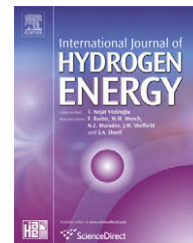


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Effect of extrinsic lactic acid on fermentative hydrogen production

Bitá Baghchehsaraee, George Nakhla*, Dimitre Karamanev, Argyrios Margaritis

Department of Chemical and Biochemical Engineering, The University of Western Ontario, 1151 Richmond Street, London, Ontario, N6A 5B9 Canada

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ABSTRACT

In this paper we report the effect of extrinsic lactic acid on hydrogen production from a starch-containing medium by a mixed culture. Study of the effect of addition of four metabolites, namely ethanol, lactic acid, butyric acid and acetic acid illustrated that lactic acid had a positive effect on both the maximum hydrogen production and hydrogen production rate. The addition of 10 mM lactic acid to a batch containing starch increased the hydrogen production rate and hydrogen production yield from 4.31 to 8.23 mL/h and 5.70 to 9.08 mmol H₂/g starch, respectively. This enhancement in hydrogen production rate and yield was associated with a shift from acetic acid and ethanol formation to formation of butyric acid as the predominant metabolite. The increase in hydrogen production yield was attributed to the increase in the available residual NADH for hydrogen production. When lactic acid was used as the sole carbon source, no significant hydrogen production was observed.

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1. Introduction

Agricultural and food wastes, as well as carbohydrate-rich industrial wastewaters are promising substrates for biological hydrogen production [1,2]. Acidogenic bacteria can ferment the carbohydrates in these feedstocks to hydrogen, carbon dioxide and volatile organic acids – mainly acetic and butyric acids. However, these bacteria are not able to further break down the organic acids to hydrogen due to the positive Gibbs free energy of the reaction.

Liu et al. [3] reported the production of hydrogen from acetate at the cathode of a microbial fuel cell. The biochemical barrier was overcome by increasing the electrochemical potential achieved by bacteria in the microbial fuel cell applying an additional voltage of at least 250 mV. Another report on hydrogen production from organic acids is from

a mixture of acetic and lactic acids [4]. The *Clostridium diolis* used in that study was not able to produce hydrogen when acetic, citric, propionic and succinic acids were the only available organic substrates. However, when a mixture of acetic and lactic acids was used hydrogen and butyric acid were produced.

In dark fermentative hydrogen production, the presence of lactic acid as a metabolite during hydrogen production is frequently regarded as a sign of lower hydrogen production [5–7]. Lactic acid has been rarely studied as substrate for hydrogen production and despite reports of methane production from lactate [8], no significant hydrogen production was reported when lactate was used as the sole substrate [4,9]. Hydrogen yield with a mixed culture using lactate as substrate was reported to be only 2.2 mL H₂/g lactate (COD basis) with a substrate conversion efficiency of 0.5% [9].

* Corresponding author. Tel.: +1 519 661 2111x85470; fax: +1 519 850 2129.

E-mail address: gnakhla@eng.uwo.ca (G. Nakhla).

Nomenclature

COD	chemical oxygen demand
TCD	thermal conductivity detector
VFA	volatile fatty acids
FID	flame ionization detector
RID	refractive index detector
VSS	volatile suspended solids
ANOVA	analysis of the variance
DF	degree of freedom
SS	sum of squares
MS	mean square
F value	Fisher's F value

P value	probability value
NADH	nicotinamide adenine dinucleotide hydride
NAD ⁺	nicotinamide adenine dinucleotide
H	cumulative hydrogen production
P	maximum hydrogen production
R _m	maximum hydrogen production rate
λ	lag phase time
t	incubation time
e	Euler's number (2.71828)
P _{H₂}	predicted maximum hydrogen production
SM	soluble metabolites
TVFA	total volatile fatty acids

The effect of the addition of lactic acid to a carbohydrate-containing medium for hydrogen production has not been investigated yet. Our study on the effect of addition of some metabolites, including acetic acid, butyric acid, lactic acid and ethanol, to a hydrogen-producing system showed that extrinsic lactic acid could enhance the hydrogen production. This paper is the first report of the enhancement in hydrogen production by addition of lactic acid to a carbohydrate-containing medium.

2. Materials and methods

2.1. Inoculum and treatment condition

Municipal waste activated sludge from the Adelaide Pollution Control Plant in London, Ontario was used as the inoculum for hydrogen production. Prior to inoculation, the sludge was dewatered and then sieved through 2 mm screen. In order to enrich hydrogen-producing bacteria, the inoculum was heat treated at 70 °C for 30 min.

2.2. Batch experiments

All hydrogen production experiments were conducted in 320 mL batch vials. The nutrient medium was prepared using 5 g/L starch as a carbon source, plus the following inorganic salts (in mg/L): NH₄Cl, 2600; K₂HPO₄, 250; MgCl₂·6H₂O, 125; FeSO₄·7H₂O, 5.0; CoCl₂·6H₂O, 2.5; MnCl₂·4H₂O, 2.5; KI, 2.5; Na₂MoO₄·2H₂O, 0.5; H₃BO₄, 0.5; NiCl₂·6H₂O, 0.5; ZnCl₂, 0.5. The solution was buffered with 0.07 M sodium phosphate and the initial pH was adjusted to 7 using 2 M NaOH or 2 M HCl. After the addition of the inoculum each vial was purged with nitrogen for 1 min. The cultures were placed in a shaker-incubator at 35 °C and 180 rpm. The experiments for this study were performed under two categories:

2.2.1. Fractional factorial experiments

These experiments included 15 batches to study the effect of addition of ethanol, lactic acid, butyric acid and acetic acid on hydrogen production. The concentration of different metabolites in each batch is presented in Section 2.4. The working volume was 120 mL and the inoculum concentration was 1.3 g VSS/L.

2.2.2. Lactic acid experiments

The experiments were conducted to study the effect of addition of lactic acid on hydrogen production. The concentration of lactic acid was 10 mM. The experiments were conducted at working volume of 140 mL with 0.8 g VSS/L inoculum.

2.3. Analytical procedures

The total gas volume was measured by releasing the gas pressure in the vials using appropriately sized glass syringes (Perfektum; Popper & Sons Inc., NY, USA) in the 5–50 mL range to equilibrate with the ambient pressure as recommended by Owen et al. [10]. Gas volumes were corrected to standard conditions (25 °C and 1 atm). Biogas composition was determined by a gas chromatograph (Model 310, SRI Instruments, Torrance, CA) equipped with a thermal conductivity detector (TCD) and a molecular sieve column (Molesieve 5A, mesh 80/100, 6 ft × 1/8 in). The temperatures of the column and the TCD detector were 90 and 105 °C, respectively. Argon was used as carrier gas at a flow rate of 30 mL/min.

The concentrations of volatile fatty acids (VFAs) were analyzed using a gas chromatograph (Varian 8500) with a flame ionization detector (FID) equipped with a fused silica column (30 m × 0.32 mm). Helium was used as carrier gas at a flow rate of 5 mL/min. The temperatures of the column and detector were 110 and 250 °C, respectively. Lactic acid concentrations were measured using a high-performance liquid chromatography system (1200 series, Agilent Technologies) equipped with Aminex HPX-87H ion exclusion column (300 mm × 7.8 mm I.D.; BIO-RAD), and a UV-detector at 210 nm. The column temperature was adjusted to 30 °C. The same instrument with a refractive index detector (RID) was

Table 1 – Experimental variables and their levels.

Variables	Levels		
	–1	0	1
x ₁ Ethanol (mM)	0	2.5	5
x ₂ Lactic acid (mM)	0	5	10
x ₃ Butyric acid (mM)	0	5	10
x ₄ Acetic acid (mM)	0	5	10

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