



Effect of carbon and nitrogen sources on photo-fermentative H₂ 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, Yuseong-gu, 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 H₂ production, the effects of carbon and nitrogen sources on nitrogenase and hydrogenase activity, poly-β-hydroxybutyrate accumulation were investigated. In succinate/ammonium sulfate medium, H₂ 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 H₂ production. In contrast, the ammonium in succinate/glutamate medium was much less, which led to rapid H₂ production in the beginning. However, H₂ evolution was repressed over time by increased ammonium. In the presence of H₂, hydrogenase activity increased with time regardless of the nitrogen source, and consequently, H₂ production was reduced. Compared with succinate, H₂ 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.

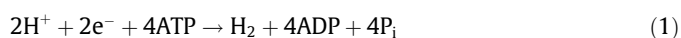
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1. Introduction

Hydrogen (H₂) 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 H₂ 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 H₂ 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 H₂ yield from various volatile organic acids (Chen et al., 2011).

During photo-fermentation, H₂ is produced by the interactions of several metabolic pathways. PNS bacteria evolve H₂ through catalysis of nitrogenase with the consumption of adenosine

triphosphate (ATP) and free electrons originating from light as follows:



The activity of nitrogenase is crucial to H₂ production by phototrophic 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 H₂ production. The substrate is also a key factor affecting cell growth and H₂ production (Khatipov et al., 1998; Fang et al., 2005). In the aspect of substrate conversion efficiency, cell growth and alternative biosynthesis are electron sinks to reduce the portion of the substrate available for H₂ production. Among metabolite alternatives to H₂, the bacterial storage compound, poly-β-hydroxybutyrate (PHB) is closely associated with H₂ production (Husted 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 (Husted et al., 1993; Khatipov et al., 1998). The total H₂ production is also limited by uptake hydrogenase activity. This enzyme catalyzes the conversion of molecular H₂ to protons and electrons to reduce a relatively high potential electron acceptor, and it consequently decreases the efficiency of H₂ 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).