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High yield single stage conversion of glucose to hydrogen by photofermentation with continuous cultures of *Rhodobacter capsulatus* JP91

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HIGHLIGHTS

- Photofermentation of glucose was carried out in a single stage continuous culture.
- Yields varied with dilution rate (HRT).
- The highest yield, 9.0 ± 1.2 mol H_2/mol glucose was 75% of theoretical.
- There is room for improvement in light conversion efficiency.

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ABSTRACT

Photofermentative hydrogen (H_2) production from glucose with the photosynthetic bacterium *Rhodobacter capsulatus* JP91 (hup^-) was examined using a photobioreactor operated in continuous mode. Stable and high hydrogen yields on glucose were obtained at three different retention times (HRTs; 24, 48 and 72 h). The H_2 production rates, varying between 0.57 and 0.81 mmol/h, and optical densities (OD_{600nm}) were similar for the different HRTs examined. However, the rate of glucose consumption was influenced by HRT being greater at HRT 24 h than HRTs 48 and 72 h. The highest hydrogen yield, 9.0 ± 1.2 mol H_2/mol glucose, was obtained at 48 h HRT. These results show that single stage photofermentative hydrogen production from glucose using photobioreactors operated in continuous culture mode gives high, nearly stoichiometric yields of hydrogen from glucose, and thus is considerably more promising than either two stage photofermentation or co-culture approaches.

1. Introduction

The purple non-sulfur photosynthetic bacteria are well known for their capacity to produce hydrogen from organic acids when grown under photoheterotrophic conditions with limiting nitrogen, a process called photofermentation. Hydrogen evolution under these conditions is catalyzed by nitrogenase, which normally functions to catalyze the reduction of dinitrogen to ammonia with the release of one H_2 per N_2 reduced. In the absence of other reducible substrates, nitrogenase continues to turnover reducing protons to hydrogen. Hydrogen production under these conditions is apparently a response to the metabolic need to maintain redox balance (Masepohl and Hallenbeck, 2010).

Light plays a key role in providing the required energy input, both high energy electrons and ATP (4 ATP/H_2), needed to drive substrate conversion to hydrogen to completion (Adessi and De Philippis, 2012; Hallenbeck, 2011; Keskin et al., 2011). Captured light energy is used to produce chemical energy (i.e. a proton gradient), which in turn is used both to drive reverse electron flow to nitrogenase, and for ATP synthesis. This energy allows in principle the complete irreversible oxidation of substrate.

Many studies have examined hydrogen production with *Rhodobacter* species growing on organic acids as substrate, converting typical fermentation products acetic, lactic, propionic, malic and butyric acids to H_2 and CO_2 under anaerobic conditions in the light. Dark, hydrogen producing fermentations convert sugars to hydrogen at maximum yields of only 33%, giving as byproducts acetate and butyrate (Abo-Hashesh and Hallenbeck, 2012a; Hallenbeck, 2009, 2012), compounds which can be completely oxidized to H_2 and CO_2 by photofermentation. Consequently, photofermentation is an attractive method for the total conversion of feedstocks that are only partially oxidized during dark fermentation and hence increases the yield of hydrogen from those substrates (Adessi et al., 2012; Hallenbeck, 2011, 2012; Hallenbeck et al., 2012; Keskin and Hallenbeck, 2012a).

Therefore several systems have been under investigation using photofermentation to extract additional hydrogen from the organic

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