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Removal of Emerging Contaminants using Microalgal Biomass

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Emerging contaminants are chemical compounds present in the environment, particularly in aquatic systems, which are currently not subjected to regulatory measures. This problem occurs when pollutants are introduced into aquatic systems through multiple sources, including wastewater discharge, agricultural runoff, and atmospheric deposition. Products that contain emerging contaminants are classified into 3 categories: pharmaceuticals, personal care products, and endocrine-disrupting chemicals. Several organisms are affected by the accumulation of the chemicals in water bodies. These emerging contaminants have been found to alter the behaviour and development of fish and organisms that form the base of the aquatic food chain. Additionally, they interfere with the ability of organisms to reproduce and grow appropriately. In the present study, a series of experiments were performed using *Stigeoclonium nanum* microalgal biomass to determine the removal efficiency of one of these contaminants, that is, caffeine. Experiments were performed in duplicate using 20 mL tubes. A 500 mg L^{-1} caffeine solution was prepared and applied at different doses at the same time as a known amount of dry biomass. The tubes were maintained at a constant temperature of 24 ± 2 °C with a stirring speed of 400 rpm. Optical absorbance readings were recorded, and calculations were performed to determine the removal percentage for each caffeine dose. At concentrations of 50, 100, and 125 mg L^{-1}, 100% removal was reached, while at 250 and 500 mg L^{-1}, removal was reduced to 79.76 % and 49.66%, respectively. These data indicated a continual decrease in caffeine up to 125 mg L^{-1}, suggesting a possible saturation point for the dry biomass used over this concentration range. Furthermore, these results emphasise the necessity of conducting adsorption isotherms to understand the relationship between caffeine concentration and its removal efficiency using microalgal biomass. This could lead to the optimisation of contaminant removal, especially at higher concentrations. Although the microalgal biomass of S. nanum appears to be effective in removing caffeine at moderate concentrations, more comprehensive experiments surrounding adsorption isotherms are required to enhance efficiency at higher concentrations.

1. Introduction

Emerging pollutants are chemical compounds that are not considered by actual environmental regulations in most countries worldwide, and for which no disposal policies have been developed. These molecules can be organic or inorganic and can easily enter the environment, causing pollution problems for flora and fauna (Gogoi et al., 2018). Emerging pollutants include diverse molecules used in pharmaceuticals, personal care, and daily-use products. Some of these compounds have toxic effects on primary producers, crustaceans, fish, and other organisms in aquatic environments, and even on human health (Vázquez-Tapia et al., 2022).
Recently, researchers have reported modifications to the growth, behaviour, fertility, and fecundity of fish and other microorganisms in the food chain, with a high risk of magnification through the same food chain (Omar et al., 2019). One chemical compound considered for daily use is caffeine, a natural alkaloid present in green, red, and black tea, coffee, and chocolate. Caffeine directly affects the central nervous system and the cardiovascular system and its abuse may cause hyperactivity, depression, and other health issues (Glade, 2010; Graham, 2009; Mersal, 2012). Research published by Rybak et al., (2015) reported that urine is the principal source of caffeine in wastewater and, consequently, in the environment. Moreover, there are discharges by industries, but

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the total amount is lower. It is important to highlight the fact that wastewater treatment plants (WWTPs) are not designed to eliminate emerging pollutants (Froehner et al., 2011).
Many technologies have been developed to remove, eliminate, or degrade emerging pollutants, including WWTPs. However, their elimination is not efficient. This is because of their low concentrations in wastewater, physicochemical properties, and toxicity (Maryjoseph & Ketheesan, 2020). Another risk derived from the presence of emerging pollutants in wastewater is the possible production of degradation-derived compounds. In some cases, these compounds have a longer half-life and are even more toxic than their original compounds (Hillebrand et al., 2012).
The development of technologies with high removal efficiencies and low risk in the presence of products derived from the degradation of emerging contaminants is stimulated by the current deficiencies of biotechnological processes that focus on degrading them (Xiong et al., 2021).
In particular, the microalgae *Stigeoclonium nanum* is a filamentous green eukaryotic microalga that can grow in large photobioreactors and in various growth media including sewage and industrial waste. The easy and economical production of biomass makes it a potential biosorbent for eliminating emerging contaminants in liquid effluents. (Figure 1).

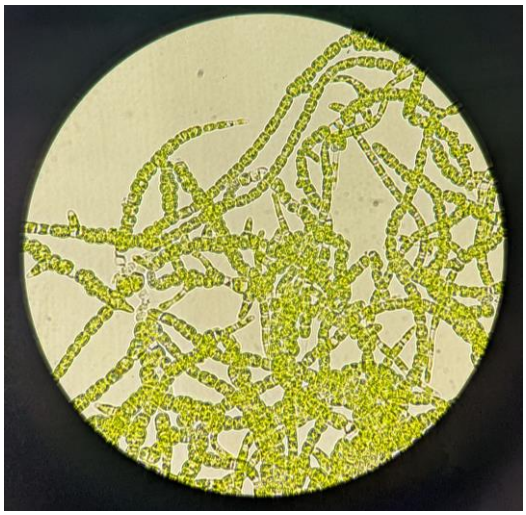

Figure 1: Cellular configuration of S. nanum (40 × magnification).

2. Materials and methods

2.1 Microalgae cultivation

The microalga *S. nanum* used in experiments was a native strain isolated by Bueno-Ramos et al., (2017) and was cultured in BG11 mineral medium for 8 d in an airlift flat- plate photobioreactor with an operational volume of 15 L, according to the methodologies reported by Martínez-Roldán et al., (2019). At the end of growth, the biomass was collected by sedimentation and centrifugation and then dried in an oven at 60 °C for 96 h. The obtained biomass was ground in a porcelain mortar, sifted, and reserved for use in subsequent adsorption experiments.

2.2 Adsorption experiments

Adsorption experiments were carried out using caffeine (Sigma ®) in a laboratory flask with an operational volume of 20 mL with constant 400 rpm rotary mixing and maintained at a constant temperature (24 ± 2 °C). After 72 h, the initial caffeine concentrations were 50, 100, 125, 250, and 500 mg L^{-1}, and the biomass concentration was 5 g L-1 under all experimental conditions. At the end of the experiments, the residual caffeine concentration was quantified using the methodology reported by Pelozo et al., (2008), consisting of spectrophotometric quantifications using 0.2 M HCl as the solvent.

3. Results and Discussion

The production of *S. nanum* biomass during growth experiments was consistent with that reported by other authors regarding this microalgae (Velasco-Flores et al., 2018). Figure 2 illustrates that the initial biomass

concentration was 0.2 ± 0.02 g L^{-1} and increased until it reached a maximum of 1.1 ± 0.05 g L^{-1} on the seventh day and exhibited a slight reduction at the experiment. Biomass productivity was calculated as biomass production after 7 d of growth, which was 133 mg L^{-1} d^{-1}.

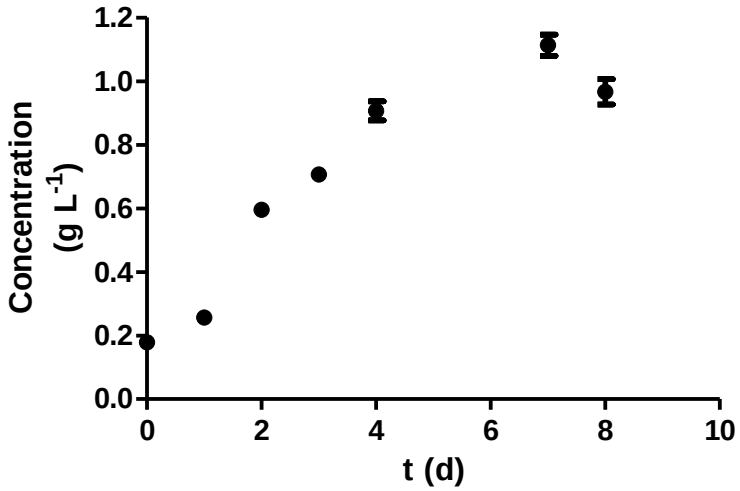

Figure 2: S. nanum biomass production in flat plate photobioreactor.

Figure 3 shows that the adsorption process was rapid and significant differences were observed after the first hour. Furthermore, the amount of residual caffeine in the supernatant decreased continuously throughout the experiment. Notably, after 96 h, the decrease in the residual amount of caffeine was minimal, which indicates that the system began to reach an equilibrium at that point, with a final concentration of caffeine in the supernatant of 35.38 ± 2.19 mg L^{-1}.

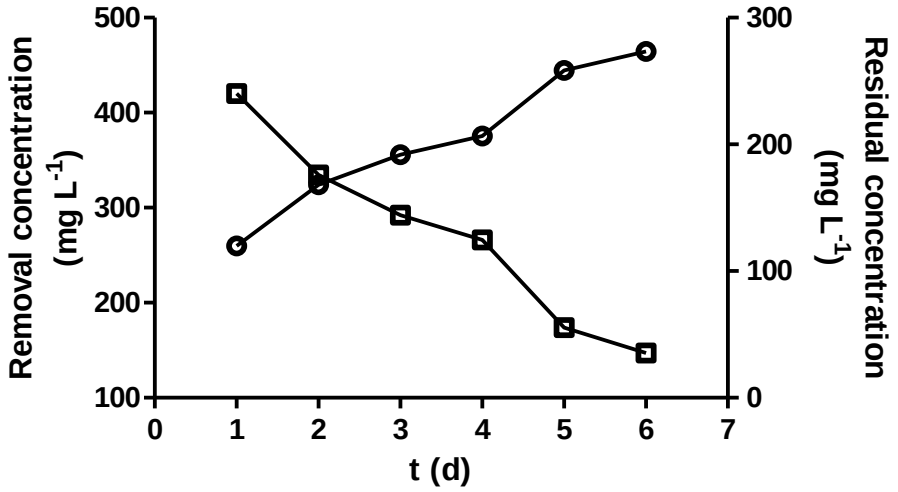

Figure 3: (○) Removal concentration of caffeine and (□) Residual concentration of caffeine.

Figure 4 shows residual caffeine concentrations assayed under different experimental conditions and total removal of the contaminant at lower concentrations. The experiments at 50, 100, and 125 mg L^{-1} achieved total caffeine removal in 72 h, and when the concentration reached 250 and 500 mg L^{-1}, the presence of residual caffeine was detected as reaching values of 33.0 and 251.6 mg L^{-1}, respectively. This indicated a saturation point at the active sites of the biomass at higher caffeine concentrations. Figure 4 also shows the removal efficiency, which reached almost 100% for 50, 100, and 125 mg L^{-1} caffeine. In experiments involving 250 and 500 mg L^{-1} caffeine, the removal efficiency decreased, reaching values of 79.7% and 49.7%, respectively.

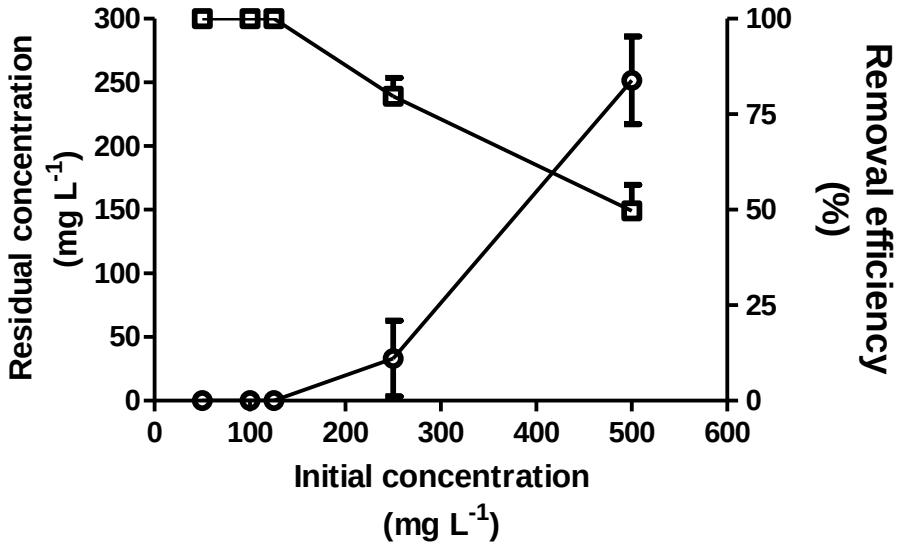

Figure 4: (○) Residual caffeine concentration and (□) caffeine removal efficiency.

Figure 5 shows the amount of caffeine adsorbed per gram of biomass. The lowest adsorption values were obtained in experiments involving lower caffeine concentrations. The ratio between caffeine removed and biomass used was non-linear, which is typical in adsorption and absorption processes. This behaviour indicates a limited capacity of the microalgal biomass to adsorb caffeine, which may be caused by saturation of active sites on the biomass surface.

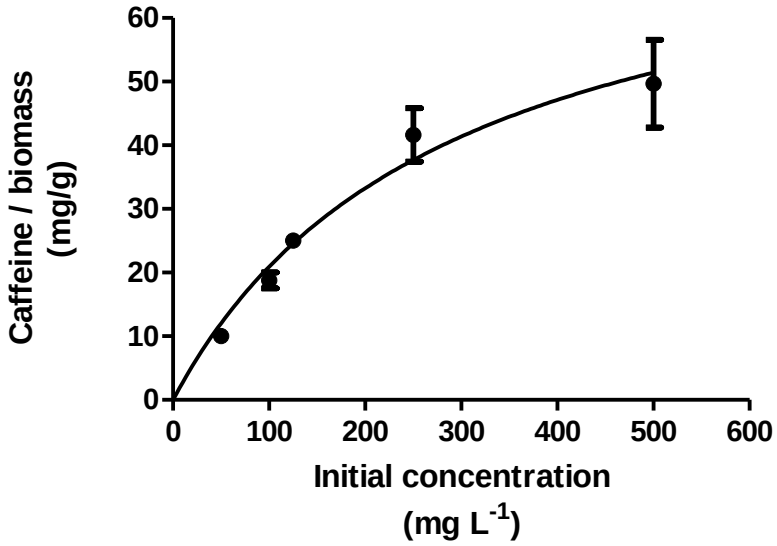

Figure 5: Amount of caffeine adsorbed per gram of biomass

The classic model used to describe the adsorption process is the Langmuir isotherm because caffeine adheres to the external surface of the dry biomass of the microalgae. This is due to interactions involving charges that exist between hydroxyl and carboxyl groups of the microalgal cell wall and the charge of the contaminant. However, the experimental conditions did not allow for adjustments. Nevertheless, the experimental data shown in Figure 5 are consistent with the Michaelis-Menten model because increases in the initial concentration of caffeine caused the adsorption system to reach saturation point, similar to that observed with enzymes in biological systems.

The data presented in Table 1 show the parameters obtained by fitting the Michaelis-Menten model. In this context, Qmax corresponds to the maximum adsorption capacity, whereas Km reflects the initial concentration at which the system achieves 50% of its maximum adsorption potential. The Qmax value was 80.91 mg caffeine

per gram of biomass. The Michaelis-Menten fit provided a satisfactory description of the adsorption process, supported by a regression coefficient of 0.86, which is in agreement with data reported by Zambrano et al., (2021), where adsorption experiments were carried out with the dry biomass of *Scenedesmus almeriensis* to remove tetracycline, ciprofloxacin, sulfadiazine, and sulfamethoxazole. The reported values of the maximum adsorption capacity with a Langmuir fit for each compound were 0.83, 0.52, 0.26, and 0.01 mg of contaminant per gram of biomass, and the regression coefficients were 0.99, 0.99, 0.99, and 0.96, respectively.

Table 1: Fit parameters to a Michaelis–Menten type model

Parameter	Value	Units
Q(max)	80.91	$mg_{caffeine}\ g_{biomass}^{-1}$
50 % Q(max)	286.8	$mg_{caffeine}\ L^{-1}$
R^2	0.8664	

Francoeur et al., (2021) described in their research the use of activated carbon from Sargassum to remove caffeine from aqueous systems. The reported Qmax value was 212.07 mg of activated carbon from Sargassum per gram of caffeine, with a correlation coefficient of 0.96. Although the values obtained in the present study are lower than those reported previously, they can be related and compared because of the rapid adsorption of caffeine in the first few minutes in both investigations. The research carried out with Sargassum revealed a removal percentage of 98.2% in the first 30 min of experiments, with an initial concentration of 20 mg L^{-1}. Other studies have reported that equilibrium is reached between 1.5 and 4 h (Anastopoulos & Pashalidis, 2019; Beltrame et al., 2018; Couto et al., 2015). Nevertheless, depending on the adsorbent used in each study, equilibrium can be reached within 24, 40, or even 90 h (Oliveira et al., 2019).

4. Conclusions

The dry biomass of *S. nanum* has demonstrated remarkable efficiency as a biosorbent for caffeine removal, particularly at concentrations below 125 mg L^{-1}, with removal rates approaching 100%. Michaelis-Menten model fitting indicated a saturation threshold of approximately 80 mg of caffeine per gram of dry biomass. However, these experiments were conducted using distilled water. This opens new avenues of research for further exploration, including testing under varying conditions, such as simulated or actual samples, which may better mimic real-world scenarios, such as wastewater. It may be possible to optimise the adsorption process by subjecting the biomass to different salinity levels, adjusting the pH, or introducing other relevant compounds. These modifications have the potential to activate functional groups within the cell wall of S. nanum, leading to enhanced caffeine adsorption capacity. Future studies exploring such variations could provide valuable insights into maximising the efficacy of *S. nanum* as a biosorbent for practical applications.

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