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Effect of shear stress on the growth of continuous culture of *Synechocystis* PCC 6803 in a flat-panel photobioreactor

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Abstract–The effect of hydrodynamic forces generated by air bubbles on cell growth of continuous culture of *Synechocystis* PCC 6803 was studied in a flat-panel photobioreactor. Keeping all relevant parameters constant enables the optimization of individual parameters, for which a continuous cultivation approach has significant advantages. Continuous culture of *Synechocystis* PCC 6803 was cultivated under different gas velocities from 0.022 m s^{-1} up to 0.128 m s^{-1}. Based on direct determination of effective growth rate at constant cell densities, cell damage due to shear stress induced by the increasing gas velocity at the sparger was directly observed. A significant decrease of effective growth rate was observed at gas velocity of 0.085 m s^{-1} generated at the gas flow rate of 200 ml min^{-1}, indicating cell damage by shear stress. Optimization of gas volume and the development of an effective aeration system corresponding to a given reactor setup is important to realize a reliable cell growth.

Keywords: Shear Stress, Bioprocess Optimization, Gas Flow Rate, Continuous Cultivation, Photobioreactor

INTRODUCTION

Microalgae and cyanobacteria are receiving increasing attention for the development of alternative renewable energy sources. Bio-hydrogen production based on the connection of water-splitting photosynthesis with hydrogenase is regarded as one of the most promising alternatives [1-3]. Along with bio-hydrogen production, another environmentally sustainable energy source from photosynthetic microorganisms is biomass. Biomass can be converted into different types of energy products, such as transportable fuels or bio-derived products including plastics and chemicals [4]. In recent decades biomass conversion to liquid biofuels including biodiesel and bioethanol has undergone substantial improvements [5]. The first-generation biodiesel was produced from only a few agricultural crop systems. Typically, only a small fraction of the solar energy captured and converted into chemical energy (biomass) is harvestable. Inefficiencies in feedstock harvesting and processing further reduce the recoverable energy and reduce the net carbon capture [6]. On the other hand, biodiesel production systems from microalgae are expected to have the lowest impact on the environment, higher productivity, greater energy return on investment, and will be directly compatible with the existing energy infrastructure. Microalgae grow rapidly, have high oil content (up to 75%), and are capable of producing 2-10 times more biomass per unit land area than any terrestrial crop system [7]. In addition, microalgae can potentially capture CO_2 as well as utilize nutrient-rich waste water [8]. However, microalgal-based biofuel production systems will face commercial competition. They must be able to produce biomass at rates that are sufficient for practical demand and at prices that can be realized on a global market.

With consideration of biotechnological approaches to overcome the low solar energy conversion efficiencies in the photosynthetic apparatus and inefficient coupling of the photosynthetic water splitting machinery with the targeted metabolisms for biomass and/or hydrogen production, it is imperative to optimize mass-cultivation systems and develop a reliable cultivation process for maximal productivity [7]. However, many photosynthetic microorganisms are affected by environmental parameters including light, temperature, media components, and pH levels, which are critical to cellular growth. Furthermore, it is difficult to predict and control their biological response to stresses related to biotic and abiotic conditions because of complex interactions between fluid dynamics, biochemical reactions, and light transfer in a photobioreactor system [9,10]. The large number of independent and mutually dependent parameters results in a huge parameter space that has to be explored to find suitable operating conditions.

Among several environmental parameters, in particular, fluid dynamics induced by pneumatic mixing restricts hydrodynamic and static pressure of the reactor system. High superficial gas velocity is necessary to achieve a high degree of turbulence for sufficient mixing of the cell suspension with light, CO_2 gas, and several nutrients [11]. High photosynthetic efficiency has been demonstrated in fast gas/liquid-circulation [9]. However, the increasing hydrodynamic force due to a fast gas-flow leads to shear stress. Shear stress is one of the possible reasons for a decline in cell growth rate or even cell death [12]. Damage of microalgae due to shear stress was demonstrated in a bubble column with a gas sparger [11]. Therefore,

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the gas volume related with superficial gas velocity is one of the important operation parameters for sustainable cell growth in terms of the balance between mixing and shear stress.

In previous research, a continuous cultivation system providing a direct approach to determine the growth rate was demonstrated to be an effective process to optimize two important parameters, light intensity and media composition, under stable cultivation conditions [13]. Here, we clearly presented the impact of the gas flow rate on cyanobacterial cell growth and optimized it by direct measurement of the effective growth rate under continuous cultivation of cyanobacterial *Synechocystis* PCC 6803 wild-type (hereafter *Synechocystis* WT).

MATERIAL AND METHODS

1. Photobioreactor Set-up

The photobioreactor was designed in the form of a flat, rectangular container with front and back windows providing a light-path of 40 mm and a working volume of 5 L. Two white LED panels are provided at both sides for illumination of the reactor. The light intensities were measured by a Li-Cor LI-189 2 π PAR quantum sensor. The voltages corresponding to each light intensity were used for regulation of light intensity. To avoid initial photodamage at low cell density, the cell culture was exposed to light intensity of 100 μmol photons m^{-2} s^{-1} following inoculation. Continuous cultures of *Synechocystis* WT have been grown at OD_{680} of 0.7 under light intensity of 100 μmol photons m^{-2} s^{-1}. The reactor was mixed by sparging gas into the liquid. A mass flow controller was used to control/measure a constant gas feed flow from 50 ml min^{-1} up to 500 ml min^{-1}. Gas bubbles enriched with 3% CO_2 (v/v) were generated from a glass-sparger embedded in the bottom of the reactor. Culture growth was monitored by an integrated turbidity sensor (Trb 8300, Toledo) and feedback-controlled by a media pump. A constant pH value of 7 was maintained by automated titration with 0.2 M HCl and NaOH. All cultivation conditions were dynamically controlled according to a user defined protocol program from LabVIEW. The plastic polymer based reactor setup was chemically sterilized by incubation with a peroxyacetic acid solution of 5 mM for 1 hour [14]. Prior to cultivation the reactor was rinsed three times with autoclaved distilled water. Air filters, HCl and NaOH solutions for pH-value regulation, and growth media were autoclaved for 20 min at 121 °C. Heat-labile solutions were filtered by sterile membrane filters with a pore size of 0.22 μm prior to application to the cell culture inside the reactor.

2. Continuous Cultivation and Optimization of Bioprocess Parameters

The photobioreactor provides reliable growth conditions for photosynthetic microorganisms to achieve conversion or production of biological products. It minimizes the demand for fresh water, permits monocultures, and offers better control over environmental parameters. Among several bioprocess systems, continuous cultivation operation is characterized by the continuous addition of a fresh medium and the withdrawal of the culture. Keeping all relevant parameters constant makes it possible to engineer an efficient bioprocess via optimization of individual factors for stable and sustainable growth [13,15]. Although more difficult to realize, continuous cultivation has several advantages over batch cultures, such as constant production rates and elimination of down time for cleaning and sterilization [16].

When considering continuous cultivation, the mass balance for biomass can be written as $dX/dT=(\mu-D)\cdot X$ with X: biomass in reactor, T: cultivation time, μ: specific growth rate, and D: dilution rate. It shows that the growth rate equals the dilution rate under steady-state conditions. Under these conditions the effective growth rate (μ_{eff}) can be calculated by a software sensor (i.e., the moving average of the media feed rate determined over a defined period) in order to focus on real-time changes of the cell growth rate [13,17].

3. Strains and Medium

The cyanobacterium *Synechocystis* sp. PCC 6803 from the Pasteur Culture Collection was used for the present study. *Synechocystis* is a freshwater unicellular cyanobacterium with capability to grow autotrophically or heterotrophically. In this work, *Synechocystis* WT was photoautotrophically cultivated in YBG11 medium [18]. This modified BG11 medium ($1.76\cdot10^{-2}$ M $NaNO_3$; $1\cdot10^{-2}$ M HEPES; $1.75\cdot10^{-3}$ M K_2HPO_4; $3\cdot10^{-4}$ M $MgSO_4$; $1.8\cdot10^{-4}$ M Na_2CO_3; $1.6\cdot10^{-5}$ M Na_2-EDTA; $4.5\cdot10^{-5}$ M Boric acid; $3\cdot10^{-5}$ M Citric acid; $9\cdot10^{-6}$ M $MnCl_2$; $6\cdot10^{-6}$ M $FeCl_3$; $1.6\cdot10^{-6}$ M Na_2MoO_4; $7\cdot10^{-7}$ M $ZnSO_4$; $3\cdot10^{-7}$ M $CuSO_4$; $1.6\cdot10^{-7}$ M $Co(NO_3)_2$; $2.5\cdot10^{-4}$ M $CaCl_2$) was introduced by Shcolnick et al. [18] to cope with the low solubility of Fe^{3+} in an oxygenated aqueous solution.

4. Offline Characterization

Optical densities were monitored in whole cells based on their absorption at 750 nm and 680 nm, by using a Beckman DU 7400 spectrophotometer. Cell count of the samples was determined with a Z2 Coulter counter (Beckman Coulter, Fullerton, CA). Cells were harvested and washed twice with deionized water by centrifugation at 7,000 rpm for 5 min. The suspensions were filtered through pre-dried and pre-weighed 0.45 μm cellulose nitrate membrane filters (Whatman, USA) and the cells were dried in an oven at 80 °C for 24 h before dry cell weight was measured.

RESULTS AND DISCUSSION

Most flat-panel photobioreactors employ a pneumatic agitation system that enables low auxiliary energy demand for mixing and supply of CO_2 in the cell suspension. In such systems hydrodynamic shear force is mainly decided by the aeration volume [19]. However, shear stress experienced by individual cell is determined by not only the superficial gas flow rate resulting from diverse aeration profiles but also biological factors including cellular morphology and cell wall rigidity.

In this study *Synechocystis* WT was cultivated continuously in our experiment setup according to a procedure reported by Kwon [13] to research the impact of gas flow rate on cellular growth. *Synechocystis* is the most widely researched cyanobacterial model organism containing a rigid cell wall structure [20]. Under turbidostatic process control a constant culture density is established by feedback loop control between measurement of actual cell density and media pump. Air bubbles enriched with 3% CO_2 (v/v) are sparged from a two glass-sparger embedded in the bottom of the reactor. Spatial circulation involving spatial lift-up and lift-down of the culture suspension in a 5 L flat-panel photobioreactor was demonstrated according to gas/liquid flow generated by air-lift with a proper distance (Fig. 1). While variation of the gas volume has no effect on

the gas composition, the pH-value of the culture is clearly affected due to change of the absolute amount of carbon dioxide. Therefore, the pH-value was adjusted by automated titration with 0.2 M hydrochloric acid and sodium hydroxide to minimize the pH effect on cellular growth of *Synechocystis* WT.

The impact of shear stress on cells in terms of cell viability is evaluated by direct measurement of the effective growth rate [13]. A rapid reduction of the cellular growth rate was clearly observed with fluctuation of cell turbidity following an increase of the gas flow rate from 100 ml min^{-1} to 500 ml min^{-1} (Fig. 2). It took 5 hours for the cell growth rate to approach zero. Significantly, the cell count decreased from 1.143±0.12×10^8 cells ml^{-1} (corresponding to biomass of 0.141 g/*l*) to 3.736±0.04×10^7 cells ml^{-1} (0.046 g/*l*) at a high aeration volume of 500 ml min^{-1}. After recovery of the gas flow

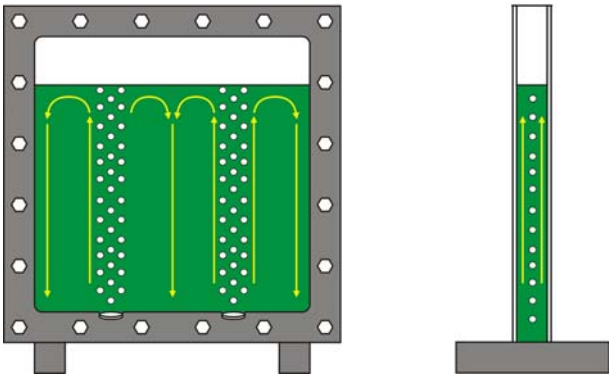

Fig. 1. A schematic view of a spatial circulation system for aeration in flat-panel photobioreactor.

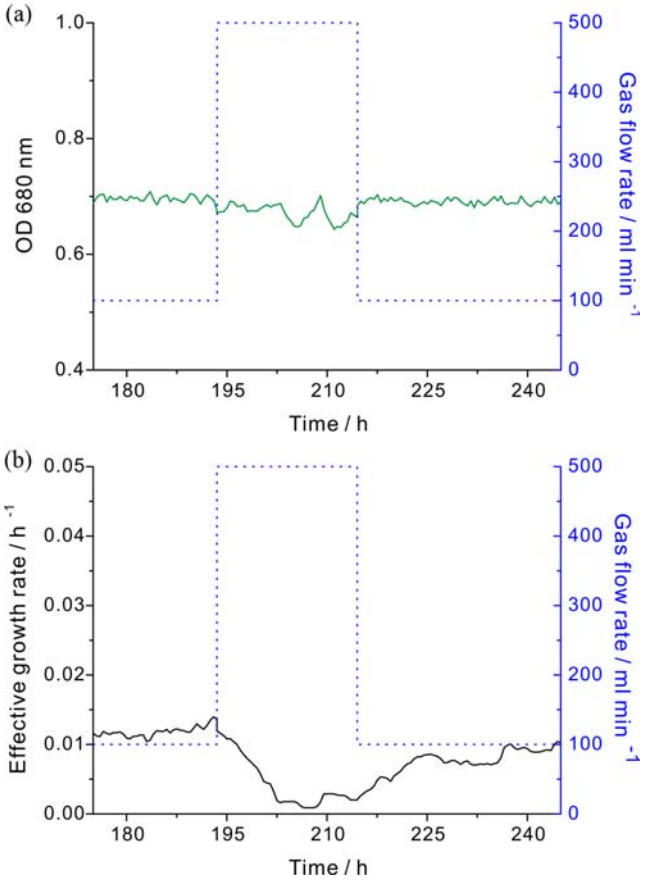

Fig. 2. Impact of shear stress on cellular growth of *Synechocystis* WT grown under continuous cultivation. Optical density at 680 nm and μ_{eff} are shown by green and black lines, respectively. The gas flow rate is shown by a blue dashed-line.

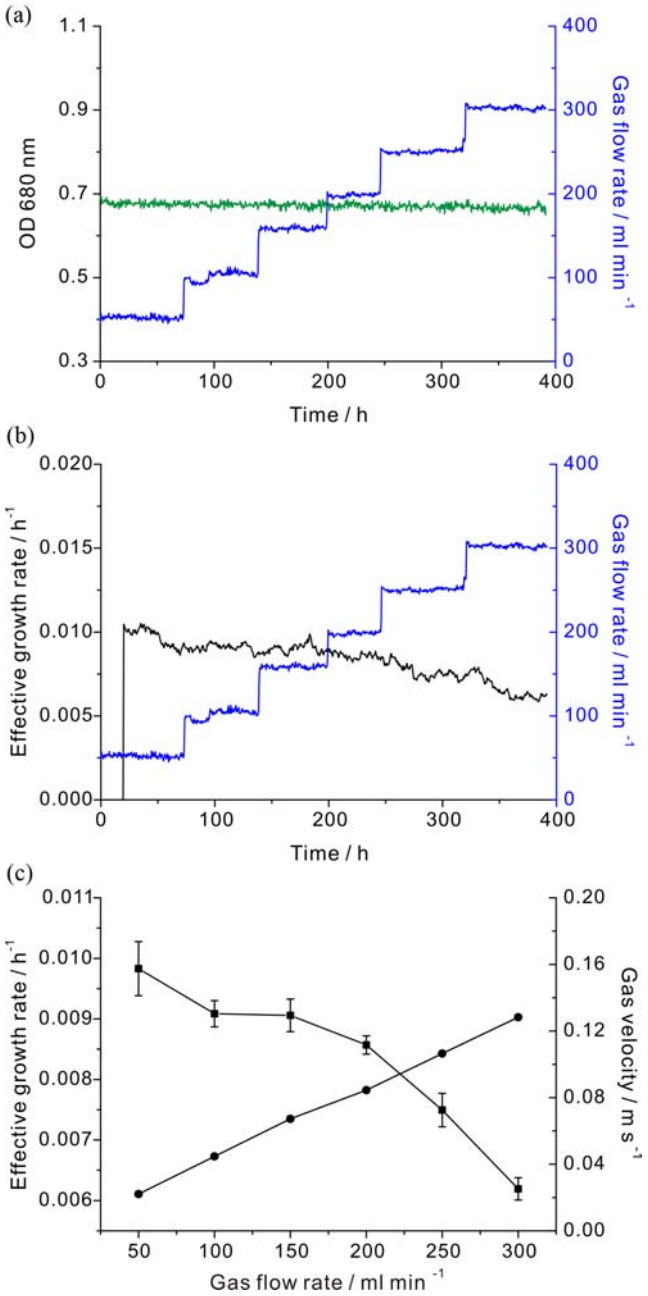

Fig. 3. Optimization of the gas flow rate for *Synechocystis* WT grown under continuous cultivation. (a) Cells were exposed to a stepwise increasing gas flow rate (from 50 to 300 ml min^{-1}) while keeping other parameters constant. (b) Effect of the gas flow rate on the effective growth rate. (c) Relationship between effective growth rate (square) and gas velocity (circle) according to the increasing gas flow rate. Turbidity and μ_{eff} are shown by green and black lines, respectively. The gas flow rate is shown by a blue line. Error bars for the points of gas velocity are smaller in height than the corresponding symbols.

rate from the stress-giving conditions, the effective growth rate increased and stabilized. This clearly indicates that the retardation of cell growth was induced by shear stress due to the high gas flow rate. Although phenotypes of cell culture including cell count and culture color seemed to be recovered, their effective growth rate did not reach the initial value (Fig. 2). Cells damaged by shear stress likely require more time-space to recover full biological stability.

The gas flow rate was optimized under turbidostat of *Synechocystis* WT (Fig. 3). Cells were exposed to a stepwise increasing gas flow rate from 50 to 300 ml min^{-1} with keeping other parameters constant. Fig. 3 shows that a stable effective growth rate above 0.009 h^{-1} was maintained up to a gas flow rate of 150 ml min^{-1}. Further increase of the gas flow rate resulted in a decrease of the effective growth rate (Fig. 3). This reflects that severe shear stress appeared at 200 ml min^{-1} in our experiment setup in spite of the relatively low gas volume as compared with the working volume of 5 L. Structurally, the focused aeration nozzle system guided by the glass sparger with a small diameter of 0.5 cm could induce high gas entrance velocities and give cell severe damage when gas and cells are mixed strongly in the reactor. When a gas flow rate of 200 ml min^{-1} was set, a gas velocity of 0.0849 m s^{-1} could be estimated at the sparger site according to Barbosa et al. [11] who demonstrated that the gas entrance velocity at the sparger is the main parameter causing hydrodynamic stress and lethal events in sparged microalgae cultures. The gas velocity increased from 0.022 to 0.128 m s^{-1} with the increasing gas flow rate in our setup.

The environmental effects of carbon dioxide are of significant interest. Carbon dioxide is an important greenhouse gas. CO_2 sequestration by photosynthetic microorganisms is another advantage together with renewable energy production. To increase the efficiency of CO_2 uptake, small gas bubbles need to be well mixed and should have a long retention time in culture media. Based on our experimental observations of culture circulation in the reactor and a short regulation response of less than 1 minute for turbidity as well as the pH-value (data not shown), the spatial circulation system introduced in our aeration setup is considered sufficient to realize effective mixing in a flat-panel photobioreactor (Fig. 1). Although a relatively low gas flow rate of 50 ml min^{-1} was applied, the growth rate matched that obtained with other gas flow rates up to 150 ml min^{-1}, thus demanding higher energy input for hydraulic mixing (Fig. 3).

Therefore, an effective aeration system with an optimized gas volume offers several advantages, including reduction of shear stress, longer retention time related with low gas entrance velocity, and reduction of the amount of CO_2 released into exhausted gas, resulting in efficient gas utilization for the cultivation of photosynthetic microorganisms.

CONCLUSIONS

During microalgae cultivation in a flat panel photobioreactor, optimization of gas volume and the development of an effective aeration system corresponding to a given reactor setup is important for minimizing shear stress and realize sufficient mixing. In the present study, shear stress induced by a high gas flow rate with gas velocity of 0.085 m s^{-1} inhibits reliable cell growth of *Synechocystis* WT, which was represented by a stable effective growth rate. The efficient optimization procedure in terms of stress parameters described here can be used for new designs and scale-up of reactors.

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ABBREVIATIONS

OD : optical density
PAA : peroxyacetic acid
PAR : photosynthetically active radiation
Synechocystis PCC 6803 wild-type : *Synechocystis* WT

REFERENCES

1. S. I. Allakhverdiev, V. Thavasi, V. D. Kreslavski, S. K. Zharmukhamedov, V. V. Klimov, S. Ramakrishna, D. A. Los, M. Mimuro, H. Nishihara and R. Carpentier, *J. Photochem. Photobiol. C: Photochemistry Reviews*, **11**, 101 (2010).
2. D. Dutta, D. De, S. Chaudhuri and S. K. Bhattacharya, *Microb. Cell Fact.*, **4**, 36 (2005).
3. B. Esper, A. Badura and M. Rögner, *Trends in Plant Science*, **11**, 543 (2006).
4. B. Vijayendran, *Journal of Business Chemistry*, **7**, 109 (2010).
5. A. J. Ragauskas, C. K. Williams, B. H. Davison, G. Britovsek, J. Cairney, C. A. Eckert, W. J. Frederick Jr., J. P. Hallett, D. J. Leak and C. L. Liotta, *Science*, **311**, 484 (2006).
6. A. Demirbas, *Prog. Energy Combust. Sci.*, **33**, 1 (2007).
7. Y. Chisti, *Biotechnol. Adv.*, **25**, 294 (2007).
8. J. Noüe, G. Laliberté and D. Proulx, *J. Appl. Phycol.*, **4**, 247 (1992).
9. M. Janssen, J. Tramper, L. R. Mur and R. H. Wijffels, *Biotechnol. Bioeng.*, **81**, 193 (2003).
10. C. Posten, *Engineering in Life Sciences*, **9**, 165 (2009).
11. M. J. Barbosa, M. Albrecht and R. H. Wijffels, *Biotechnol. Bioeng.*, **83**, 112 (2003).
12. A. Contreras, F. Garcia, E. Molina and J. Merchuk, *Biotechnol. Bioeng.*, **60**, 317 (2000).
13. J. H. Kwon, M. Rogner and S. Rexroth, *J. Biotechnol.*, **162**(1), 156 (2012).
14. S. Oie, A. Obayashi, H. Yamasaki, H. Furukawa, T. Kenri, M. Takahashi, K. Kawamoto and S. Makino, *Biological and Pharmaceutical Bulletin*, **34**, 1325 (2011).
15. V. Czitrom, *American Statistician*, 126 (1999).
16. H. Tang, M. Chen, K. Simon Ng and S. O. Salley, *Biotechnol. Bioeng.*, **109**(10), 2468 (2012).
17. I. Havlik, P. Lindner, T. Scheper and K. F. Reardon, *Trends in Biotechnology*, **31**(7), 406 (2013).
18. S. Shcolnick, Y. Shaked and N. Keren, *Biochim. Biophys. Acta (BBA)-Bioenergetics*, **1767**, 814 (2007).
19. X. Zhang, S. Liu and X. Chen, *Ener. Procedia*, **11**, 2121 (2011).
20. M. Liberton, R. H. Berg, J. Heuser, R. Roth and H. B. Pakrasi, *Protoplasma*, **227**, 129 (2006).