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Urban wastewater treatment by *Tetraselmis* sp. CTP4 (Chlorophyta)

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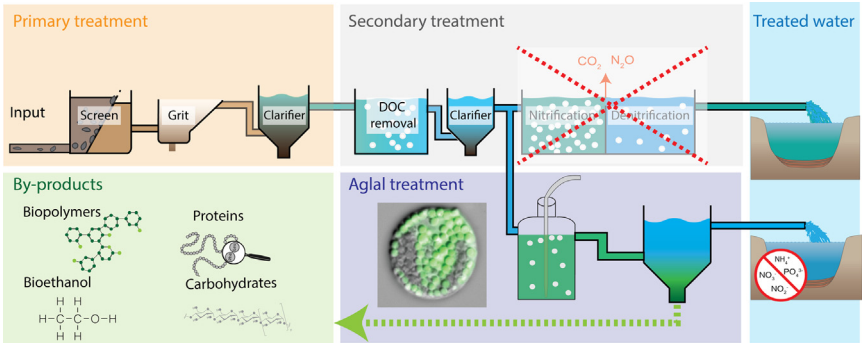
HIGHLIGHTS

- *Tetraselmis* sp. CTP4 is suitable for urban wastewater treatment.
- Wastewater N and P were 100% removed in batch and continuous conditions.
- Produced biomass displayed high protein and carbohydrate contents.
- Aging cultures showed decreased protein and increased carbohydrate contents.
- Cells grown in low saline media had low PUFA but high EPA content.

GRAPHICAL ABSTRACT



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ABSTRACT

The ability of a recent isolate, *Tetraselmis* sp. CTP4, for nutrient removal from sewage effluents before and after the nitrification process under batch and continuous cultivation was studied. Biomass productivities in both wastewaters were similar under continuous conditions (0.343 ± 0.053 g L^{-1} d^{-1}) and nutrient uptake rates were maximal 31.4 ± 0.4 mg N L^{-1} d^{-1} and 6.66 ± 1.57 mg P-PO_4^{3-} L^{-1} d^{-1} in WW before nitrification when cultivated in batch. Among batch treatments, cellular protein, carbohydrate and lipid levels shifted with aging cultures from 71.7 ± 6.3 to 29.2 ± 1.2%, 17.4 ± 7.2 to 57.2 ± 3.9% and 10.9 ± 1.7 to 13.7 ± 4.7%, respectively. In contrast, CTP4 cultivated continuously in Algal medium (control) showed lower biomass productivities (0.282 g VSS L^{-1} d^{-1}) although improved lipid content (up to 20% lipids) in batch cultivation. Overall, *Tetraselmis* sp. CTP4 is promising for WW treatment as a replacement of the costly nitrification process, fixating more nutrients and providing a protein and carbohydrate-rich biomass as by-product.

1. Introduction

Combining wastewater (WW) treatment with microalgal production has already been researched since 1950s (Oswald et al., 1957) and has received increasing interest in science and industry over the last decades. Currently, microalgal based WW treatment is considered to be an economically and environmentally sustainable procedure to remove dissolved nutrients from effluents and to produce valuable biomolecules to offset water treatment costs. Particularly, the usage of high rate algal ponds (HRAPs) fed by wastewater and/or CO_2 exhausts is considered as the most promising strategy to clean waste water streams and produce a feedstock for microalgal based by-products such as biofuels (Craggs et al., 2014; Park et al., 2011). Although the main focus of using microalgae has been the removal of nutrients (Christenson and Sims,

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2011), the reintroduction of nutrients into the market as transformed bio-products would follow the circular economy principle, a requirement to sustain the present life standards in industrial nations (European commission 2015, IP/15/620). In Europe, on average, 0.51 kg P and 2.52 kg N per inhabitant and year are discharged in WW (EU-EEA, 2015). These nutrients are valuable and/or finite resources that can substitute expensive fertilizers for production of crops and algae (Vaccari, 2009). Nitrogen and phosphorus are currently removed through treatments involving biological nitrification followed by denitrification or precipitation (US-EPA, 2013). The nitrification step receives ammonia-rich WW from a biological oxygen demand (BOD) removal step. Ammonia is transformed into nitrate by nitrifying bacteria under an oxygen-rich environment. Subsequently, the nitrate-rich effluent enters the denitrification step where denitrifying bacteria transform nitrate into molecular nitrogen under anoxic conditions, which is stripped out as gas by gentle aeration. However, denitrifying bacteria require external carbon sources that are often of fossil origin (e.g. methanol). WWs with high phosphorus concentrations must be treated through a phosphorus removal step before discharge into protected areas. Here, effluents coming from the denitrification step are supplemented with flocculants (e.g. aluminium salts, lime stone) allowing the precipitation of phosphorus as insoluble salts. However, these procedures present additional costs and can cause deterioration of the biomass quality as a feedstock of nutrients in a functional fertilizer. One reason for this is the contamination with toxic, metal-containing flocculants that remain bound to phosphorus (Christenson and Sims, 2011). Recent reports showed that different microalgal strains use these nutrients from urban, industrial and aquaculture effluents to produce biomolecules that can be further upgraded for the production of bioplastics, feed, or biodiesel, among others (Christenson and Sims, 2011; Zeller et al., 2013). Moreover, contrary to standard biological treatments, algae were found to further improve the final effluent quality through natural disinfection and incorporation of other contaminants, such as heavy metals, pharmaceuticals and endocrine disrupters (Correa-Reyes et al., 2007; Craggs et al., 2014; Devi et al., 2012). The separation of microalgae cells from the treated water, however, remains a major bottleneck for the large-scale implementation of microalgal-based bioremediation facilities, as current technologies (e.g. centrifugation and flocculation) for biomass recovery have high costs in terms of energy and/or chemicals (Christenson and Sims, 2011).

The present work aimed to treat urban sewage water (before and after nitrification processes) in batch and continuous cultivation systems, using a novel, robust, euryhaline microalgal strain (*Tetraselmis* sp. CTP4) that naturally settles down to the bottom of the containing vessel, reducing significantly the dewatering costs (Pereira et al., 2016). The biochemical composition (e.g. total lipids, proteins, fatty acid profile), best culturing strategies and applications for the biomass to offset the costs of nutrient removal are also discussed.

2. Methods

2.1. Microalgal strain and growth conditions

Tetraselmis sp. CTP4 belongs to the *T. striata/convolutae* clade (Chlorophyta, Chlorodendrophyceae) and was previously isolated from the Ria Formosa lagoon, located in the south of Portugal (Algarve) near a WW stream using a high throughput method combining a pre-enrichment step for robust microalgae, fluorescence activated cell sorting and direct plating onto 96-well plates as described by Pereira et al. (2016).

Urban sewage effluent waters were collected at a WW treatment plant located in Quinta do Lago, Algarve, Portugal (WWTP Quinta do Lago) between May and July 2015. In this WWTP, urban WW goes through primary/secondary screens and grit chambers; a primary clarifier and aeration tank for removal of dissolved organic carbon (DOC) and finally undergoes a nitrification/denitrification step prior to sterilization and discharge. For this study, WW samples were collected before (Pre-N) and after (Post-N) the nitrification step, and CTP4 was cultivated in both effluents to allow a direct comparison. The effluents were not sterilized and were stored at 4 °C until use. As control, CTP4 was also grown in a previously established standard medium composed of sterile seawater from the Atlantic shoreline of Faro (Portugal, salinity of *ca* 35) enriched with Modified Algal Medium (MAM; 2 mM $NaNO_3$, 0.1 mM, KH_2PO_4, 20 μM EDTA-Na, 20 μM $FeCl_3$*6 H_2O, 2 μM $MgSO_4$*7H2O, 1 μM $ZnCl_2$, 1 μM $ZnSO_4$, 1 μM $MnCl_2$, 0.1 μM Na_2MoO_4, 0.1 μM $CoCl_2$, 0.1 μM $CuSO_4$ (Pereira et al., 2016).

All cultures were initially grown in 5L reactors filled up to a volume of 4L, until the stationary phase was reached. Experiments were conducted in a climate chamber (Aralab Fitoclima s 600 PL clima plus 400), in triplicates, at 20 °C and exposed to a light intensity of 100 μmol photons s^{-1} m^{-2} using continuous illumination (Osram cool white 840). Cultures were aerated with 0.2 μm-filtered air with a flow rate of ~1 L min^{-1}. For continuous cultivation conditions, cultures were grown in batch until the late exponential phase (biomass concentrations of ~0.7 g L^{-1}) with a subsequent shift to continuous growth. A hydraulic retention time (HRT) of 6.6 d^{-1} was set by controlling the influent flow rate. This HRT equalled $2 \times \mu^{-1}$, where μ is the average growth rate estimated during the batch growth among all treatments to obtain highest biomass productivity (Ruiz et al., 2013a).

2.2. Analytical methods

At different time points during the batch and continuous culturing period, aliquots of the cultures were harvested and centrifuged (5000*g*, t = 5 min) to separate algal biomass from the growth medium. The algal pellet was freeze dried and stored in a desiccator until analysis, whereas the supernatant was filtered (pore ∅ = 1.2 μm) and stored at −20 °C for the analyses of the dissolved nutrients.

2.2.1. Monitoring of algal growth

Cell growth was monitored by cell counting (Neubauer chamber), optical density (OD_{750}) and volatile suspended solids concentration (VSS), which is equivalent to ash free dry weight (AFDW). Algal suspension was filtered (pore ∅ = 0.47 μm) and VSS was determined using 0.5 M sodium bicarbonate as washing reagent and processed according to standard methods. Briefly, 10–30 mg biomass was dried at 105 °C for 24 h to obtain total suspended solids (TSS). Subsequently, the moisture free biomass was incinerated at 560 °C for 8 h and the ash content was determined. The VSS of the samples was calculated by subtracting the moisture weight and the TSS from the initial biomass weight. Upon plotting VSS and cell concentration (cells mL^{-1}) against the OD_{750} of different experiments and time points (n = 93), significant linear correlations were found and used to estimate VSS ($r \geqslant 0.97$, $p < 0.01$) and cellular concentrations ($r \geqslant 0.90$, $p < 0.01$) on a daily basis (Eqs.(1) and (2)):

$$VSS\ (g\ L^{-1}) = 1.991 * OD_{750} - 0.075 \quad (1)$$

$$Cell\ concentration\ (Cells\ mL^{-1}) = 3.57 * 10^6 * OD_{750} + 1.67 * 10^5 \quad (2)$$