



# Modelling of light and temperature influences on cyanobacterial growth and biohydrogen production



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## ABSTRACT

Dynamic simulation is a valuable tool to assist the scale-up and transition of biofuel production from laboratory scale to potential industrial implementation. In the present study two dynamic models are constructed, based on the Aiba equation, the improved Lambert–Beer's law and the Arrhenius equation. The aims are to simulate the effects of incident light intensity, light attenuation and temperature upon the photo-autotrophic growth and the hydrogen production of the nitrogen-fixing cyanobacterium *Cyanothece* sp. ATCC 51142. The results are based on experimental data derived from an experimental setup using two different geometries of laboratory scale photobioreactors: tubular and flat-plate. All of the model parameters are determined by an advanced parameter estimation methodology and subsequently verified by sensitivity analysis. The optimal temperature and light intensity facilitating biohydrogen production in the absence of light attenuation have been determined computationally to be 34 °C and 247  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively, whereas for cyanobacterial biomass production they are 37 °C and 261  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively. Biomass concentration higher than 0.8  $\text{g L}^{-1}$  is also demonstrated to significantly enhance the light attenuation effect, which in turn inducing photolimitation phenomena. At a higher biomass concentration (3.5  $\text{g L}^{-1}$ ), cyanobacteria are unable to activate photosynthesis to maintain their lives in a photo-autotrophic growth culture, and biohydrogen production is significantly inhibited due to the severe light attenuation.

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## 1. Introduction

Carbon dioxide,  $\text{CO}_2$ , is the major source of environmental concern for causing global warming and it is mainly released by burning carbon-based energy resources such as petrol, coal and natural gas [21]. Meanwhile, it is accepted that it is unsustainable to continuously rely on limited and nonrenewable conventional fuels [8]. To reduce the production of  $\text{CO}_2$  and fulfil the increasing demand for energy, novel sustainable and environmentally friendly energy sources are being sought. Biofuels such as biodiesel, biohydrogen, and ethanol are expected to provide new opportunities to replace conventional fossil fuels and diversify sustainable energy sources [22]. Biofuels are produced by microalgae mainly through photosynthesis, whereby  $\text{CO}_2$  is fixed into carbohydrates such as lipids, starch and sugars via different metabolic pathways. Lipids are usually used to generate biodiesel, while starch and sugars are always converted to ethanol, hydrogen and other biofuels [30]. Conventional diesel and gasoline can be replaced by biodiesel and bioethanol, respectively.

Biohydrogen is mainly used within fuel cells and as a transport fuel due to its high heat of combustion [9,22].

Biohydrogen is considered to be the most promising bio-energy carrier as there is no release of  $\text{CO}_2$  during its combustion [17]. The  $\text{CO}_2$  fixed during microalgal photosynthesis is mainly used to compose the cell structure of microalgae. Hence, theoretically, biohydrogen generated in bioprocesses can be considered to be carbon-neutral. Another attractive advantage of generating biohydrogen from microalgae is that microalgae have been utilised as healthy food because of their high nutritional value. For instance, microalgae have been served as food in China and Mexico from ancient times [10]. Therefore, the  $\text{CO}_2$  derived microalgal biomass can be concurrently sold as a by-product of the biohydrogen generation process, which could make the process more economical [13].

Recent research found that *Cyanothece* sp. ATCC 51142 (*Cyanothece* 51142), a type of nitrogen-fixing unicellular cyanobacterium, offers remarkably high rates of  $\text{H}_2$  production, which have never been observed before in any other hydrogen-producing strain [5]. Although *Cyanothece* 51142 contains both hydrogenase and nitrogenase for biohydrogen production, previous research has demonstrated that the nitrogen-fixing process regulated by nitrogenase is predominant reaction, and hydrogen production rates employing this metabolic process are

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## Nomenclature

$\alpha_g$	Bubble volume fraction
$\tau_c$	Light extinction coefficient by cell absorption
$\mu_{\max}$	maximum specific growth rate
$A_c$	Pre-exponential factor for cyanobacterial growth
$A_d$	Pre-exponential factor for cyanobacterial decay
$d_b$	Average bubble diameter
$E_a$	Activation energy for cyanobacterial growth
$E_{a,H_2}$	Activation energy for hydrogen production
$E_b$	Inactivation energy for cyanobacterial decay
$H_2$	Hydrogen production
$I$	Light intensity cells experience
$I_0$	Incident light intensity
$k_i$	Light photoinhibition coefficient
$k_{i,H_2}$	Nitrogenase light photoinhibition coefficient
$K_N$	Nitrate half-velocity coefficient
$k_s$	Light saturation coefficient
$k_{s,H_2}$	Nitrogenase light saturation coefficient
$N$	Nitrate concentration
$T_a$	Reference temperature for cyanobacterial growth
$T_{a,H_2}$	Reference temperature for enzyme activation
$T_b$	Reference temperature for cyanobacterial decay
$T_{b,H_2}$	Reference temperature for enzyme inactivation
$X$	Biomass concentration
$z$	Thickness of photobioreactor

significantly higher than those in photosynthesis processes stimulated by hydrogenase [24]. Additionally, compositional analyses of *Cyanothece* 51142 show that this strain contains high levels of protein (60%) and carbohydrate (29%) with low levels of fat (less than 1%) [28], which indicates its high potential of being refined to health food. As a result, this species is being extensively studied at present.

However, challenges of biohydrogen production from *Cyanothece* 51142 still greatly restrict its further development, and the evolution of biofuel production from laboratory scale to industrial scale has to be implemented [27]. High biomass density cultivation is seen as the major challenge at the current stage [8]. In a lab scale photobioreactor (PBR), biomass concentration usually reaches densities of  $1 \text{ g L}^{-1}$ – $3 \text{ g L}^{-1}$  where the culture is highly dense [3,12]. Even when excess nutrients are present in the culture medium, cyanobacteria are not able to grow because of the serious light attenuation observed in PBRs. Although recent research [3] demonstrated that cyanobacteria can be cultivated up to  $12 \text{ g L}^{-1}$  in a laboratory scale, the incident light intensity provided in that case was relatively high ( $457 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ) which would add cost for the necessary lighting.

While a number of studies have been conducted to analyse the influence of light intensity and temperature on *Cyanothece* 51142 growth and biohydrogen production [3,14,16,24], much less research has focused on the aspects of cell growth and hydrogen production with regard to light attenuation, which always exists and is difficult to be eliminated and analysed in experiments. Furthermore, it is also very time consuming to seek the optimal temperature and light intensity for cell growth and biohydrogen production rate purely by experiments. As a result, the current research aims to explore the effect of light intensity, light attenuation and temperature on the *Cyanothece* 51142 growth rate and biohydrogen production rate by dynamic simulation.

## 2. Experimental setup and data selection

### 2.1. Experimental setup

Input data for our simulation were obtained from experimental studies using two different geometries of photobioreactor: tubular and flat-plate. To investigate the cyanobacterial growth kinetics, a tubular flow Biostat PBR 2S was our reactor choice [12], whereas an Imperial College flat-plate PBR [32] was utilised for the study of  $H_2$  production. The reason behind our PBR selection is based on the main features of each PBR. Specifically, the tubular PBR enables an automated and consistent control of cultivating conditions and was thus used for the study of growth kinetics. In the case of  $H_2$  production kinetics study, our customised flat-plate PBR was employed instead, due to its gas-tight design as well as ability to in-situ measure  $H_2$ . During the growth study [12], *Cyanothece* 51142 culture was cultivated in an artificial sea water (ASP2) with supplement of  $1.5 \text{ g L}^{-1} \text{ NaNO}_3$  and 10% volume  $\text{CO}_2$  volume air<sup>-1</sup> under continuous illumination of cool-white fluorescent using seven light regimes (23, 46, 92, 138, 207, 275 and  $320 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ) and constant temperature at  $35 \text{ }^\circ\text{C}$ . In the case of temperature effects, a fixed light intensity of  $92 \mu\text{mol m}^{-2} \text{ s}^{-1}$  was employed and our investigated regimes were 25, 30, 32, 35, 37 and  $40 \text{ }^\circ\text{C}$ .

For  $H_2$  productivity study [14], a non-heating illumination provided by a panel of cool-white 5000 K light-emitting diodes (LED) was used. As an anaerobic environment is necessary for the onset of cyanobacterial  $H_2$  production, glycerol was chosen to replace  $\text{CO}_2$  as carbon source. In the presence of glycerol, photosynthetic activities of *Cyanothece* 51142 are inhibited, whereas the respiratory activity becomes enhanced [4,15]. The culture of *Cyanothece* 51142 was initially subjected into the ASP2 medium with a supplement of 50 mM glycerol. As soon as  $H_2$  production was observed, the switch in operating conditions was made. The temperature regimes were 20, 25, 30, 35, 40, 47 and  $55 \text{ }^\circ\text{C}$  and the light intensity regimes were 46, 92, 138, 229,  $320 \mu\text{mol m}^{-2} \text{ s}^{-1}$ . The dry biomass concentration of the *Cyanothece* 51142 culture was derived from its spectrophotometrically measured chlorophyll concentration, using a previously determined correlation in our work [12]. The hydrogen production rate of the cyanobacterial culture was in situ measurement using a membrane-inlet mass spectrometry (MIMS) system [32]. Details of the experimental setup and the execution of these experiments can be found in [12,14].

### 2.2. Data selection

In order to truly simulate light intensity and temperature influences on cyanobacterial growth, it is vital to ensure that nutrients are in excess and not the growth-limiting factors. During our experimental studies, sufficiency of the carbon source was ensured by a continuous provision of  $\text{CO}_2$ , whereas the nitrogen source, nitrate, always ran out at the end of experiments (except during the runs with incident light intensity  $I_0 = 23 \mu\text{mol m}^{-2} \text{ s}^{-1}$  and  $I_0 = 46 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ) [12]. Therefore, the data for our simulation were selected during the nitrate-sufficient period where nitrate concentration was higher than  $500 \text{ mg L}^{-1}$ .

A similar principle was applied to simulate the environmental effects on hydrogen production rate. Our previous research showed that the gas is mainly produced during the cyanobacterial second growth and the decay phases [15]. However, during the latter phase, the activity of nitrogenase was not only affected by light intensity and temperature, but also significantly affected by acidity (low pH environment) of the culture phase [15]. As a result, the data were selected during the cyanobacterial second growth phase and the early decay phase, where the pH was still in an appropriated range (between 6 and 8) and cyanobacterial decay rate was not significant.

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