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

Article

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Abstract: The absorption efficiency and kinetic parameters (V_{max}, K_s and V_{max}:K_s) of the seaweed *Gracilaria cervicornis* for the nutrients NH_4^+, NO_3^- and PO_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_4^+, NO_3^- and PO_4^{3-}, respectively. Kinetic parameters show that *G. cervicornis* exhibits greater ability to take up high concentrations of NH_4^+ (V_{max}=158.5 µM g_{dw}^{-1} h^{-1}) and low concentrations of PO_4^{3-} (K_s=5 µM and V_{max}:K_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.

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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_{max} and K_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_4^+, NO_3^- and PO_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 (06º01'S-35º06'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_4^+<1 µM, NO_3^-<1 µM and PO_4^{3-} undetectable) with constant aeration and illuminated (180 µmol photon $m^{-2}\cdot s^{-1}$) in a 10 h light-14 h dark cycle at a temperature of

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_4Cl (NH_4^+), KNO_3 (NO_3^-) and KH_2PO_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 ºC) 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}^{-1} 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 (≈ 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}^{-1} h^{-1}) and Ks is the half-saturation constant.

Statistical analyses

All data were tested for normality (Kolmogorov-Smirnov test) and homoscedasticity (Levene's test). One-way ANOVA was used to determine the variations between the time intervals monitored, followed by the Student–Newman–Keuls post-hoc test for multiple comparisons of means (Zar, 1999). The Michaelis-Menten function was analyzed for significance by using the Table Curve® 2D 5.0, 2000 program. Statistical analyses were conducted with SigmaStat v2.03 software.

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).

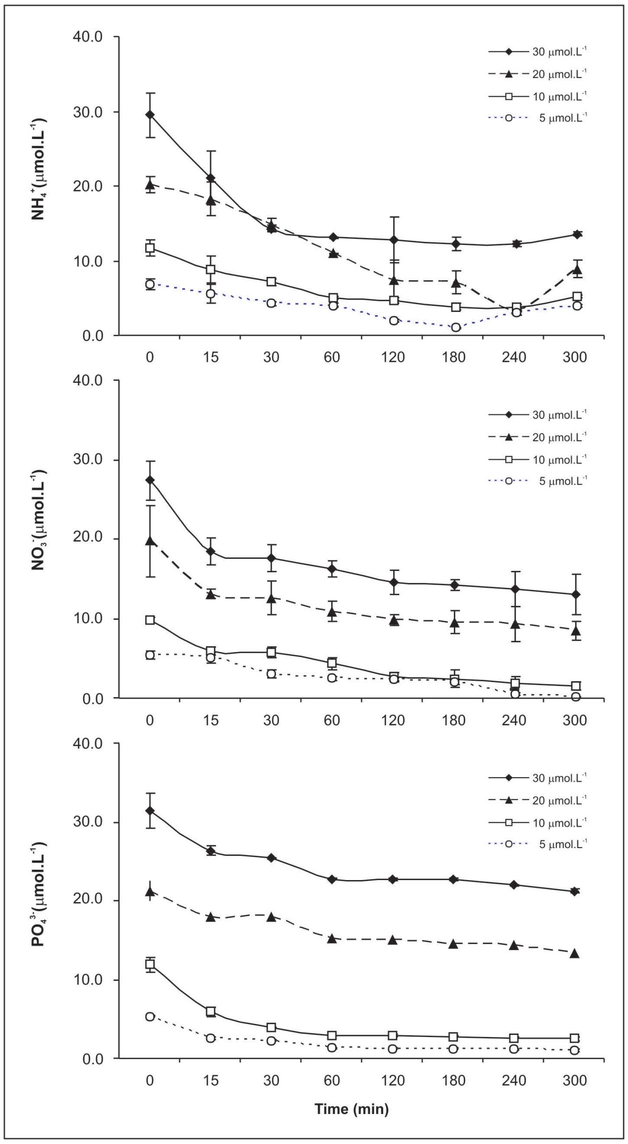

Figure 1. Depletion of the concentrations of NH_4^+, NO_3^- and PO_4^{3-} during the 5 h experiment with *G. cervicornis*.

Table 1. Initial and final concentrations of NH_4^+, NO_3^- and PO_4^{3-} 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).

		Control ($C_{initial}$-C_{final})	Treatments ($C_{initial}$-C_{final})	Absorption efficiency (%)
NH_4^+	5 μM	5.2-5.4	6.8-1.0	85.3
	10 μM	11.5-11.8	11.7-3.7	68.0
	20 μM	20.4-20.5	20.2-3.5	82.9
	30 μM	30.5-30.3	29.5 - 12.3	58.3
NO_3^-	5 μM	5.2-5.6	5.4-0.1	97.5
	10 μM	10.8-10.3	9.7-1.5	84.6
	20 μM	20.7-20.7	19.7-8.4	57.3
	30 μM	30.0-30.5	27.3-13.0	52.6
PO_4^{3-}	5 μM	5.3-5.3	5.3-1.0	81.6
	10 μM	11.7-11.5	11.8-2.5	79.1
	20 μM	20.7-20.4	21.2 -13.2	37.5
	30 μM	30.8-30.1	31.3-21.2	32.5

Higher uptake rates (*V*) were obtained from 0 to 15 min. Uptake rates for the three nutrients decreased over the course of the experiment and are illustrated on a hyperbolic curve (Figure 2). As the external concentration of nutrients increased, the velocity showed a tendency to saturation (Figure 2A, B and C). Kinetic parameters from the Michaelis-Menten equation obtained for the three nutrients are shown in Table 2. The seaweed *G. cervicornis* exhibited the highest V_{max} and K_s values in the experiment with NH_4^+ (158.5 μM g_{dw}^{-1} h^{-1} and 41.6 μM). On the other hand, PO_4^{3-} had the lowest V_{max} and K_s values (51.5 μM g_{dw}^{-1} h^{-1} and 5 μM, respectively). The V_{max}:*Ks* ratio (10.3) was higher for PO_4^{3-}.

Table 2. Kinetic parameters (V_{max}, K_s and V_{max}:K_s) of the Michaelis-Menten equation obtained from the rates for uptake of NH_4^+. NO_3^- and PO_4^{3-} by *G. cervicornis.*

	V_{max} (μM g_{dw}^{-1} h^{-1})	K_s (μM)	V_{max}:K_s
NH_{4+}	158.5	41.6	3.8
NO_{3-}	67.9	19.6	3.5
PO_4^{3-}	51.5	5.0	10.3

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% - NH_4^+; 97.5% - NO_3^- and 81.2% - PO_4^{3-}) 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 NH_4^+ concentration by 74% (2 h), NO_3^- by 97.7% (4 h) and PO_4^{3-} by 95.1% (10 h). In an experiment using *Gracilariopsis longissima*, Hernández et al. (2006) recorded mean filtration efficiencies of 93.2% for NH_4^+ and 62.2 % for PO_4^{3-} after 7 h of incubation.

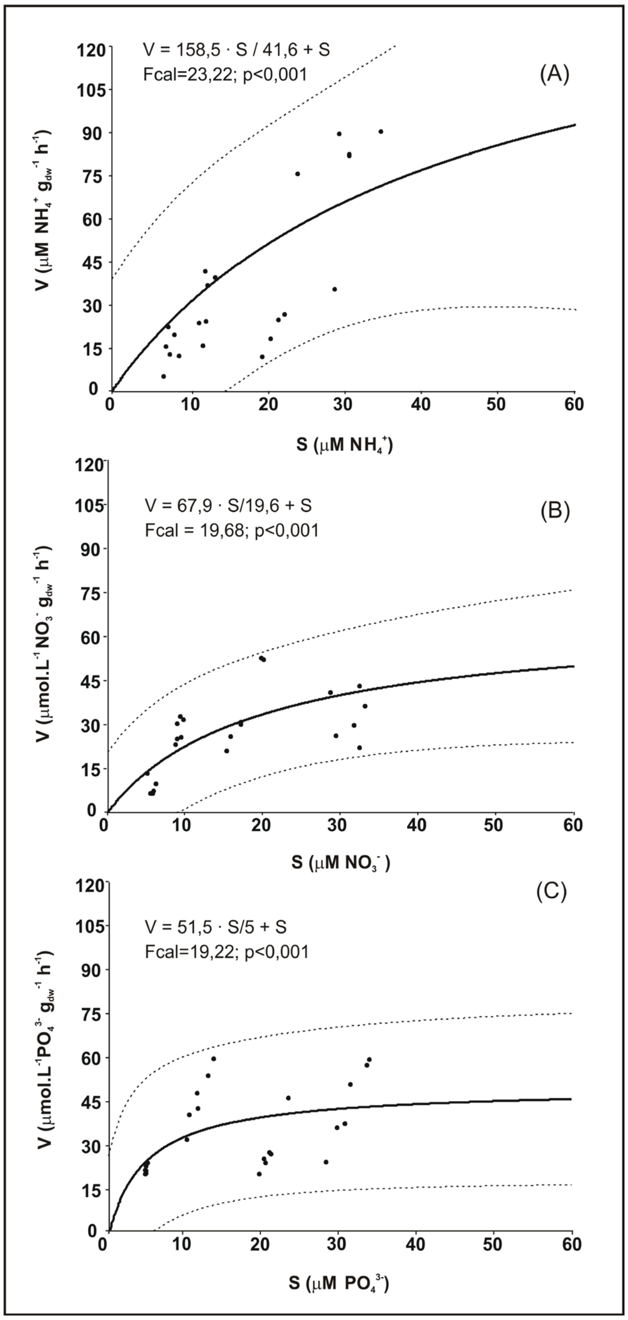

Figure 2. Uptake rates (V) for NH_4^+ (A), NO_3^- (B) and PO_4^{3-} (C) as a function of substrate concentrations (S). Curves fitted to the Michaelis-Menten equation. *p*-value <0.001 indicates that the independent (S) and dependent (V) variables follow this function. The dashed lines delimit the prediction 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_{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_{max} estimates the maximum uptake rate at high concentrations (Raven & Taylor, 2003). Table 3 shows V_{max} and K_s values for various seaweed species. The V_{max} values for *G. cervicornis* are higher than those recorded for other species of *Gracilaria*. The high V_{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_{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 phosphorous 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_{max} values, such as the species *Ulva* and *Chaethomorpha*, which exhibit K_s values of 3.5-10 μM and V_{max} values of 8.5-20.8 μM g_{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

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

Species	NH_4^+		NO_3^-		PO_4^{3-}		Reference
	V_{max}	K_s	V_{max}	K_s	V_{max}	K_s	
Gracilaria pacifica	21.5	50.9	6.0	26.8	---	---	Thomas & Harrison (1987)
Gracilaria foliifera	23.8	1.6	9.7	2.5	---	---	D'Élia & DeBoer (1978)
Agardhiella subulata	---	---	---	---	0.5	0.4	DeBoer (1981)
Cladophora montagneana	130	20.7	42.1	1.4	3.6	0.5	Gordon et al. (1981)
Enteromorpha compressa	---	---	---	----	48.7	10.6	Raven & Taylor (2003)
Gracilaria cervicornis	158.5	41.6	67.9	19.6	51.5	5.0	Present study

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