Algal Research 33 (2018) 173–181

Contents lists available at ScienceDirect

Algal Research

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

Shaping colour changes in a biofilm-forming cyanobacterium by modifying the culture conditions

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ARTICLE INFO

Keywords:
Modelling
Response surface methods
Cyanobacteria
Light and nutrients

ABSTRACT

Cyanobacteria, pioneering microorganisms that create a conditioning layer on substrates and act as a source of nutrients for successive colonization by heterotrophic microbes, are commonly studied in relation to the microbial ecology of stones in indoor and outdoor habitats. They are well known for producing greenish-yellow, bluish-green or occasionally pink or pinkish-orange discolouration of surfaces. Such discolouration may be desirable for integrating new elements in landscapes and could be achieved by inoculating surfaces with cyanobacteria of a specific colour. In the present study, with the aim of producing cultures of perceptibly different hues, we modelled the colourimetric response of the biofilm-forming cyanobacterium *Nostoc* sp. PCC 9104 to variations in light intensity (L) and the concentrations of phosphorus (P) and nitrogen (N). The model obtained, which was validated from both mathematical and perceptual perspectives, enables production of cultures of a particular hue, within a range of 18° and with an efficacy of 92%. Coloured cultures of hue between 129° and 147°, corresponding to yellowish-green to bluish-green tones, were obtained by modifying nutrients inputs and the amount of light, without the need to resort to genetic manipulation.

1. Introduction

Cyanobacteria are ecologically and economically important in environmental research and also in bioindustrial processes and the field of material conservation [1]. Cyanobacteria have simple metabolic requirements, tolerate marginal environments and undergo rapid genetic changes. They are easy to handle in the laboratory and large amounts of biomass can be produced at low cost. Because of their considerable metabolic versatility and flexibility they have been engineered to produce valuable bioindustrial compounds, such as polysaccharides [2] and hydrocarbons [3], as well to generate hydrogen [4] and treat polluted water [5]. Environmental applications in which cyanobacteria are used to remediate water and soils have also been widely investigated. For example, cyanobacteria are used to remove excess fluoride from industrially polluted water [6], to trigger land rehabilitation in arid and semiarid environments [7], and to remove nitrogen from aquatic ecosystems [8].

Cyanobacteria, along with other phototrophic and heterotrophic microorganisms, play an important role in the formation of subaerial biofilms (SAB) on solid surfaces [9]. Biofilm formation can be induced on the surface of new elements in landscapes to mitigate the corresponding visual impacts. This is done in open cast quartz mining, in which the bright white colour of the mine walls has a strong visual impact against a background of wooded areas. Mining companies are required by law to restore the appearance of any landscape affected by opencast mining, and assessment of the visual impact considers two aspects: (i) the area occupied by the quarry as observed from a specific location, and (ii) the chromatic contrast between the landscape and the mining exploitation [10]. Traditional revegetation techniques are unsuccessful on quartz surfaces because of the resistance of quartz to weathering, and the surrounding areas thus become strongly eroded. Our research group has carried out studies aimed at evaluating the induction of epilithic photoautotrophic biofilms on quartz surfaces as an alternative remediation technique [11–13]. Successful results, in terms of biofilm development and reduction on the visual impact, were obtained in laboratory tests with quartz samples. Biofilm formation was induced on quartz despite the low porosity, smooth surface and purity of the material, which lacks elements considered nutrients. Moreover, the biofilm caused a substantial change in the colour of the surface of the quartz samples, turning them greenish-yellow or reddish-yellow.

The colour of organisms is determined by the type and abundance of biological pigments that they possess, i.e. chlorophyll *a*, carotenoids and phycobiliproteins, which in turn vary with environmental factors such as nutrient availability, light, temperature, ultraviolet radiation

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https://doi.org/10.1016/j.algal.2018.05.010
Received 27 December 2017; Received in revised form 16 April 2018; Accepted 12 May 2018

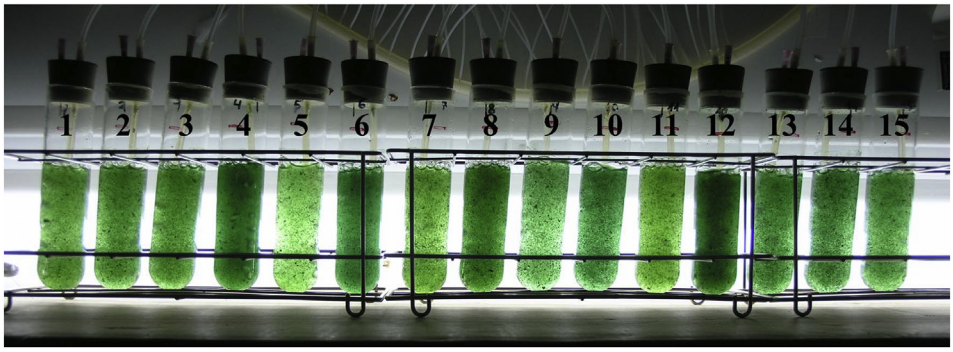

Fig. 1. Appearance of the photobioreactors cultured for 14 days under the conditions considered in the optimization experiment (Box-Behnken design). The numbers correspond to the different assays.

and pH. Numerous studies have demonstrated the relationship between the colour of cyanobacteria and the surrounding environment [14–17]. Our research group was the first to report the objective quantification of the colour of cyanobacteria [18] and to later develop a standardized protocol for defining the colour [19]. Use of the protocol enabled our group to establish relationships between i) the colour of cyanobacteria and the pigment content and ii) the changes in both colour and pigment contents and environmental variable including adverse conditions such as those resulting from the application of biocidal products [16,20,21]. Moreover, the close relationship between the pigment content and the colour of cultures was considered in developing linear equations to estimate the pigment contents (chlorophylls and carotenoids) in the cyanobacterium *Nostoc* sp. PCC 9104 via measurement of colour parameters [17].

The objective of the present study was to produce cultures of cyanobacterial cells of specific hues, without the need to resort to genetic manipulation, with the overall aim of using the cultures to mitigate the chromatic contrast of new elements in landscapes. With this aim, we analysed the effects of light intensity and nutrient concentration on the colour of the biofilm-forming cyanobacterium *Nostoc* sp. PCC 9104 and used response surface methods to produce a model that could be used to control the colour in the colourimetric range of the organism. We designed screening experiments to determine which the parameters initially considered (light intensity and concentrations of N, P, S, C and Fe) had the greatest influence on the colour of the cultures. We then designed an optimization experiment to generate a model relating the colour and the culture conditions. The model was validated using experimental data, and a psychophysical study was also carried out to validate the model from a visual perspective.

2. Material and methods

2.1. Batch experiments with cyanobacterial cultures

The experimental work was carried out with *Nostoc* sp. PCC 9104, an aerobic, terrestrial, mesophilic, filamentous N_2-fixing heterocyst-forming cyanobacterium. The growth, pigment content and colour of this strain have already been characterized by our research group [16–18]. Thus, regarding the pigment contents, chlorophyll *a* contents of between 10.76 and 72.63 $mg\,l^{-1}$, total carotenoids contents of between 1.20 and 4.36 $mg\,l^{-1}$ and phycocyanin contents of between $0.3{\cdot}10^{-2}$ and $6.7{\cdot}10^{-2}\,mg\,l^{-1}$ were determined in 14 day-old cultures subjected to different growth conditions in terms of nutrients and light intensity [16]. The colour data obtained in the same study also indicated the area of CIELAB space in which this strain defines its colour: from 41.47 to 86.34 for L*, from −23.92 to −2.50 for a*, from 1.30 to 23.57 for b*, from 2.94 to 41.00 for C^*_{ab} and from 113.60 to 158.80 for h_{ab} [16]. The close relationship between the pigment contents and colour of this cyanobacterium has also been reported [16,17].

Axenic cultures of *Nostoc* sp. PCC 9104 were grown in N-free $BG11_0$ liquid medium [22] in a climatic chamber (SCLAB PGA-1228/2 HR) under stationary conditions at 20 °C and with a 12 h light/dark photoperiod (100 μmol photon $m^2\,s^{-1}$). Cells in the exponential growth phase were collected and used as the inoculum in the experiments.

2.2. Experimental procedure for modelling the colourimetric response of cyanobacterial cells under different culture conditions

The response surface methodology [23] was used to model the colourimetric response of the cyanobacterial cells to different culture conditions.

Initial screening was carried out with the aim of identifying the most important factors influencing the colour of the cyanobacterial cells. An optimization experiment was then carried out to generate a model relating the colour of the cyanobacteria and the culture conditions. The experimental procedure was carried out with aerated batch cultures (Fig. 1), each containing 60 mL of various different culture media and 20 mL of the *Nostoc* sp. PCC 9104 stock culture. The culture medium consisted of modified $BG\text{-}11_0$ medium (containing 20 mM HEPES buffer), to which different amounts of nutrients were added in each tube (see Table 1). The batch cultures were maintained with aeration for 14 days under different light intensities (see Table 1) and a 12 h:12 h light:dark photoperiod. Lighting was provided by fluorescent lamps (Mazda Fluor Lumiere du Jour C9 TF 65, 85 W), and the light intensity was measured with a radiometer (DHD 2302.0, HERTER). Water loss by evaporation was corrected daily by the addition of sterilized distilled water.

a) Initial screening

An incomplete factorial design was used to analyse the influence of environmental conditions on the colour of the cyanobacterial cultures [24]. The design enabled the number of experiments required to estimate the effect of each considered factor on the response (in this case the cyanobacterial colour) to be minimized. The design included 6 factors, each with 2 levels, and a total of 8 assays were carried out (with 2 independent replicates of each assay).

On the basis of previous findings on the relationship between environmental conditions and colour changes in cyanobacteria [14,15], including our own findings [16,17], the following factors were selected for study: light intensity (L) and the concentrations of the various nutrients in the culture medium: $NaNO_3$ (N), K_2HPO_4 (P), $MgSO_4$ (S), Na_2CO_3 (C) and $(NH_4)_5[Fe(C_6H_4O_7)_2]$ (Fe). The different intensities/concentrations of each factor are shown in Table 1. The ranges are also based on the findings of the aforementioned studies.

The cyanobacterial biomass and colour were determined after 14 days. The optical density of each culture was measured at 750 nm (OD_{750}) in a spectrophotometer (UV–Vis Varian Cary 100) and used to