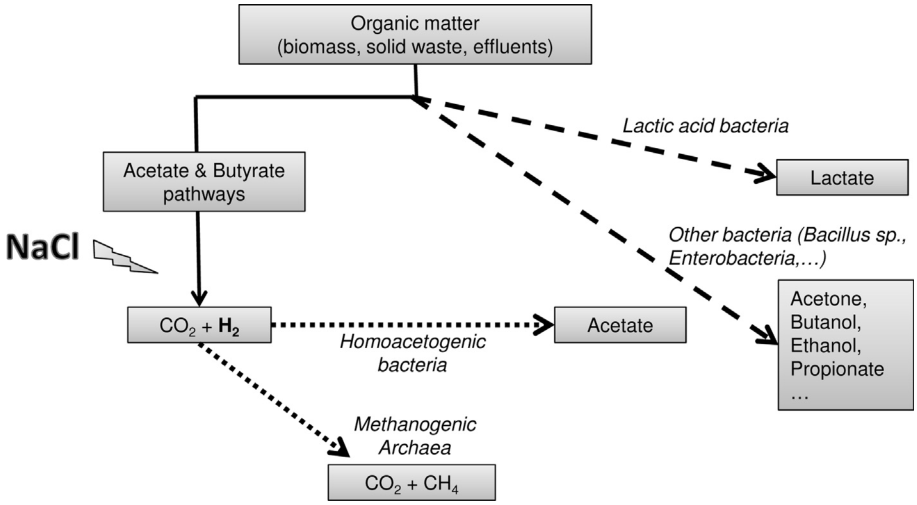

Fig. 1 – General pathways for hydrogen production. Bold arrows are represented for hydrogen production pathways, dotted arrows for hydrogen consumption pathways and dashed arrows for hydrogen production concurrent routes (adapted from Guo et al. [3]).

using ion-exchange membrane and reverse-osmosis processes may also contain high amounts of salts [11]. Overall, saline wastewaters represent more than 5% of the worldwide effluent treatment requirements [12]. Discharging those saline wastewaters directly to the environment leads to high risks of soil, surface water and groundwater salinization. In most cases, high salinity wastewaters have to be diluted before any biological treatment to reduce their salinity. Indeed, a high salinity can disturb the osmotic balance across microbial cell walls and cause plasmolysis of microbial cells, making unefficient any biological treatment [13,14]. Dilution of saline wastewaters implies a high increase in water consumption of the treatment bioprocess and, as a consequence, its operating costs.

Although Na^+ concentration has a strong inhibitory effect on anaerobic digestion processes [12,14,15], natural microbial communities can nevertheless well adapt to high salt concentrations to finally exhibit efficient activity for anaerobic treatment of saline wastewaters [10]. Some anaerobic microbial communities were reported as halophilic [16,17], whereas some others were non-halophilic and had to be adapted by increasing salt concentrations prior to using them as inoculum [12,18,19].

While many species from *Clostridium*, *Enterobacter* and *Escherichia* genera have been described in the literature as hydrogen-producing bacteria in mixed cultures in non halophilic environment [2,3,8,9,20–23], only few studies have dealt with dark fermentation of saline effluents in pure and mixed cultures [24,25]. Regarding fermentative hydrogen production under moderate halophilic conditions with pure cultures, only few bacteria such as *Bacillus megaterium* [26], *Halocella cellulolytica* [27] and *Clostridium acetobutylicum* [28] were reported previously to produce H_2 at pH7 and under saline conditions up to 2% w/v NaCl [26–28]. Interestingly, Alshiyab et al. [28] showed that hydrogen productivity of *C. acetobutylicum* decreased by 18% while increasing NaCl concentration from 0 to 5 $g_{NaCl}\ L^{-1}$. Simankova et al. [27] reported that a halophilic bacteria, *H. cellulolytica*, isolated from hypersaline lagoons with high NaCl concentrations (50–200 $g_{NaCl}\ L^{-1}$), was also capable of hydrogen production of about 4 $mmol_{H_2}\cdot L^{-1}$ during microcrystalline cellulose breakdown. Liu et al. [26] studied the capability of *B. megaterium (Bacillus sp B2)* to produce hydrogen within a range of 4–70% $g_{NaCl}\ L^{-1}$. They observed a maximum hydrogen production of 1.65 $mol_{H_2}\cdot mol_{Glucose}{}^{-1}$ in marine conditions (30 $g_{NaCl}\ L^{-1}$). Kivisto et al. [24] showed that *Halanaerobium saccharolyticum* sspp. *saccharolyticum* (Hssa) and *senegalensis* (Hsse) produced respectively 0.6 and 1.6 $mol_{H_2}\cdot mol_{Glycerol}{}^{-1}$, at pH7 and 150 $g_{NaCl}\ L^{-1}$. Similarly, Brown et al. [29] described *Halanaerobium hydrogeniformans* as a fermentative hydrogen producer in haloalkaline conditions at pH11 and 70 $g_{NaCl}\ L^{-1}$. Those results emphasize that hydrogen producers do exist and can produce hydrogen efficiently in pure cultures under halophilic conditions, mainly in neutral or alkaline conditions unlike classical dark fermentation processes that are operated under acid conditions.

The aim of the present study is to evaluate the capability of mixed cultures to produce biohydrogen by dark fermentation under increasing NaCl concentrations. Experiments were performed in mixed culture, inoculated with a microbial ecosystem adapted to saline conditions, to provide new insights about using dark fermentation in moderate halophilic condition for producing hydrogen and treating saline waste streams at the same time, that cannot be feasible with pure cultures. A series of batch experiments was thus carried out with increasing NaCl concentrations from 9 to 75 $g\ L^{-1}$ at pH 8, and using a saline sediment well adapted to halophilic conditions as inoculum.

2. Materials and methods

2.1. Source of inoculum

The seed sediment used for hydrogen production was sampled in a lagoon collecting wastewaters from a salt factory. The sediments were filtrated through a 2 mm sieve and

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