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Effects of nitrogen on growth and carbohydrate formation in *Porphyridium cruentum*

Research Article

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Abstract: The microalga *Porphyridium cruentum* (Rhodophyta) has several industrial and pharmaceutical uses, especially for its polysaccharide production. This study aimed to investigate the influence of nitrogen levels as reflected by altered N:P ratios on the production and content of biomass and carbohydrate. N:P molar ratios were altered in batch cultures to range from 1.6 to 50 using the Redfield ratio of 1:16 as reference. Algal growth (estimated as final cell number, biomass concentration and maximum specific growth rate) was negatively affected at low N:P ratios. The optimal N:P ratio for growth was identified at 35-50, with specific growth rates of 0.19 day^{-1} and maximum cell concentrations of $59 \cdot 10^8$ cells L^{-1} and 1.2 g dry weight of biomass L^{-1}. In addition, variation in cell size was seen. Cells with larger diameters were at higher N:P ratios and smaller cells at lower ratios. The cellular carbohydrate content increased under reduced nitrogen availability. However, because accumulation was moderate at the lowest N:P ratio, 0.4 g per g dry weight biomass compared to 0.24 at the Redfield ratio of 16:1, conditions for increased total carbohydrate formation were identified at the N:P ratios optimal for growth. Additionally, carbohydrates were largely accumulated in late exponential to stationary phase.

Keywords: *Rhodophyta • Red algae • Redfield ratio • Nitrogen-to-phosphorous ratio*

1. Introduction

In a bio-based society, sustainable production processes should be based on renewable raw materials. Starch-based biomass of phototrophic organisms helps to fulfill this criterion. In this respect, studies on how culture conditions, such as nutrient availability, that would enhance cellular carbohydrate content are important. The microalga *Porphyridium* sp. (Rhodophyta) is a potential source for several products, such as fatty acids and lipids [1,2], pigments [3], and cell-wall polysaccharides [4]. These polysaccharides are sulphated and their structure gives rise to some unique properties that could lead to a broad range of industrial and pharmaceutical applications [5]. Polysaccharides surround the algal cell as an amorphous capsule. In the marine species *P. cruentum*, this capsule consists of glucose, galactose, xylose, glucuronic acid and methyl-glucuronic acid as sugar monomers [6]. The outer part of the capsule can be partly excreted to the surroundings, *i.e.* exopolysaccharides, thereby increasing the viscosity of the medium [7]. Additionally, *P. cruentum* biomass contains starch [7] and cellular contents of carbohydrates of up to 57 % have been reported [8]. Thus, the combined amount of carbohydrates in biomass and exopolysaccharides of this microalga could potentially provide the carbon source for fermentation processes, such as bioethanol production [9].

Culture conditions affect carbohydrate content in microalgae. Limited levels of macro-nutrients, such as nitrogen and phosphorous, in the growth medium have been shown to affect formation of carbohydrates in several algae species. For instance, nitrogen limitation increased carbohydrate formation in *Chlamydomonas mexicana* [10] and *Chlorella* spp. [11] and increased starch production in *Tetraselmis subcordiformis* [12]. Phosphorus limitation induced an increase of carbohydrate formation

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