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Development of an optimal light-feeding strategy coupled with semi-continuous reactor operation for simultaneous improvement of microalgal photosynthetic efficiency, lutein production and CO_2 sequestration

Ramalingam Dineshkumar[a], Ganeshan Subramanian[a], Sukanta Kumar Dash[b], Ramkrishna Sen[a,*]

[a] Department of Biotechnology, Indian Institute of Technology Kharagpur, India
[b] Department of Mechanical Engineering, Indian Institute of Technology Kharagpur, India

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ABSTRACT

In addition to leveraging some environmental and energy benefits, microalgae can also be used as a potentially sustainable source for carotenoids like lutein for healthcare applications. As light influences biosynthesis of lutein in microalgae, it would be interesting to investigate the role of incremental illumination in enhancing photosynthetic efficiency and consequent lutein production, while sequestering CO_2 simultaneously. Thus, in this study, an optimal light feeding strategy coupled with a semi-continuous reactor operation was developed for achieving the above-mentioned goals. At a light intensity of 260 $\mu mol\, m^{-2}\, s^{-1}$ as optimized in batch mode, baseline lutein productivity of 4.32 $mg\, L^{-1}\, d^{-1}$ was obtained, when culturing *Chlorella minutissima* under optimal process conditions. On switching over to incremental illumination modes, the linear light-feeding (Strategy-III) resulted in higher lutein productivity of 5.35 $mg\, L^{-1}\, d^{-1}$ with photosynthetic efficiency of 8.38%. When Strategy-III was integrated with semi-continuous mode involving 20% medium replenishment, lutein productivity, photosynthetic efficiency and CO_2 sequestration rate were further enhanced by 19%, 41% and 34% respectively. Moreover, on this process optimization and integration, light-energy consumption was significantly reduced by 32%, in comparison with constant-illumination. Thus, this optimal production strategy resulted in significantly higher lutein productivity, content and photosynthetic efficiency, as compared to the relevant studies reported in literature.

1. Introduction

The carotenoid lutein has potential nutraceutical and pharmaceutical applications including prevention and treatment of age related blindness, cataracts, atherosclerosis and some types of cancers [1–3]. In recent years, microalgae have emerged as a potential and sustainable source for lutein production, while addressing some environmental and energy challenges like CO_2 sequestration, waste water remediation and biofuels production [4–6]. Moreover, these photosynthetic microorganisms have better capability to meet the increasing demands of lutein in future and provide consistent supply of lutein throughout the year. While the conventional source i.e. marigold flowers have inherent limitations such as seasonal harvest, low productivity, high land requirements and intense human labor [2,3].

Among the physicochemical parameters that influence microalgal lutein synthesis, light intensity, CO_2 and some medium components (nitrate, manganese and copper) were identified as the primary factors critically influencing the growth rate and accumulation of lutein [7–9]. The main roles of lutein and other carotenoids are to protect the photosynthetic components from detrimental effects of reactive oxygen species, and capture light energy so as to transfer it to chlorophyll [10,11]. The biosynthesis of lutein can be improved by providing optimal mode of light intensity, as the carotenoid lutein is present in the photosynthetic antenna complexes [12]. This is evident from the study of Vaquero et al. [13] which reported that sudden change in light intensity either from low to moderate level or from moderate to low level enhanced the

* Corresponding author at: Department of Biotechnology, Indian Institute of Technology Kharagpur, West Bengal, 721 302, India. Tel.: +91–3222–283752.
E-mail address: rksen@yahoo.com (R. Sen).

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lutein content of microalga *Coccomyxa onubensis*. In another study, Ho et al. [14] carried out a two-stage process involving cultivation of microalgae under high light intensity (300 $\mu mol\ m^{-2}\ s^{-1}$) in the first stage and then the culture was shifted to low illumination intensity (75 $\mu mol\ m^{-2}\ s^{-1}$) for increased lutein synthesis. Although this strategy resulted in significant enhancement in lutein content ($mg\ g^{-1}$), the lutein productivity ($mg\ L^{-1}\ d^{-1}$) was reduced due to lower biomass production in the second stage. Therefore, it is essential to develop optimal cultivation strategies to significantly improve both lutein content and productivity, in order to make the microalgal lutein production commercially feasible.

In photoautotrophic mode, the light energy required by microalgae varies with respect to biomass growth. For instance, a lower level of light intensity is adequate during initial stages of microalgal growth, whereas, a higher level is needed for moderate-high density cultures to avoid mutual shading of cells and enhance photosynthesis [15,16]. The studies on light-mediated lutein enhancement indicate that the microalgal lutein synthesis is greatly affected at higher light intensities [13,14]. In literature, various light feeding approaches have been demonstrated for the improvement of photosynthetic efficiency and biomass productivity. The sequential increase in light intensity was generally achieved by maintaining some indirect biological or physical empirical parameters including specific light uptake rate (lumostat) [17], specific irradiation rate [18], increase in illumination when there is decline in growth [19], and increase in light intensity at different time intervals or at various growth stages [20]. Recently, Xie et al. [21] demonstrated an innovative combined strategy (i.e. step-wise light and nutrient feeding combined with repeated fed-batch operation) that resulted in remarkable enhancement of lutein productivity and CO_2 sequestration of *Desmodesmus* sp. F51. However, no studies have been reported to evaluate the role of incremental illumination strategies for improving photosynthetic efficiency and consequent lutein production, while sequestering CO_2 simultaneously. Accordingly, appropriate light-feeding strategies that were different with that of reported by Xie et al. [21] were attempted in the current study for achieving the above-mentioned goals. The main advantages of using incremental light feeding approach in this study are achieving higher lutein productivity and content with reduced light-energy consumption, and subsequently offsetting the downstream processing costs due to higher product yield and lower light-energy requirements.

In this context, the present study is proposed to improve the performance of the microalga *Chlorella minutissima* for lutein production by implementing the following cultivation strategies: (1) development of a suitable incremental light-feeding approach for enhancing photosynthetic efficiency and lutein synthesis without hampering the microalgal growth rate, and (2) integration of optimized illumination strategy with the semi-continuous cultivation for further improvement of lutein productivity, photosynthetic efficiency and CO_2 fixation rate. Thus, the potential implication of this study lies in systematic design and development of an optimal algal cultivation strategy for simultaneous improvement of carotenoid production and photosynthetic efficiency with consequent CO_2 sequestration by *Chlorella minutissima*.

2. Materials and methods

2.1. Microalgae and operating conditions of photobioreactor

The microalga *Chlorella minutissima* (MCC-27) was maintained and sub-cultured using 150 ml Erlenmeyer flasks containing modified Bold's Basal Medium (BBM) in a temperature controlled shaking incubator (LSI-2005RL, Daihan Lab Tech, Korea) at $27 \pm 2^{\circ}$ C, 120 rpm with a light intensity of 50 $\mu mol\ m^{-2}\ s^{-1}$. The seed culture was developed by inoculating *Chlorella minutissima* into 500 ml bubble column photobioreactors containing modified BBM with a light intensity of 100 $\mu mol\ m^{-2}\ s^{-1}$ and air flow rate of 0.425 vvm, and it was grown till it reached mid-logarithmic phase. The required amount of inoculum was then aseptically transferred to 2-L airlift photobioreactors. The composition of modified BBM ($mg\ L^{-1}$) is as follows: $NaNO_3$, 1152; KH_2PO_4, 34; K_2HPO_4, 75; $MgSO_4{\cdot}7H_2O$, 150; NaCl, 25; $CaCl_2{\cdot}2H_2O$, 25; EDTA, 63.7; KOH, 31; H_3BO_3, 11.42; $FeSO_4{\cdot}7H_2O$, 10; $ZnSO_4{\cdot}7H_2O$, 4.0; $CuSO_4{\cdot}5H_2O$, 2.35; $MnCl_2{\cdot}4H_2O$, 4.57; $Co(NO_3){\cdot}6H_2O$, 1.0; MoO_3, 0.71.

All the experiments were carried out in an indigenously designed airlift photobioreactor (2-L) with the following cultivation conditions: inoculum concentration, 50 $mg\ L^{-1}$; inoculum age, mid-log phase; pH, 7–8; temperature, $30 \pm 2\ ^{\circ}C$; CO_2 concentration, 3.5% (v/v) and air flow rate, 0.425 vvm. The design configuration of the customized 2-L airlift photobioreactor are as follows: height/diameter, 3.6; illuminated surface area/volume, 0.465 cm^{-1}; area of downcomer/area of riser, 1.25 and perforated ring shaped spargers with $\Phi_{sparger}$, 5.5 cm and Φ_{pore} = 0.5 mm. The photobioreactor was continuously illuminated with external light supply mounted on both sides and the light intensity was measured using quantum meter (LX 102 Lux Meter, HTC Instruments, India). All experiments were performed in triplicates and expressed as mean with standard deviation. The microalgal cultures were harvested upon depletion of the nitrogen source in the medium.

2.2. Influence of different light intensity and biomass concentration on lutein production

Our previous study on the optimization of critical process parameters in batch mode indicated that the optimal light intensity for improved lutein productivity by *C. minutissima* is 260 $\mu mol\ m^{-2}\ s^{-1}$ [8]. However, it is presumed that the incremental exposure of light intensity may further enhance the synthesis of lutein in microalgae. Hence, a preliminary study involving the exposure of different light intensities on low and high biomass concentration levels (0.2 and 2 $g\ L^{-1}$), was carried out to demonstrate the importance of incremental light-feeding strategy for improving lutein production. The required amount of microalgal biomass was produced using the 2-L airlift photobioreactors with a light intensity of 100 $\mu mol\ m^{-2}\ s^{-1}$ and other cultivation conditions as mentioned in Section 2.1, and the produced biomass was then appropriately diluted with modified BBM so as to obtain 0.2 and 2 $g\ L^{-1}$ biomass concentrations. According to our earlier batch experiments, the low, moderate and high levels of light intensity were considered as 50, 100 and 250 $\mu mol\ m^{-2}\ s^{-1}$ respectively. As the light intensity of $\geq$300 $\mu mol\ m^{-2}\ s^{-1}$ affects lutein synthesis in *Chlorella minutissima*, the high illumination level was set close to optimal light intensity range, which was observed during our previous batch studies [8]. For these experiments, the microalgal cells were cultured in a 2-L airlift photobioreactor in batch mode under the specified biomass concentration and light intensity conditions. The time required to observe the change in lutein levels was fixed to be 8 h and termed as induction time. Subsequently, the samples collected from different induction experiments were analyzed for biomass and lutein concentration as per the protocols mentioned in Section 2.5.

2.3. Incremental light feeding strategies

In photo-autotrophic mode of microalgal cultivation, light is generally considered as one of the major limiting factors [17,22], as it directly influence the specific growth rate and product synthesis e.g. lutein. Hence, in this study, two different light-feeding strategies which are analogous to fed-batch mode were attempted with the aim of enhancing microalgal lutein productivity and pho-