Different nitrogen sources and growth responses of *Spirulina platensis* in microenvironments

Jorge Alberto Vieira Costa*, Karla Leal Cozza, Lucielen Oliveira and Glênio Magagnin

Laboratory of Biochemical Engineering, Department of Chemistry, Federal University Foundation of Rio Grande, P.O. Box 474, RS 96201-900, Brazil

*Author for correspondence: Tel.: +55-53-233 8653, Fax: +55-53-232 9716, E-mail: dqmjorge@super.surg.br

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Summary

*Spirulina platensis* was cultivated, in comparative studies, using several sources of nitrogen. The standard source used (sodium nitrate) was the same as that used in the synthetic medium Zarrouk, whereas the alternative nitrogen sources consisted of ammonium nitrate, urea, ammonium chloride, ammonium sulphate or acid ammonium phosphate. The initial nitrogen concentrations tested were 0.01, 0.03 and 0.05 M in an aerated photobioreactor at 30 °C, with an illuminance of 1900 lux, and 12 h-light/12 h-dark photoperiod over a period of 672 h. Maximum biomass was produced in medium containing sodium nitrate (0.01–0.03–0.05 M), followed by ammonium nitrate (0.01 M) and urea (0.01 M). The final biomass concentrations were 1.992 g l⁻¹ (0.03 M sodium nitrate), 1.628 g l⁻¹ (0.05 M sodium nitrate), 1.559 g l⁻¹ (0.01 M sodium nitrate), 0.993 g l⁻¹ (0.01 M ammonium nitrate) and 0.910 g l⁻¹ (0.01 M urea). This suggested that it is possible to utilize nitrogen sources other than sodium nitrate for growing *S. platensis*, in order to decrease the production costs of scaled up projects.

Introduction

The first tribes of hunter-collectors used to pick up microalgae for food on margins of lakes, many of them saline, e.g., the collection of *Spirulina* in Lake Chad, Africa (Henrikson 1994). Many advantages are recognized in microalgae cultivation, such as: high protein biomass (above 60%), absence of parts to be discarded during processing, use of arid or semi-arid areas around the world, and growth in saline waters without competing with traditional agriculture. However, there are some disadvantages, mainly due to the costs of the mineral sources required (Richmond 1990).

The microalga *Spirulina platensis* is a multicellular filamentous cyanobacterium, whose cells (diameter from 1 to 12 μm) are organized in helical trichomes without ramifications (Richmond 1990). Several species of *Spirulina* are able to inhabit hostile environments, such as saline lakes (Henrikson 1994).

According to Richmond (1990) the first known *Spirulina* pills are going to be substituted slowly by candies, gums, biscuits and pastas enriched with this microalga. The presence of free amino acids, vitamin B₁₂, thiamin, riboflavin, niacin, phycocyanin, carotenoids, and essential unsaturated fatty acids make *Spirulina* a promising product to the food industry. Many therapeutic applications, such as stimulus to the immune system (Hayashi 1996), regulation of blood pressure and cholesterol synthesis (Nayaka 1988; Iwata 1990), and zinc deficiency (Huang et al. 1982) could also be quoted.

The aim of this study was, through using different nitrogen sources and varying them quantitatively, to obtain new alternative substrates for *S. platensis* cultivation.

Material and methods

Microorganism and media

*Spirulina platensis* LEB-52 was supplied by the Faculty of Pharmaceutical Sciences, University of São Paulo (Costa et al. 2000). Medium composition was the one established by Zarrouk in which the nitrogen source available to the microorganism (sodium nitrate) was replaced by ammonium nitrate, urea, ammonium chloride, ammonium sulphate or acid ammonium phosphate. The concentrations of nitrogen tested were 0.01, 0.03 and 0.05 M in all media.

Cultivation

The experiment was carried out at a scale of 2000 ml and started with a biomass concentration of 0.1 g l⁻¹. It was run in a sterile aerated photobioreactor at 30 °C. The aeration rate was 201 h⁻¹, and a 12 h-light/12
h-dark photoperiod was used. Illumination was obtained from fluorescent lights at an illuminance of 1900 lux. A schematic diagram of the experimental device used is shown in Figure 1.

**Analytical and statistical procedures**

Samples were taken aseptically each 24 h, to monitor pH, carbonates and bicarbonates according to AOAC (1995), and the cellular concentration growth was determined by optical density measurements at 750 nm. All experiments were carried out in duplicate or triplicate. Statistical treatment was done with Newman–Keuls analysis within the 95% confidence level.

**Results and discussion**

Results obtained with different nitrogen sources could help our understanding of the development of these microalgae over 672 h. Since nitrogen is an important nutritional source, *S. platensis* response varied with different sources and amounts. *Spirulina platensis* grew successfully in six out of 18 media, which can be seen in Table 1. There were no statistical differences (at the 95% confidence level) in the Newman–Keuls test among media with sodium nitrate in all three concentrations tested. However, there were statistical differences between the nitrate and the three other media tested. The final *S. platensis* biomass concentrations in different nitrogen sources were given in Figure 2.

<table>
<thead>
<tr>
<th>Medium</th>
<th>Final biomass concentration (g l⁻¹)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0.01 M</td>
</tr>
<tr>
<td>Acid ammonium phosphate</td>
<td>0.924</td>
</tr>
<tr>
<td>Ammonium chloride</td>
<td>0.056</td>
</tr>
<tr>
<td>Ammonium nitrate</td>
<td>0.993</td>
</tr>
<tr>
<td>Ammonium sulphate</td>
<td>0.081</td>
</tr>
<tr>
<td>Sodium nitrate</td>
<td>1.559</td>
</tr>
<tr>
<td>Urea</td>
<td>0.910</td>
</tr>
</tbody>
</table>

The best environmental phosphate concentrations for growth of microorganisms follow a wide range of tolerance according to the specific species used and do not depend on the concentrations of the other nutrients (Becker 1994). The average phosphorus tolerance of many species, according to Becker (1994) is situated between 0.050 and 20 mg l⁻¹. During this study, the