Algal Research 11 (2015) 85–89

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

Effects of plasmonic film filters on microalgal growth and biomass composition

Bendy Estime [a], Dacheng Ren [a,b,c,d], Radhakrishna Sureshkumar [a,e,*]

[a] Department of Biomedical and Chemical Engineering, Syracuse University, Syracuse, NY 13244, USA
[b] Department of Civil and Environmental Engineering, Syracuse University, Syracuse, NY 13244, USA
[c] Department of Biology, Syracuse University, Syracuse, NY 13244, USA
[d] Syracuse Biomaterials Institute, Syracuse University, Syracuse, NY 13244, USA
[e] Department of Physics, Syracuse University, Syracuse, NY 13244, USA

ARTICLE INFO

Article history:
Received 20 February 2015
Received in revised form 29 May 2015
Accepted 10 June 2015
Available online xxxx

Keywords:
Microalgae
Plasmon resonance
Lipid
Carbohydrate
Microalgal pigments

ABSTRACT

Polymer films consisting of spherical silver nanoparticles were fabricated and used as plasmonic filters that selectively enhance blue light absorption in microalgal cultures. For the microalgal species *Chlamydomonas reinhardtii*, after ten days of cultivation the use of plasmonic filters led to an increase in the microalgal dry biomass by more than 25% and an increase in chlorophyll and carotenoid pigments by more than 35%, compared to the control cultures without using these films. Further, light enhancement by plasmon resonance did not affect lipid and carbohydrate accumulation within individual algal cells. However, higher cell densities obtained with plasmonic filters resulted in enhanced overall carbohydrate and lipid production.

1. Introduction

In the context of renewable energy production, microalgae represent a promising viable and sustainable feedstock that can replicate the traditional refinery approach, often referred to as the bio-refinery [20]. Through thermal and biochemical conversions, microalgal feedstock can be used to produce biodiesel, bioethanol, biohydrogen and other types of biofuels [7]. Moreover, chlorophyll and carotenoid pigments from microalgae have several applications in food and pharmaceutical industries as food additives, cosmetic agents and antioxidants for medical purposes [10,11]. Furthermore, algal extract is rich in proteins, carbohydrates and free amino-acids and can serve as a replacement for yeast extract used to feed microorganisms [17]. However, commercial utilization of microalgae for such purposes is still far from being financially feasible due to high extraction costs and low productivity in confined environments. Therefore, strategies for improving algal biomass growth, carbohydrate and lipid production, as well as pigment accumulation have been extensively investigated. For instance, transcription factor engineering and genetic engineering approaches have been applied to enhance lipid overproduction [6]. Physiological stresses such as high salinity and nutrient deprivation are also often used to induce accumulation of carbohydrate, lipid or other valuable compounds [20].

Light intensity dependency and wavelength specificity of microalgal photosynthetic activity have also been studied with the goal of developing technologies to improve biomass growth in microalgal cultures. The effect of light intensity on microalgal growth has already been well described [5]. It is also known that blue and red lights are optimal for photosynthetic activity whereas other wavelengths of the electromagnetic spectrum may in some cases cause photo-inhibition [23,26]. Based on these facts, Wondraczek et al. [30] developed a photoluminescence converter that enables conversion of undesired green light in the incident spectrum to desired red light by photoactive pigments. Our earlier work [29] has shown that localized surface plasmon resonance by metallic nanoparticles can effectively enhance light irradiance in microalgal cultures. Specifically, the use of a silver nanoparticle suspension, as a plasmonic filter that backscatters blue light into microalgal culture, was shown to result in an increase in photosynthetic growth by more than 30%, compared to a control without nanoparticle suspension. Recently, experiments were conducted on microalgal species *Chlorella vulgaris* to show how nanometallic suspensions can be used to increase photosynthetic pigment accumulation [10]. Wavelength selective scattering from nanopatterned surfaces have been shown to enhance the growth rate of cyanobacterium *Synechococcus elongatus* in modular bioreactors by 6.5% while improving the power efficiency by 52% as compared to systems that utilize broadband reflectors [24]. These

* Corresponding author at: 329 Link Hall, Syracuse, NY 13244, USA.
E-mail address: rsureshk@syr.edu (R. Sureshkumar).

http://dx.doi.org/10.1016/j.algal.2015.06.004
2211-9264/

techniques may be impractical to outdoor culture due to potential loss of efficiency through light saturation [25] that may be induced by the additional light provided. However, their application to large scale enclosed microalgal photo-bioreactors is very promising.

Concomitant effects of plasmon-enhanced growth on microalgal growth, accumulation of photosynthetic pigments and production of lipids and carbohydrates remain largely unexplored. Investigating such effects is important because the way an improvement in any given parameter impacts the others highly depends on the type of enhancement. For instance, stress conditions such as high salt concentration and nitrogen deprivation that increase lipid production have been observed to have a negative effect on microalgal growth [16,20]. Similarly, nutrient starvation that increases lipid production in cyanobacteria has been shown to either increase or have little effect on carbohydrate generation, depending on the bacterial species [1,8]. Further, enhanced microalgal growth by light enhancement could be accompanied either by an increase [22] or decrease [12] in triglyceride production. It has been also shown that, in many cases, light enhancement can cause alteration of fatty acid synthesis by inducing the production of mono-unsaturated fatty acids and concomitantly disfavoring the formation of poly-unsaturated fatty acids [16].

In this work, we developed a plasmonics-based technology to improve microalgal biomass production. Specifically, flexible polymeric films consisting of spherical silver nanoparticles were fabricated and used as filters that selectively enhance the irradiance of blue light in microalgal cultures. This technology is much more easily implemented, scalable, durable and safer compared to that based on nanoparticle suspensions [29]. Previous simulation results have demonstrated how plasmon resonance by spherical silver nanoparticles increases the available irradiance in microalgal culture [29]. By virtue of this, the plasmonic film would enhance photosynthetic activity. It was also hypothesized that the additional blue light provided by plasmon resonance would increase photosynthetic pigment contents through chromatic acclimation [21]. Further, it was important to assess potential effects on lipid and carbohydrate production. The plasmonic film was therefore tested for a *Chlamydomonas reinhardtii* culture and its effects were assessed through analyses of biomass, photosynthetic pigment, lipid, and carbohydrate production.

2. Materials and methods

2.1. Plasmonic film preparation

Silver nanoparticles were synthetized by using sodium borohydride ($NaBH_4$) as a reducing agent for silver nitrate [28]. Using a pipette, 20 ml of 1.0 mM silver nitrate solution was gently added to a 60 ml solution of 2.0 mM sodium borohydride. The reaction was performed at 4 °C on a magnetic stir plate. Synthetized spherical silver nanoparticles had diameters of 12 ± 2 nm, as confirmed by dynamic light scattering measurements. Plasmonic films (Fig. 1A) were prepared by adding polyvinyl alcohol (PVA) powder to the nanoparticle suspension such that final PVA content in the mixture was 5% by weight. The final mixture was dried in a petri-dish for two days. Then, the film was peeled off and stored at room temperature until use. The resulting plasmonic film was about 1 mm thick when 20 ml of the mixture was put to dry in a 100 mm diameter petri-dish. The thickness may vary slightly if more volume is added or the PVA content is higher. The absorption spectrum of the plasmonic film exhibited a single peak near 400 nm but was broad enough to cover the entire blue region of the electromagnetic spectrum (Fig. 1B). Note that chlorophyll a, chlorophyll b, and carotenoids are able to absorb light at 410–430, 450, 400–500 nm respectively [10].

2.2. Microalgal strain and culture conditions

The wild type microalgae *C. reinhardtii* CC-124 obtained from the Chlamydomonas Resource Center (University of Minnesota, St. Paul, Minnesota) was used in this study. The experimental setting consisted of a 250 ml conical flask with the base wrapped with a plasmonic film. The base of the flask was then covered with black tape (Scotch Super 33 + Vinyl Electrical Tape, 3 M, St. Paul, Minnesota) so that incident light could enter only from the top of the culture. Another conical flask wrapped with only black tape (without the film) was used as a control. Algal cells were allowed to grow in a 50 ml minimum medium enriched with 20 mM of sodium bicarbonate [29], with the flasks placed on a rotary shaker (100 rpm). The space was continuously illuminated by full spectrum compact fluorescent lamps (CFL 60 W, Fancierstudio, San Francisco, California). The photosynthetic active radiation at the top surface of the culture was at 100 ± 5 μE/m^2/s, as measured by quantum meters (MQ, Apogee Instruments, Inc., Logan, Utah). The temperature was controlled at 23 ± 1 °C. Triplicate samples were collected every two days to measure the optical density of the culture at 675 nm (OD_{675}) [29]. After ten days of cultivation, algal cells were harvested for dry mass determination and compositional analyses.

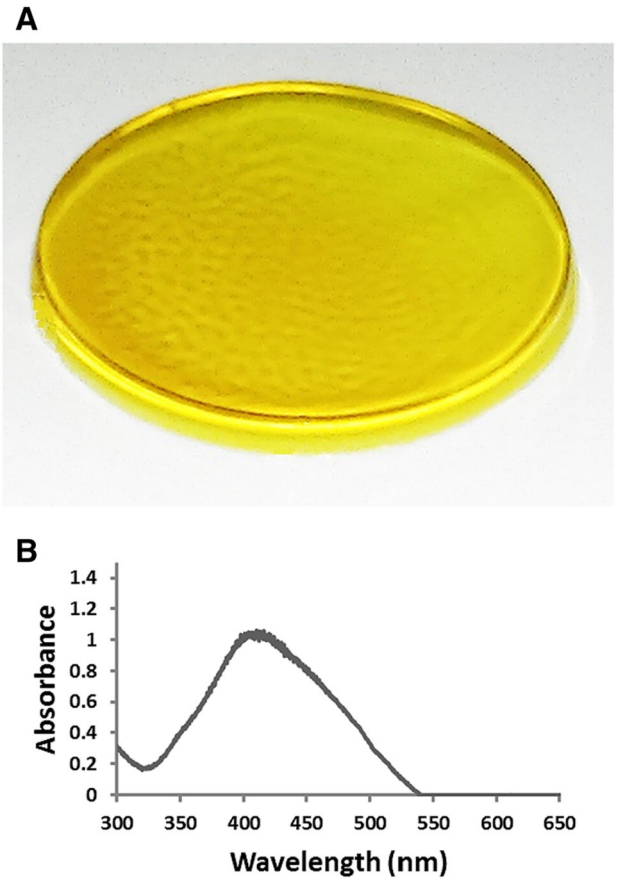

Fig. 1. Photograph of a plasmonic film after being peeled off from petri-dish (A) and absorbance spectrum of the plasmonic film consisting of spherical silver nanoparticles (B).

2.3. Lipid analyses

Lipid generation was assessed using three techniques: gravimetric quantification, fluorometric scanning, and gas chromatography. For gravimetric quantification, a modified Bligh and Dyer method was used [2]. Briefly, each algal sample was centrifuged (4800 *g*, 5 min, 20 °C) to remove excess water. Cell lysis for lipid release was assured by sonication (Q500 Sonicator, Qsonica, LLC., Newtown, Connecticut) with a 20/10 s ON/OFF cycle for 2 min; 5 ml of chloroform, 10 ml of methanol and 5 ml of deionized water were then added to the sonicated samples. To facilitate phase separation, the samples were placed on a