Study on biofiltration capacity and kinetics of nutrient uptake by *Gracilaria cervicornis* (Turner) J. Agardh (Rhodophyta, Gracilariaceae)

Marcella A. A. Carneiro,¹ Fúlvio Aurélio de M. Freire,² Eliane Marinho-Soriano*¹

¹Departamento de Oceanografia e Limnologia, Universidade Federal do Rio Grande do Norte, Brazil, ²Departamento de Botânica, Ecologia e Zoologia, Universidade Federal do Rio Grande do Norte, Brazil.

Abstract: The absorption efficiency and kinetic parameters (V\text{max}, K\text{s} and V\text{max}:K\text{s}) of the seaweed *Gracilaria cervicornis* for the nutrients NH\text{4}⁺, NO\text{3}⁻ and PO\text{4}³⁻ were evaluated. Absorption efficiency was measured by monitoring nutrient concentrations for 5 h in culture media with initial concentrations of 5, 10, 20 and 30 μM. Kinetic parameters were determined by using the Michaelis-Menten formula. Absorption efficiencies for this algae were greater in treatments with lower concentrations, as evidenced by a reduction of 85.3, 97.5 and 81.2% for NH\text{4}⁺, NO\text{3}⁻ and PO\text{4}³⁻, respectively. Kinetic parameters show that *G. cervicornis* exhibits greater ability to take up high concentrations of NH\text{4}⁺ (V\text{max}=158.5 μM g\text{dw}⁻¹ h⁻¹) and low concentrations of PO\text{4}³⁻ (K\text{s}=5 μM and V\text{max}:K\text{s}=10.3). These results suggest that this algal species has good absorption capacity for the nutrients tested and may be a promising candidate as a bioremediator of eutrophized environments.

Keywords: *Gracilaria* nutrient uptake macroalgae

Introduction

Seaweeds are particularly efficient nutrient absorbers and possess specific mechanisms for storing large amounts of nitrogen and phosphorous in their tissues (Lobban & Harrison 1997). These nutrients may be used in the future when, for example, the environmental concentrations fall to levels lower than those required by the seaweeds for growth (DeBoer, 1981).

Studies related to nutrient removal by seaweeds have been conducted to select species that can be used as biofilters for the treatment of eutrophized environments (Chopin et al., 2001; Buschmann et al., 2001; Troell et al., 2003; Neori et al., 2004). The seaweeds most widely used in this type of experiment belong to the genus *Gracilaria*. For example, in an integrated salmon/seaweed system, Buschmann et al. (1996) demonstrated that *Gracilaria chilensis* was capable of removing 95% of ammonium and 32% of phosphorous. Other integrated studies with *Gracilaria gracilis* also demonstrated a high capacity for removing these nutrients: around 93% and 62% of ammonium and phosphorous, respectively (Hernández et al., 2002; Martínez-Aragón et al., 2002). More recently, Marinho-Soriano et al. (2009) showed that *Gracilaria caudata* was capable of reducing the concentrations of ammonium, nitrate and orthophosphate to 59.5, 49.6 and 12.3%, respectively, in only 4 h.

Nutrient uptake rates achieved by seaweeds are generally based on the reduction of the concentration of a given nutrient in the culture medium. In these studies, kinetic parameters (V\text{max} and K\text{s}) are quite useful for identifying the physiological abilities of a seaweed (Phillips & Hurd, 2004), supplying valuable data to help in the selection of species that can be used as biofilters in eutrophized environments. The aim of the present study was to obtain information on the filtration capacity and the kinetics of uptake of NH\text{4}⁺, NO\text{3}⁻ and PO\text{4}³⁻ by the seaweed *Gracilaria cervicornis* (Turner) J. Agardh.

Materials and Methods

Algal material and preculture conditions

The seaweeds used in this experiment were collected at the Buzios beach in the Northwest of Brazil (06°01’S-35°06’W) and taken to the laboratory, where epiphytes and sediment were removed. Before the experiment, the seaweeds were maintained for 24 h in seawater (NH\text{4}⁺<1 μM, NO\text{3}⁻<1 μM and PO\text{4}³⁻undetectable) with constant aeration and illuminated (180 μmol photon m\text{2}·s⁻¹) in a 10 h light-14 h dark cycle at a temperature of
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28.6±0.5 °C and salinity of 37.8±0.2 PSU. The samples used in the absorption experiment were selected from this stock.

**Experimental design**

The absorption efficiency of *G. cervicornis* for NH$_4^+$, NO$_3^-$ and PO$_4^{3-}$ was determined by monitoring these nutrients at initial concentrations of 5, 10, 20 and 30 μM. For each concentration, transparent cylindrical recipients (triplicate) containing 5 g of *G. cervicornis* and filled with 1 L of filtered seawater were used. To obtain the desired concentrations, previously prepared solutions containing NH$_4$Cl (NH$_4^+$), KNO$_3$ (NO$_3^-$) and KH$_2$PO$_4$ (PO$_4^{3-}$) were added to the seawater. A recipient containing only enriched seawater served as the control. The experiment lasted for 5 h, with samplings at 15 min, 30 min, 1 h, 2 h, 3 h, 4 h and 5 h. To ensure that none of the nutrients (N and P) would become limiting for *G. cervicornis*, a molar ratio of 10:1 (N:P) was used (Friedlander & Dawes, 1985). Water samples were analyzed according to Strickland & Parsons (1972). After the last sampling, the seaweeds were immediately removed and oven dried (60 °C) to obtain the dry weight. Absorption efficiency was calculated based on the reduction in the concentration of nutrients for each sampling period and expressed in percentages.

Uptake rates (μM g$_{dw}$ h$^{-1}$) were calculated for each time interval during the depletion according to Pedersen (1994): $V = [(S_0 \cdot Vol_0) - (S_1 \cdot Vol_1)]/(B \cdot t)$, where $S_0$ and $S_1$, are the substrate concentrations and Vol$_0$ and Vol$_1$, the volumes before and after a sampling period ($t$), and $B$ is algal dry weight biomass ($\approx 0.5$ g).

Uptake rates ($V$) between 0-15 min were plotted against each corresponding mean substrate concentration and the Michaelis-Menten function: $V = V_{max} S/(K_s+S)$, was fitted to the data by regression, where $V_{max}$ is the maximum uptake rate (μM g$_{dw}$ h$^{-1}$) and $K_s$ is the half-saturation constant.

**Results**

Absorption of nutrients by *G. cervicornis* was significant at all concentrations analyzed (ANOVA, $p<0.05$), with the lowest values being recorded in the first 15 min of incubation and the maximum values at the end of the experiment (3-5 h) (Figure 1). The absorption efficiency of *G. cervicornis* was greatest at the lowest initial concentration (5 μM), showing the greatest percent reduction for NO$_3^-$ (97.5%), followed by NH$_4^+$ (85.3%) and PO$_4^{3-}$ (81.6%). In the treatments with initial concentrations of 20 and 30 μM, higher NH$_4^+$ absorption was observed, followed by NO$_3^-$ and PO$_4^{3-}$. No variations in nutrient concentrations were observed in the controls (ANOVA; $p>0.05$) (Table 1).

![Figure 1. Depletion of the concentrations of NH$_4^+$, NO$_3^-$ and PO$_4^{3-}$ during the 5 h experiment with *G. cervicornis*](image-url)
Table 1. Initial and final concentrations of NH₄⁺, NO₃⁻ and PO₄³⁻ in the control and G. cervicornis groups for treatments with 5, 10, 20 and 30 μM of the nutrient and the absorption efficiency (%) of the seaweed at the end of the experiment (3-5 h).

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Control (C_initial-C_final)</th>
<th>Treatments (C_initial-C_final)</th>
<th>Absorption efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₄⁺</td>
<td>5 μM 5.2-5.4</td>
<td>10 μM 11.5-11.8</td>
<td>85.3</td>
</tr>
<tr>
<td></td>
<td>20 μM 20.4-20.5</td>
<td>30 μM 30.5-30.3</td>
<td>68.0</td>
</tr>
<tr>
<td></td>
<td>5 μM  5.2-5.6</td>
<td>10 μM 10.8-10.3</td>
<td>82.9</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>20 μM 20.7-20.7</td>
<td>30 μM 30.0-30.5</td>
<td>84.6</td>
</tr>
<tr>
<td></td>
<td>5 μM  5.3-5.3</td>
<td>10 μM 10.8-10.3</td>
<td>57.3</td>
</tr>
<tr>
<td>PO₄³⁻</td>
<td>20 μM 20.7-20.4</td>
<td>30 μM 30.8-30.1</td>
<td>79.1</td>
</tr>
<tr>
<td></td>
<td>5 μM  5.3-5.3</td>
<td>10 μM 11.7-11.5</td>
<td>37.5</td>
</tr>
</tbody>
</table>

Table 2. Kinetic parameters (V_max, K, and V_max:K) of the Michaelis-Menten equation obtained from the rates for uptake of NH₄⁺, NO₃⁻ and PO₄³⁻ by G. cervicornis.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>V_max (μM g⁻¹ h⁻¹)</th>
<th>K (μM)</th>
<th>V_max:K</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₄⁺</td>
<td>158.5</td>
<td>41.6</td>
<td>3.8</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>67.9</td>
<td>19.6</td>
<td>3.5</td>
</tr>
<tr>
<td>PO₄³⁻</td>
<td>51.5</td>
<td>5.0</td>
<td>10.3</td>
</tr>
</tbody>
</table>

Discussion

This study showed a significant removal of the three nutrients by the seaweed G. cervicornis, with greater biofiltration efficiency for the two forms of nitrogen than for orthophosphate. The maximum absorption efficiency values obtained in this experiment (85.3% - NH₄⁺; 97.5% - NO₃⁻ and 81.2% - PO₄³⁻) were similar and at times higher than data found in the literature. Jones et al. (2001) observed that, in wastewater treatment using seaweeds, Gracilaria edulis was capable of reducing the NH₄⁺ concentration by 74% (2 h), NO₃⁻ by 97.7% (4 h) and PO₄³⁻ by 95.1% (10 h). In an experiment using Gracilaria longissima, Hernández et al. (2006) recorded mean filtration efficiencies of 93.2% for NH₄⁺ and 62.2% for PO₄³⁻ after 7 h of incubation.

Figure 2. Uptake rates (V) for NH₄⁺ (A), NO₃⁻ (B) and PO₄³⁻ (C) as a function of substrate concentrations (S). Curves fitted to the Michaelis-Menten equation. p-value <0.001 indicates that the independent (S) and dependent (V) variables follow this function. The dashed lines delimit the prediction interval at the 95% confidence level.
With respect to uptake rate, \( G. \ cervicornis \) showed reduced values as the incubation time increased. At the end of the experiment, rates were less than 15% of those obtained at the beginning. This pattern was also observed by other authors for several seaweed species (Pedersen, 1994; Campbell, 1999; Dy & Yap, 2001; Pedersen et al., 2004).

The rate of uptake of \( NH_4^+ \) by \( G. \ cervicornis \) was greater than that of \( NO_3^- \) at all concentrations tested. This behavior has been generally observed for a number of seaweed species (D’Elia & DeBoer, 1978; Wallentinus, 1984; Phillips & Hurd, 2004), given that the process of \( NO_3^- \) absorption and assimilation is more costly to seaweeds than those for \( NH_4^+ \) (McGlathery et al., 1996).

Maximum uptake rates (\( V_{\text{max}} \)) and half-saturation constants (\( K_s \)) are generally the most widely used parameters for comparing and contrasting uptake rates in seaweeds. \( K_s \) is normally used to estimate the ability of a species to absorb a nutrient at low concentrations, while \( V_{\text{max}} \) estimates the maximum uptake rate at high concentrations (Raven & Taylor, 2003). Table 3 shows \( V_{\text{max}} \) and \( K_s \) values for various seaweed species. The \( V_{\text{max}} \) values for \( G. \ cervicornis \) are higher than those recorded for other species of \( Gracilaria \). The high \( V_{\text{max}} \) obtained for \( NH_4^+ \) suggests that \( G. \ cervicornis \) performs better in environments where the concentration is high and where it can uptake \( NH_4^+ \) at a rate proportional to its concentration in the water column. This ability may be an advantage for seaweeds cultivated in eutrophized environments.

In this study, \( G. \ cervicornis \) also exhibited high \( V_{\text{max}} \) values for nitrate. This rapid absorption of \( NO_3^- \) from the medium suggests that this species is highly competitive in removing this nutrient from water. The nitrogen uptake rate was always higher than that of phosphorus at the same concentrations. In relation to \( PO_4^{3-} \), there are few studies on the uptake kinetics of this nutrient, probably reflecting the fact that orthophosphate concentrations are often near the detection limit (Lobban & Harrison, 1997).

However, seaweeds found in eutrophic environments may exhibit very high \( K_s \) and \( V_{\text{max}} \) values, such as the species \( Ulva \) and \( Chaetomorpha \), which exhibit \( K_s \) values of 3.5-10 \( \mu M \) and \( V_{\text{max}} \) values of 8.5-20.8 \( \mu M \) \( g_{\text{dw}}^{-1} \) h\(^{-1} \) (Lavery & McComb, 1991). The high absorption rate recorded in this study for the three nutrients should represent an advantage for this species when nutrient availability in the environment is high. This being so, \( G. \ cervicornis \) can be considered to be an important species for bioremediation in eutrophized environments and in integrated aquaculture.

### Acknowledgements

This research was supported by the CNPq and CAPES.

### References


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### Table 3. Comparison of \( V_{\text{max}} \) (\( \mu M \) \( g_{\text{dw}}^{-1} \) h\(^{-1} \)) and \( K_s \) (\( \mu M \)) values for several species of seaweeds.

<table>
<thead>
<tr>
<th>Species</th>
<th>( NH_4^+ )</th>
<th>( NO_3^- )</th>
<th>( PO_4^{3-} )</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( V_{\text{max}} )</td>
<td>( K_s )</td>
<td>( V_{\text{max}} )</td>
<td>( K_s )</td>
</tr>
<tr>
<td>( G. \ pacifica )</td>
<td>21.5</td>
<td>50.9</td>
<td>6.0</td>
<td>26.8</td>
</tr>
<tr>
<td>( G. \ foliifera )</td>
<td>23.8</td>
<td>1.6</td>
<td>9.7</td>
<td>2.5</td>
</tr>
<tr>
<td>( Agardhiella subulata )</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>( Cladophora montagneana )</td>
<td>130</td>
<td>20.7</td>
<td>42.1</td>
<td>1.4</td>
</tr>
<tr>
<td>( Enteromorpha compressa )</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>( G. \ cervicornis )</td>
<td>158.5</td>
<td>41.6</td>
<td>67.9</td>
<td>19.6</td>
</tr>
</tbody>
</table>
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*Correspondence*

Eliane Marinho-Soriano
Departamento de Oceanografia e Limnologia, Universidade Federal do Rio Grande do Norte 59014-100 Natal-RN, Brazil eliane@ufrnet.br
Tel.: +55 84 3342 4950
Fax: +55 84 3423 4951