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A strategy for urban outdoor production of high-concentration algal biomass for green biorefining

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HIGHLIGHTS

- C. sorokiniana's adaptability in 12-h interchanging trophic conditions was studied.
- Cell productivity and C-N-P removals under various conditions were determined.
- Microalgae flocculation using chitosan is dependent on chitosan: cell (w/w) ratio.
- Achieved reproducible >99% flocculation and >20% solids algae broth.

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ABSTRACT

The present study was to investigate the feasibility of carrying out effective microalgae cultivation and high-rate tertiary wastewater treatment simultaneously in a vertical sequencing batch photobioreactor with small areal footprint, suitable for sustainable urban microalgae production. For 15 consecutive days, *Chlorella sorokiniana* was cultivated in synthetic wastewater under various trophic conditions. A cycle of 12-h heterotrophic: 12-h mixotrophic condition produced 0.98 g l^{-1} d^{-1} of algal biomass in tandem with a 94.7% removal of 254.4 mg l^{-1} C-acetate, a 100% removal of 84.7 mg l^{-1} N-NH_4 and a removal of 15.0 mg l^{-1} P-PO_4. The cells were harvested via cost-effective chitosan flocculation with multiple dosing (3 times) applying established chitosan:cell ratio (1:300 w/w) and pH control (6.3–6.8). Reproducible flocculation efficiencies of greater than 99% and high-concentration algal broths (>20% solids) were achieved.

1. Introduction

Biorefining feedstock ranges from forest raw materials, whole crops to green grasses and algae. Most of these could not be abundantly available or cultivated in cities due to urbanisation, but with one exception. Microalgae cultivation does not require arable land. Studies on photoinhibition (Ogbonna and Tanaka, 2000) and truncation of chlorophyll antenna (Polle et al., 2002) suggested that microalgae do not require the high sunlight intensity nor long duration of exposure to the sun to maximise productivity. Therefore, microalgae cultivation could potentially be scaled up vertically to minimise areal footprint. While the achievable photosynthetic algal productivity was well debated (Waltz, 2009), mixotrophic microalgae species can utilise organic carbon streams for growth and are therefore capable of productivity surpassing that of purely photoautotrophic cultures, which is bound by maximum theoretical photosynthetic efficiency. According to the Food and Agriculture Organisation of the United Nations, about 1.3 billion ton per year or a third of edible parts of food produced was wasted and the extent of wastage is much greater in urban cities. This vast amount of urban nutrient-rich wastes could provide not just cheap sources of organic carbon, but also a steady stream of nitrogen and phosphorus. The supply of both was identified to be a very significant challenge for algae production scale-up (Pate et al., 2011).

There is a surging interest in Singapore to recover energy and resources from urban organic wastes, namely human faeces (brown water), human urine (yellow water) and food wastes, through source-separation and decentralised anaerobic digestion in urban communities (www.nrf.gov.sg). The effluent of anaerobically digested brown water with food waste (ADBF) could provide a steady

Abbreviations: CDW, cell dry weight; ADBF, anaerobically digested brown-water and food-waste; TOC, total organic carbon.

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source of cultivation medium for algae production. Anaerobes present in this effluent are unlikely to thrive in the aerobic algae culture, thereby lowering chances of bacterial contamination. Algae cultivation would proceed simultaneously with the tertiary treatment of the ADBF effluent, with the organic carbon, ammonium and phosphate assimilated by the microalgae cells for accelerated growth. Such a coupling could potentially improve the economic feasibility of microalgal biomass production (Pittman et al., 2011).

However, there are challenges facing simultaneous algae cultivation with tertiary wastewater treatment. Tertiary treated wastewater (culture medium) has to be discharged after the cell harvesting process. The remaining suspended cell concentration needs to be kept low to prevent unwanted downstream algal blooms or related issues. At less than 1% solids content, centrifugation of the culture for cell harvesting is generally considered uneconomical at this stage (Sukenik et al., 1988). Immobilising the cells during cultivation is one alternative to retain them, but cell leakage from alginate beads was observed after only 5 days in use (Robinson et al., 1985). Even if leakage is prevented, the significant amounts of matrix-forming materials required and the immobilisation processes may be too costly for large-scale productions. It is also unclear how the algae cells could be extracted from the matrix and how the matrix could be reused in a simplified and scalable way.

Flocculation followed by gravitational settling provides an initial concentrating of the microalgal culture into a broth. Inorganic salt aluminium sulphate (alum) is the widely used coagulant especially in wastewater treatments primarily due to its low cost. However, the possible toxicity to human health and the environment of its application is still debatable. The use of organic polymer would have less impact for downstream green refining processes. Organic polymer chitosan is abundant, non-toxic, biodegradable, renewable, and is an effective coagulant-cum-flocculant due to its high cationic charge density and long polymer chains (Renault et al., 2009). Many works have shown that chitosan could be utilised for effective microalgae flocculation but the optimal dosages reported were wide-ranging (Divakaran and Sivasankara Pillai, 2002; Heasman et al., 2000; Sukenik et al., 1988) and their applicability for varying algal concentrations (common in microalgae production facilities) were not reported. Due to the high costs of chitosan, a more practical chitosan dosing method should be established.

In this study, *Chlorella sorokiniana* was selected due to its temperature tolerance (de-Bashan et al., 2008) and excellent mixotrophic capability (Lee et al., 1996). With exposure to the equatorial sun, temperature of closed outdoor cultures could reach as high as 40–50 °C without temperature controls (Geier et al., 2011). Feasibility of cultivating *C. sorokiniana* with synthetic wastewater (simulating ADBF effluent) using sequencing batch vertical photobioreactor was investigated. Algae productivity, removal rates of organic carbon, nitrogen, and phosphorus in different conditions (i.e., photoautotrophic, heterotrophic, and mixotrophic) under 12-h light phases/12-h dark phases (to mimic the tropical sunlight provision) were discussed in depth. Subsequently, coagulation/flocculation of varying concentrations of *C. sorokiniana* using chitosan, via dosing and pH control strategies was studied to minimise chitosan usage and maximise flocculation efficiency. It is anticipated that the overall findings could extend the understanding on cost-effective urban outdoor production of high-concentration algal biomass for green biorefining.

2. Methods

2.1. Microalga and inoculum preparation

The green alga *C. sorokiniana* 211/8k used in this study was obtained from the Culture Centre of Algae and Protozoa, Cambridge. The alga inoculum was cultivated in 8 glass bottles (250 ml) plugged with culture plugs (ShinEtsu Bio-Silico N42). The cultures experienced magnetic stirring at 100 rpm, continuous illumination from white fluorescent tubes, temperature at 27 ± 2 °C, and were supplied with 5% CO_2 in air at a rate of 10 ml min^{-1}. The culture medium used was BG-11 (Stanier et al., 1971).

2.2. Synthetic wastewater

The synthetic wastewater contained modified BG11 medium and varying concentrations of Na-acetate and NH_4Cl (from 0–2000 mg l^{-1} and 0–200 mg l^{-1} respectively in each individual feed) to mimic the fluctuating total organic carbon and N-ammonium levels found in ADBF effluents. The modified BG11 medium was composed of the following (in mg l^{-1}): $K_2HPO_4{\cdot}3H_2O$ 250; $MgSO_4{\cdot}7H_2O$ 75; $CaCl_2$, $2H_2O$ 36; citric acid 6; Ferric ammonium citrate 6; EDTA 1; and 1 ml l^{-1} of trace elements stock. The trace elements stock was made up (in mg l^{-1}) of: H_3BO_3 2860; $MnCl_2{\cdot}4H_2O$ 1810; $ZnSO_4$, $7H_2O$ 222; $CuSO_4{\cdot}5H_2O$ 79; $NaMoO_4{\cdot}2H_2O$ 39; $Co(NO_3)_2{\cdot}6H_2O$ 49.

2.3. Culture system

The sequencing batch photobioreactor was a vertical cylindrical glass vessel with 6 l of working volume, sterilised via autoclaving at 121 °C for 20 min prior to experimentation. Air enriched with 5% CO_2 was bubbled through the centre of the vessel at 600 ml min^{-1} (0.1 vvm) continuously, creating an airlift system that provided the only form of mixing. The temperature was maintained at 27 ± 2 °C. Continuous 12-h light/12-h dark cycles were imposed. Twelve LED light tubes were placed vertically and well-spaced around the cylindrical photobioreactor, providing 175.2 ± 11.1 μmol photons m^{-2} s^{-1} of photosynthetically available radiance (PAR) to light-facing surfaces and 80.4 ± 6.2 μmol photons m^{-2} s^{-1} to non-light-facing surfaces during the light phases. During the dark phases, PAR provision was negligible at 0.4 μmol photons m^{-2} s^{-1}. PAR was measured with Li-192SA underwater quantum sensor attached to Li-250A light metre (Li-COR, USA). The average light intensities were determined from respective measurements of 40 selected points on the inner surface of the photobioreactor.

2.4. Cultivation

The inoculum prepared in Section 2.1 was added into the photobioreactor and mixed with synthetic wastewater to begin a 3-d acclimatisation, allowing the cells to adapt especially to the light/dark cycles and the new medium with organic carbon and ammonium. A 15-d experiment followed after the acclimatisation. One 10 ml sample was extracted each at the 1–l, 3–l and 5–l level outlets of the reactor at the end of each 12-h phase. A fresh feed consisted of a withdrawal of culture from the photobioreactor (ranging from 2.0 to 4.0 l) and its replacement of equivalent volume of fresh synthetic wastewater. Varying withdrawal volumes were required to adjust the concentrations of the cells, organic carbon and ammonium for the next-in-line test cycle. A full cycle started with a fresh feed, and consisted sequentially of either light-dark phases or dark-light phases. A total of 15 light phases, 15 dark phases and 13 full cycles were evaluated in this study. In this study, the trophic condition in a phase of 12 h was considered photoautotrophic when C-acetate concentration was less than 50 mg l^{-1} at the start of any light phase, mixotrophic when C-acetate concentration was greater than 50 mg l^{-1} at the start of any light phase, and heterotrophic when C-acetate concentration was greater than 50 mg l^{-1} at the start of any dark phase. Biomass losses were also observed during dark phases without organic carbon sources. The pH of