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Kinetic analysis of photosynthetic growth, hydrogen production and dual substrate utilization by *Rhodobacter capsulatus*

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ABSTRACT

Rhodobacter capsulatus is purple non-sulfur (PNS) bacterium which can produce hydrogen and CO_2 by utilizing volatile organic acids in presence of light under anaerobic conditions. Photofermentation by PNS bacteria is strongly affected by temperature and light intensity. In the present study we present the kinetic analysis of growth, hydrogen production, and dual consumption of acetic acid and lactic acid at different temperatures (20, 30 and 38 °C) and light intensities (1500, 2000, 3000, 4000 and 5000 lux). The cell growth data fitted well to the logistic model and the cumulative hydrogen production data fitted well to the Modified Gompertz Model. The model parameters were affected by temperature and light intensity. Lactic acid was found to be consumed by first order kinetics. Rate of consumption of acetic acid was zero order until most of the lactic acid was consumed, and then it shifted to first order. The results revealed that the optimum light intensities for maximum hydrogen production were 5000 lux for 20 °C and 3000 lux for 30 °C and 38 °C.

1. Introduction

Hydrogen is an environmentally friendly energy carrier because it produces only water when it is consumed in the fuel cells for electricity production. Biological hydrogen production is a sustainable way since it utilizes biomass under ambient conditions [1]. Our group was involved in 6th framework EU project, Hyvolution, where the aim was to produce H_2 from biomass with integration of dark and photofermentation. Effluents obtained from dark fermentation were used to produce H_2 by photofermentation [2]. These effluents contained both acetic acid and lactic acid as carbon sources. In photofermentation purple non-sulfur (PNS) bacteria perform anoxygenic photosynthesis and produce hydrogen as a by-product in the presence of light [3]. *Rhodobacter capsulatus* is selected PNS bacterium since it produces hydrogen most effectively by breaking down organic acids such as acetic acid and lactic acid under anaerobic conditions and illumination [4].

Carbon and nitrogen sources, C/N ratio, pH, light intensity and temperature are some factors that affect photofermentative hydrogen production. These environmental factors may also affect the regulation of nitrogenase enzyme [5]. Since photofermentation process is an enzymatic process, hydrogen production by bacteria is strongly affected by temperature. Özgür et al. [6] demonstrated that hydrogen

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production is significantly decreased at fluctuating temperatures (15–40 °C) when it is compared with constant temperature at 30 °C.

In addition, light intensity is one of the most important factors that affect hydrogen production by PNS bacteria. Hydrogen production by PNS bacteria is mediated by nitrogenase enzyme and the required energy for hydrogen production is provided by the conversion of light energy to ATP by photosynthetic membrane apparatus. Nitrogenase synthesis, which affects hydrogen production, is strongly stimulated by light [3]. Therefore, the light intensity to which the cells are exposed is a very important factor for hydrogen production. There are several studies conducted with PNS bacteria to understand the effect of light intensity. These studies showed that increasing light intensity increases hydrogen production [7–10]. Outdoor production of hydrogen with photosynthetic bacteria is also strongly affected by fluctuations in temperature and light intensity due to the day–night cycle and due to seasonal, geographic and climatic conditions. In order to forecast the hydrogen productivity at different places throughout the world and based on that to estimate the cost-effectiveness for a certain location a model describing the dependency of hydrogen production from the natural parameters is necessary.

Light intensity and temperature are also expected to affect the bacterial growth rate and, consumption rates of acetic acid and lactic acid during the biohydrogen production process. In literature there is no work reporting the dual consumption kinetics of acetic acid and lactic acid during the growth and the hydrogen production by *R. capsulatus*. In the present study we present models for growth, cumulative hydrogen production, and dual consumption of acetic acid and lactic acid at different temperatures (20, 30 and 38 °C) and light intensities (1500, 2000, 3000, 4000 and 5000 lux) which are suitable for biohydrogen production process. The main objective of this study is to shed insight into the relations between growth, hydrogen production and substrates consumption in *R. capsulatus*.

2. Materials and methods

2.1. The microorganism and media composition

In this study *R. capsulatus* DSM 1710 was used. The microorganism was obtained from Deutsche Sammlung von Mikroorganismen (DSM, Braunsweig Germany). Modified Biebl and Pfennig medium [11] was used in experiments. The medium contained a mixture of 40 mM acetic acid/7.5 mM lactic acid/2 mM glutamate.

2.2. Photobioreactors

The photobioreactors were 55 ml transparent circular glass bottles containing 50 ml culture. The media was inoculated with 10% bacteria. In order to keep the temperature constant, all photobioreactors were kept in a cooling incubator (Nüve, ES250). Photobioreactors were illuminated by a tungsten lamp (100 W). Light intensity measurements were done by luxmeter (Lutron LX-105 Light Meter). The conversion factor was determined as 1 W/m^2 = 14.8 lux for indoor experiments [7]. The selected light intensities were 1500, 2000, 3000, 4000, 5000 lux and selected temperatures were 20, 30 and 38 °C. The experiments were carried out in duplicates. The error bars indicate the standard deviation.

2.3. Analytical methods

Liquid samples were taken from photobioreactors in certain time intervals. Cell concentration, pH and gas composition and organic acid concentrations were determined. The cell concentrations were determined by spectrophotometer (Shimadzu UV-1201) at 660 nm pH measurements were performed by a pH meter (Mettler Toledo 3311). The amount of the evolved gas was determined volumetrically by water replacement method. The composition of the gas was analyzed by a Gas Chromatograph (GC), (Agilent Technologies 6890N). The column used in GC was Supelco Carboxen 1010. Hydrogen content of the gas was between 90 and 100% for all conditions. For organic acid analyses filtered liquid samples which contain organic acids were analyzed by High Performance Liquid Chromatography (HPLC). The analyses were done by an Alltech IOA-1000 (300 mm × 7.8 mm) HPLC column. Samples were injected to the system with an autosampler (Shimadzu SIL-10AD) and the detection of organic acids was determined by an UV detector (Shimadzu FCV-10AT) at 210 nm. The oven temperature was kept at 66 °C. As a mobile phase 0.085 M H_2SO_4 was used. Flow rate of mobile phase was adjusted to 0.4 ml/min.

3. Results and discussion

3.1. Modeling of growth of R. capsulatus at different temperatures and light intensities

Growth curves were obtained by plotting cell concentration (gdw/L) versus time for all temperatures and light intensities. *R. capsulatus* DSM 1710 growth curve fit to the logistic model using a program (Curve Expert 1.3). The model equation is given below:

$$X = \frac{X_{max}}{\left[1 + \exp(-k_c \cdot t)\left(\frac{X_{max}}{X_o} - 1\right)\right]} \quad (1)$$

where X is the cell concentration (gdw/L) and t is time (h). The model parameters; k_c is the apparent specific growth rate (h^{-1}), X_o is the initial cell concentration and X_{max} is the maximum cell concentration (gdw/L). Extent of the fit, r, and the model parameters are tabulated for 20, 30 and 38 °C in Table 1.

Fig. 1(a)–(c) illustrate the cell concentration versus time data for 20, 30 and 38 °C, respectively, at all light intensities. The lines correspond to the fitted logistic model curves for 1500, 3000 and 5000 lux. Growth data were found to fit well to the logistic model with extent of the fit (r) values close to 1.

The maximum cell concentration, X_{max}, decreased with increase in light intensity at 20 °C, but it was not affected significantly with light intensity at 30 and 38 °C. The smallest specific growth rate (k_c = 0.022 h^{-1}) was found at 20 °C and