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Short Communication

Biological hydrogen production by immobilized cells of *Clostridium tyrobutyricum* JM1 isolated from a food waste treatment process

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Abstract

A fermentative hydrogen-producing bacterium, *Clostridium tyrobutyricum* JM1, was isolated from a food waste treating process using 16S rRNA gene sequencing and amplified ribosomal DNA restriction analysis (ARDRA). A fixed-bed bioreactor packed with polyurethane foam as support matrix for the growth of the isolate was operated at different hydraulic retention time (HRT) to evaluate its performance for hydrogen production. The reactor achieved the maximal hydrogen production rate of 7.21 $H_2\,l^{-1}\,d^{-1}$ at 2 h HRT, where hydrogen content in biogas was 50.0%, and substrate conversion efficiency was 97.4%. The maximum hydrogen yield was 223 ml (g-hexose)$^{-1}$ with an influent glucose concentration of 5 g l^{-1}. Therefore, the immobilized reactor using *C. tyrobutyricum* JM1 was an effective and stable system for continuous hydrogen production.

Keywords: Hydrogen production; *Clostridium tyrobutyricum*; Immobilized bioreactor; Food waste; ARDRA

1. Introduction

Global climate change and environmental pollution due to the abuse of fossil fuels as well as those shortfall make it important to search for alternative energy sources that are cost-effective, environmentally-friendly and renewable. Hydrogen (H_2) satisfies the above requirements because it has a higher energy yield (122 kJ g^{-1}) by 2.75 times than hydrocarbon fuels and produces only water, not carbon combined end-products, when it is combusted as a fuel or converted to electricity. Among various hydrogen production processes, biological methods are known to be less energy intensive than chemical or electrochemical ones since they are carried out at ambient temperature and pressure (Cheong and Hansen, 2007; Elam et al., 2003). Dark fermentation of organic compounds might be the most feasible one since the rates of hydrogen production and microbial growth were higher than those of photo fermentation and bio-photolysis (Levin et al., 2004). The anaerobes responsible for fermentative hydrogen production can produce hydrogen all day long without light and can use various kinds of substrates such as refuse and food waste products. Until recently, clostridia and enteric bacteria such as *Clostridium butyricum* and *Enterobacter aerogenes* have been known to be strong and efficient producers of hydrogen (Nath and Das, 2004; Tanisho et al., 1987). Saccharolytic clostridia represent one of the largest genera of prokaryotes and satisfy the following four criteria: a) able

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to form endospores; b) must rely on the energy metabolism of obligate anaerobes; c) unable to carry out a dissimilatory sulfate reduction; and d) the cell wall must be gram-positive (Balow et al., 1992).

In this work, a hydrogen-producing anaerobe was isolated from a food waste treatment process and designated as *Clostridium tyrobutyricum* JM1. *C. tyrobutyricum*, one of the saccharolytic clostridia, is a low G + C gram-positive anaerobe and exhibits special metabolic routes to produce short-chain fatty acids and hydrogen from carbohydrates and amino acids (Balow et al., 1992; Dürre, 2005). To improve the efficiency of substrate utilization and hydrogen productivity, the isolate was immobilized in a packed-bed reactor using polyurethane foam as support media. To the best of our knowledge, there has been no report on the pure *C. tyrobutyricum*-immobilized bioreactor system for continuous hydrogen production (Table 1), unlike the immobilizations of facultative anaerobes such as *E. aerogenes* and *E. cloacae* (Kumar and Das, 2001; Palazzi et al., 2002; Yokoi et al., 1997). Hydrogen production rate, hydrogen content in biogas, soluble metabolites compositions, and hydrogen yield were investigated to identify for the proper HRT condition for continuous hydrogen production by the isolate.

2. Methods

2.1. Isolation of H_2-producing anaerobe from a food waste treating reactor

The bacterium used in this study, *C. tyrobutyricum* JM1, was isolated from effluent of a food waste treating reactor in our laboratory (Jo et al., 2007). The strain was stored at −80 °C in sterile 20% (v/v) glycerol solution.

2.2. Amplified ribosomal DNA restriction analysis (ARDRA) and determination of 16S rRNA gene sequence

To isolate anaerobes responsible for hydrogen production, serial dilutions of the broth from the food waste treating reactor were plated and cultivated on RCM (Reinforced Clostridial Medium, Merck) plates in the Gas-Pak anaerobic cultivation jar (BBL, UK) at 35 °C. The RCM was composed of the following materials (g l^{-1}): meat extract, 10.0; peptone, 5.0; yeast extract, 3.0; D(+) glucose 5.0; starch, 1.0; sodium chloride, 5.0; sodium acetate, 3.0; and L-cysteinium chloride, 0.5. The agar plates were prepared by adding 1.5% (w/v) agar to the RCM broth. Single colonies obtained on RCM agar plates were re-streaked three times to ensure the purity of the strains.

Table 1
The experimental conditions and hydrogen productivity by pure *Clostridium* species

Microorganism	Carbon	Type	Experimental condition	SHPR[a]	VHPR[a]	Yield	References
C. butyricum	Sucrose	Batch	pH 5.5, temperature 37 °C and sucrose 17.8 g l^{-1}	–	–	2.78 mol mol^{-1}	Chen et al. (2005)
C. butyricum	Sucrose	Batch	pH 6.0, temperature 37 °C and sucrose 17.8 g l^{-1}	–	209 ml l^{-1} h^{-1}	–	
C. butyricum	Glucose	Batch	Temperature 30 °C and glucose 9 g l^{-1}	–	4.92 ml ml^{-1} d^{-1}	–	Fang et al. (2006)
C. butyricum	Glucose	CSTR	pH 6.7, temperature 30 °C and HRT 8 h	–	–	1.4–2.3 mol mol^{-1}	Kataoka et al. (1997)
C. butyricum	Glucose	Batch	–	–	–	2.29 ± 0.21 mol mol^{-1}	Lin et al. (2007)
C. acetobutylicum	Glucose	Batch	–	–	–	1.8 ± 0.02 mol mol^{-1}	
C. beijerinckii	Glucose	Batch	–	–	–	2.81± 0.04 mol mol^{-1}	
C. tyrobutyricum	Glucose	Batch	–	–	–	1.47 ± 0.73 mol mol^{-1}	
C. beijerinckii	Glucose	Batch	Temperature 36 °C and glucose 10 g l^{-1}	–	660 ml h^{-1}	1.3–2.0 mol mol^{-1}	Taguchi et al. (1992)
	Starch	Batch	Temperature 36 °C and starch 10 g l^{-1}	–	410 ml h^{-1}	–	
C. thermocellum	Cellulosic substrates	Batch	Substrate 0.1 g l^{-1}	–	–	1.6 mol mol^{-1}	Levin et al. (2006)
C. acetobutylicum	Glucose	Trickle bed	Temperature 30 °C and glucose 10.5 g l^{-1}	–	220 ml l^{-1} h^{-1}	–	Zhang et al. (2006)
C. thermolacticum	Lactose	CSTR	–	5 mmol (g-DW d)$^{-1}$	–	–	Collet et al. (2004)
C. saccharoperbutyl-acetonicum	Disaccharide	Batch	pH 7.5 and temperature 30 °C	–	–	2.81 mol mol^{-1}	Ferchichi et al. (2005)
	Monosaccharide	Batch	pH 7.5 and temperature 30 °C	–	–	1.29 mol mol^{-1}	
C. tyrobutyricum	Glucose	Fed-batch	pH 6.0, temperature 37 °C	–	–	1.35 mol mol^{-1}	Liu et al. (2006)
C. tyrobutyricum	Glucose	IBR	HRT 2 h and glucose 5 g l^{-1}	–	7.2 l l^{-1} d^{-1}	223 ml g-hexose^{-1}	This study

[a] SHPR: specific hydrogen production rate; VHPR : volumetric hydrogen production rate.