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Uptake of caprolactam and its influence on growth and oxygen production of *Desmodesmus quadricauda* algae☆

Jana Pexová Kalinová [a, *], Jan Tříska [b], Naděžda Vrchotová [b], Jan Novák [c]

[a] Faculty of Agriculture, University of South Bohemia, Studentská 13, 370 05 České Budějovice, Czech Republic
[b] Laboratory of Metabolomics and Isotopic Analyses, Global Change Research Centre, Academy of Sciences of the Czech Republic, Branišovská 31, České Budějovice 370 05, Czech Republic
[c] Institute of Complex Systems, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Faculty of Fisheries and Protection of Waters, University of South Bohemia in Ceske Budejovice, Zámek 136, 373 33 Nové Hrady, Czech Republic

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ABSTRACT

The consumption of polyamides produced from caprolactam is increasing continuously, and for that reason the danger of environmental contamination by this lactam is also rising. This study's aim was to evaluate the influence of caprolactam on the growth and oxygen production of the green alga *Desmodesmus quadricauda* and on caprolactam uptake by this alga. The presence of caprolactam in water was observed to cause the algae significantly to increase its oxygen production. Caprolactam concentration of 5,000 mg/L stopped algae growth after 6 days and influenced coenobia structure (seen as disappearance of pyrenoids, deformation of cells) but did not decrease the number of cells in the coenobia. Caprolactam uptake is probably passive but relatively rapid. Maximum concentration in the algae was reached after 18–24 h.

1. Introduction

Caprolactam (ε-caprolactam), a cyclic amide of caproic acid, is one of the raw materials most used in producing polyamides. It is utilized in manufacturing nylon 6 fibres (accounting for about 60% of its total production), resins and film processing in technical plastics for the automobile industry (25%), as well as coverings in the food industry (dishes, packaging for sausages and meat, cooking bags, packaging for use in microwave ovens or for roasting food) and cable insulation (Bustos et al., 2009). Global caprolactam output reached 4.7 million tonnes in 2013 and demonstrates steady growth (MRC, 2015). Inasmuch as this also increases caprolactam concentrations in the environment, studying the effects of caprolactam on the environment is gaining in importance.

Wastewater generated during caprolactam production contains about 5%–10% caprolactam (He et al., 2004). Products from nylon 6 may be another source of caprolactam because they contain about 10% of the residual caprolactam (Sanches et al., 2006). Caprolactam can therefore contribute to soil and water contamination, either directly or indirectly through washing away from the surfaces of various nylon 6 products (Bradley et al., 2004; Canova and Muthig, 1991). For instance, Canova and Muthig (1991) noted caprolactam contamination of groundwater connected with the use of nylon cords. Caprolactam has been found also in some plants (Hasegawa et al., 1983; Kalinová et al., 2014; Tin et al., 2009). Caprolactam is easily water soluble, as 4560 g/L of caprolactam can be dissolved in water at 20 °C (OECD, 2001). It is relatively stable in water, with a half-life of more than 1 year. Nevertheless, OECD 301C testing determined caprolactam biodegradation to be about 82% after 14 days (OECD, 2001).

Microalgae of the genus *Desmodesmus* (family *Scenedesmaceae*) are among the most common freshwater phytoplankters. The coenobial structure (usually 2–16 cells free-connected into one unit) of members of this genus can change with shifting environmental conditions (Chia et al., 2015). This can serve as a biomarker for monitoring the effect of various pollutants in aquatic ecosystems. The exposure of a microalga to toxic substances may cause an assortment of physiological alterations (e.g. increase of biomembrane permeability, reduction of electron transport and carbon dioxide fixation, inhibition of respiration, changes in nutrient

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* Corresponding author.
E-mail address: janak@zf.jcu.cz (J.P. Kalinová).

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uptake, inhibition of several enzymes, inhibition of protein synthesis) that can be readily observed via changes in growth, morphological changes, pigment degradation, decrease in biomass production, etc. (Chia et al., 2015; Pinto et al., 2003; Van Assche and Clijsters, 1990).

Caprolactam's effects on an autotrophic organism can be qualitatively evaluated using a growth curve or by monitoring the speed and efficiency of the photosynthetic processes. Such evaluation is used, for example, for the induced fluorescence of chlorophyll molecules (Maxwell and Johnson, 2000). Another possibility is to measure oxygen dissolved in the medium, whereby an oxygen electrode is used to determine changes in the concentration of oxygen released into that medium (Krejčí et al., 2011). The aims of this study were to evaluate the influence of caprolactam on the growth and oxygen production of the green alga *Desmodesmus quadricauda* and caprolactam uptake by this alga.

2. Material and methods

2.1. Material

Desmodesmus quadricauda syn. *Scenedesmus quadricauda* (Turpin) Brébisson (CCALLA 464 Strain number by isolator: GREIFSWALD/15) was obtained from the Culture Collection of Autotrophic Organisms (CCALA), Institute of Botany, Czech Academy of Sciences in Třebon, Czech Republic. The alga has been maintained at the Institute of Complex Systems, University of South Bohemia in České Budějovice since 2005.

The alga was cultivated under continuous stirring (150 rev/min) at 22 °C and continuous light with intensity 40 μmol photons/m^2/s in Šetlík–Simmer medium (Setlik, 1966). The culture used for all experiments had optical density greater than 5 McFarland units (about 2.75 × 10^6 cells/mL) as determined using a Densilameter III (Erba Lachema, Czech Republic).

2.2. Determination of algae growth

An algae sample (1.5 mL) with density of 40,000 cells/mL was pipetted into a plate with 25 wells having 1.8 mL volume. Cultivation was run at 22 °C and continuous light with intensity 40 μmol photons/m^2. Cell numbers and number of cells per coenobium were counted using a Bürker chamber under a light microscope (LM556SP, Intaco Micro, Czech Republic) after 24, 48, 72, 96, 144, and 168 h of cultivation.

Average growth rate (μ_r) was calculated using the following equation in terms of the parameter C (cell density) measured at times t_1 and t_2

$$\mu_r = \ln(C_2) - \ln(C_1)/(t_2 - t_1),$$

where C_1 and C_2 are cell densities at the start (t_1) and end (t_2) of the experiment, respectively.

2.3. Photosynthetic oxygen measurements

Dissolved oxygen concentration in the medium as an indicator for photosynthetic activity was measured using an Algareact algae growth analyser (BVT Technologies, Brno, Czech Republic) with an oxygen electrode.

The algae culture was pipetted into a cell at 25 ± 0.1 °C and caprolactam (Sigma–Aldrich, stock solution in the concentration 50,000 mg/L and 10,000 mg/L) was immediately added during continuous stirring of the aqueous solution so that its concentration reached levels of 0, 10, 50, 100, 500, 1,000, and 5,000 mg/L. The final sample amount was 10 mL. The pH was checked prior to the start of the experiment after the addition of caprolactam to the sample.

Because pH was in the range of 7.0–7.2 for all concentrations used, pH was not adjusted. Distilled water was used to calibrate the analyser. If the value of oxygen in the water was not between 8.2 and 8.3 mg/L after 5 min of a run at 25 ± 0.1 °C, the analyser was calibrated using a saturated solution of sulphite sodium.

After insertion into the analyser, the sample was held in darkness for 1,200 s (one dark cycle) and then illuminated for 2,400 s with light intensity 450 μmol photons/m^2 (one light cycle). One run took 3 h (10,800 s). Oxygen content was recorded every second. Each run with 3 light/dark cycles was repeated 3 times.

The maximum oxygen production was found for each light phase and was decreased by the minimum oxygen production in the dark phase of the given period. At 25 °C, the minimum oxygen production was always very close to 8.2 mg/L, which is natural oxygen saturation at this temperature. Mean oxygen production during the light phase was also determined and decreased by the relevant minimum oxygen production.

2.4. Caprolactam uptake

To 1.9 L of algae culture with density 2,000 mg/L medium was added 100 mL of water solution with 2,000, 200, and 0 mg of caprolactam. After 30 min, 60 min, 90 min, 130 min, 1 day, and 8 days of cultivation at 22 °C and continuous light intensity of 40 μmol photons/m^2 and under continuous stirring (150 rev/min), 600 mL of algae culture was sampled and centrifuged (2,600 g) for 12 min. The precipitate was resuspended in water for 10 min and again centrifuged. The precipitate was lyophilized (Heto PowerDry LL 3000, Thermo Scientific) at −50 °C for 7 days.

2.5. HPLC analysis

Freeze-dried material (300 mg) was extracted using 3 mL of 80% acetone for 5 min in an ultrasonic bath and then centrifuged (1,800 g) for 10 min. The supernatant was removed and the sediment was resuspended in 1 mL of 80% acetone, shaken, then centrifuged again. After removing the supernatant the sediment was washed again. All three supernatants were collected into the same graduated tube and shaken, and the volume was adjusted to 4 mL of 80% acetone.

Extracts were analysed using an HP 1050 HPLC instrument (Hewlett-Packard, USA) and connected to a G1315B DAD detector (Agilent). The analyses used a Luna C18 (2) column (150 × 2 mm, 3 μm) (Phenomenex, Torrance, CA, USA). As mobile phase, a mixture of water, acetonitrile, and 0.1% *o*-phosphoric acid was used as follows: mobile phase A: 5% acetonitrile +0.1% *o*-phosphoric acid, mobile phase B: 80% acetonitrile +0.1% *o*-phosphoric acid.

The analyses were performed using a linear gradient from 0% B to 12% B for 15 min with a flow rate of 0.25 mL/min at wavelength 220 nm. Raw data was scanned in the range 190–600 nm.

2.6. Data analysis

The following parameters were calculated to complete information on the uptake kinetics of caprolactam:

Initial uptake of caprolactam (mg/kg dry matter (DM)/min) was calculated according to Malea et al. (2013) as $C_{max}/(2 \times K_m)$, where C_{max} is the maximum concentration of caprolactam reached in the algae tissue and K_m is time to reach half of C_{max} as determined from the best-fit equation.

Mean velocity of uptake (mg/kg DM/min) was calculated as C_{max}/time to reach C_{max}.

The best-fit equation for caprolactam uptake to maximum