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The First Evidence of the Water Bioremediation Potential of *Ficopomatus enigmaticus* (Fauvel 1923): From Threat to Resource?

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Abstract: Each year, a staggering 700,000 tons of synthetic dyes are manufactured globally, leading to the release of dye-laden wastewater into aquatic systems. These synthetic dyes resist biodegradation, endangering human and environmental health. Since traditional wastewater treatments are basically unable to remove dyes, exploring the potential of alternative solutions, such as bioremediation, is crucial to reduce dye contamination in aquatic ecosystems. *Ficopomatus enigmaticus* (Fauvel 1923), listed as one of the 100 worst invasive species in Europe, is considered an invasive ecosystem engineer capable of causing economic and ecological losses. Despite this negative status, the literature suggests its positive contributions to aquatic ecosystems as habitat former and water bioremediator. However, existing evidence on the potential of *F. enigmaticus* to improve water quality is fragmented and lacks experimental data from laboratory tests. This study examined the potential of *Ficopomatus* reefs, both living and dead, to enhance water quality by removing contaminants, focusing on methylene blue (MB), one of the most common synthetic dyes. Bioaccumulation and bioadsorption were identified as key mechanisms for dye removal, supported by ATR-FTIR and microscopic analyses. *Ficopomatus* efficiently removed up to 80% of MB within 24 h. Bioaccumulation in the soft body accounted for 18% of the total removal, while complex adsorption phenomena involving carbonaceous, microalgal, and organic reef components accounted for 82%. Surprisingly, bioremediated solutions exhibited significant effects in ecotoxicological tests on bacteria, indicating the potential of *F. enigmaticus* to disrupt bacterial quorum sensing related to biofilm formation, and suggesting a possible antifouling action. This study underscores the intricate interplay between *F. enigmaticus*, water quality improvement, and potential ecological consequences, stressing the need for further investigation into its multifaceted role in aquatic ecosystems.

Keywords: bioremediation; water purification; methylene blue; synthetic dye; bioadsorption; bioaccumulation; ecosystem service; invasive species; antifouling; toxicity

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1. Introduction

Ficopomatus enigmaticus (Fauvel, 1923) is a tube-building polychaete worm belonging to the family Serpulidae. The origin of *F. enigmaticus* still remains unknown, although most probably, it is native of temperate regions of the Indian Ocean [1]. Today, *F. enigmaticus* is found worldwide, between the northern hemisphere isotherm of 16 °C and the 21 °C isotherm of the southern hemisphere [2], preferentially inhabiting coastal brackish water lagoons at temperate latitudes. *F. enigmaticus* was discovered in 1921 in northern France by Fauvel (1923) [3] and it is listed as one of the 100 worst invasive species in Europe [4]. However, there are currently no unified strategies for its control or management. This species exhibits a fast growth rate and high tolerance to variable environmental conditions,

leading to significant ecological impacts. It hosts a pollution-resistant invertebrate community [5] and alters the surrounding environmental conditions by modifying water flow regimes, affecting sediment grain size [6], and enriching the sediment with organic carbon and nitrogen compounds through the release of *feces* and *pseudofeces* [7].

The proliferation of this species may also result in economic losses as it can negatively impact ships, buoys, and harbor structures [1] and build extensive reefs that can physically impede navigation and hinder the deployment of fishnets [8]. There is an ongoing debate about the invasiveness or naturalization of this species and, despite its putative negative effects, *F. enigmaticus* has also been suggested to enhance the status of aquatic ecosystems, acting as a habitat former. Indeed, serpulid reefs are generally considered a biodiversity hotspot capable of supporting a higher abundance and diversity of associated fauna [9–11]. For example, *Ficopomatus* reefs not only provide refuge, substrate, and feeding ground to a number of aquatic invertebrates, but may also serve as resting sites for some birds, such as swans [12]. A further effect of the presence of large populations of *F. enigmaticus* may concern the improvement of water quality by reducing the organic and nutrient load of water bodies as a result of their filter-feeding activity [13,14]. However, the potential role of this species as a bioremediator has been more advocated rather than actually documented.

In this study, experiments in aquaria were carried out to explore the ability of *Ficopomatus* reefs (both living reefs and their dead biogenic structures) to enhance water quality and remove harmful substances from the aquatic medium. We focused on the synthetic dye methylene blue (MB) as an emblematic example of aquatic contaminants. Every year, about 7×10^5 tons of synthetic dyes are produced worldwide, the majority of them being currently used in the textile industry, totaling approximately ten thousand varieties [15–17]. Dyes deteriorate the environmental quality of water bodies by increasing the biochemical and chemical oxygen demand, hindering photosynthesis, inhibiting plant growth, entering the food chain, displaying resistance and bioaccumulation, and potentially contributing to toxicity, mutagenicity, and carcinogenicity in aquatic organisms [17–19]. MB is one of the most used substances in the dye industry, which is commonly used to dye silk, wool, cotton, and paper [18]. Moreover, the food, cosmetics, and pharmaceutical industries also contribute significantly to the use of MB [15,20,21]. Given its extensive industrial usage, a substantial volume of wastewater containing MB is discharged into groundwater and surface water (including brackish, marine, and freshwater bodies), since conventional water treatment processes are typically unable to remove dye contamination [22]. Once released into the environment, MB and dyes in general can persist for a long time due to their high stability against degradation from temperature, light, and water actions, posing severe threats to aquatic environments and human health [15].

We also investigated the possible mechanisms underlying the process of MB decontamination mediated by *Ficopomatus* reefs, including both bioaccumulation and bioadsorption, and tested the toxicity of the bioremediated aquatic medium to understand whether *Ficopomatus* reefs may effectively remove and detoxify cationic dyes, as well as to shed light on their potential contribution to water purification functions in aquatic ecosystems.

2. Materials and Methods

2.1. Animal Sampling and Acclimatization

Ficopomatus enigmaticus was collected in May 2023 within a brackish coastal lake near Koper (Slovenia) recognized as having an established population of this species (45°32′29″ N 13°43′24″ E [23]). Once in the laboratory, the animals were kept in 6 L aquaria filled with 0.45 µm filtered natural seawater at reduced salinity (20 ppt, f-NSW-20; pH 8.0 ± 0.1) and maintained under constant environmental conditions conducive to the well-being of the animal (temperature: 20 °C; light: 12 h/12 h; weekly feeding with *Isochrysis galbana*) for 40 days. During this depuration period, the water was changed weekly and the associated vagile fauna were carefully removed to prevent potential interference in the experiments.

One day (24 h) before the start of the experiment, the main reef was divided into small blocks, each containing approximately 60 individuals (corresponding to 13.22 ± 1.12 g of wet weight, mean ± standard deviation), in analogy to other ecotoxicological studies focusing on *F. enigmaticus* adults (e.g., Ref. [24] used 50 g of reef per 1 L of solution, which corresponds to the 12–13 g of reef per 250 mL used in this study). These blocks were transferred to 250 mL glass beakers for a 24 h acclimatization under the same experimental conditions described earlier (20 °C; 12:12 light/dark; pH 8.0 ± 0.1; continuous stirring at 200 rpm). No food was provided 48 h before or during the experiment.

2.2. Methylene Blue Solution

The tests were performed with an MB (No. M 9140, Sigma-Aldrich, St Louis, MO, USA) solution prepared with f-NSW-20. MB is a well-known primary thiazine, a highly water-soluble cationic dye [18]. MB has a characteristic deep-blue color when oxidized and it is colorless in the reduced form [25]. Therefore, the color attenuation of the MB water solution in the experimental treatments, expressed in terms of the reduced optical density (OD), can be used as an indirect measure of the MB removal capacity of the tested organisms [26,27]. The maximum absorbance of the MB solution was investigated by scanning different sample solutions (0.5–5–10–15–20–25 mg/L) between 400 and 800 nm using a UV–visible spectrophotometer (DU 730, Beckman Coulter, Brea, CA, USA). In agreement with previous evidence [26,27], the maximum absorbance value for each concentration is achieved at λ = 660 nm, which was the value used to perform the experiment.

2.3. Bioremediation Experiment

In the experiments, both living and dead reefs of *F. enigmaticus* were used for the comparison of their bioremediation capacity in order to disentangle the contribution of bioaccumulation associated with the soft body of the animals and their biogenic hard structures. Concentrations of MB ranging from 50 to 200 mg/L are generally well tolerated by aquatic organisms in analogous experiments [26–28]. However, since the potential toxicological effects of these concentrations on *F. enigmaticus* are unknown, a precautionary approach was preferred and the test concentration was reduced to 25 mg/L, with a total exposure time of 24 h, to prevent excessive stress for the animals.

For the experiment on living reefs, eight small blocks of *F. enigmaticus* (approximately 460 individuals in total) were randomly selected and submerged in 200 mL of a filtered seawater solution of MB at a concentration of 25 mg/L in separate 250 mL glass beakers. The samples were kept in constant agitation (200 rpm) for 24 h at 20 °C. For the experiment on dead reefs, since the physical removal of the worms from their tubes would have damaged the bioconstruction, dead reefs were obtained by drying the living reef blocks for 20 h at a constant temperature of 40 °C to kill the worms while leaving untouched their biogenic structure. Five random replicate blocks of dead reefs were then used for the experimental exposure to MB following the exact same protocol as for the living reefs.

Methylene blue is a widely recognized cationic thiazine dye that is highly water-soluble and exhibits a deep-blue color when solubilized. Consequently, it is customary in the literature to employ color attenuation, expressed in terms of optical density (OD), as an indicator of methylene blue removal capacity [26,27]. Accordingly, the ability of *F. enigmaticus* to remove MB from the aquatic medium was evaluated spectrophotometrically using a UV/Vis spectrophotometer (DU 730 Beckman Coulter) by monitoring, at regular intervals (0–1–2–4–24 h), the decrease in the optical density of the water at a wavelength of 660 nm. In each time of sampling, three replicates of the treatment solution of 3 mL each were sampled in the center of the beaker with constant stirring. After the completion of the spectrophotometric measurements, the samples were returned to their respective beakers to prevent an excessive drop in the water level during the whole experiment.

A negative control with living reef blocks in beakers filled with filtered natural seawater at 20 ppt salinity (hereafter referred to as CN, with five replicates) was implemented. This control was used to evaluate possible spectral interferences due to the release of mucus

under the experimental conditions and to correct, through subtraction, the optical density (OD) of the MB-treated samples. A second positive control, consisting of beakers filled with 25 mg/L of the MB seawater solution without animals (hereafter referred to as CP, with four replicates) was used to evaluate the contribution of the possible light photo-degradation process [27] and the possible changes in the solubility of MB in a solution with a high ionic load (i.e., seawater at 20 ppt salinity).

The adsorbent dose, pH, initial MB concentration, and temperature are known to be operating conditions and parameters that can influence the uptake of MB on various adsorbents [15]. For these reasons, the mass of the *Ficopomatus* reef, the initial concentration of the contaminants, and the temperature were maintained constant over the 24 h of exposure (i.e., 13.22 ± 1.12 g of wet weight, 25 mg/L, and 20 °C, respectively). The pH of the dye solutions was checked at the beginning and end of the experiments; a slight average decrease in pH (<0.5 units) was recorded at the end of the experiments for all treatments (Table S1 in Supplementary Material).

2.4. Expression of the Bioremediation Performance

The ability of *F. enigmaticus* to remove MB was assessed by calculating both the decolorization percentage (*D*%) and the quantity of dye eliminated per unit of biomass at time *t* (*qt*).

The decolorization percentage was calculated using Equation (1) [19] as follows:

$$Decolorization\ \% = D\% = \left(\frac{A_{CP} - A_S}{A_{CP}}\right) \times 100 \quad (1)$$

where A_{CP} is equal to the absorbance at *t* hours of the positive control; A_S is the absorbance at *t* hours of the samples (living or dead); and both corrected by the negative control (CN) (i.e., by subtracting the absorbance of CN from the absorbance of A_{CP} and A_S).

As the decolorization rate expressed in Equation (1) may be affected by the volume of water and grams of biomass present in the experimental unit, we also calculated the amount of dye eliminated per unit of biomass at time *t* using the Formula (2) [29] as follows:

$$qt = \left(\frac{C_{CP} - C_S}{m}\right) \times V \quad (2)$$

where *V* is the volume of water in the beaker (200 mL); *m* is the wet weight in grams of reef (both for living and dead); C_{CP} is the positive control concentration after *t* hours; and C_S is the MB concentration after *t* hours. The optical density was converted to MB concentrations using an equation obtained by fitting a linear regression of MB concentrations against OD (ranging from 0.5 to 25 mg/L, R^2 = 0.978).

2.5. Reef Surface Characterization

The reef surface was observed under a stereomicroscope (Nikon—P-DSL32, Landsberg, Germany), and high-definition images were captured with a digital camera (Nikon—DSFi3, Landsberg, Germany). Since a strong MB adsorption was observed, the surface of the reefs was analyzed by means of attenuated total reflectance (ATR) Fourier-transform infrared spectroscopy (FTIR), and collected spectra were matched with reference materials in order to identify the components likely involved in the adsorption process. The infrared spectra were recorded within the 3800–800 cm^{-1} range using an FTIR spectrometer from ThermoFisher—iN10 MX (Waltham, MA, USA). The ATR-FTIR analysis was carried out only on dead reefs. Tube samples from a single, randomly selected reef were pulverized with the aid of an agate pestle and mortar and placed on glass supports for the acquisition of the spectrum via ATR analysis with germanium iridium crystals. The spectra obtained were analyzed using the OMINC Software (version 8.2.0.403), applying components through the matching of libraries acquired from ThermoFisher and internally generated at the BsRC laboratory.

2.6. Toxicity Test

The toxicity of the solutions bioremediated by living and dead *Ficopomatus* reefs was assessed using a bioluminescence inhibition assay that employed the marine Gram-negative bacterium *Aliivibrio fischeri* as the test species. *A. fischeri* is known to be sensitive to a wide range of toxicants and is used in standard acute bioassays [30–32]. Light production is directly proportional to the metabolic activity of the bacterial population, and any inhibition of enzymatic activity due to toxicity or cell death results in a corresponding decrease in the bioluminescence produced by the colony.

Test samples were exposed to freshly prepared bacteria (NRRL B-11177 strain and vial from internal propagation) for a predetermined period (15 and 30 min), and the degree of light emission inhibition was compared to a negative control. This assay provided a measure of both the sub-lethal response and lethality through the degree of inhibition of bioluminescence.

More specifically, a standard Microtox® acute assay was performed following a procedure that is sensitive to changes in bioluminescence and yields highly reproducible results (UNI EN ISO 11348-2:2019 [32]). Briefly, MB and bioremediated MB solutions were tested alongside positive controls (3.5- Dichlorophenol) and negative controls (ASW, artificial sea water, UNI EN ISO 11348-2:2019 [32]. Each treatment was run in duplicate, and the relative bioluminescence was measured using a Microtox® Model 500 Analyzer (ModernWater, 5003054, New Castle, DE, USA) after an incubation period of 15–30 min. Bioluminescence inhibition was expressed as the percentage of the initial concentration reduction in luminescence compared to the luminescence intensity of the control sample.

2.7. Statistical Analysis

A statistical analysis was performed using the GraphPad Prism software (GraphPad Software version 6, San Diego, CA, USA,). Data on the bioremediation performance are represented as mean ± standard deviation. The normality of the data was previously examined using a D'Agostino and Pearson tests, and the homoscedasticity was checked. Consequently, the statistical significance ($\alpha = 0.05$) of the comparisons between living and dead reefs was studied using parametric or nonparametric (Mann–Whitney) unpaired *t*-tests depending on the case. Data on the bioassay are expressed as mean ± standard deviation.

3. Results

3.1. Bioremediation Experiment

MB was partially removed by both living and dead reefs. The changes in color of the solutions, the reef structure, and the naked worm between the start ($t = 0$) and after 24 h of exposure were clearly visible for both the living reef (Figure 1) the dead reef samples (Figure 2).

For both the living and dead reefs, the percentage of removal (D%) increased over time (Figure 3). The living reefs were capable of removing 32.75% of the dye in the first hour, with the maximum percentage of decolorization, 80.43%, achieved after 24 h (Table 1). The dead reefs were less efficient, reaching a maximum percentage of dye removal (64.61%) after 24 h. The performance of the living and dead reefs was significantly different at all times ($p < 0.05$), with the largest differences in decolorization observed at 1 h (17.70%, $p = 0.0016$).

The capacity of dye removal per unit of biomass at time t (qt) reached 0.53 mg/g and 0.31 mg/g after 24 h for the living and dead reefs, respectively (Table 1). The performance of the living and dead reefs was always statistically different ($p < 0.05$), with the largest difference in dye removal per unit of biomass observed at 24 h (0.22 mg/g, $p = 0.0016$).

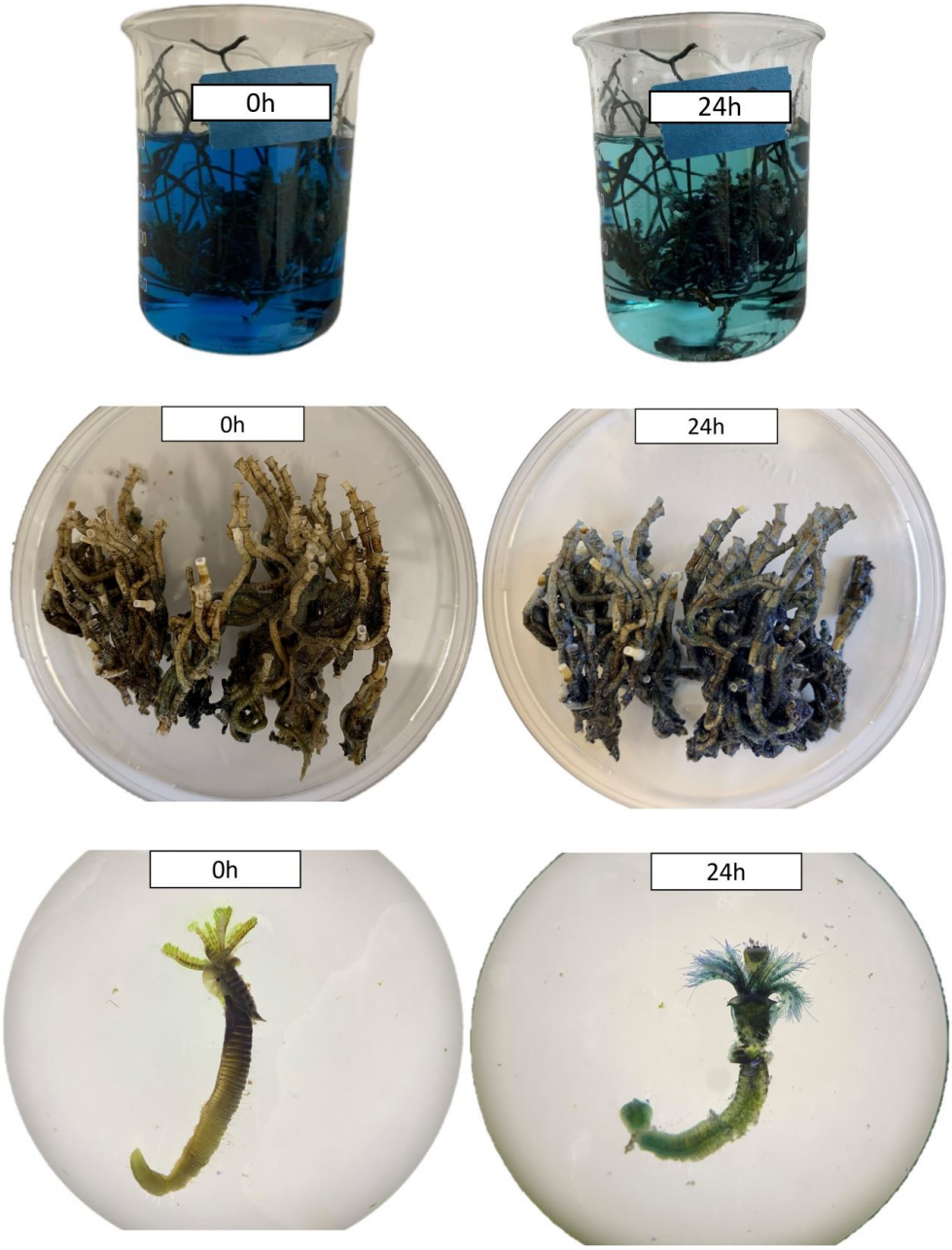

Figure 1. Change in the color of the MB seawater solutions (top pair of photos), living *Ficopomatus* reef blocks (middle pair of photos), and the naked bodies of the worms (bottom pair of photos) at the start of the experiment (0 h) and at the end of the experiment (after 24 h).

Table 1. Decolorization percentage (*D*%; mean ± standard deviation) and dye removal capacity (*qt*; mean ± standard deviation) of living and dead reefs of *F. enigmaticus* exposed to 25 mg/L of MB for 24 h. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

	Reef	1 h	2 h	4 h	24 h
D%	Living	32.75 ± 8.57	43.71 ± 11.73	50.20 ± 12.11	80.43 ± 8.83
	Dead	15.06 ± 4.19	27.66 ± 5.61	33.49 ± 4.28	64.61 ± 5.94
	Difference	17.70 **	13.44 *	15.20 *	15.22 *
qt (mg/g)	Living	0.17 ± 0.04	0.21 ± 0.05	0.30 ± 0.08	0.53 ± 0.04
	Dead	0.08 ± 0.02	0.13 ± 0.02	0.15 ± 0.02	0.31 ± 0.03
	Difference	0.09 ***	0.08 **	0.15 **	0.22 **

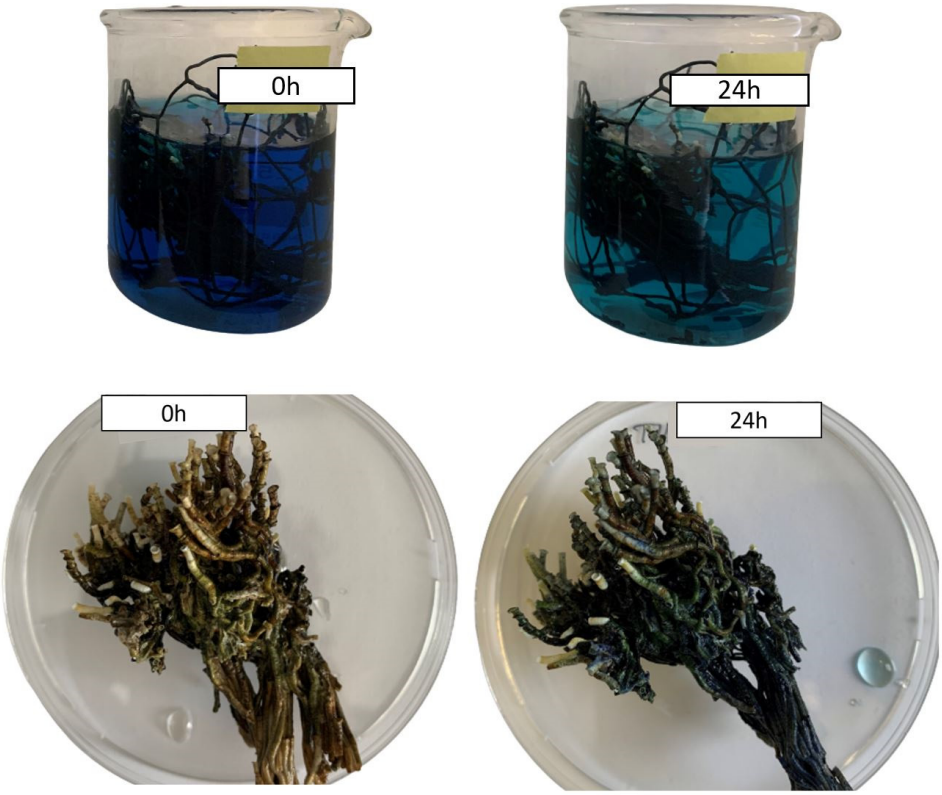

Figure 2. Change in the color of the MB seawater solutions (top pair of photos) and in dead *Ficopomatus* reef blocks (bottom pair of photos) at the start of the experiment of exposure to MB solution (0 h) and at the end of the experiment (after 24 h).

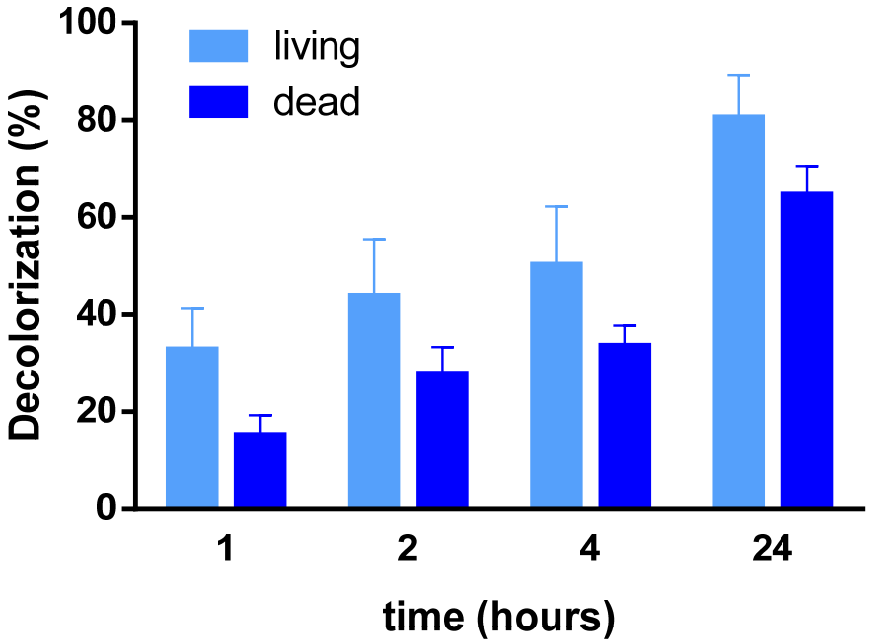

Figure 3. Percentage of decolorization (*D*%) of dead and living reefs over time.

3.2. Reef Surface Characterization

The observation of the surface of the dead and living reefs under a stereomicroscope highlighted a strong MB adsorption, as shown in Figure 4.

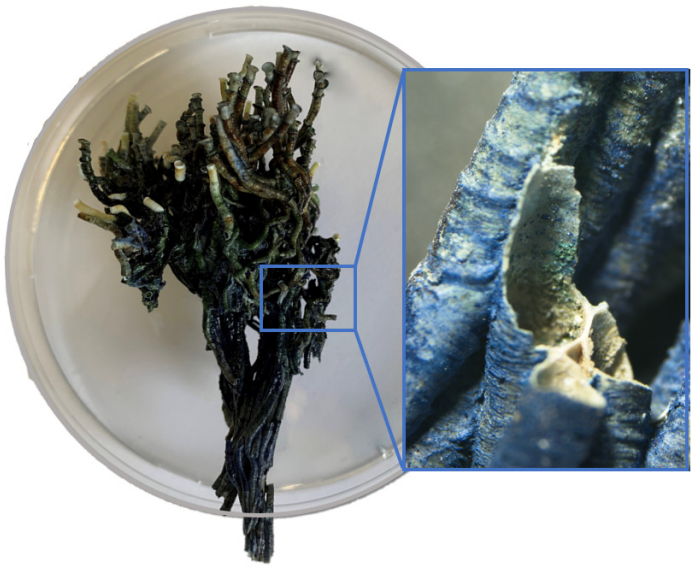

Figure 4. High-resolution details of tube surface after 24 h of exposure to MB seawater solution. Sample from dead reef.

The results of the ATR-FTIR analysis, necessary to identify the components likely involved in the adsorption process, are reported in Table 2, and the relative spectra are depicted in Figure 5. A high match (84.27%) was obtained with a reference composite material whose main components were found to be cellulose (contributing 60.52%), travertine (contributing 22.64%), and calcite (contributing 13.27%). The signal of MB was detectable and accounted for 3.57%.

Table 2. Percentage of matching of *F. enigmaticus* reef with reference composite materials and their relative percentage composition.

Total Match with Reference (%)	Name Composite	Composite Relative %
84.27	Cellulose	60.52
	Travertine	22.64
	Calcite	13.27
	Methylene blue, certified	3.57

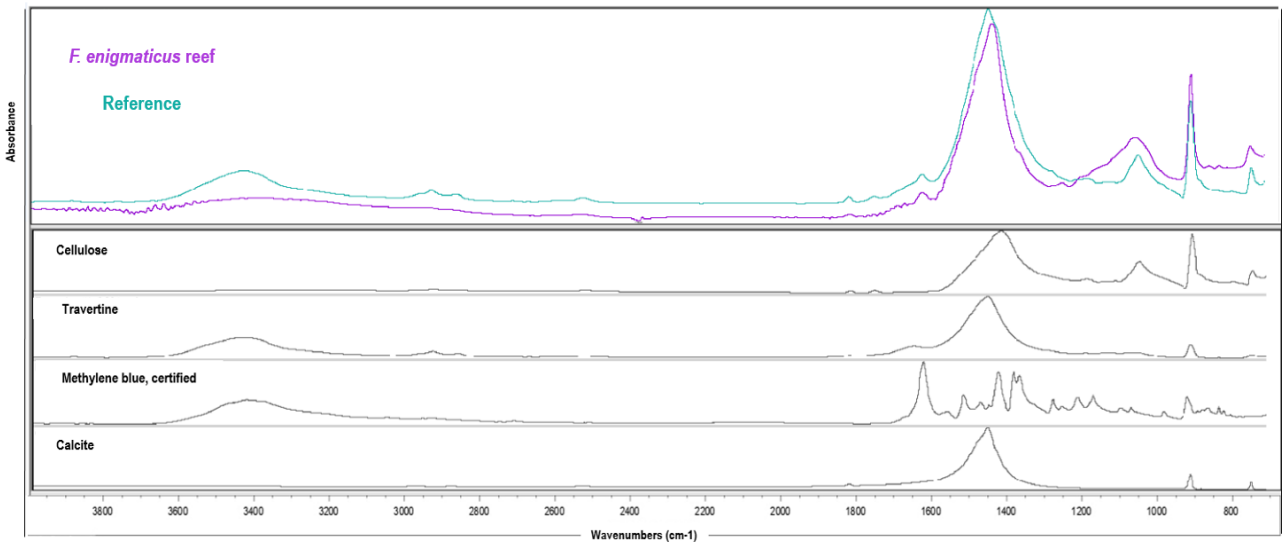

Figure 5. ATR-FTIR spectra of dead reefs of *F. enigmaticus* after exposure to 25 mg/L of methylene blue solution.

3.3. Toxicity Test

Higher toxicity values were observed in the non-bioremediated MB sample, resulting in a 59.8% inhibition of *A. fischeri* bioluminescence (Table 3). The solutions bioremediated by living reefs demonstrated toxicity levels ranging from 21.5% to 58.2%, with a dose-dependent response where increased residual concentrations of MB corresponded to greater toxicity. The samples bioremediated by dead reefs exhibited a toxic effect ranging from 30.5% to 38.2%. Seawater samples from the negative control (without MB but exposed to the reefs) displayed a significant ecotoxicological response, which was more pronounced in the case of the living reef (43.2%) than in the case of the dead reef (33.1%).

Table 3. Results of the toxicological test with *A. fischeri* (15 min, 90%) conducted on 24 h bioremediated solutions of MB using living (L) and dead (D) reefs. Values are listed in decreasing order of the tested residual MB concentrations. CP refers to the solution with non-bioremediated MB, while CN denotes seawater without MB exposed to living (CN_L) and dead (CN_D) reefs.

Samples	Residual MB Concentration Tested (mg/L)	*Aliivibrio fischeri* Bioluminescence Inhibition (%)	
		Mean	SD
CP	22.50	59.8	3.4
L2	8.39	58.2	0.0
L1	8.04	37.2	1.5
L5	6.09	38.5	0.0
L8	5.43	31.3	0.1
L7	2.99	31.1	0.0
L4	1.89	21.5	1.4
CN_L	0.00	43.4	1.5
D1	11.61	33.0	1.5
D2	9.58	30.5	1.9
D3	8.76	35.1	0.0
D5	8.04	31.3	0.7
D4	7.90	38.2	1.8
CN_D	0.00	33.1	1.9

4. Discussion

F. enigmaticus efficiently removed up to 81% of methylene blue (MB), a potential contaminant in aquatic systems resulting from industrial and domestic activities, within 24 h. Previous research has primarily focused on the removal of MB using biological systems, such as bacteria [28,33,34], fungi [35,36], marine algae [37–39], fruit [40,41], and snail shells [42], which were employed as biotechnology to treat dye-contaminated water after undergoing various processes like transformation, grinding, and encapsulation with nanomaterials. Less is known about the capacity of aquatic organisms to remove synthetic dyes once they are introduced into the environment, with limited information in the literature mostly focusing on plants, macroalgae, and microalgae. For instance, Manghabati et al. (2014) [43] reported a removal rate of 50–75% of MB (initial concentration: 40 mg/L) by the marine alga *Bifurcaria bifurcata* within 24 h. Al-Fawwaz et al. (2016) [44] observed a 30% removal of the dye by the green alga *Desmodesmus* sp. after 48 h of contact time. Santaeufemia et al. (2021) [29] investigated the percentage of dye removed by the marine algae *Phaeodactylum tricornutum* at different initial concentrations of MB and reported a 26.5% removal after 11 h (initial concentration of 20 mg/L). Also, Al-Baldawi et al. (2018) [45] studied the phyto-transformation of MB from water using the aquatic plant *Azolla pinnata* and reported a maximum degradation efficiency of 90% after 5 days at an initial concentration of 25 mg/L. The only study reporting performance similar to that obtained with *F. enigmaticus* in the present study is Imron et al. (2019) [26], where they achieved an 80.5% removal of MB within 24 h using the duckweed *Lemna minor* (initial concentration: 50 mg/L). Therefore, the efficiency of *F. enigmaticus* in removing the synthetic

dye MB within just 24 h of contact time is comparable to, if not higher than, what has been obtained with other organisms, highlighting the high potential of this organism for bioremediation.

Besides the quantification of the capacity of *Ficopomatus* to remove MB, our findings also shed light on the processes underlying bioremediation. Two primary mechanisms have been hypothesized as the basis for the bioremediation capacity of *Ficopomatus*: bioaccumulation and bioadsorption. Evidence of the former can be qualitatively observed in microscopic images of the de-tubed worm body, which is distinctly colored blue after 24 h. This ability of MB to impart color is well-known and has contributed to its popularity in biological research, earning it the distinction as a vital dye par excellence (it stains negatively charged cellular components like nucleic acids). Notably, MB is recognized as one of the most valuable tools in various fields of biology, including histology, cytology, histochemistry, parasitology, mycology, microbiology, and virology, among others [46]. A comparison between the results obtained with the living and dead reefs, however, allowed us to estimate a limited contribution (18%) of bioaccumulation to decolorization after 24 h, supporting the hypothesis that the removal of MB could mostly depend on bioadsorption.

Bioadsorption was demonstrated through the microscopic and ATR-FTIR analysis of the reef surface, which facilitated the identification of the composition of the surface layer characterizing *Ficopomatus* tubes, thereby revealing the components likely involved in the process. The main component consisted of 60.52% cellulose followed by calcium carbonate in the form of calcite and travertine (altogether accounting for 35.91%). The MB signal was identified as further evidence of its adsorption. Therefore, the tubes predominantly featured cellulose as the main component, likely attributable to the layer of microalgae that develops over time on the external surface of the tube, imparting its characteristic brownish color [47]. Evidence of MB biosorption by microalgae is extensively documented in the literature. For example, Seoane et al. (2022) [38] used the biomass of the microalga *Chlamydomonas moewusii* for the removal of MB and obtained satisfactory results [38]; Yang et al. (2021) investigate the adsorption of MB on biochars derived from *Chlorella* sp. and *Spirulina* sp. [48], while Moghazy and Mahmoud (2023) used a macro-hollow loofah fiber, both with and without bio-attaching with the green microalga *Chlamydomonas reinhardtii*, for the removal of MB from an aqueous solution [49]. Such findings suggest that the hard structure of the reef itself, which was responsible for 61.51% of the removal of MB, could play a crucial role for contaminant sequestration, largely due to the presence of microalgae. Therefore, even when transient or persistent declines in *F. enigmaticus* population occur, such as after irregular river floods [50], their complex dead bioconstructions still ensure the continuity of much of the water remediation function, serving as collectors and catalysts for contaminant sequestration based on their associated microbiota.

No attempts have been made in this study to quantify the desorption of MB from *Ficopomatus* reefs, which requires further investigations to understand the potential of these organisms as dye bioremediators. Evidence from studies conducted on by-products of vegetal origin (therefore more similar to the surface composition of the *Ficopomatus* reefs of our study) suggest that the desorbing percentage of MB is generally limited and often requires the addition of solvents and several cycles of extraction. For instance, Daneshvar et al., (2017) [51] conducted a desorbing experiment with the brown macroalga *Nizamuddinia zanardinii*, which was used as a natural sorbent for MB removal from aqueous solutions. They achieved a desorption yield of only 2.36% using simple distilled water and had to prepare a mixture of 1 M HCl (25%)/1 M 1-butanol (75%) to enhance the performance to 64%. Similarly, Oladoja et al. (2009) [52] studied the batch desorption of MB from loofah using different solvents; NaOH and water yielded scarce results (<5.6% of MB desorbed), while acidic solvents (i.e., CH3COOH and HCl) were necessary to achieve 33% and 42%, respectively.

The acute toxicity tests on *Aliivibrio fischeri* revealed surprising and unexpected findings. Generally, the solutions proved to be toxic to *A. fischeri*, which is not surprising considering the well-documented antiseptic properties of MB in the literature [53]. How-

ever, bioremediated solutions from living reefs were found to be more toxic than those from dead reefs and, in relation to the residue, even more toxic than the positive control. In the first case, samples with similar residues from living (L1) and dead (D5) reefs caused a 37.2% and 31.3% inhibition of bioluminescence, respectively. In the second case, a similar response (58.2% vs. 59.8%) was triggered by 8.39 mg/L of bioremediated solutions and 22.50 mg/L of non-bioremediated solutions. Based on these data, it could be hypothesized that 8 mg/L may represent a threshold concentration beyond which the response curve of *A. fischeri* to MB no longer exhibits linear growth but instead becomes exponential. However, it is worth noting that the mere presence of *Ficopomatus* (CN) has proven to be harmful in itself (43.4% inhibition).

Two potential mechanisms of toxicity can be hypothesized to explain the observed pattern. The first is that MB may have been partially or completely metabolized into by-products, which are also toxic. MB is a basic phenothiazine dye that can undergo demethylation, resulting in the formation of azure A, azure B, azure C, benzidine and methylene [17,54]. For example, azure B has been shown to delay the development of the gastrula and blastula stages in the starfish *Marthasterias glacialis* [55]. However, which and how many substances of MB could be biotransformed still remain an essential gap in bioremediation experiments, requiring much more effort in future research. In this regard, techniques such as liquid chromatography (LC) and liquid chromatography coupled with mass spectrometry (LC/MS) can be capable of simultaneously determining a number of dyes, as reported in the review of Hakami and colleagues [56]. The second possible explanation is that *Ficopomatus* has the ability to produce compounds that interfere with quorum sensing (QS) in *A. fischeri*. QS is a synchronization mechanism within a bacterial population. Bacteria employ QS to communicate, regulate their behavior, assess their population density, and generate a synchronized behavior such as bioluminescence, virulence, or aggregation to form biofilms [57]. Here, we detected an effect of *Ficopomatus* reefs to inhibit the QS related to bioluminescence, but it is conceivable that the production of *Ficopomatus* secretions capable of disrupting bacterial QS is related to the formation of bacterial biofilms, suggesting a possible antifouling action. Despite evidence of the antifouling effects of metabolites from marine organisms, mostly focused on sponges (e.g., [58]), cnidarians (e.g., [59]), bryozoans (e.g., [60]), and ascidians (e.g., [61]), marine polychaetes are known to produce a wide range of antifouling, antipathogen, and defensive chemicals [62], reinforcing the hypothesis that *F. enigmaticus* could produce bioactive compounds. However, we may not exclude toxic effects on *A. fischeri* due to allelopathic compounds produced by the microbiota associated with reef structures (e.g., diatoms, [63]).

5. Conclusions

Our study on *F. enigmaticus* demonstrates its efficient removal of the synthetic dye methylene blue (MB) within just 24 h of contact time. Such findings unveil the potential role of this suspension-feeding and reef-building species, so far mostly considered as a threat to marine environments due to its invasiveness, to contribute to ecosystem services by enhancing water quality.

The slight difference in MB removal capacity between living and dead reefs (less than 18%) highlighted the ability of the calcified portion of reefs to sequester contaminants even after the colonies die. Moreover, dead reefs, by providing a suitable substrate, may represent natural collectors of microalgal biofilms that seemed to be involved in the process of adsorption of the dissolved dye. The high performance of *F. enigmaticus* in removing MB with respect to other organisms thus probably stems from the combination of direct removal by the worms and the indirect effects of reducing dye concentrations related to its nature as a habitat former.

The results of our preliminary assessment bode well for a possible employment of *F. enigmaticus* as a bioremediator, at least in specific environmental contexts (e.g., confined environments characterized by high levels of pollution) where potential negative effects of its presence are of relatively minor importance or the eradication of this species is not

strictly necessary. Further investigations to extend the assessments to other classes of contaminants and to delve into the spatio-temporal dynamics underlying bioaccumulation are needed to fully explore the putative bioremediation abilities of *F. enigmaticus* and to help the management of this invasive species.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/w16030368/s1: Table S1: Composition and physical-chemical properties of the synthetic seawater used for bioassay with *Aliivibrio fischeri*; Table S2: pH values measured at time 0 and after 24 h of contact time with 25 mg/L methylene blue. CN = negative controls; L = living reefs; D = dead reefs; CP = positive controls.

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