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Effect of light spectrum on isolation of microalgae from urban wastewater and growth characteristics of subsequent cultivation of the isolated species

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ABSTRACT

Light quality plays an important role in regulation of microalgae growth. The aim of this work was to investigate the role of light spectrum in isolation of different strains of microalgae from urban wastewater. Screening and isolation under three light spectrums of white, red, and blue resulted in three different strains of *Chlorella sorokiniana* IG-W-96, *Chlorella vulgaris* IG-R-96, and *Chlorella* sp. IG-B-96, respectively. The strains were identified and named based on morphological characterization under light and confocal microscopy followed by molecular characterization. It was revealed that the light spectrum had significant influence on microalgae cell size. The largest and smallest cells were observed under blue and red light, respectively. The isolated strain under blue light when subsequently cultured under blue light in suspension mode showed the highest biomass density of $1.21 \pm 0.00\ g\,l^{-1}$, as well as lipid density and productivity of $0.29 \pm 0.02\ g\,l^{-1}$ and $40.24 \pm 4.02\ mg_{lipid}\,l^{-1}\,day^{-1}$, respectively. Due to its shorter growth time, however, maximum biomass productivity was obtained for the isolated and cultured strain under red lights. The proposed illumination methodology in isolation and cultivation improved growth characteristics, in such a way that similar light spectra in both steps led to the highest amounts of biomass, lipid, and productivities for each strain.

1. Introduction

Various parameters such as light, temperature, nutrients, agitation, and pH influence microalgae growth [1]. Among these, light is the most significant factor that controls productivity of photosynthetic culture [2–4]. Intensity and quality of the irradiated photons are two important characteristics of a light source that definitely regulate the growth of microalgae [5]. Extensive irradiation would lead to photo oxidation and photo inhibition, whereas low intensities cause a decrease in growth rate due to light limitation [6,7]. The effect of light intensity on microalgal culture has been extensively studied. The findings showed that light intensity controls not only growth rate [8], but also storage and structural lipid distribution [9,10], cellular composition (e.g., proteins and essential fatty acids) [11], and pigment synthesis [12].

Although most studies have been focused on the effects of light intensity, it has been shown that light quality also plays a critical role on algal metabolism [13]. The effect of light wavelength on growth is species specific, because of the differences in metabolic pathways [14], pigmentation, and photo receptors among different species [15]. For instance, when *Porphyra umbilicalis* was illuminated by blue and red light sources, the highest growth rate was observed with red light [16]. Conversely, the highest level of growth by *Nannochloropsis* sp. was experienced under blue light when cultured in both phototrophic and mixotrophic conditions [17]. The quality of light also affects the synthesized pigment content by microalgae. For example, higher astaxanthin levels were obtained by *Haematococcus pluvialis* under blue than red light emitting diode (LED) [18]. In addition, a large number of enzymes that are involved in photosynthetic carbon metabolism and chlorophyll synthesis have been reported to be influenced by blue light [19]. Moreover, it has been reported that light quality regulates cell division of *Chlamydomonas reinhardtii* when cultured under different light wavelengths. The cell size of the microalgae grown under blue light was larger than the red source due to a delay in cellular division processes [20].

Whilst many researches have focused on the effect of light quality on growth of a certain strain of microalgae, to the best of our knowledge, there has been no study to unravel the dependency of isolates on light spectrum in the screening and isolation processes. The aim of the present study was to fill this gap by altering light colors (i.e., white, red, or blue) in the screening step to isolate a strain that could utilize a specific light wavelength more efficiently. Furthermore, it was postulated that a spectrum-specific isolated strain would behave differently

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when subsequently cultivated in suspension under different light color illumination.

2. Materials and methods

2.1. Microalgae and culture conditions

A wastewater sample was collected from the stabilization pond of Parkand Abad wastewater treatment facility (Mashhad, Iran). The sample was maintained in refrigerator after transferring it to the laboratory. Three different light sources of red, blue, and white were used. The following steps were carried out under a red LED, blue LED, or white LED light source for each isolated strain of algae.

The pond sample of 1000 ml was cultured in a photobioreactor for 7 days. An aliquot of 10 μm of each culture was plated on sterile agar plate prepared by adding 1.5% agar to BG11 medium including following component: $NaNO_3$ (1.5 g l^{-1}), $K_2HPO_4{\cdot}3H_2O$ (0.04 g l^{-1}), $MgSO_4{\cdot}7H_2O$ (0.075 g l^{-1}), $CaCl_2{\cdot}2H_2O$ (0.036 g l^{-1}), Na_2CO_3 (0.02 g l^{-1}), citric acid (0.006 g l^{-1}), Ferric ammonium citrate (0.006 g l^{-1}), EDTA (0.001 g l^{-1}) and 1 ml trace metal solution per liter that consisted of H_3BO_3 (2.86 g l^{-1}), $MnCl_2{\cdot}H_2O$ (1.81 g l^{-1}), $ZnSO_4{\cdot}7H_2O$ (0.222 g l^{-1}), $CuSO_4{\cdot}5H_2O$ (0.079 g l^{-1}), $Na_2MoO_4{\cdot}2H_2O$ (0.390 g l^{-1}) and $Co(NO_3)_2{\cdot}6H_2O$ (0.049 g l^{-1}) and incubated for 10 days. The grown algal culture was streaked using sterile technique onto nutrient agar plate and placed back into the incubator for isolation. The streaking method was repeated until pure algal cultures were obtained. Following the isolation of the individual algal colonies under white, red, or blue light source, for the sake of simplicity, each strain was respectively labeled as AW, AR, or AB. Thereafter, the colonies were transferred into 500 ml fresh sterile BG11 medium and cultured for 1–2 days and used for inoculation purposes.

Each batch experiment was carried out in a photobioreactor containing 900 ml BG11 medium and 100 ml inoculum. The contents of the bioreactor were continuously aerated at a flow rate of 1 vvm containing 6% pure cylindrical CO_2 and stirred at 300 rpm, maintained at temperature 25 ± 2 °C, and illuminated with light regime of 16:8 h light/dark. All experiments were carried out in duplicate. The pH of the cultures was monitored and measured throughout the time course of the experiments.

2.2. Morphological and molecular identification

Identification of each isolated algal strain was primarily based on morphological characteristics using selected reference materials [21]. Morphological identification of the new isolates was then confirmed via molecular identification by sequencing the complete 18S rRNA gene and ITS (Internal Transcript Spaces) region. DNA was extracted from each isolate by the Genomic DNA extraction Kit (Denazist Company, Iran). PCR amplification performed using 5′-GTCAGAGGTGAAATTCT TGGATTTA-3′ (F) and 5′-AGGGCAGGGACGTAATCAACG-3′ (R) primers for AW, universal primer pairs ITS (F)/ITS4 (R) for AR, and ITS5 (F)/ITS4 (R) for AB isolates [22]. The PCR reaction was carried out with a thermal program, which consisted of initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation step at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 45 s, followed by another 7 min extension at 72 °C. To ensure the specificity of the primers and to have an estimation of the basepair numbers of the PCR products, gel electrophoresis was utilized. The PCR products were sequenced at Macrogen Inc., Korea. Following the cleaning up and editing using BioEdit 7.2.1 software, the sequences were aligned with those from GenBank database using the BLAST program.

2.3. Analytical methods

The amount of biomass was determined by a spectrophotometric method in which the culture absorbance was measured at a wavelength of 680 nm. Cell dry weight was measured by centrifuging 500 ml of each sample at 4400 rpm for 15 min, draining the supernatant, and drying the algal paste in the oven at 45 °C overnight. Eventually, the dried samples were placed in a desiccator and weighed for determination of biomass density. Calibration curves were obtained for each isolates showing the relationship between the cell dry weight biomass density and optical density:

$$AW\ Dry\ weight\ (g\ l^{-1}) = 0.2750 \times OD_{680} \qquad R^2 = 0.9991 \tag{1}$$

$$AR\ Dry\ weight\ (g\ l^{-1}) = 0.2468 \times OD_{680} \qquad R^2 = 0.9963 \tag{2}$$

$$AB\ Dry\ weight\ (g\ l^{-1}) = 0.3194 \times OD_{680} \qquad R^2 = 0.9981 \tag{3}$$

Lipids were extracted in a chloroform-methanol-water system according to Bligh and Dyer [23]. Extracted lipids were transferred into pre-weighed aluminum weighing cups, evaporated in a water bath (45 °C) under a stream of nitrogen. The dried residue was placed under nitrogen and weighed.

Based on the fact that changes in cell size are often associated with the cell cycle and the stage of growth, therefore, all cell size measurements have been carried out by samples taken at the start of the stationary phase of each culture with 8 replicates in order to avoid systematic error. The corresponding growth curves are presented in Fig. 2 for the nine experiments carried out in the research. Live cell sizes, after light treatment, were measured with an Olympus BH-2 microscope coupled with an Olympus C-35AD-4 camera at 100 × 10 magnification.

2.4. Photobioreactor design

The photobioreactors were constructed with 3-mm Pyrex glass, internal diameter of 8.8 cm and nominal working volume of 1.0 l and covered by a cylindrical mirrored stainless steel shield to exclude external light sources. Three photobioreactors were used in the experiments. Each photobioreactor was illuminated by a single color LED of red (12v, 620–750 nm), blue (12v, 440–470 nm), or cool white (12v, 350–750 nm) with the same intensity of 85 μmol m^{-2} s^{-1}.

The gas flow into each photobioreactor was provided via 0.20 μm filter through silicone tubing and bubbled at the bottom of the photobioreactor using a stainless steel ring sparger (nine holes, each 0.8 mm diameter). The photobioreactor contents were stirred by two pitched five-blade turbine (pitch angle of 45°) impellers. One impeller was located 2 cm above the sparger ring and the other 3 cm under the culture surface. The schematic diagram of the photobioreactor is depicted in Fig. 1. Different parts of each photobioreactors were sterilized separately and then assembled in a laminar flow chamber before being used in each experiment.

2.5. Statistical analysis

The cell size of each microalgal species under the three different light qualities was analyzed by one-way ANOVA with a significance level of $P < 0.05$. The individual means were compared using the Tukey's method, in which the distribution of the studentized range statistic was employed to check whether two means were significantly different [24].

3. Results and discussion

3.1. Isolation and identification of microalgae

Microscopic analysis of the samples allowed preliminary identification of the isolates as genus *Chlorella*. The species were spherical and each had a solitary parietal, visible cell wall, cup-shaped chloroplast, and true nucleus. Due to the huge diversity of microalgae and the fact that most of the species of a certain genus (e.g., *Chlorella*) have analogous morphology and characteristics in the cell forms, molecular