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Bioremediation Potential of the Macroalga *Ulva lactuca* (Chlorophyta) for Ammonium Removal in Elastomer Industry Wastewater

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Abstract

During the production of nitrile rubber, significant amounts of nitrogen in the form of ammonium are generated in the wastewater. The discharge of this high-nitrogen wastewater can lead to serious environmental issues, including eutrophication, disruption of aquatic ecosystems, and groundwater contamination. To mitigate these impacts, this research explored the bioremediation capabilities of the macroalgae *Ulva lactuca* (Chlorophyta) for removing nitrogen from nitrile rubber production wastewater. The study employed single-phase and Michaelis-Menten decay models based on ammonium consumption, using various dilutions of wastewater to identify the optimal concentration for treatment. The physiological state of the macroalgae was monitored by measuring the photosynthetic capacity and specific growth rate during the experiments. In the presence of *U. lactuca*, ammonium concentrations decreased in all treatment groups, confirming that the ammonium kinetics conformed to both applied models. Our results show that *U. lactuca* effectively reduces ammonium concentrations, with an approximate removal rate of 0.020 $\mu M \cdot g^{-1} \cdot min^{-1}$ across different wastewater concentrations (70%, 80%, 90%, and 100%). Notably, the treatments with 70%, 80%, and 90% wastewater strength achieved about 67% reduction in ammonium, demonstrating the alga's capacity to treat high-nitrogen wastewater. The photosynthetic performance of *U. lactuca* initially declined in control conditions but stabilized across all treatments, highlighting its adaptability. The kinetic analysis using the Michaelis-Menten model indicated a Vmax of 1342 μM·g^{-1}·DMh^{-1}, suggesting a robust capacity for ammonium uptake when fully saturated. Our study underscores the potential of *Ulva lactuca* as a cost-effective and efficient agent for wastewater bioremediation, particularly in settings with high nitrogen loads.

Keywords

Photosynthetic Quantum Yield, One-Phase Decay Model, Michaelis-Menten Model Nitrogen, Physiological Parameters, Elastomers

1. Introduction

Synthetic polymers play a fundamental role in various aspects of our lives, being extensively utilized across a range of products and industrial sectors. While they have facilitated technological advances and provided substantial benefits, they also pose an environmental challenge due to their adverse impacts on ecosystems [1]. Synthetic polymers such as styrene-butadiene, polyisoprene, chloroprene, and nitrile are widely used in elastomers industry for tires, gloves, condoms, and shoe production [2]. The production of these synthetic elastomers depends on non-renewable resources including coal, oil, natural gas, and acetylene. Consequently, during manufacturing processes, fossil fuels are burned, leading to greenhouse gas emissions and contributing to global warming and climate change [2] [3].

Furthermore, the elastomers industry produces a complex wastewater composition. The elastomers industry wastewater is characterized by high levels of total solids, suspended solids, dissolved solids, and turbidity, alongside significant biochemical oxygen demand (BOD), chemical oxygen demand (COD), ammonia, nitrate, phosphate values [4] [5] [6] [7] and dyes indicating high pollution potential [8].

As a result, the excessive nitrogen input, such as ammonia and nitrates, into aquatic systems can lead to eutrophication, which depletes oxygen levels and harms aquatic life [9] [10]. This nutrient overload alters the nitrogen cycle, affecting the abundance and diversity of denitrifying bacterial communities, which are crucial for nitrogen removal processes in aquatic environments [11]. Elevated nitrogen levels also disrupt the trophic dynamics within fish and phytoplankton communities, leading to unrealistic estimates of trophic positioning and niche space, as seen in polluted river basins [12] [13] [14]. Moreover, studies have shown that nitrogen from various sources, including but not limited to wastewater, atmospheric deposition, and agricultural runoff, leads to nutrient pollution in coastal and freshwater systems [15] [16].

Continuous monitoring and innovative treatment technologies, such as subsurface infiltration systems, are essential for managing nitrogen levels in wastewater and protecting aquatic ecosystems from the adverse effects of nitrogen pollution [8]. Furthermore, the development of sensors for real-time detection of nitrate and ammonium in water can aid in the continuous monitoring and management of nitrogen levels in aquaponic systems and other aquatic environments [9]. Overall, addressing nitrogen pollution through advanced treatment processes and continuous monitoring is crucial for maintaining the health and biodiversity of aquatic ecosystems [9].

While techniques such as anaerobic digestion, membrane technologies, coagulation-flocculation, advanced oxidation processes, and integrated methods are capable of efficiently removing contaminants from wastewater, the quest for new, more sustainable technologies remains imperative [17].

Given the environmental challenges associated with synthetic polymer production and in alignment with the United Nations' Sustainable Development Goals (SDGs), the development of innovative and sustainable wastewater treatment systems is increasingly vital [18] [19]. In this context, macroalgae have emerged as a promising solution for wastewater bioremediation, offering potential resolutions to challenges faced by conventional methods, including issues related to circular economy principles, the use of toxic chemicals, and the utilization of sustainable raw materials [20] [21]. The utilization of macroalgae for wastewater treatment presents a natural and sustainable solution by efficiently removing excess nutrients such as nitrogen and phosphorus, crucial for eutrophication control in water bodies [22] [23] [24]. Additionally, macroalgae possess the ability to sequester heavy metals, including cadmium and lead, through their biomass, thereby aiding in bioremediation efforts [25]. Their photosynthetic activity not only contributes to carbon sequestration, mitigating CO_2 emissions, but also provides a renewable resource for biofuel production [25]. The valorization of macroalgae biomass for biofuel not only offers an alternative energy source but also reduces dependency on fossil fuels. Furthermore, the residual biomass from the treatment process can be utilized as biofertilizer, enriching soils with nutrients and promoting sustainable agriculture [19] [25] [26].

In this context, many marine macroalgae species have already been indicated as appropriate bioremediation agents, where species of the genus *Ulva* have been highlighted for their capacity to treat a broad variety of effluents [27] [28] [29]. The physiological and metabolic characteristics of *Ulva spp*. allow the efficient removal of nitrogen and phosphorus dissolved in enriched water, resulting in increased biomass, tissue nutrient content and pigment. When considering a year-round period and a cultivated area of one square kilometer, *Ulva spp*. demonstrates nutrient recovery potential, recovering approximately 0.17 $kg \cdot km^{-2} \cdot year^{-1}$ for phosphorus and 11.35 $kg \cdot km^{-2} \cdot year^{-1}$ for nitrogen [30]. Among *Ulva* species, *U. lactuta* demonstrates potential for bioremediation due to their efficient uptake and removal of pollutants like phthalates from the environment [31]. These seaweeds can thrive in nutrient-rich conditions, making them suitable for Integrated Multi-Trophic Aquaculture (IMTA) systems to mitigate fish waste effluents [24] [29]. Furthermore, *U. lactuca* demonstrated effectiveness in the absorption of heavy metals, showing strong affinity for bioaccumulative metals such as Iron (Fe), Manganese (Mn) and Lead (Pb), indicating its potential in mitigating heavy metal pollution [32] [33] and the ability to remove of nitrogenous compounds from oilfield wastewater [22]. In this study, the efficiency of bioremediation in reducing ammonium concentrations in elastomer wastewater using the green macroalgae *Ulva lactuca* Linnaeus was evaluated. This research highlights *U. lactuca* as a promising treatment option and explores the potential uses of the generated algal biomass.

2. Materials and Methods

2.1. Macroalgae Sampling and Acclimation

The macroalgae were collected from the intertidal zone at Piratininga Beach in Niterói, Brazil (22˚52'51"S; 43˚6'15"W). In the laboratory, the macroalgae were initially sorted, undergoing a manual cleaning process to remove epiphytes and encrusting animals, followed by alternating washes with filtered seawater (0.72 μm GF/F-Whatman®) and distilled water to eliminate diatoms and cyanophytes. Subsequently, the algae were placed in two 5 L Erlenmeyer flasks containing oligotrophic seawater for acclimatization. During the 96-hour acclimation period, the macroalgae were maintained under the following conditions: 0.2 μM NH_4, 1.5 μM NO_3, 14 μM PO_4, 22˚C, 200 μmol photons $m^2 \cdot s^{-1}$ PAR, with a 12:12 h photoperiod (light/dark), and 31 PSU salinity. Germanium dioxide (GeO_2) at a concentration of 4.5 μM was added to suppress diatom growth. Water movement was generated by air compressors (Boyu 7500 model), propelling water through air bubbles, and promoting algae circulation in the Erlenmeyer flasks. The flasks were partially closed to minimize water evaporation.

2.2. Elastomer Industry Wastewater

The elastomer industry wastewater was sourced from the nitrile rubber production process at the Brazilian Petrochemical Center in Duque de Caxias, Brazil. At the Multiuser Environmental Analysis Unit (UMAA), physical-chemical tests were conducted to characterize the elastomer wastewater. The wastewater exhibited specific physical-chemical characteristics, including a salinity of 0.9 PSU, pH levels ranging from 7.4 to 8.5, dissolved oxygen concentration of 5.4 $mg \cdot L^{-1}$, temperature recorded at 22˚C, and concentrations of 1700 μM dissolved ammonium (NH_4^+), 240 μM nitrite (NO_2^-) as well as less than 0.10 μM nitrate (NO_3^-) and less than 0.05 μM phosphate (PO_4^{3-}). These analyses were conducted in triplicate using specific methods: method 5220 for Ammonium determination, method 5201 for Nitrate determination, method 5200 for Nitrite determination, and method 5240 for Orthophosphate determination, all utilizing the FIAstar 5000 analyzer.

2.3. Experimental Design

Four treatments (70%, 80%, 90% and 100% concentration) of EW were made by adding ultra-pure water and salinized with artificial seawater (RedSea™).

Oligotrophic seawater was used as control treatment. There were four experimental replicates per treatment and all treatments were conducted under the same conditions of light, salinity and temperature that had been used in the acclimation period. The proportion of 2.0 g fresh weight·L^{-1} of macroalgae was used for each treatment. The experiment lasted (360 min), pH, temperature and salinity were monitored.

2.4. Bioremediation Calculation

The experimental design was established to measure the reduction in the concentration of ammonium ion (NH_4^+) for each treatment. The assessments were carried out with samplings at 0, 15, 30, 45, 60, 120 and 360 minutes. Aliquots of 20 ml sample of each treatment were removed and immediately filtered (0.52 µm GF/F-Whatman™) and placed in polyethylene bottles (25 ml) for subsequent analysis [34].

Ammonium bioremediation rates (ABR) and removal efficiencies (RE) were calculated based on the method described by Pedersen [35], as expressed in the following equation:

$$\text{ABR} = \frac{\left(S_t \cdot Vol_t\right) - \left(S_{t+1} \cdot Vol_{t+1}\right)}{B \cdot \Delta t} \quad (1)$$

$$\text{RE} = \frac{\left(S_{t+1} - S_t\right)}{100} \quad (2)$$

In these equations, S_t and S_{t+1}, represent the substrate concentrations before and after the sampling period (Δt), and Vol_t and Vol_{t+1}, denote the volumes before and after sampling. The approximate algal dry weight biomass (B) was set at 2.0 g.

To compare the efficiencies of each treatment, the data on ammonium bioremediation rates were analyzed using One-phase decay and Michaelis-Menten models. The One-phase decay model was represented by the formula:

$$C = \left(C_0 - \text{Plateau}\right) \cdot \exp\left(-\lambda \cdot X\right) + \text{Plateau} \quad (3)$$

where C is the bioremediation capacity, C_0 is the initial ammonium concentration, Plateau is a constant value of Y near or equal to zero, and λ is the rate constant in minutes.

The Michaelis-Menten model was expressed as:

$$V_i = \frac{V_{\max} \times \left[S_i\right]}{K_m + \left[S_i\right]} \quad (4)$$

where V_i is the velocity of the reaction at substrate concentration (i), $V_{\max}$ is the saturation velocity, $[S_i]$ is the substrate concentration, and K_m is the Michaelis constant. These models allowed for a detailed analysis of the efficiency of each treatment in terms of ammonium bioremediation rates.

2.5. Photosynthetic Analysis

To assess the photosynthetic health of the algae, the effective quantum yield of photosystem II (Y) was measured concurrently with the water samples using a submersible diving pulse amplitude-modulated (PAM) fluorometer (Walz™). This measurement was performed by chlorophyll-a fluorescence. The effective quantum yield (Y) was calculated using the Equation (5).

$$Y = \frac{\left(F_m' - F_t\right)}{F_m'} \tag{5}$$

where, F_m' is the maximum fluorescence in the light and F_t is the steady state of fluorescence in the light [36]. Additionally, biomass variation was assessed at the end of the experimental period to determine the specific growth rate (SGR). The SGR was calculated using the formula:

$$\text{SGR} = 100\ln\left(\frac{W_{t+1}}{W_t}\right)\cdot t^{-1} \tag{6}$$

where, W_t is the initial wet weight and W_{t+1} is the wet weight at time t (hour). This formula was derived from the method outlined by Lobban and Harrison [37].

2.6. Statistical Analyses

One-way analyses of variance (ANOVA) were conducted to compare the ammonium reduction by *Ulva lactuca* among different treatments, which varied by concentrations of elastomer wastewater. The same statistical procedure was applied to analyze the physiological parameters based on the effective quantum yield of PSII and specific growth rate (SGR). Subsequently, post hoc comparisons were carried out using multiple comparison Tukey tests to identify significant differences among the treatments, with a significance level set at $P < 0.05$.

3. Results and Discussion

Different parameters, including but not limited to desiccation, water velocity, and nitrogen limitation, can affect the growth rates and nutrient removal capabilities of seaweeds. This indicates that optimal conditions are essential for efficient bioremediation [38] [39] [40]. For example, *Ulva lactuca* has shown the highest reduction in nitrogenous compounds such as ammonia, nitrite, and nitrate in treated wastewater [41]. However, the efficiency of seaweed bioremediation for nitrogenous compounds in fish wastewater can be influenced by crucial parameters such as pH, dissolved oxygen, and biological oxygen demand (BOD) [41].

Throughout the experiment, the salinity, pH, temperature, and biomass parameters were evaluated daily. A significant effect of the salinized wastewater on the salinity concentration (**Figure 1A**) was observed on the second day compared to the control. Following the second day, the salinity concentration was gradually increased and stabilized on the fifth day, remaining in a range between 28% and 30%. Besides salinity, the pH of the medium was also influenced by different wastewater concentrations (**Figure 1B**). In the control treatment, the pH ranged between 7.9 - 8.0 on the first day, gradually increasing until it stabilized between 8.0 - 8.1 on the fifth day. On the other hand, a pH between 7.9 and 8.0 was maintained by the 70% and 80% wastewater during the five days. The 90% wastewater remained between 8.0 - 8.1, and the 100% wastewater remained in the highest pH range, 8.1 - 8.2. Regarding temperature (Figure 1C), a variation of just 1˚C was observed throughout the experiment, demonstrating the stability of the experiment. During the first four days, the temperature was between 19.5˚C - 20.5˚C; it was only on the fifth day that the temperature dropped and was between 19˚C - 20˚C. Finally, it was observed that variations in salinity can affect the biomass production of *U. lactuca* (Figure 1D). Similar to the salinity graph (Figure 1A), the biomass also reduced significantly on the second day but gradually increased again, coinciding with the increase of salinity at the end of the 5-day experiment. Although some variations in parameters were observed, the bioremediation performance of *U. lactuca* was not affected.

The pursuit of cost-effective and efficient methods for removing pollutants has spurred industries to promote the development of the bioremediation process. In this context, our study demonstrates that the application of *U. lactuca* is capable of effectively removing ammonium from elastomer wastewater over a short period of time (Figure 2A). Furthermore, the evident adaptation capacity

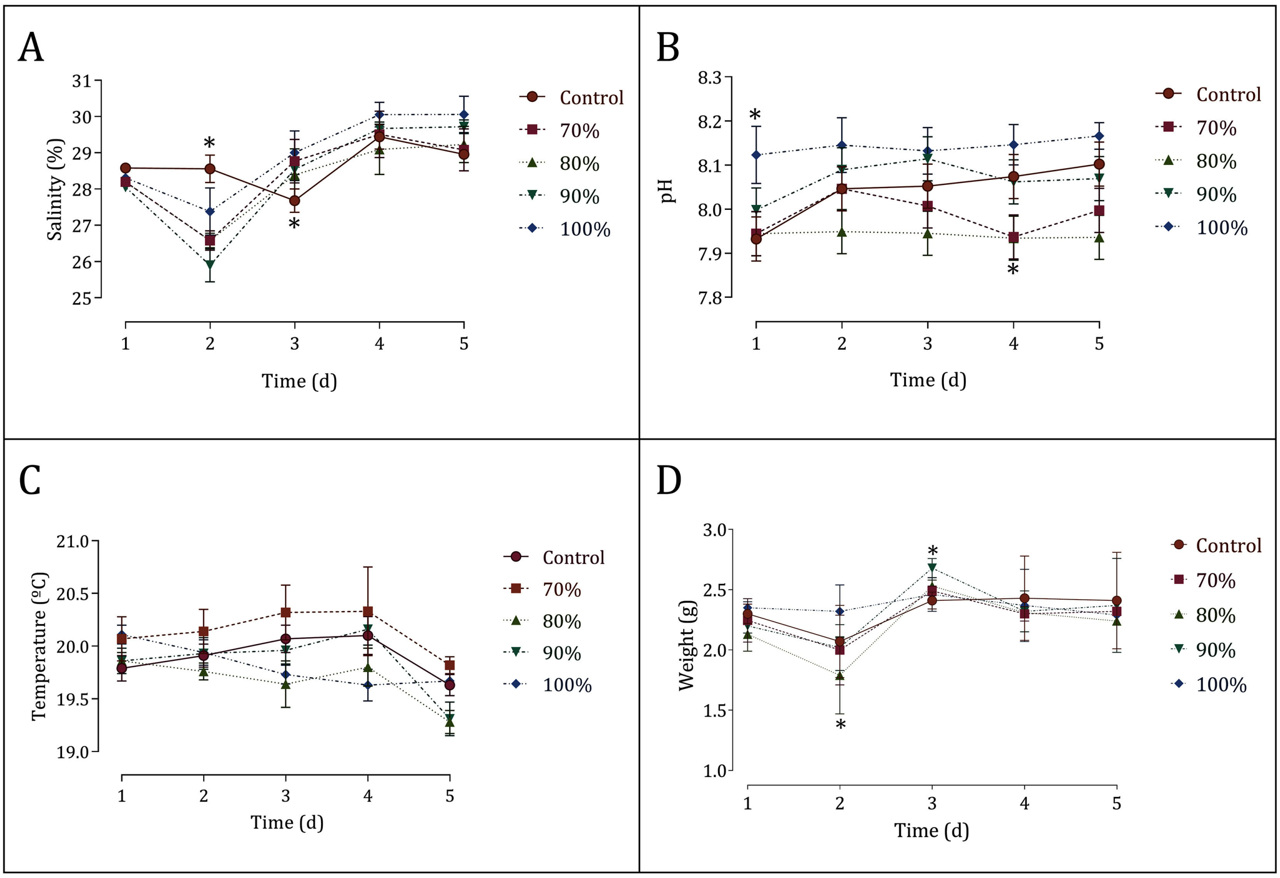

Figure 1. Variation of key parameters over the 5-day experiment period. (A) Salinity shows a significant change (P < 0.0001). (B) pH levels remain relatively stable (P = 0.7098). (C) Temperature exhibits a non-significant fluctuation (P = 0.3470; F = 5.4), and (D) biomass shows significant variation (P < 0.0001). Vertical bars represent ± standard deviation for the mean (n = 4).

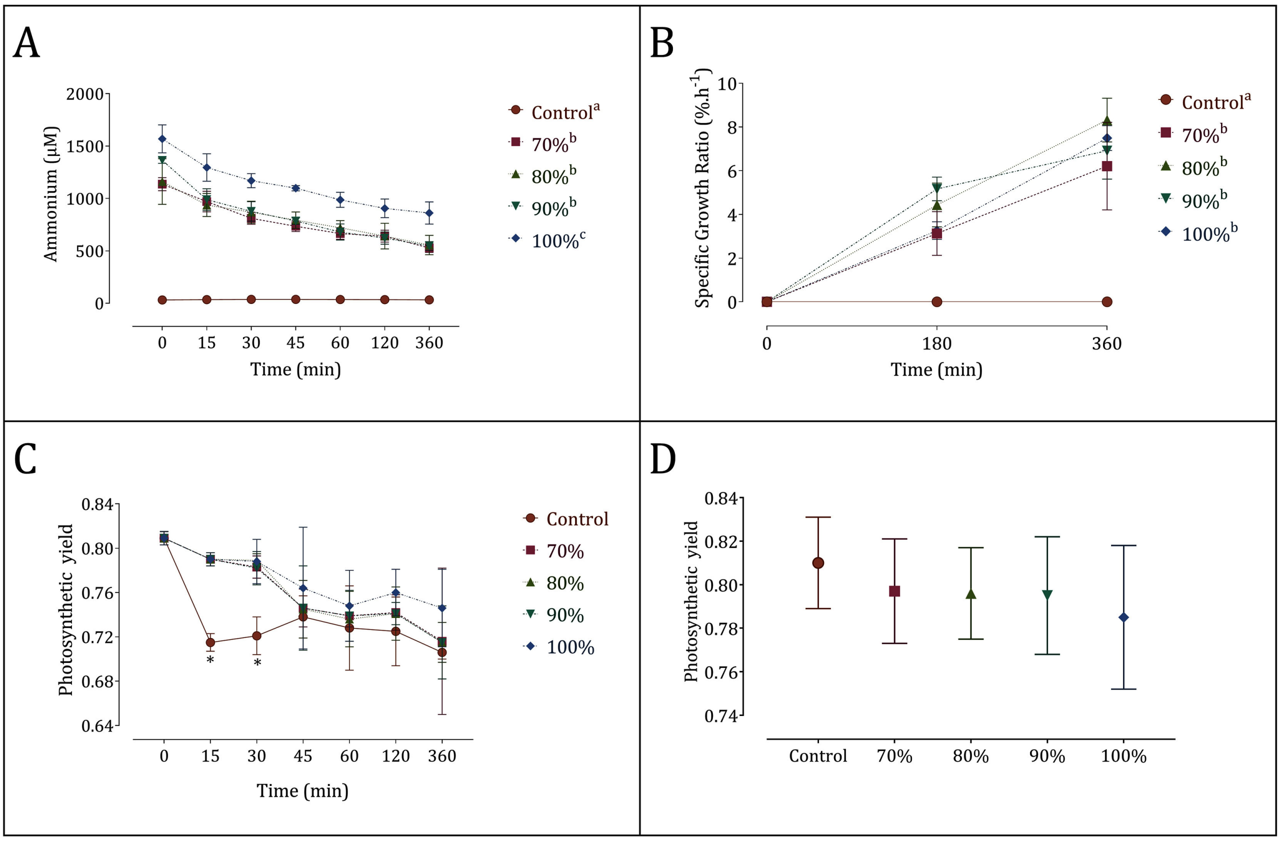

Figure 2. Evaluation of bioremediation performance. (A) Ammonium reduction by the macroalga *Ulva lactuca* in artificial water (control) and various concentrations of wastewater over a 360-minute period. (B) Specific growth of *U. lactuca* over the 360-minute experimental duration. (C) Effective quantum yield of Photosystem II (PSII) in *U. lactuca* across different wastewater concentrations during the 360-minute experiment. (D) PSII effective quantum yield of *U. lactuca* at the conclusion of the 5-day experiment. Vertical bars represent ± standard deviation for the mean (n = 4). Superscript letters indicate Tukey (ANOVA) results, with significant differences denoted as ($P < 0.0001$).

of *U. lactuca* is highlighted by the absence of any significant difference in the specific growth rate between the wastewater treatments and the control group. Additionally, our observations have revealed that the photosynthetic performance of *U. lactuca* decreased significantly after the first 15 minutes but only in the control treatment. This might have happened because the macroalgae from all treatments were moved from a medium composition of 0.2 μM NH_4^+, 1.5 μM NO_3^-, 14 μM PO_4^{3-} to a wastewater composition of 1700 μM NH_4^+, 240 μM NO_2^-, less than 0.10 μM NO_3^- and less than 0.05 μM PO_4^{3-}.

It has been observed that under high light conditions, the initial decrease in photosynthetic performance of *U. lactuca* during adaptation is influenced by the replacement of inorganic nitrogen (nitrate) by organic nitrogen (urea) [42]. Additionally, the growth, pigment content, and photosynthetic performance of *Ulva* species can be altered by the combined effects of nitrogen sources and salinity levels [43]. Therefore, it is crucial to choose the right nitrogen source and its concentration to determine the photosynthetic efficiency and overall performance of *U. lactuca.* Subsequently, there was no significant difference in the photosynthetic yield, both after 360 minutes (**Figure 2C**) and at the experiment's conclusion (**Figure 2D**), indicating the remarkable adaptability of *U. lactuca* to thrive in environments abundant in nitrogen compounds.

Ulva lactuca can employ several strategies to recover its photosynthetic efficiency, including adjusting the quantum yields of photosystem II [44], adjusting carbohydrate metabolism to utilize ammonia metabolism, and increasing fermentative metabolites [45], regulating antioxidant activity through superoxide dismutase and ascorbate peroxidase, along with phenolic compounds [46], utilizes the xanthophyll cycle and increases lutein concentration to recover photosynthetic performance [47], and by high nitrogen consumption [22]. These combined strategies allow *U. lactuca* to maintain and recover its photosynthetic performance in response to shifting environmental conditions.

The concentration of 2.0 g fresh mass·L^{-1} of *Ulva spp.* applied during this study removed a large amount (~1500 μM) of dissolved ammonium from the wastewater under all experimental conditions (**Figure 2A**). However, none of the treatments exhausted all the nitrogen within five hours, suggesting the need for an initial inoculum with more algal biomass or a longer time scale.

This concentration is often selected for bioremediation experiments due to its optimal balance between growth rate, nutrient uptake efficiency, and practical handling in aquaculture systems. Studies have shown that *Ulva* species, such as *U. ohnoi* and *U. fasciata*, exhibit significant growth and nutrient removal capabilities at this concentration, making it a practical choice for bioremediation in aquaculture effluents [28]. Additionally, this biomass concentration, also aligns with findings that indicate optimal growth and nutrient removal rates in integrated multi-trophic aquaculture (IMTA) systems, where *Ulva spp*. are used to mitigate nutrient loads from fish effluents [28] [29] [48]. Furthermore, this concentration has been effective in various environmental conditions, including different temperatures and nutrient levels, highlighting the adaptability and robustness of *Ulva spp*. for bioremediation purposes [22]. However, it's important to consider not only the weight of biomass, but also the volume of water used, as it can affect photosynthesis and nutrient absorption [49]. Exploring the relationship between biomass weight and time is crucial for refining the experiment and enhancing the results of large-scale analyses.

There were no significant differences ($P > 0.05$; **Table 1**) between the ammonium bioremediation rates for the 100%; 90%; 80%; and 70% (≈0.020 μM·g^{-1}·min^{-1}) treatments, showing that high concentrations of elastomer wastewater did not affect the rate of ammonium bioremediation by the algae, appearing to be constant for the different concentrations (**Table 1**). Additionally, a higher proportion of ammonium removal was recorded for the treatments 70%, 80% and 90% (≈67%; **Table 1**), which reiterates the capacity of *Ulva* in the treatment of this wastewater. Although it is difficult to provide unambiguous comparisons across other studies with *Ulva* species using this metric (due to the specificity of ammonium concentration present in the wastewater), we can consider that values above 60% would be significant for bioremediation processes. [50] also obtained a high percentage of ammonium reduction at different concentrations (6, 12, 25 and 100 μM NH_4) of ammonium in the reject water from anaerobically digested wastewater, in which all ammonium was removed during 18 days by *U. lactuca*. The same authors also obtained good bioremediation results at concentrations of 50 and 100 μM NH_4 representing 94% and 64% of removal respectively [50].

Table 1. Ammonium bioremediation rate ($\mu M \cdot g^{-1} \cdot min^{-1}$) and removal efficiencies (%) after 360 minutes of *Ulva lactuca* submitted at different elastomer wastewater. Different overwritten letters represent significant differences (ANOVA).

Treatment	ABR ($\mu M \cdot g^{-1} \cdot min^{-1}$)	RE (%)
Control	0.001 ± 0.001^a	0.08 ± 0.14^a
100%	0.020 ± 0.002^b	48.33 ± 6.64^b
90%	0.020 ± 0.001^b	67.24 ± 0.25^c
80%	0.018 ± 0.002^b	66.69 ± 5.52^c
70%	0.014 ± 0.001^c	68.38 ± 2.46^c

Standard deviation (±).

Applying numerical models that generate comparable parameters is one of the tools available to help with planning and obtaining the best estimates of a bioremediation process with saturable uptake kinetics. These models facilitate cross-study comparisons between wastewater, independent of the concentrations of the substance to be remediated. The results from the one-phase decay model analysis of ammonium bioremediation by *U. lactuca* offer valuable insights into the efficiency and dynamics of pollutant removal by this macroalgae species (**Table 2**). It was observed that the initial ammonium concentrations (C_0) decreased progressively from 29.66 ± 0.69 at 100% concentration to 19.84 ± 0.54 at 70% concentration. The final steady-state concentrations, or plateau values, were found to be lower with decreasing treatment concentration, ranging from 15.89 ± 0.54 for 100% concentration to 9.962 ± 0.50 for 70% concentration. The decay rate constants (K) indicated a slower decay at lower treatment concentrations, with values ranging from 0.0331 ± 0.01 at 100% concentration to 0.0232 ± 0.00 at 70% concentration. Correspondingly, the half-life and tau (τ) values increased as the treatment concentration decreased, indicating a longer time for ammonium to decay at lower concentrations. The spans, representing the overall reduction in ammonium concentration, decreased from 13.77 ± 0.82 at 100% concentration to 9.875 ± 0.68 at 70% concentration. High R^2 values, ranging from 0.8527 to 0.9369, suggested that the one-phase decay model fitted the data well across all treatment concentrations. In summary, the results suggest that higher concentrations of the treatment lead to more effective and faster bioremediation of ammonium by *Ulva lactuca*.

Table 2. Parameters of a one-phase decay model derived from the bioremediation rates of ammonium by *Ulva lactuca.*

One-phase decay values	100%	90%	80%	70%
C_0	29.66 ± 0.69	24.16 ± 0.59	22.06 ± 0.77	19.84 ± 0.54
Plateau	15.89 ± 0.54	10.45 ± 0.46	10.65 ± 0.65	9.962 ± 0.50
K	0.0331 ± 0.01	0.0330 ± 0.01	0.0284 ± 0.01	0.0232 ± 0.00
Half Life	20.94	20.94	24.35	29.84
Tau	30.21	30.22	35.13	43.06
Span	13.77 ± 0.82	13.71 ± 0.71	11.4 ± 0.95	9.875 ± 0.68
R^2	0.9172	0.9369	0.8527	0.8924

C0: Initial concentration; Plateau: Steady-state concentration; K: Rate constant; Half Life: Time to reduce concentration by half; Tau: Time constant; Span: Range between maximum and minimum concentrations; R^2: Coefficient of determination, measures fit of model to data. Standard deviation (±).

The growth rate of *U. lactuca* is significantly influenced by the type of nitrogen source available, with ammonia (NH_4^+) being a key factor in its growth dynamics [51]. Studies have shown that *U. lactuca* exhibits a rapid uptake of ammonia when it is the sole nitrogen source, which can lead to a quick production of protein-rich biomass [52]. This rapid uptake of ammonia and its positive effect on growth rates suggest that environments rich in ammonia could potentially support faster growth of *U. lactuca* compared to those with other forms of nitrogen [53] [54]. In general, high concentrations of dissolved ammonium favor cell absorption by passive diffusion, but frequently the rate of transport by this process is faster than diffusion and can saturate this pathway [55] [56] [57]. In this sense, treatments with lower initial ammonium concentrations could reach a plateau near ≈10 μM NH_4, favoring treatments with 90%, 80% and 70% strength wastewater.

The results from both **Table 1** and **Table 2** shed light on the intricate dynamics of *U. lactuca*-mediated bioremediation in elastomer wastewater with varying concentrations of ammonium. Interestingly, while the initial bioremediation rates (ABR) in **Table 1** indicate a robust start, with the highest rate observed at 100% concentration (0.020 ± 0.002 $\mu M \cdot g^{-1} \cdot min^{-1}$), the subsequent removal efficiency (RE) is notably lower compared to slightly lower concentrations (70% - 90%), where ABR values range from 0.014 to 0.020 $\mu M \cdot g^{-1} \cdot min^{-1}$. In contrast, **Table 2** provides a deeper understanding, revealing that although *U. lactuca* demonstrates a strong initial capability to process ammonium at higher concentrations, its capacity to sustain this process over time is compromised when faced with excessive pollutant levels. For instance, at 100% concentration, the initial ammonium concentration (C_0) is highest (29.66 ± 0.69 μM), but the plateau concentration remains higher (15.89 ± 0.54 μM), indicating a higher residual concentration compared to lower concentrations. Conversely, at lower concentrations, while the bioremediation process starts slower, *U. lactuca* exhibits a more sustained capability to reduce ammonium levels over time, resulting in higher removal percentages. These findings underscore the importance of considering both the initial bioremediation rates and the overall capacity to achieve significant pollutant reduction when evaluating the effectiveness of *U. lactuca* in bioremediation efforts.

Using the Michaelis-Menten model, which integrates all controls to determine the parameters V_m and K_m, values of 1342 μM·g^{-1}·h^{-1} and 84.4 μM were observed, respectively, up to sixty minutes (**Table 3**). These high values must be specified for the specific capacity to store large amounts of nitrogenous compounds [58]. In a study with *Sargassum hemiphyllum*, the use of seedlings grown in private environments with abundant N obtains higher rates than those cultivated in environments with abundant N, in part this is because algae with N limitation, with reduced rates pools of intracellular N, the initial increase in uptake rate represents a filling phase [49] [59]. In addition, we note that macroalgae sufferers were maintained for 96 hours in the absence of ammonia, or that favor the high values of these parameters.

Table 3. Kinetic parameters (V_{max}, K_s, V_{max}/K_s) of the Michaelis-Menten equation obtained from the absorption rates of *Ulva lactuca* for ammonium.

Parameters	N- NH_4^+
V_{max} (μM·g^{-1}·DMh^{-1})	1342
K_s (μM·L^{-1})	84.4
V_{max}/K_s	15.8
*DM-dry matter; R square 0.77	

Although other studies corroborate this uptake feature, values of V_m and K_m above 400 μM·g^{-1}·h^{-1} and 25 μM are not common in the literature [60] [61] [62]. Low values of Km show good absorption at low concentrations of nutrients (**Table 3**), in this study the concentrations of ammoniac nitrogen were very high, which influenced the high value of K_m. It has already been reported that algae found in eutrophic environments can achieve high V_m and K_m values [63]. The values for *U. lactuca* reported here confirm the viability of using this species for the treatment of elastomer wastewater. These findings indicate that this wastewater affected the photosynthetic functions, but not negatively as assessed by the minimum value required for photosynthetic maintenance. In general, nutritional increments are invariably reflected in photosynthetic processes and growth rates and long experimental periods may favor biomass gain [64] [65].

The kinetic parameters obtained from studying the ammonium uptake by *U. lactuca* were analyzed using the Michaelis-Menten model to determine the efficiency of this algal species in nutrient removal, particularly under conditions varying in ammonium concentration. The parameters measured were the maximum substrate removal rate (V_{max}), the half-saturation constant (K_s), and the efficiency ratio (V_{max}/K_s). The V_{max} recorded was 1342 $\mu M \cdot g^{-1} \cdot DMh^{-1}$, indicating a high maximal rate of ammonium uptake when the enzyme systems involved in the uptake are fully saturated. This value is substantial, suggesting that *Ulva lactuca* possesses a robust capacity to absorb ammonium when it is abundantly available in the environment. This characteristic is particularly beneficial for bioremediation applications where the bio load of ammonium is high, such as in eutrophic waters or in wastewater treatment facilities [66].

4. Conclusion

The results of this study highlight the ability of *Ulva lactuca* to bioremediate nitrogen-rich wastewater, particularly elastomer wastewater, through its ammonium uptake capabilities. The seaweed's ability to thrive and maintain high bioremediation performance despite fluctuations in environmental parameters such as salinity, pH, and temperature highlights its adaptability. Our findings indicate that higher concentrations of wastewater enhance the effectiveness and speed of ammonium removal, supported by strong kinetic data and one-phase decay model analysis. *Ulva lactuca* demonstrated no significant adverse effects on its photosynthetic yield, even when exposed to high ammonium concentrations, thanks to its adaptive strategies, including adjustments in quantum yields. These adaptations ensure sustained photosynthetic performance and growth under variable conditions. The study also emphasizes the need for optimizing initial biomass and treatment duration for large-scale applications to achieve complete nitrogen removal. In conclusion, our findings support *Ulva lactuca's* potential as a viable and efficient agent for the bioremediation of nitrogenous compounds in wastewater treatment, offering a cost-effective and environmentally friendly solution for managing industrial effluents. Future research should focus on fine-tuning the balance between biomass weight and treatment duration to maximize bioremediation outcomes on a larger scale.

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Availability of Data and Material

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Authors' Contributions

All authors contributed to the study's conception and design. Material preparation and data collection, by Camile Chaves. Writing, review, editing and statistical analysis were conducted by Diego Lelis and Thuane Anacleto. Writing, review and editing were carried out by Roberta Pereira. Supervision, funding acquisition, and final review were undertaken by Alex Enrich-Prast and Vinicius Peruzzi. The first draft of the manuscript was written by Camile Chaves, but the second and final version of the manuscript was written by Diego Lelis. All authors read and approved the final manuscript.

Competing Interest

All authors certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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