Bioresource Technology 116 (2012) 179–183

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Effect of carbon and nitrogen sources on photo-fermentative H_2 production associated with nitrogenase, uptake hydrogenase activity, and PHB accumulation in *Rhodobacter sphaeroides* KD131

Mi-Sun Kim [a,b,*], Dong-Hoon Kim [a], Jaewhan Cha [a], Jeong K. Lee [c]

[a] Clean Fuel Department, Korea Institute of Energy Research, 102 Gajeong-ro, Yuseong-gu, Daejeon 305-343, Republic of Korea
[b] Division of Renewable Energy Engineering, University of Science and Technology, 217 Gajeon-ro, Yuseongu, Daejeon 305-350, Republic of Korea
[c] Department of Life Science and Interdisciplinary Program of Integrated Biotechnology, Sogang University, Mapo, Shinsu 1, Seoul 121-742, Republic of Korea

ARTICLE INFO

Article history:
Received 18 January 2012
Received in revised form 3 April 2012
Accepted 5 April 2012
Available online 13 April 2012

Keywords:
Biohydrogen
Photo-fermentation
Nitrogenase
Uptake hydrogenase
Poly-β-hydroxybutyrate

ABSTRACT

During photo-fermentative H_2 production, the effects of carbon and nitrogen sources on nitrogenase and hydrogenase activity, poly-β-hydroxybutyrate accumulation were investigated. In succinate/ammonium sulfate medium, H_2 was not detected for the first 6 h because high ammonium concentration considerably reduced the nitrogenase activity to below 5 nmol/g-dcw/h. After 24 h, 99% of the ammonium was consumed, and the nitrogenase activity increased to 296 nmol/g-dcw/h, accelerating H_2 production. In contrast, the ammonium in succinate/glutamate medium was much less, which led to rapid H_2 production in the beginning. However, H_2 evolution was repressed over time by increased ammonium. In the presence of H_2, hydrogenase activity increased with time regardless of the nitrogen source, and consequently, H_2 production was reduced. Compared with succinate, H_2 production in acetate media was severely limited due to increased pH over 9. During extended cultivation, the PHB accumulated in acetate media was 7 times higher than in succinate media.

1. Introduction

Hydrogen (H_2) is regarded as an environmentally clean fuel to overcome the adverse effect of hydrocarbon fuels where carbon dioxide and other pollutants are released to the atmosphere. Since H_2 carries high energy per unit mass and produces only water vapor at the end point of use, extensive studies have been done on the hydrogen energy system, including production, storage, use, and safety issues. However, most hydrogen fuels currently are produced by reforming of fossil fuels using physicochemical methods which are usually expensive and energy intensive. Thus, biological H_2 production from renewable energy sources, such as biomass, is considered the most environmental friendly technology. It has advantages of reducing organic waste and generating clean energy, i.e. hydrogen. In particular, phototrophic fermentation using purple non-sulfur (PNS) bacteria is of great interest due to high theoretical H_2 yield from various volatile organic acids (Chen et al., 2011).

During photo-fermentation, H_2 is produced by the interactions of several metabolic pathways. PNS bacteria evolve H_2 through catalysis of nitrogenase with the consumption of adenosine triphosphate (ATP) and free electrons originating from light as follows:

$$2H^+ + 2e^- + 4ATP \rightarrow H_2 + 4ADP + 4P_i \quad (1)$$

The activity of nitrogenase is crucial to H_2 production by photosynthetic bacteria, and it is strongly inhibited by high ammonia concentration (Koku et al., 2002). Thus, the concentration and type of nitrogen source is critical to the performance of phototrophic H_2 production. The substrate is also a key factor affecting cell growth and H_2 production (Khatipov et al., 1998; Fang et al., 2005). In the aspect of substrate conversion efficiency, cell growth and alternative biosynthesis are electron sinkers to reduce the portion of the substrate available for H_2 production. Among metabolite alternatives to H_2, the bacterial storage compound, poly-β-hydroxybutyrate (PHB) is closely associated with H_2 production (Hustede et al., 1993; Kim et al., 2006). The polymer, which serves as a reserve of carbon and energy, is accumulated in cytoplasm under unbalanced growth conditions. The amount of PHB accumulated in photosynthetic bacteria depends on physiological conditions, such as pH and the type of substrate (Hustede et al., 1993; Khatipov et al., 1998). The total H_2 production is also limited by uptake hydrogenase activity. This enzyme catalyzes the conversion of molecular H_2 to protons and electrons to reduce a relatively high potential electron acceptor, and it consequently decreases the efficiency of H_2 production (Kars et al., 2008).

* Corresponding author. Address: Clean Fuel Department, Korea Institute of Energy Research, 102 Gajeong-ro, Yuseong-gu, Daejeon 305-343, Republic of Korea. Tel.: +82 42 860 3554; fax: +82 42 860 3739.
E-mail address: bmmskim@kier.re.kr (M.-S. Kim).

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http://dx.doi.org/10.1016/j.biortech.2012.04.011