Electronic Supplementary Material (ESI) for Environmental Science: Water Research & Technology.

Photosynthetic bioconversion of hydroponics effluent into biochemical-rich biomass for microalgal biorefinery

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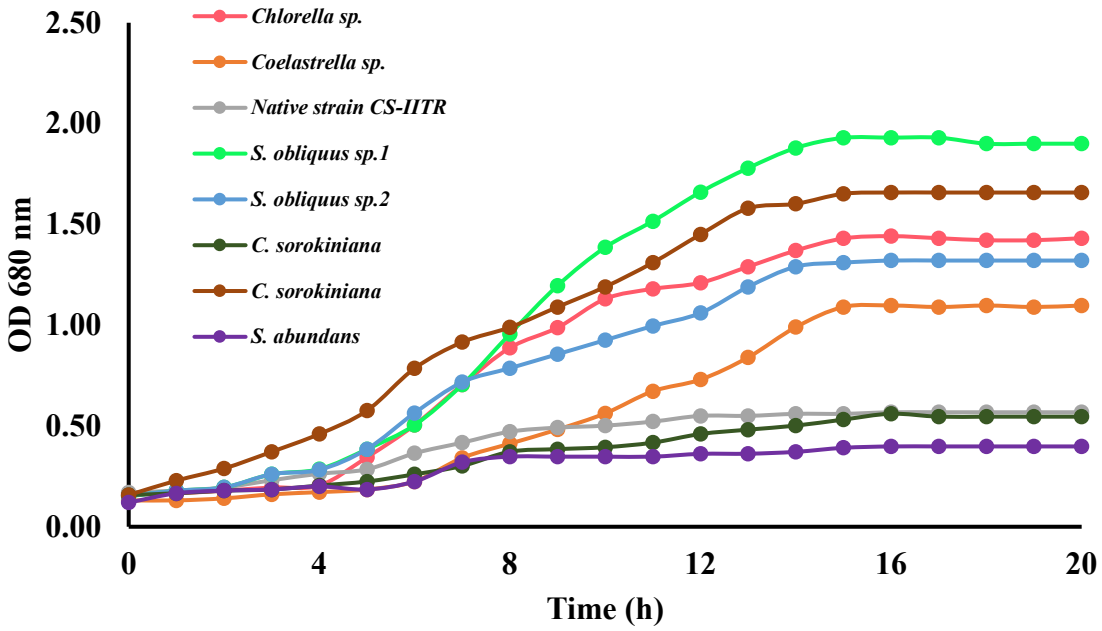

Figure S1. Daily Growth potential (OD_{680}) of tested microalgae cultivated in hydroponics effluent under controlled conditions.

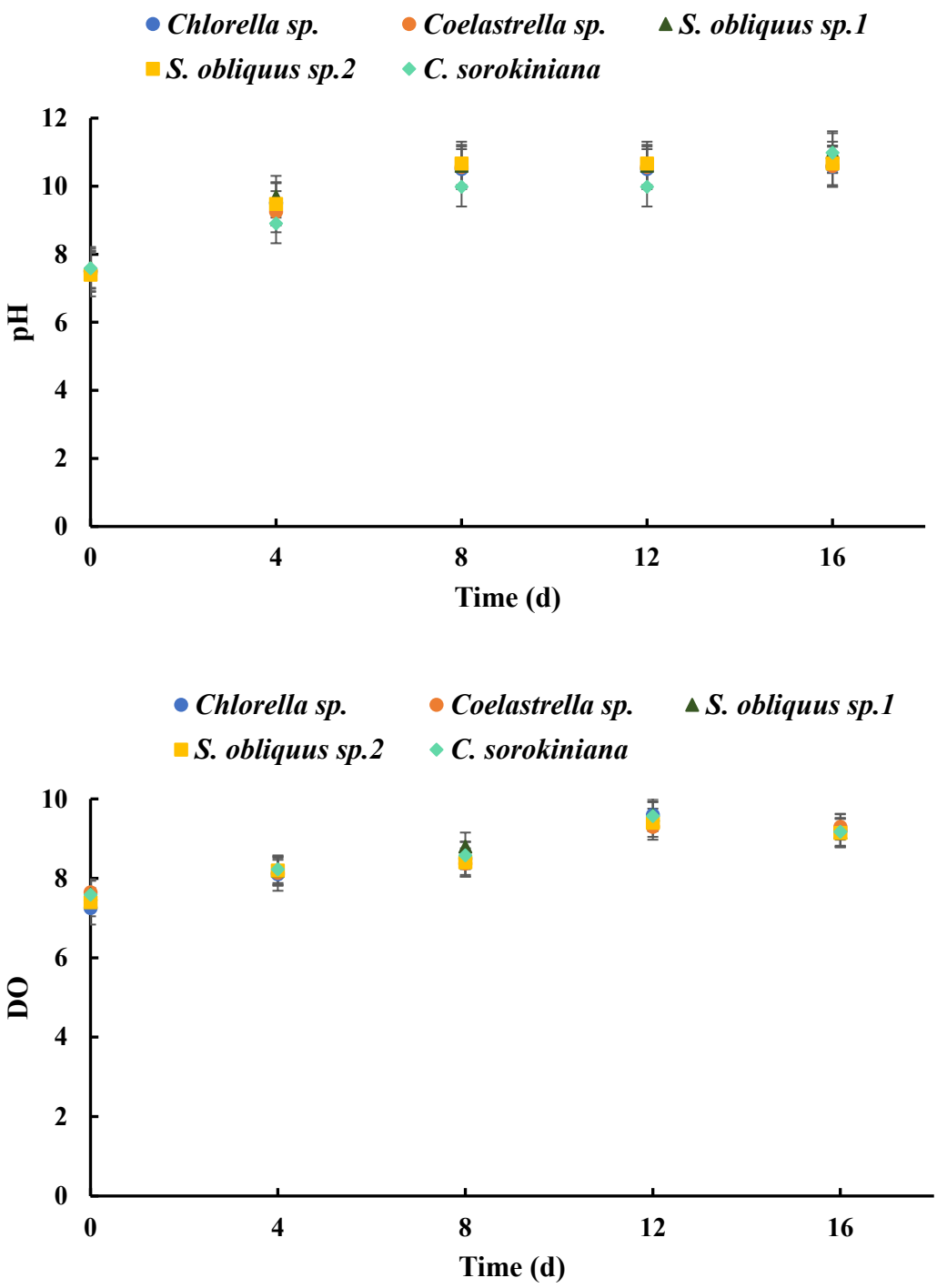

Figure S2. Variation in (a) pH and (b) dissolved oxygen concentrations of HE during the period of microalgal cultivation.

Biomass concentration

The Bx, (B_x) of each microalgae was estimated using the calibration equations (1), (2), (3), (4), and (5).

For *C. sorokiniana*

$$B_x\ (g\ L^{-1}) = 0.83 * OD_{680} - 0.125\ (R^2 = 0.997) \quad (1)$$

For *S. obliquus* sp.1

$$B_x\ (g\ L^{-1}) = 0.8 * OD_{680} - 0.12\ (R^2 = 0.996) \quad (2)$$

For *S.obliquus* sp.2

$$B_x\ (g\ L^{-1}) = 0.5 * OD_{680} - 0.04\ (R^2 = 0.991) \quad (3)$$

For *Chlorella* sp.

$$B_x\ (g\ L^{-1}) = 0.925 * OD_{680} - 0.025\ (R^2 = 0.995) \quad (4)$$

For *Coelastrella* sp.

$$B_x\ (g\ L^{-1}) = 0.775 * OD_{680} - 0.025\ (R^2 = 0.992) \quad (5)$$

Where, OD_{680} is the absorbance at 680nm.

Carbohydrates and Lipid analyses of microalgal biomass

For carbohydrates, 5 mg of dried biomass was hydrolyzed with 5 mL of 2.5 N HCl at 100°C for 3 h using preheated DRB200 reactor. Thereafter, the hydrolyzed biomass slurry was cooled to room temperature and neutralized with sodium carbonate. Afterward, 2 mL of supernatant from neutralised hydrolyzed slurry was withdrawn in a clean glass vial and diluted with 0.5 mL using HQ-pure water. The diluted sample was then mixed with 0.5 mL of 4 % phenol solution followed by 2.5 mL of concentrated sulphuric acid and further incubated for 10 min at room temperature. Further, the absorbance of the sample was recorded at 490 nm using a

microplate reader (Synergy HTX Multi-Mode Microplate Reader, BioTek India, Mumbai). The absolute concentration of carbohydrates was determined by using developed calibration equation (13) obtained from D-glucose standard curve.

$$Glucose\ concentration\ (mg.L^{-1}) = \frac{OD_{490} + 0.0186}{0.0303} \qquad (R^2 = 0.99) \tag{6}$$

Where, OD_{490} is the absorbance at 490 nm.

For lipid extraction, 20 mg of dried biomass was added to 10 mL of deionised water to obtain a final concentration of 2.0 g L^{-1}. Afterward, the microalgal slurry was subjected to microwave pre-treatment under the irradiation of 700 W for 5 min [28]. The pre-treated biomass was then mixed with chloroform and methanol (1:2) and continuously mixed for 1 hour using an orbital shaker at 200 RPM. Subsequently, 5 mL of deionised water was added to the mixed solution, and the suspension was kept static until the organic phase separated. The lipid-containing organic phase was then carefully withdrawn into a clean falcon tube, and the amount of lipid extracted was recorded (V). Besides, the known volume (v) of the lipid-containing organic layer was placed in pre-weighted foil cups, and then oven dried for 1 h at 105 ° C. Lastly, the weight of dried foil cups was noted, and the absolute amount of lipid was quantified using equation (14).

$$Lipid\ (mg) = \frac{(w_f - w_i)}{v} \times V \tag{7}$$

Where, w_f and w_i is the final, and initial weight of the foil cups (mg), respectively; V and v are the total volume of extracted lipid, and the aliquot volume, respectively.