Utilizing anaerobic digestion byproducts for sustainable production of microalgae-based biosorbents: a strategy for environmental remediation and resource recovery

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Introduction

Exopolysaccharide-producing microalgae and cyanobacteria, here generally referred to as “microalgae”, represent potential biosorbents for environmental contaminants. In particular, the presence of uronic acids and negatively charged groups (e.g. SO_4^{3-}, PO_4^{3-}) in exopolysaccharides (EPS), including capsular (CPS) and released (RPS) exopolysaccharides, promotes the binding to positively charged ions, including heavy metals (Ciani and Adessi, 2023). Since biosorption is a passive process, spent biomasses can be adopted and pre-treated to maximize the removal and may undergo several adsorption-desorption cycles, increasing their shelf-life (Ramírez Calderón et al., 2020). Nevertheless, microalgae production is often expensive ranging from some to hundreds of euros per kilogram of biomass, depending on the cultivation conditions (Tredici et al., 2016; Valdovinos-García et al., 2021). In order to improve the economic and environmental feasibility of the process of pollutant adsorption, microalgae-based biosorbent can be produced adopting wastewater as nutrients source and supplying biogas as a carbon source. In this way, other outcomes can be reached: nutrients can be removed and recovered from wastes, while the biogas can be upgraded to biomethane, sustaining sustainability challenges (García et al., 2017). Thus, this study aims to use byproducts of anaerobic digestion, i.e., centrate (the liquid fraction of digested sludge) and biogas, for the production of microalgae-based biosorbents.

Materials and Methods

Organisms and standard growth conditions

Six cyanobacteria and two microalgae belonging to Cyanobacteria and Microalgae Collection of the University of Florence (Italy) were selected for their known ability to adsorb heavy metals, produce EPS, and/or grow in wastewater. The cultures were kept in 250-mL Erlenmeyer flasks containing standard growth media (BG11, $BG11_0$, and seawater enriched medium) inside a rotatory shaker (Innova 44B, New Brunswick, USA) at a constant temperature of 30°C, light intensity of 30 μmol photons m^{-2} s^{-1}, and stirring speed of 100 rpm.

Experimental set-up

First, a pre-screening was carried out to select the strains able to remove Cu(II). To this aim 1 mg of biomass per replicate of each strain was pre-treated with HCl 0.05M. The acidic solution was replaced by a Cu(II) solution 20 mg L^{-1}. The samples were kept in continuous agitation (150 rpm) and after 16 hours residual Cu concentration in the solution was quantified by an adaptation of the bicinchoninate method (kit HI 93702, Hanna Instruments Srl, Italy). Then, the strains able to remove Cu(II) were cultivated adopting centrate (730 mg L^{-1} N-NH_4^+, 138 mg L^{-1} P-PO_4^{3-}, 650 mg L^{-1} inorganic carbon) and synthetic biogas composed of CO_2 (30 %) and CH_4 (70 %) (Carburos Metalicos; Spain). Batch assays were performed in 0.2 L glass bottles filled with 50 mL of medium composed by standard growth medium (control), raw centrate or centrate diluted (1:2, 1:5, 1:10, 1:20, v:v) with standard growth medium and 150 mL of biogas headspace and inoculated with microalgae to reach a final optical density of 0.3 OD_{680}. Then, the bottles were incubated at 35°C under continuous magnetic stirring (300 rpm) and illumination (150 μmol photons m^{-2} s^{-1}) provided by LED lights, evaluating optical density at 680 nm to assess the growth.

Results and discussion

The higher Cu uptake (up to 0.19 mmol Cu g^{-1} DW, Fig. 1a) was shown by three cyanobacteria: *Cyanothece* sp. CE4, *Dactylococcopsis salina* 16Som2 and *Nostoc* sp. PCC 8109. These cyanobacteria are known to produce high quantity of CPS and RPS (De Philippis et al., 2000, 1998). Within the two microalgae *Parachlorella* sp. N9 showed the higher Cu uptake (Fig. 1a). These strains were selected for the cultivation adopting centrate and biogas as nutrient sources. *Parachlorella* sp. N9 resulted the most performant strain in growing adopting centrate and biogas as nutrients and carbon source even with 1:2 centrate dilutions (Fig. 1b). No difference was observed adopting different centrate dilutions, while the growth was inhibited with raw centrate, probably due to the high N-NH_4^+ concentration. The microalga has been isolated from a harsh environment in a salty lake in Algerian desert, characterized by high irradiance, temperature, and salinity, and it has already shown metal removal capability (Guehaz et. al., unpublished). The cells are surrounded by a thick capsule (Fig. 1c) that may represent a protection against abiotic and biotic stresses, facilitating the growth in adverse conditions.

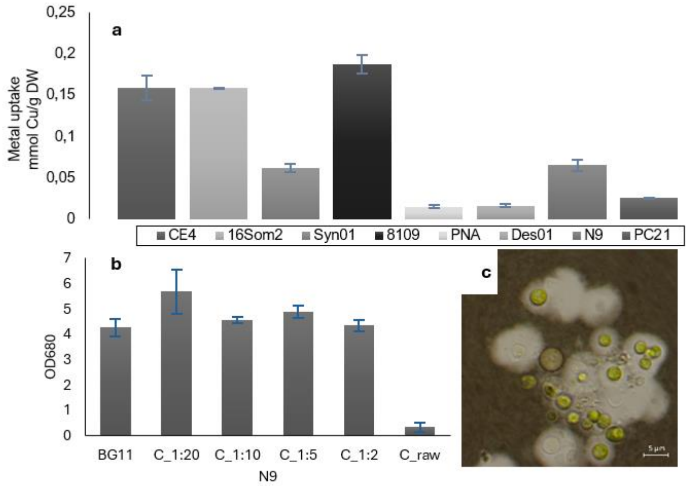

Fig. 1. Metal uptake of different cyanobacteria and microalge, expressed as mmol Cu per gram of dry weight (a). *Parachlorella* sp. N9 optical density at 680 nm after 4 days of growth adopting BG11 medium, centrate diluted with BG11 medium, raw centrate and supplying biogas as carbon source (b). Micrographs (100x) of *Parachlorella* sp. N9 cells stained with China Ink (c).

Parachlorella sp. N9 has been selected for the cultivation in 1.2L glass bottles to define the conditions to get a EPS-rich biomass with high metal-removal capability, by evaluating biomass growth, cellular and soluble carbohydrate productivity, nutrients consumption, biogas upgrading and metal uptake.

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