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Effect of nitrogen limitation on starch accumulation in *Chlorella vulgaris*

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Abstract

Due to the limited stocks of fossil fuels and the production of greenhouse gas carbon dioxide on their combustion alternative sources of energy are being investigated. Recently, microalgae have received much attention as a renewable energy resource because these photosynthetic microorganisms can convert sunlight, water and CO₂ into potential biofuels (1). The microalga *Chlorella vulgaris*, particularly, has been considered as a potential raw material for bioethanol production because it can accumulate high levels of starch when grown under optimized culture conditions (2).

The aim of the present work was to study the effect of nitrogen limitation on starch production by the microalgae *C. vulgaris*.

C. vulgaris CCAP 211/1e (P12 strain) was obtained from the Culture Collection of Algal Laboratory, Institute of Botany, Academy of Sciences of the Czech Republic. The original (nitrogen sufficient) growth medium based on the elementary composition of algal biomass had the following initial composition (mg l⁻¹): 1,100 (NH₂)₂CO, 237 KH₂PO₄, 204 MgSO₄·7H₂O, 40 C₁₀H₁₂O₈N₂NaFe, 88 CaCl₂, 0.83 H₃BO₃, 0.95 CuSO₄·5H₂O, 3.3 MnCl₂·4H₂O, 0.17 (NH₄)₆Mo₇O₂₄·4H₂O, 2.7 ZnSO₄·7H₂O, 0.6 CoSO₄·7H₂O, and 0.014 NH₄VO₃ in distilled water (3). Nitrogen limited growth medium was formulated by omitting urea from the original growth medium. The microalgae were grown in 1 l Schott flasks with 0.4 l of medium. Cultures were maintained at 30°C under continuous, cool white fluorescent lamps. Light intensity was approximately 100 mmol m⁻² s⁻¹ at the surface of the photobioreactors. The concentration of suspended algal biomass was determined by optical density measurement at 700 nm. Starch content in the microalgae was determined colorimetrically by the anthrone reaction.

The results showed that starch accumulation in *C. vulgaris* was strongly related to nitrogen concentration. Under nitrogen limited growth conditions, starch constituted 36% of the algal biomass after 118 h of cultivation, whereas nitrogen sufficient microalgae contained 8% of their dry weight as starch. On the other hand, nitrogen sufficient condition led to an increase in biomass concentration, with the highest biomass concentration of 2.06 g l⁻¹. It can be concluded that accumulation of starch is enhanced in nitrogen limited cultures of *C. vulgaris* P12.

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