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# The Effect of Temperature and Light Intensity on Hydrogen Production by *Rhodobacter capsulatus*

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## Abstract

*Rhodobacter capsulatus* is a purple non-sulfur photosynthetic bacterium which can produce hydrogen by photofermentation on acetate and lactate. Hydrogen productivity depends on several parameters such as medium composition, pH, light intensity and temperature. In the present study, the effects of temperature and light intensity on hydrogen production were investigated. The cell growth curve has been fitted to the logistic model and hydrogen productivity was interpreted by Modified Gompertz Equation. The maximum productivity was obtained at 30°C and light intensity of 4000 lux.

## 1 Introduction

Hydrogen production by purple non sulphur (PNS) bacteria is mediated by nitrogenase enzyme. There are several factors affecting the activity of nitrogenase enzyme. The required energy for hydrogen production is provided by the conversion of light energy to ATP by photosynthetic membrane apparatus [1]. Nitrogenase enzyme synthesis is strongly stimulated by light. Hence, the light intensity to which the cells are exposed is a very important factor for hydrogen production [2]. Another parameter that strongly affects hydrogen production is temperature. Sasikala et al, [3] have reported the maximum productivity at 5000 lux and 30°C. Özgür et al. [4] have simulated the outdoor conditions and approved that the hydrogen productivity decrease with fluctuating temperatures compared to the productivity obtained at 30°C. Therefore, temperature and light intensity have simultaneous affects on the nitrogenase enzyme synthesis and the activity. The objective of the present study is to obtain phenomenological models to interpret the kinetics of the cell growth and the hydrogen productivity by *Rhodobacter capsulatus* on acetate and lactate, for scale-up purposes in the outdoor applications.

## 2 Materials & Methods

In this study *Rhodobacter capsulatus* (DSM 1710) was used. Bacteria were inoculated to modified Biebl and Pfennig medium [5] for activation (20 mM acetate, 7.5 mM lactate, 10 mM glutamate). For hydrogen production experiments the activated bacteria were inoculated into hydrogen production medium (40 mM acetate, 7.5 mM lactate, 2 mM glutamate) with 10% inoculation. All experiments were done in 50 ml glass bottles (photobioreactors). In order to

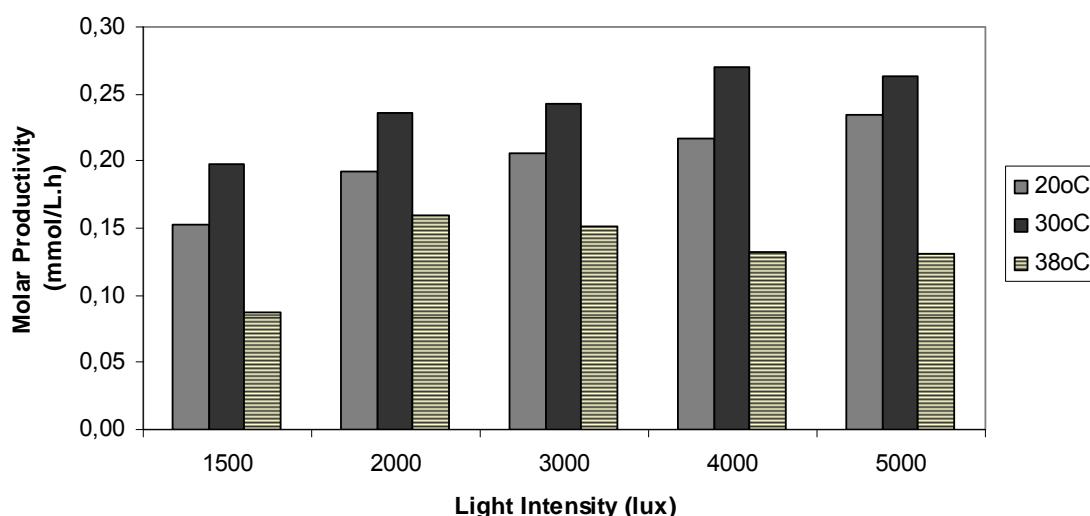
keep the system anaerobic, argon gas was flushed to photobioreactors. All photobioreactors were connected to water filled graduated cylinders by capillary tube and produced hydrogen was measured volumetrically by water replacement method [2].

The photobioreactor (PBR) was placed in an incubator. The incubator temperature was adjusted to 20°C, 30°C and 38°C. The medium temperature of the photobioreactors were determined by a digital thermometer and it was 22±2, 32±2 and 40±2 respectively. The light intensities exposed to the PBR were 1500, 2000, 3000, 4000 and 5000 lux. PBR was illuminated by a tungsten lamp (75-100W). Light intensity was measured by a luxmeter. Liquid and gas samples were taken periodically from the PBR. pH was analysed by using a pH-meter (Testo 830 T-2). Biomass was determined spectrophotometrically at 660nm (Shimadzu UV-1201 Spectrophotometer). Gas composition was analyzed by a Gas Chromatography. (Agilent Technologies 6890N Supelco Carboxen 1010 column). Curves in kinetic analyses were drawn by use of CurveExpert 1.3.

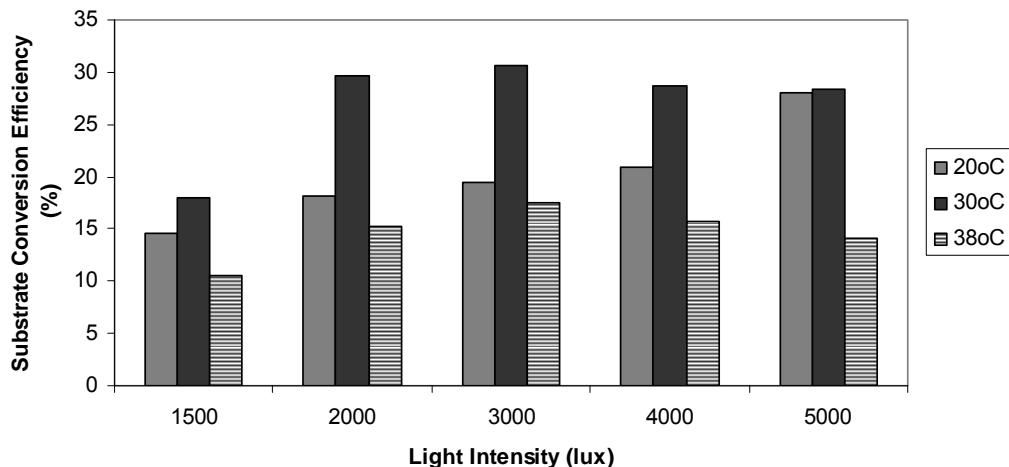
### 3 Results

#### 3.1 The effect of light intensity and temperature on hydrogen productivity and substrate conversion efficiency

Figure 1 illustrates the hydrogen productivity in terms of mmoles/L.h at different temperatures and at different light intensities. The results indicate that the highest hydrogen productivity is achieved at 30°C, at all light intensities. The substrate conversion efficiency is seen to steadily increase in parallel with increase in light intensity at 20°C. The highest value is obtained at 30°C for 3000 lux light intensity (Figure 2). Substrate conversion efficiency at any light intensity is seen to be the lowest at 38°C.



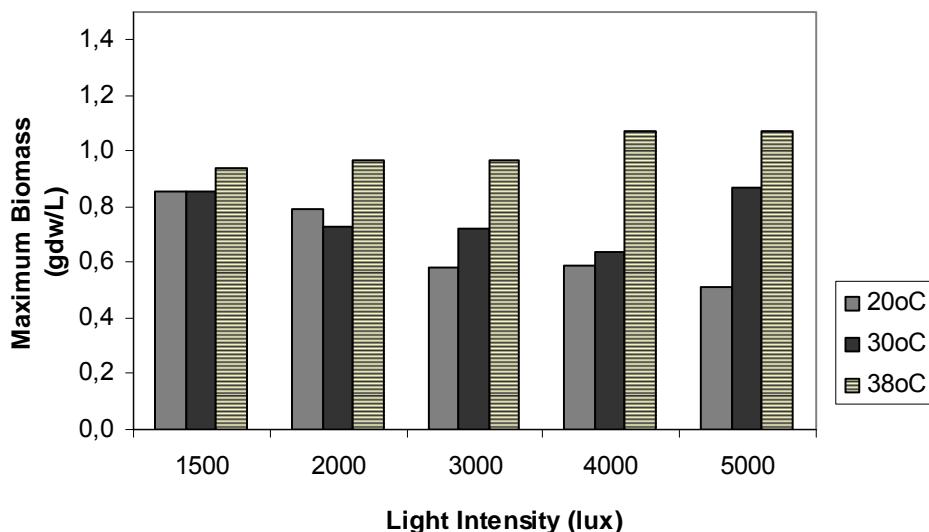
**Figure 1:** Hydrogen Productivity obtained at different temperatures and light intensities.



**Figure 2:** Substrate Conversion Efficiencies for hydrogen production by *R. capsulatus* at different temperatures and light intensities.

### 3.2 The effect of light intensity and temperature on cell growth

Figure 3 illustrates the maximum biomass values for different light intensities at 20°C, 30°C and 38°C. The maximum biomass concentration decreases with increasing light intensity at 20°C. Whereas, at 38°C biomass concentration increases with increasing light intensity. At 30°C, maximum biomass concentration decreases with increasing light intensity up to 4000 lux, however it increases at 5000 lux. The trends for hydrogen production and maximum cell growth values appear to be in opposite directions.



**Figure 3:** Maximum biomass values of *R. capsulatus* at different temperatures and light intensities.

### 3.3 Cell growth kinetics

The cell growth of PNS bacteria generally fits to the logistic model [6, 7]. The growth rate for the logistic model is expressed as:

$$dX/dt = k_c X (1 - X/X_{\max})$$

After integration of this equation the model equation becomes,

$$X = X_{\max} / [1 + \exp(-k_c \cdot t) (X_{\max}/X_0 - 1)]$$

where  $X$  is the dry cell weight (g/L),  $X_{\max}$  is the maximum dry cell weight (g/L),  $X_0$  is the initial bacterial concentration (g/L),  $t$  is time (h) and  $k_c$  is the apparent specific growth rate ( $h^{-1}$ ).

In this study cell growth data were tested whether they would fit to the model equation given above. Correlation of determination ( $r$ ) values obtained between 0.93 and 0.98 which indicated that the logistic model can be used to express the cell growth in the photobioreactors for all light intensities and all temperatures.

### 3.4 Hydrogen production kinetics

There are different models to express hydrogen production in literature. Among them Modified Gompertz Equation widely used to describe the changes on hydrogen production [7]. The Modified Gompertz Equation is given below:

$$H = H_{\max} \exp \left[ -\exp \left( \frac{R_{\max} \cdot e}{H_{\max}} \cdot (\lambda - t) + 1 \right) \right]$$

Where  $H$  is cumulative hydrogen produced (mmole),  $H_{\max}$  is hydrogen production potential (mmole),  $R_{\max}$  is the maximum production rate (mmole/h)  $\lambda$  is the lag time (h).  $e$  is a constant (2.718282)

In this study Modified Gompertz Equation was used to express the effects of light intensity and temperature on hydrogen production. The extends of correlation values ( $r$ ) were found to be above 0.98 for all temperatures and light intensities, which indicated a good fit to the model.

## 4 Discussion

Increasing light intensity resulted in an obvious increase in hydrogen production in comparison to 1500 lux exposure at 30°C. These results are in consistence with previous studies. Uyar et al. [2] demonstrated that the rate of hydrogen production increased with increasing light intensity up to 4000 lux at 30°C for *Rhodobacter sphaeroides* on malate.

Hydrogen production and maximum biomass values appear to be in opposite directions. Maximum biomass is highest for all light intensities at 38°C. This high temperature condition may cause a shift from hydrogen production to cell growth.

There are only a limited number of studies demonstrating the effects of temperature and light intensity on hydrogen production efficiency and kinetics of *R. capsulatus*. The studies of cell growth and hydrogen production kinetics of *R. capsulatus* may provide an insight for further studies and guide for large scale hydrogen production processes.

### **Acknowledgement**

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