Abstract

Marine cyanobacteria could be promising feedstocks for bioethanol production due to their high photosynthetic efficiency, lignin-free nature and ability to reduce water footprint. The current study, therefore, explored the potential of fifteen marine cyanobacterial species, where *Leptolyngbya valderiana* BDU 41001, a non-nitrogen fixing, filamentous cyanobacterium, exhibited higher biomass and carbohydrate accumulation under laboratory as well as semi-outdoor conditions, demonstrating its suitability for further investigation.

To improve the innate carbohydrate pool of *L. valderiana*, a two-stage cultivation strategy was employed, combining the standardised physiochemical parameters (90 µmol photon m^{-2} s^{-1} PAR, initial culture pH 8.5, 35℃ temperature and mixing) at the first stage for biomass production followed by nitrate starvation at the second stage, resulted in augmentation of carbohydrate pool up to 86% than control. Prior to fermentation, an effective pretreatment process of biomass was applied, involving dilute sulphuric acid and Lewis acid coupled with autoclaving, achieving 88% hydrolysis yield of total carbohydrate. Subsequently, a comparative evaluation revealed that, unlike the single-use free yeast cells, immobilised cells could be used for three consecutive cycles without impeding bioethanol yield with a reduced time scale.

In order to adhere to the principles of green chemistry, a novel dark-static incubation protocol was developed for C-phycocyanin (C-PC) extraction from *L. valderiana* biomass with 0.8 purity, which was further enhanced to 2.8 by the combined applications of ammonium sulfate precipitation and dialysis. This partially-purified C-PC also showed significant antioxidant activity.

Eventually, the current investigation was extended to semi-outdoor condition, where a predictive growth model was developed with the help of Principal Component Analysis and Multiple Linear Regression with selected predictors, showing a high predictive R^2 of 0.97 for *L. valderiana*. Notably, 92-94% prediction efficiency was also achieved for biomass prediction of three other marine species, highlighting the potential of the developed model. Moreover, a 10% rise in C-PC along with an equivalent bioethanol production under the semi-outdoor cultivation unknot the potential of *Leptolyngbya valderiana* BDU 41001 as a model cyanobacterium for multifaceted pilot-scale investigations within the biorefinery frame-work.

Keywords: Bioethanol; Carbohydrate augmentation; C-phycocyanin; *Leptolyngbya valderiana* BDU 41001; Marine cyanobacteria; Predictive growth model; Semi-outdoor cultivation.