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Subarctic microalgal strains treat wastewater and produce biomass at low temperature and short photoperiod

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ABSTRACT

In Northern countries, microalgal-based processes are challenging due to low light and temperature conditions during a significant part of the year. Three natural strains from Northern Sweden (*Chlorella vulgaris*, *Scenedesmus* sp., *Desmodesmus* sp.) and a collection strain (*Scenedesmus obliquus* UTEX 417) were cultured in municipal wastewater, comparing their performances, biomass composition and nutrients removal under continuous light at standard (25 °C) and low temperature (5 °C), short photoperiod (3 h light, 25 °C), or moderate winter conditions (6 h light, 15 °C). Only the natural strains grew at low temperature, highly consuming total nitrogen and phosphate (> 80% and > 70%, respectively) even during cold- and dark-stress. At reduced growth rates, *C. vulgaris* and *Scenedesmus* sp. produced similar amounts of biomass (> 1 g/l) as in standard conditions. *Scenedesmus* sp. and *Desmodesmus* sp. showed phenotypic plasticity and increased carbohydrate content. Short photoperiod strongly reduced growth rates, biomass and storage compounds and induced flocculation in *C. vulgaris*.

1. Introduction

Large volumes of wastewater are produced worldwide due to household activities, industrial processes, farming and agriculture. Being rich in nitrogen and phosphorous, i.e. the nutrients responsible for eutrophication of the aquatic ecosystems [1], wastewaters need to be treated before their discharge into fresh or marine water bodies. Microalgal-based wastewater treatment represents a low-cost alternative to traditional wastewater treatment systems, as these photosynthetic microorganisms have been shown to efficiently uptake not only nutrients, but also heavy metals and pharmaceuticals [2–4]. Additionally, this approach can be coupled to the generation of biomass and biofuels (e.g. biodiesel, bioethanol, and biogas) [5].

The growth rate of microalgae and their efficiency in biomass production and wastewater remediation is highly influenced by light and temperature conditions, especially when open systems (e.g. raceway ponds) are used [6]. Light is the main energy source in photochemical reactions; short day-length, prolonged low irradiance or even darkness generally reduce algal metabolism and cell division, possibly inducing a dormancy state [7]. Temperature influences macromolecular structures, energy absorption and consumption, as well as the functioning of photosynthetic and metabolic enzymes [7–9]. Cell division will drop by 50% when the temperature decreases by 10 °C below the optimal growth temperature [10].

At high latitudes (> 50°), light availability is limited in the autumn/winter season and temperatures are often below or close to zero, forcing severe constraints on microalgal growth. In Umeå, Northern Sweden (63°49′42 N, 20°15′34 E), the average temperature is approx. −3 °C in winter, with an average solar radiation of only 24 W/m^2 and a minimum day-length of 4.30 h (Umeå University, November–March years 2004–2015; http://www8.tfe.umu.se/weather-new/). The establishment of microalgal-based processes in Northern regions is therefore challenging. In addition, the algal culture is exposed to abiotic and biotic stress in the wastewater medium due to the presence of various toxic compounds (e.g. heavy metals, pharmaceuticals) and other organisms (e.g. bacteria, fungi, other microalgae or even grazers) competing with the inoculated strain. However, wild photosynthetic microorganisms are naturally adapted to their northern habitats, coping with the drastic changes in light quality and quantity and tolerate extreme cold temperatures for most of the year [7]. Those able to grow in wastewater therefore might be able to produce biomass and at the same time remove nutrients and pollutants even during autumn/winter.

In the present study, the potential for simultaneous biomass production and wastewater treatment during the winter season was

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compared between three natural, North-Swedish, microalgal strains and a culture collection strain. The long-term response of *Chlorella vulgaris* (13-1), *Scenedesmus* sp. (B2-2) and *Desmodesmus* sp. (RUC-2) [11] cultured in municipal wastewater was evaluated in laboratory scale with respect to short day-length and/or low temperature in order to determine their biomass concentration and composition, as well as their ability to remove nitrogen and phosphate during these unfavorable conditions. The performances of the three wild strains were compared to the culture collection strain *S. obliquus* (UTEX 417). Results from these batch-scale experiments are important for the application at the local algae pilot located in Umeå (Sweden) and aid future perspectives to prolong the wastewater treatment season in subarctic climate.

2. Materials and methods

2.1. Algal cultivation

Three microalgal strains were isolated from Umeå, Northern Sweden, and genetically identified as *Chlorella vulgaris* (strain 13-1, municipal wastewater), *Scenedesmus* sp. (strain B2-2, municipal wastewater) and *Desmodesmus* sp. (strain RUC-2, freshwater) [11]. The collection strain *Scenedesmus obliquus* (UTEX 417) had been inoculated to a pond in Bäckhammar (Southern Sweden) three years before recollection [11] and was used as a control. Microalgal cultures were maintained in BG11 [12] or BBM [13] media before cultivation in municipal wastewater.

Five ml of inoculum with an initial cell number of $3{\cdot}10^6$/ml were added to a final working volume of 80 ml. The algae were then allowed to grow at either standard conditions (25 °C, continuous light), moderate winter conditions (15 °C, 6:18 [L:D]), cold-stress (5 °C, continuous light) or dark-stress (25 °C, 3:21 [L:D]); light intensity was kept at 100 μmol m^{-2} s^{-1} PAR (photosynthetically active radiation). At continuous light, the algae were grown in a Multi-Cultivator MC 1000-OD (Photon Systems Instruments, Czech Republic, www.psi.cz/products/photobioreactors/multi-cultivator-mc-1000) equipped with eight independent autoclaved glass test-tubes and bubbled with air. To test the influence of the photoperiod, a floor standing incubated shaker AlgaeTron AG 230 (Photon Systems Instruments, Czech Republic, http://www.psi.cz/products/growth-chambers-and-incubated-shakers/algaetron-ag-230) was used, in order to prevent interferences from external light sources; 100 ml autoclaved Erlenmeyer flasks equipped with cotton plugs were used and shaken at 150 rpm. The photobioreactors were programmed accordingly to light intensity, photoperiod and temperature conditions.

2.2. Growth rates, biomass concentration and total lipids

Experiments were continued until the various cultures reached stationary growth phase (13 days at standard conditions; 40–45 days at moderate winter conditions; 20–30 days at cold-stress; 50–65 days at dark-stress). In the low temperature experiment, *Scenedesmus* sp. was intentionally cultivated for a longer time period (approx. 15 days in stationary phase) in order to evaluate the lipid accumulation under this conditions. All the experiments were performed with three biological replicates.

Cell density was measured at day 3, 5, 7, 10 and 13 of growth in standard conditions and with intervals of 2 to 6 days when grown in the other conditions, using a cell counter (Multisizer 3 Coulter Counter, Beckman, USA). Growth rates were calculated with the statistical software R (www.r-project.org) based on the logistic growth model equation. Cell morphology and size were studied regularly with an inverted microscope (Leica DMi1, Leica Microsystems, Germany) fitted with a phase contrast objective. Pictures were taken at 400× magnification (MC170 HD, Leica Microsystems, Germany).

Biomass concentration and total lipids were determined gravimetrically at the end of the experiments [11,14]. The analyses were performed in technical duplicates for each of the three biological replicates.

2.3. Quantum yield (Fv/Fm) and pigments analysis

The photosynthetic performances of the algal cultures were evaluated by regularly monitoring the maximum quantum yield of photosystem II (Φ or Fv/Fm); fluorescence of the cells was measured after 10 min of dark incubation using an AquaPen-C AP-C 100 (Photon Systems Instruments, Czech Republic). The analyses were performed in duplicates for each biological replicate.

After the final harvesting of each culture pigments were extracted in 100% methanol; 1 ml of fresh culture was centrifuged, ultrasound-treated for 10 min and incubated at 60 °C for 30 min. Pigments were determined spectrophotometrically (Cary 50 Bio, Varian, USA) according to the equations reported by Lichtenthaler and Buschmann [15]. The analyses were performed in technical triplicates for each biological replicate.

2.4. FTIR (Fourier transformed infrared) spectroscopy and PCA (principal component analysis)

Algal biomass was analysed by diffuse reflectance (DR) FTIR spectroscopy, using a Bruker IFS 66 v/S instrument under vacuum conditions (4 mbar) according to the protocol by Gorzsás and Sundberg [16]. Dried samples (5–10 mg) were mixed with potassium bromide (KBr, VWR International, infrared spectroscopy grade, approx. 390 mg) and manually ground in an agate mortar to obtain a uniform mixture. Spectra were recorded in the range of 400–4000 cm^{-1} at 4 cm^{-1} spectral resolution, using pure KBr as background. 128 scans were co-added to obtain a high signal to noise ratio.

Spectra were exported as MATLAB (version 2017a, MathWorks Inc., CA, USA) .mat files and processed using the open source MATLAB-based script provided by the Vibrational Spectroscopy Core Facility at Umeå University (Umeå, Sweden, http://www.kbc.umu.se/english/visp/download-visp/). Spectra were cut to 800–1900 cm^{-1} (fingerprint region), as this region was shown to contain most of the information relevant for biomass composition [17] and is the least sensitive to potential baseline correction and normalization errors. Baseline correction was performed using asymmetric least squares [18], with lambda = 10,000,000 and $p = 0.001$ values. A very mild smoothing was performed by Savitzky-Golay filtering [19], using a first order polynomial and a frame of 5. Total area normalization (after cut) to total protein content (by the intensity of the amide I peak at 1655 cm^{-1}) were used. Consequently, the determined lipid and carbohydrate contents are semi-quantitative, expressed either as proportion of the total, or relative to protein amounts, respectively. Lipid and carbohydrate contents were derived by band intensities at 1745 cm^{-1} (−C═O, lipids), 1655 cm^{-1} (amide I, proteins) and 1150 cm^{-1} (−C−O−C−, carbohydrates). The band assignments proposed by Duygu et al. [20] were used for interpretation.

For multivariate data analysis, total area normalised spectra (3 per condition) were imported to SIMCA-P (version 14, Umetrics AB, Sweden), centred and analysed by principal component analysis (PCA), using two principal components. Spectra from *S. obliquus* were omitted from PCA comparisons due to its failure to grow during cold-stress. The following models were obtained: *Chlorella vulgaris*: R2X(cum) = 0.982, Q2(cum) = 0.973; *Scenedesmus* sp.: R2X(cum) = 0.962, Q2(cum) = 0.956; *Desmodesmus* sp.: R2X(cum) = 0.974, Q2(cum) = 0.947.

2.5. Wastewater analyses

The microalgae were grown on municipal wastewater provided by the local wastewater treatment plant (Vakin AB, Umeå, Sweden). The

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