Antimicrobial Ceramic Filters for Water Bio-Decontamination

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Abstract: Bio-contamination of water through biofouling, which involves the natural colonization of submerged surfaces by waterborne organisms, is a global socio-economic concern, allied to premature materials bio-corrosion and high human health risks. Most effective strategies release toxic and persistent disinfectant compounds into the aquatic medium, causing environmental problems and leading to more stringent legislation regarding their use. To minimize these side effects, a newly non-biocide-release coating strategy suitable for several polymeric matrices, namely polydimethylsiloxane and polyurethane (PU)-based coatings, was used to generate antimicrobial ceramic filters for water bio-decontamination. The best results, in terms of antimicrobial activity and biocide release, showed an expressed delay and a decrease of up to 66% in the population of methicillin-resistant *Staphylococcus aureus* bacteria on ceramic filters coated with polyurethane (PU)-based coatings containing grafted Econea biocide, and no evidence of biocide release after being submerged for 45 days in water. Biocidal PU-based surfaces were also less prone to *Enterococcus faecalis* biofilm formation under flow conditions with an average reduction of 60% after 48 h compared to a pristine PU-based surface. Biocidal coated filters show to be a potential eco-friendly alternative for minimizing the environmental risks associated with biofouling formation in water-based industrial systems.

Keywords: micro-foulers; ballast waters; cordierite monoliths; non-biocide release coating; dynamic biofilm assays; antimicrobial filtration

1. Introduction

Water is the most vital resource for all living forms on earth. However, surface water and groundwater are globally impacted by diverse pollutants where the presence of fecal bacteria (e.g., *Escherichia coli*, *Enterococcus faecalis*) is a good indicator of water contamination [1–3]. Furthermore, only less than 1% of the freshwater is drinkable, and, still, at least one billion people around the world do not have access to potable and microbiologically-safe water [2]. Water is a vulnerable resource and its quality is a worldwide top-priority

issue for suppliers, consumers, and regulatory bodies. Water storage and distribution systems, composed of diverse infrastructure networks, that transport water targeting several uses, are crucial to guarantee a safe water supply. Ballast waters from ships constitute a representative and increasing concern of water use. Those waters, stored in ships and often released in harbors, can, in the absence of any treatment, contaminate the surface waters by transferring microorganisms from one part of the world to another [4]. The presence of microorganisms and their interaction with those waterborne systems, namely the production of biofilms, can lead to serious economic penalties and health threats, which is enhanced by the current lack of control on their discharge, reinforced by the continuous increase at a global scale, of the shipping transport traffic, including transport in lakes and rivers, which is estimated to transfer about three to five billion tonnes of ballast water per year [5,6].

The biofilm matrix is an aggregation of different types of biopolymers produced by microorganisms that are responsible for the integrity of a structural and functional biofilm [7,8]. The formation of a complex multispecies biofilm, always comprises a sequence of events over different length scales [5,9]. Studies also reported that biofilms provide the habitat for pathogenic microorganisms, such as *Pseudomonas aeruginosa*, *Escherichia coli*, or even multidrug resistant bacteria [10,11]. Most accepted stages of biofilm development on a surface [12,13] comprise the primary adhesion of colonizers (planktonic microorganisms) in a conditioning film rich in organic matter, followed by a secondary adhesion of bacterial cells, with the production of extracellular polymeric substances, which act as nutrient concentration and storage promoters, as well as reactive transport barriers to chemical biocides. This secondary adhesion will further develop to a macrofouling level, depending on the physical-chemical and biological characteristics of the environment, which can be reached in just a few days or weeks. This is a phenomenon known as biofouling [14,15]. Biofouling is mostly composed of water, bacteria, suspended solids, and macro-foulants (e.g., algae and yeast/fungi), which, as a whole, are intensive promoters of biocorrosion [14], leading to premature equipment deterioration. Another issue associated with this natural phenomenon is its contribution to flow resistance in water system circuits (e.g., pipes and filters) as a result of biomass clogging effects and subsequent retrofitting consequences [5,16]. On the other hand, bio-detachment from biofilms is one of the least studied events in these systems. However, it is also one of the most concerning events, particularly in dynamic systems where hydrodynamics plays an important role. This may have severe detrimental impacts on water quality and safety, since it is a promotor of water bio-contamination and possible human waterborne diseases [4,17,18] posing serious public health risks.

Several methodologies were developed, and new technologies were implemented in the search for solutions to mitigate this bio-burden [1,6,19]. One of the most addressed targets in antifouling strategies is the development of surfaces that are not readily colonized by microorganisms and are ideally easy to clean. To date, the most effective is the chemical disinfection by toxic agents or disinfectants, such as bactericides or oxidizing biocides, directly applied to inactivate microbial cells and to limit microbial regrowth and contamination, and are commonly combined with a prior filtration or separation. Among those, most conventional treatments are based on the use of chlorine dioxide disinfectants [6,20]. The success of disinfection practices led to the virtual elimination of the deadliest waterborne diseases in developed countries, particularly during the 1940s and 1950s, suppressing typhoid fever and cholera diseases [1]. Unfortunately, the by-products originating from the disinfection processes constitute themselves a concern. This is due to their intrinsic toxicity and ineffectiveness against biofilm development since the dead biomass can still serve as a substratum for new microorganism deposition and regrowth.

Additionally, stringent international regulations (e.g., Biocidal Products Directive (BPD) EU Regulation, 2012) were issued, limiting or even banning the use of the most effective employed biocidal agents and related products currently in the market. Alternative commercially available methods include filtration and UV treatment, which can also be

an alternative eco-friendly cleaning process [5,21], but it is expensive and fails to prevent some microorganisms' colonization (e.g., fungi), particularly on organic materials, such as polymers [5,22].

There is, thus, a need for new non-toxic and long-lasting antifouling strategies in these waterborne systems. In this direction, we report an original antimicrobial non-biocide release strategy, able to provide antimicrobial monolithic ceramic filters through their surface protection with antifouling coating films containing grafted Econea biocide [23,24].

For this purpose, polydimethylsiloxane (PDMS) and polyurethane (PU)-based marine coatings were selected to confirm the feasibility of coated filters for the different polymeric matrices. PDMS-based coatings have been of increasing interest, not only due to their eco-friendly nature, as no biocidal agents are used in their formulations [25], but also due to their low surface energy and relatively higher elasticity (low Young's modulus), able to weaken the adhesion of organisms and promote their removal under dynamic conditions. However, these coatings also exhibit relatively low mechanical properties, becoming easily damaged (e.g., by cutting, tearing, puncturing) under more aggressive conditions [26], reducing their performance and service lifetime, making them fully effective only under dynamic conditions [27,28]. On the other hand, PU-based coatings provide relatively higher physical-mechanical properties, thermal stability, and chemical resistance, becoming preferred under more demanding conditions, such as those found in water treatment systems, but its antifouling action is still provided through the release of biocides [29].

The potential application of non-biocide release coatings as coated filters for bio-fouling prevention in both quasi-static and flow conditions is demonstrated for the first time in this work. Hydrodynamic conditions are crucial, since they may vary daily and seasonally from stagnation to high flow as demand varies [30], and these changes are reflected in the resultant biofilm. Furthermore, there are critical zones in piping or storage systems such as crevices, depressions, or dead-zones, which are difficult to clean due to limited access and where lower fluid velocities are found, making these places suitable for biofilm build-up [31]. According to the numerical simulations previously performed by the group [32], the flow rate chosen to operate the flow cell system in the present work (300 L/h) reproduces the hydrodynamics of these critical zones where shear stress values around 0.25 Pa can be attained [31,33].

2. Materials and Methods

2.1. Monolithic Filters Coated with Biocide Non-Release Coatings

Biocide non-release coatings were prepared following a prior developed methodology [23,24] in order to graft the booster Econea (4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)-1H pyrrole-3-carbonitrile > 97%) biocide (E), provided by Janssen PMP (Beerse, Belgium), in two representative commercial paints, a foul-release polydimethylsiloxane, X3-PDMS, (HEMPASIL X3+ 87500) and a polyurethane (PU), (Ref. F0038), both provided by Hempel A/S, Copenhagen, Denmark, and composed by two-component systems, comprising a base resin and curing agent: Hempasil Base 87509 and Crosslinker 98951 for the PDMS paint, and base F0032 and curing agent 95580 for the PU system. Briefly, an isocyanate-functional derivative (EM), possessing a grafting ability into polymeric compatible matrices, was obtained from the functionalization of the biocide with a di-isocyanate reagent (Supplementary Figure S1). The biocidal coating formulations were further obtained through the addition and blending of a solution of the biocide derivative in 1-methyl-2-pyrrolidone (99.5% Acros Organics, Geel, Belgium), at a solvent/biocide (w/w) ratio of 0.48/0.23, in the components of the paint at a base resin/curing agent (w/w) ratios of 12.30/1.40 and 13.20/1.50 for the X3-PDMS and PU systems, respectively.

A second PDMS-based coating system (RTV-PDMS), known as a room-temperature-vulcanizing (RTV) polydimethylsiloxane (PDMS) coating (RTV11, MOMENTIVE, Waterford, NY, USA), was also included in the studies. This particular two-component coating system was selected for pre-screening antimicrobial bioassays, since it allows an evaluation of the single biocide influence in the coating behavior, avoiding, for instance, any potential

overlapping or synergistic effects, which may occur for commercial paints, particularly the ones associated with the intrinsic foul-release properties of the X3-PDMS. However, these are only significant under specific dynamic conditions [24]. For the optimized RTV-PDMS formulations, a biocide solution in 1-methyl-2-pyrrolidone at a solvent/biocide (*w/w*) ratio of 0.48/0.23, and a base resin/curing agent ratio of 13.37/0.08 were employed. The coating formulations were then used to coat filter samples ($1.5 \times 1.5 \times 2$ cm^3), obtained by disc cutting from a cordierite-based commercial monolithic filter (200 cpsi) commonly applied in fluid purification industrial systems [34]. The filter samples were then cleaned with compressed air and coated with the coating formulations by using a conventional dip-coating methodology. For the PDMS-based formulations, and in accordance with coating components supplier recommendations, a prior coating of the filters was also carried out with a silicone-tie coat (Base XA17 and curing agent XA18, Hempel A/S, Copenhagen, Denmark) to guarantee the proper adhesion of the PDMS smooth coating matrices on the filters' surface.

The grafting methodology between the polymeric matrices (X3-PDMS and PU) with the isocyanate Econea derivative and the dip-coating procedure for the painting of the monolithic filters are illustrated in Figure 1.
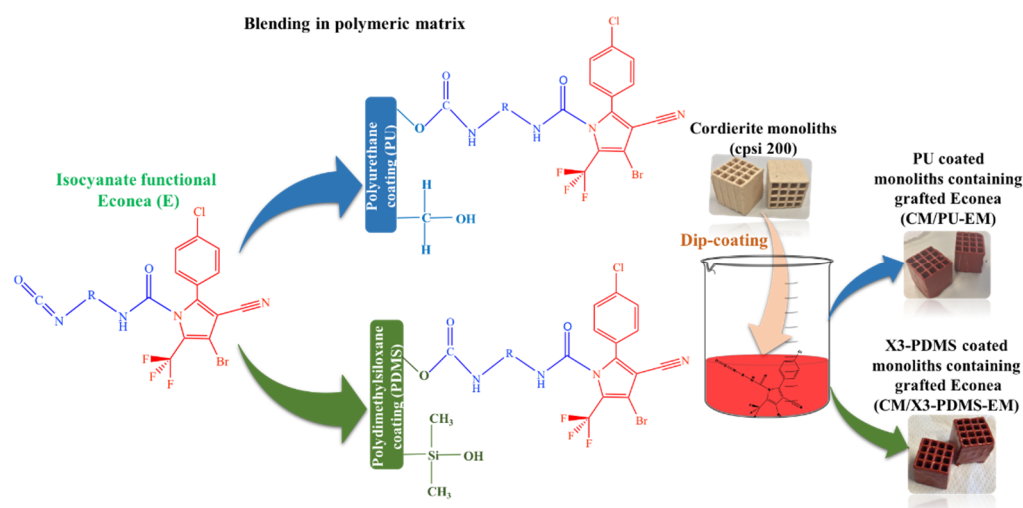
Figure 1. Eco-friendly strategy comprising the development of isocyanate-reactive biocides and their subsequent grafting in different representative polymeric matrices, polyurethane (PU)-based, and polydimethylsiloxane (X3-PDMS)-based paints to originate non-release antifouling coatings used to coat cordierite monolithic filters.

Coating formulations of the pristine polymeric matrices and containing incorporated Econea (E) biocide were also prepared to be used as controls and for comparative purposes. The pristine coatings preparation followed the suppliers' instructions, using base resin/curing agent (*w/w*) ratios of 6.99/0.90, 7.52/0.84, and 17.83/0.11 for the X3-PDMS, PU, and RTV-PDMS systems, respectively. For the Econea biocide incorporation in those coatings, the addition and blending procedure was similar to the prior one described for the biocide derivative immobilization, using a solvent/biocide (*w/w*) ratio of 0.48/0.23, and base resin/curing agent ratios of 9.09/1.17, 6.90/0.79, and 13.66/0.09 for the X3-PDMS, PU, and RTV-PDMS systems, respectively.

2.2. Structural Characterization and Antimicrobial Activity of Biocidal Agents

The biocides structural characterization was performed by standard techniques such as Fourier Transform Infrared Spectroscopy (FTIR) and Nuclear Magnetic Resonance (NMR). The isocyanate-free content on the functional biocide derivative was determined by an adapted procedure from the standard ASTMD2572, already described in previous publications [24,35].

For the antimicrobial activity evaluation of both Econea biocide and its isocyanate-functional derivative, direct contact and microdilution methodologies were used, as the following described.

2.2.1. Antimicrobial Susceptibility by Direct Contact

A direct contact methodology was used to assess the effect of the biocides on the microorganism's cultural growth. This contact was promoted by growing a pure culture of methicillin-resistant *Staphylococcus aureus* (MRSA) strain (ATCC 33591) at $36 \pm 1\ ^\circ$C in Tryptic Soy Broth (TSB) (Becton Dickinson, Franklin Lakes, NJ, USA) until the exponential phase. Furthermore, 100 µL of this culture were spread on Tryptic Soy Agar (TSA) (Biokar, Allonne, France) plates. Each of the prepared plates was further covered with the biocide samples in the powder state and incubated at 37 °C for 48 h. After incubation, plates were checked for the presence of bacterial growth.

2.2.2. Microdilution Method—Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs)

Minimal inhibitory concentrations (MIC) were determined by the broth dilution method, following the recommendations of the Clinical and Laboratory Standards Institute [36] and against the Gram-positive bacteria *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), and MRSA (CIP 106760). Stock solutions of Econea (E) and isocyanate-functional derivative (EM) (10 mg/mL) were prepared in dimethyl sulfoxide (DMSO) and kept at $4 \pm 1\ ^\circ$C.

In each well of a 96 U-shaped microplate, 100 µL of Mueller-Hilton (MH) broth were introduced. In the first well, 100 µL of E and EM were added at a concentration of 1 mg/mL (diluted from the appropriate stock solution). The former (E) acts as a positive control. A two-fold serial broth microdilution within the concentration range of 500–0.49 µg/mL was further performed using a multichannel micropipette. In each well, 10 µL of a standardized bacterial suspension equivalent to 0.5 McFarland of each microorganism was placed. Finally, the microplates were incubated at 37 °C for 24 h. The microbial growth was evaluated using an absorbance microplate reader (Multiskan FC Thermo Scientific, Loughborough, UK) at 620 nm, and the MIC was taken as the lowest concentration of the biocide that inhibited the visible growth of microorganisms by comparison with the controls.

The MBCs values were also evaluated to verify the bactericidal/bacteriostatic properties of the biocides against MRSA bacteria. For this purpose, after MIC determinations and for each set of wells that did not show any growth of MRSA bacteria, a loopful of broth was collected and inoculated on sterile MH agar. The MBC was taken as the lowest concentration of the biocide that killed 99.9% of the initial inoculum after 24 h of incubation at 37 °C.

Three independent bioassays were performed for all tested microorganisms and methodologies.

2.3. Antimicrobial Potential of Developed Biocidal Coatings

Prior to the evaluation of the antimicrobial potential of the coated filters, a screening of the biocidal coatings' antimicrobial susceptibility against *E. faecalis* (ATCC 29212) and MRSA (ATCC 33591) was performed by a direct contact methodology. Briefly, in a sterile environment, the bottom of 90 mm Petri dishes (Greiner bio-one, Frickenhausen, Germany) was coated with Econea-based polymeric coatings (X3-PDMS, PU, and RTV-PDMS), comprising biocide contents ranging from 0.56 to 1.30 wt.% for the PDMS-based coating and from 2 to 3 wt.% for the PU-based coatings. On each coated Petri dish, 20 mL of TSA (Biokar, Allonne, France) were poured and left to solidify. After solidification, the bacteria were spread into the agar plate surfaces and incubated for 48 h at $36 \pm 1\ ^\circ$C. Reaching the incubation period, the bacterial growth extension on the coating surface was visually evaluated as a yellowish, opaque film formation, and its extension in the tested area registered.

2.4. Biocide Grafting Efficacy on Coated Monolithic Filters

After the successful development of bioactive coatings, the most promising were used for the first time to coat monolithic filters. The biocide immobilization efficacy on the monolithic filters coated with X3-PDMS and PU-based coatings, containing either incorporated Econea (CM/X3-PDMS-E and CM/PU-E) and grafted Econea (CM/X3-PDMS-EM and CM/PU-EM), was evaluated through a leaching test methodology adapted from the standards of OECD 313-2007 [37] and ISO 15181 [38]. The leaching tests comprise the submersion of coated filters in distilled water (250 mL) for at least 45 days, under continuous stirring ($\cong$ 200 rpm). The test was performed with an average pH of 7 ± 1, and temperatures ranging from 20 to 24 °C. The obtained leaching waters were then filtered and stored in the refrigerator for at least 30 days to ensure the complete degradation of the potentially released Econea into a more stable compound in water, which allows its further accurate quantification in leaching waters through ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS). The chromatographic analyses were conducted by using an ELUTE autosampler UHPLC (Bruker, Bremen, Germany), using an Intensity Solo 2 C18 RP column (100 mm × 2.1 mm, 2.0 µm, Bruker, Bremen, Germany). A volume of 5 µL was injected (auto injector) into the system using a gradient composed of water (Milli-Q) with 0.1% formic acid (eluent A, 99.5+%, Optima® LC/MS grade 50ML Fisher Chemical, Porto Salvo, Portugal) and Acetonitrile (ACN, Optima LC/MS 2.5 L Fisher Chemical, Porto Salvo, Portugal) with 0.1% formic acid (eluent B), as follows: 0 min—95% A, 1.5 min—95% A, 13.5 min—25% A, 18.5 min—0% A, 21.5 min—0% A, 23.5 min—95% A, and 30 min—95% A, with a flow rate of 0.250 mL/min and a column temperature of 35 °C.

For mass spectrometry, an Impact II QToF (Bruker, Bremen, Germany) was used. The method consisted of MS/MS scans, in both positive and negative ionization modes, in the 50–1500 *m/z* range. The capillary voltage was set to 3500 and 4000 V, for the negative and positive ionization modes, respectively, and the collision cell energy to 5.0 eV. The end plate offset was set to 500 V, the dry gas (N_2) was kept at 8.0 L/min at 200 °C, and the nebulizer gas (N_2) was set to 2.0 bars. Internal calibration was performed on the high precision calibration mode (HPC) with a solution of 250 mL of ultra-pure water (Milli-Q Water Purification System, Merck Millipore, Burlington, MA, USA), 250 mL of isopropanol (Isopropanol LC/MS OPTIMA Grade 1 L Fisher Chemical, Porto Salvo, Portugal), 750 µL of acetic acid (Glacial HPLC, Fisher Chemical, Porto Salvo, Portugal), 250 µL of formic acid, and 0.5 mL of sodium hydroxide (Sodium hydroxide, for analysis, pellets, Fisher Chemical, Porto Salvo, Portugal) solution 1N. The data was acquired through the DataAnalysis 4.4 software (Bruker, Bremen, Germany).

For the leaching water, at least two replicates were performed by UHPLC-MS/MS.

2.5. Water Bio-Decontamination Potential of Non-Biocide-Release-Coated Substrates

2.5.1. Antimicrobial Susceptibility of Coated Monolithic Filters

For the antimicrobial susceptibility evaluation of the coated monolithic filters at quasi-static conditions ($\leq$200 rpm), a time-kill test was performed to assess their bioactivity as a function of time against MRSA. The method includes the monitoring of the bacterial growth of a known population (10^6 CFU/mL) of MRSA (CIP 106760) in the absence and presence of immersed uncoated and coated monolithic filters.

Aliquots of bacteria medium were taken every 60 min of testing and monitored by absorbance measurements at 620 nm in a Microplate Reader (Multiskan FC Thermo Scientific, Loughborough, UK) to evaluate the bacterial growth population, expressed by absorbances, until reaching a steady bacterial content. The control consisted of the same buffer solution containing the bacteria without the monolithic filter. Three independent bioassays were performed for the tested microorganism.

The data of bioassays were analyzed non-parametrically using the Friedman test to detect statistical differences in the bacterial absorbance across the monolithic filters. Results were reported as a mean $\pm$ standard deviations (SDs) and considered statistically different for a confidence level greater than 95% ($p < 0.05$). The data were analyzed using statistical software (IBM SPSS Statistics, version 26).

2.5.2. Biofilm Formation on Coated Substrates under Dynamic Conditions

In water treatment systems, severe conditions are employed (e.g., UV light, aggressive chemicals, flow stresses), and a PDMS-based coating is not the most suitable for this specific use, mostly due to its relatively low mechanical properties, becoming easily damaged (e.g., by cutting, tearing, and puncturing) [26]. For that reason and to prove the water bio-decontamination concept in conditions closer to the real ones, the polyurethane coating formulations were used to coat acrylic prototypes (2 cm $\times$ 1 cm) and the antifouling effect of these surfaces against *E. faecalis* was evaluated at hydrodynamic conditions prevailing in water-based industrial systems.

An overnight culture of *E. faecalis* (ATCC 29212) was obtained by inoculation of 500 µL of a glycerol stock (kept at –80 °C) to a total volume of 200 mL of TSB [39] and incubation at 30 °C with orbital agitation (AGITORB 200, Aralab, Portugal). TSB was used as a culture medium for dynamic assays given its relevance in the development of biofilms in industrial systems [39].

The culture was then harvested by centrifugation (10 min, 3202 g) and the pellets were resuspended in fresh TSB to reach a final optical density (OD) of 0.23 at 620 nm, which corresponds to 7.6×10^7 CFU/mL.

A semi-circular flow cell system was used for these assays to simulate the formation of biofilms in industrial pipes [32]. It is composed of a recirculating tank, a vertical flow cell, and peristaltic and centrifugal pumps. This system allows the sampling of individual coupons placed in the vertical flow cell, with surface materials glued to them, without disturbing the biofilm formed on the other coupons [40]. The bacterial suspension was circulated in the system at a flow rate of 300 L/h in order to obtain an average wall shear stress of 0.25 Pa that can be found in critical piping zones (e.g., crevices, corners, joints, and valves [32]). The recirculating tank was continuously fed with sterile TSB (0.025 L/h) and biofilms were formed on pristine PU coatings (control), PU coating with incorporated Econea (PU-E), and PU coating with grafted Econea (PU-EM). The temperature was kept at 30 °C and the antibiofilm properties of these coatings were evaluated after 48 h by determining colony forming units (CFUs). The biofilms were mechanically detached (by vortexing) from the surfaces and homogenized in 5 mL of sterile saline solution (8.5 g/L NaCl). To assess cell counts, 10-fold serial dilutions of the biofilm suspensions were prepared, spread on plate count agar (PCA, Merck, Darmstadt, Germany), and incubated at 30 °C for 24 h for colony enumeration. Biofilm cell counts are presented as CFU per unit area of surface (CFU/cm^2).

Two independent experiments with five technical replicates of each surface were performed. Paired *t*-test analyses were done to evaluate if statistically significant differences were obtained between the two PU modified coatings (PU-E and PU-EM) and pristine PU. Results were reported as mean $\pm$ standard deviation (SD) and considered statistically different for a confidence level greater than 99% ($p < 0.01$).

3. Results and Discussion

3.1. Structural Characterization and Antimicrobial Activity of Biocidal Agents

The Econea biocide was successfully functionalized with 4,4′-methylene diphenyl diisocyanate (MDI) (Supplementary Figure S1) and its structure were confirmed by FTIR-ATR and NMR, as reported in previous publications [23,24,35], with conversions as high as 95% $\pm$ 5% and a free isocyanate content average in the isocyanate-functional Econea derivative (EM) of 9 $\pm$ 2 wt.%.

The antimicrobial activities of Econea (E) and its isocyanate-functional derivative (EM) were evaluated against methicillin-resistant *Staphylococcus aureus* (MRSA) (ATCC 33591) by performing direct contact bioassays. The MRSA growth could be visually identified by a yellowish, opaque surface on the TSA medium, and the biocides that were in direct contact with the medium covered with bacteria could be identified as a dispersed powder (Supplementary Figure S2). The antimicrobial activity of the tested biocides was confirmed by the reduction of the yellowish opaque surface area on the TSA surface medium and the following order was obtained in terms of bacterial growth: MRSA culture > Econea biocide (E) > functional Econea derivative (EM). These results confirmed the antimicrobial potential of the biocide Econea (E) and its isocyanate-functional derivative (EM) against MRSA bacteria (Supplementary Figure S2). However, a more pronounced effect was obtained for EM, which can be associated with the dispersion effect of the biocide on the culture media, since, for the biocidal agent E, higher dispersion of the biocide along the MRSA culture media was obtained, whereas, for the EM, at a similar content, some agglomeration occurred, promoting a higher local concentration of the biocide, which was expressed by an increased antimicrobial effect on the tested bacteria media (Supplementary Figure S2c). This agglomeration of the Econea derivative is inherent to its isocyanate group, which is highly reactive with any active hydrogen-containing molecules [41], including the urea found in the EM derivative or formed by its reaction with water (moisture), which is impossible to remove completely during bioassays, and which promotes the self-reaction of the isocyanate-EM derivative and subsequent agglomeration.

From the MIC values of the biocides obtained against *S. aureus* and *E. faecalis* bacteria and a selected resistant bacterium, MRSA (CIP 106760), the E and EM biocides showed similar MIC values of 3.91 μg/mL against *S. aureus and E. faecalis*, but different for MRSA, with MIC values of 0.49 and 3.91 μg/mL, respectively. These values suggest that the Econea biocide has a significantly higher antimicrobial activity (eight-fold) against MRSA when compared with its isocyanate derivative (EM), showing that the intrinsic structural bioactivity of E was affected by the derivatization reaction. However, a MIC value of 3.91 μg/mL is still considered a potential antimicrobial activity against MRSA bacterium, which encouraged its use for the generation of non-biocide-release protective coatings. Additionality, MBC assays were performed to E and EM biocides against MRSA bacteria (Supplementary Figure S3) in order to address the bacteriostatic and bactericidal properties of the biocides. Relatively high MBC values, 15.64 μg/mL (E) and 125 μg/mL (EM), were obtained for the biocides, expressed by their MBC/MIC ratios, 31.92 (E) and 31.97 (EM), which is a clear indicator of bacteriostatic properties of these biocides against MRSA bacteria, since MBCs exceed four-fold the MICs values, being in accordance with French [42].

3.2. Biocidal Coating Formulations

Several trials and iterative formulations of the polydimethylsiloxane (PDMS) and polyurethane (PU) coating formulations were tested, including at different biocide contents. The optimized formulations listed in Table 1 were then evaluated in terms of their antimicrobial potential, particularly when used as a protective coating on monolithic ceramic filters. The RTV-PDMS coating was exclusively used for the pre-screening bioassays.

Table 1. Polymeric coating formulations containing either incorporated or grafted Econea biocide, and the respective pristine coating's controls (free of biocide).

Coating Formulation [1]	Polymeric Matrix	Biocide Immobilization	Base Resin/Curing Agent Ratio (w/w)	Solvent/Biocide Ratio (w/w) [2]	Biocide Content in Wet Formulation (wt.%)
Polymeric coatings formulations					
X3-PDMS (control)	PDMS	-	7.74	-	-
X3-PDMS/E	PDMS	Incorporation	7.77	1.19	0.58 ± 0.01
X3-PDMS/EM	PDMS	Grafting	7.36	1.72	0.60 ± 0.01
PU (control)	PU	-	8.61	-	-
PU/E	PU	Incorporation	9.23	1.34	2.06 ± 0.02
PU/EM	PU	Grafting	9.03	1.17	1.96 ± 0.02
Polymeric coatings formulations for pre-screening bioassays					
X3-PDMS (control)	PDMS	-	7.92	-	-
X3-PDMS/E	PDMS	Incorporation	7.92 8.94	2.28 2.09	0.57 ± 0.01 1.31 ± 0.01
X3-PDMS/EM	PDMS	Grafting	8.86 8.39	1.60 1.91	0.59 ± 0.01 1.24 ± 0.01
PU (control)	PU	-	9.31	-	-
PU/EM	PU	Grafting	9.27 9.21	2.79 2.79	1.96 ± 0.02 3.03 ± 0.02
RTV-PDMS (control)	PDMS	-	162.11	-	-
RTV-PDMS/E	PDMS	Incorporation	151.76	1.92	1.36 ± 0.01
RTV-PDMS/EM	PDMS	Grafting	190.97	2.27	1.32 ± 0.01

[1] X3-PDMS—Hempasil X3+, a polydimethylsiloxane (PDMS) based paint. PU—Hempel F0038 polyurethane-based paint. RTV-PDMS, an RTV11 room-temperature-vulcanizing-polydimethylsiloxane coating. E—Econea. EM—Isocyanate-functional Econea derivative. [2] Solvent: 1-methyl−2-pyrrolidone.

3.2.1. Antimicrobial Potential of Developed Biocidal Coatings

Antimicrobial activities of PDMS-based and PU-based coatings containing either Econea (PDMS-E) and grafted Econea (PDMS-EM, PU-EM) against *E. faecalis* (ATCC 29212) and MRSA (ATCC 33591) were obtained.

From the bacterial growth responses obtained, expressed by a yellowish opaque film, the contact of Gram-positive bacteria *E. faecalis* and MRSA with the PDMS-based coatings (the red-colored X3-PDMS and the white-colored RTV-PDMS coatings containing incorporated Econea (E) and grafted Econea (EM)) affected their normal growth, whereas no significant effect was observed when in contact with the reference coating without biocide (Pristine X3-PDMS and Pristine RTV-PDMS) (Figure 2). These results are indicative of inhibitory effects by the biocidal coatings on the growth of MRSA. In addition, for the PDMS biocide coating containing the highest biocide content (1.30 wt.%), a slightly higher inhibitory effect, expressed by a lesser extent of the yellowish opaque film on the coated Petri dish, was observed, suggesting a content effect on the action of the antimicrobial coatings.

Similar behavior was obtained for the biocidal PU-based coatings where the inhibitory growth outcomes against MRSA of the PU coating containing grafted Econea (PU-EM), Supplementary Figure S4, showed a more pronounced effect for higher biocide contents (3 wt.%), whereas no significant inhibition was observed on the PU-based coating containing grafted Econea against the tested bacterium *E. faecalis* (Supplementary Figure S4). These results suggest that higher biocide contents in the formulations can promote improvement in the final antimicrobial properties, but they are also related to the intrinsic bioactivity of the biocide to a specific bacterium. In the case of *E. faecalis*, the bioactivity of the Econea biocide expressed in MIC values was relatively low against this bacterium (3.91 µg/mL) when compared with its MIC against the MRSA bacterium (0.49 µg/mL), suggesting that a higher biocide content would be needed to enhance the inhibition effect.

Overall, these results confirm the antimicrobial potential of the biocidal coating formulations, as well as the maintenance of the intrinsic bioactivity of the biocides when incorporated or grafted into polymeric-coating matrices.
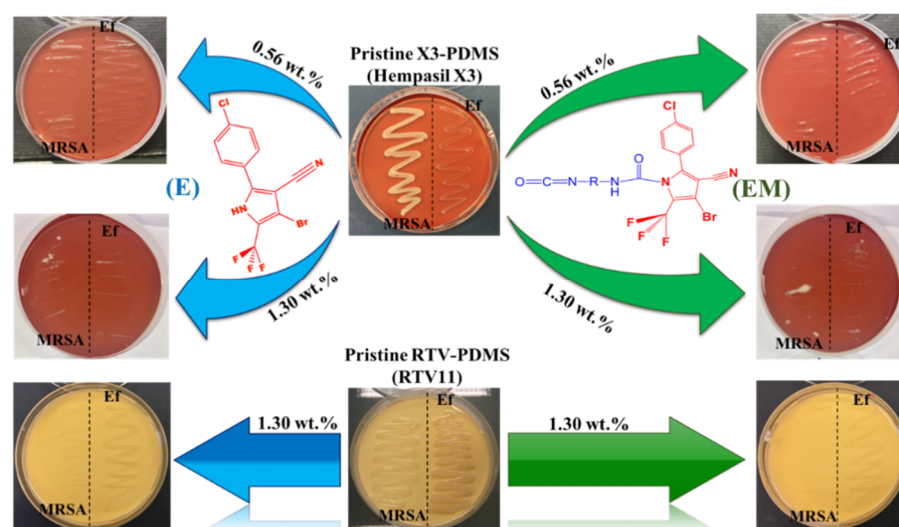
Figure 2. Antimicrobial susceptibility against MRSA and *E. faecalis* (Ef) of polydimethylsiloxane (PDMS)-based coatings, X3-PDMS, and RTV-PDMS, containing incorporated Econea, E (left side), and grafted Econea, EM (right side), at different biocide contents, 0.56 wt.% and 1.30 wt.%.

3.2.2. Biocide Grafting Efficacy on Coated Monolithic Filters

To confirm whether the Econea biocide grafting was effective in the different polymeric-based coating matrices (X3-PDMS and PU), optimized coating formulations were used to coat monolithic ceramic filters, and the biocide release from the coatings into the water (leaching waters obtained after submersion tests of 45 days) was evaluated by UHPLC-MS/MS. These analyses, performed either in the negative and positive ionization modes (Supplementary Figure S5a,b), allowed the identification of 3-bromo-5-(4-chlorophenyl)-4-cyano-1H-pyrrole−3-carboxylic acid (BCCPCA) in the leaching waters (Table 2 and Supplementary Figure S6). Nevertheless, the negative mode seemed to be more suitable for the analysis of these samples and, therefore, it was selected for the subsequent quantification of the leached compound. This BCCPCA (Econea degradation by-product [43]), was found in leaching waters of the PDMS-based filters containing incorporated (CM/X3-PDMS-E) or grafted Econea (CM/X3-PDMS-EM), as well as for the PU-based filter containing incorporated Econea (CM/PU-E). For the PU-based filter containing grafted Econea (CM-PU-EM), no leached compounds were detected.

Table 2. Identification of a leached compound from monoliths coated with polydimethylsiloxane (X3-PDMS) and polyurethane (PU) coatings containing incorporated (X3-PDMS-E and PU-E) and grafted Econea (X3-PDMS-EM and PU-EM), by UHPLC-MS/MS in negative ([M − H]$^-$) and positive ([M + H]$^+$) ESI modes. The compound was identified by the retention time (Rt).

Coated Monolith Filters (CM) [1]	Rt (min)	[M]	[M − H]$^-$/[M + H]$^+$	Error (Δppm)	Formula	Proposed Compound
ESI negative mode						
CM/X3-PDMS-E$^-$	11.6	323.9296	322.9220	2.6	$C_{12}H_6BrClN_2O_2$	BCCPCA
CM/X3-PDMS-EM$^-$	11.4	323.9296	322.9239	−3.3	$C_{12}H_6BrClN_2O_2$	BCCPCA
CM/PU-E$^-$	11.3	323.9296	322.9232	−1.1	$C_{12}H_6BrClN_2O_2$	BCCPCA
CM/PU-EM$^{-\ 2}$	-	-	-	-	-	-

Table 2. *Cont.*

Coated Monolith Filters (CM) [1]	Rt (min)	[M]	$[M - H]^-/[M + H]^+$	Error (Δppm)	Formula	Proposed Compound
ESI positive mode						
CM/X3-PDMS-E$^+$	11.2	323.9296	324.9376	−0.6	$C_{12}H_6BrClN_2O_2$	BCCPCA
CM/X3-PDMS-EM$^+$ [2]	-	-	-	-	-	-
CM/PU-E$^+$ [2]	-	-	-	-	-	-
CM/PU-EM$^+$ [2]	-	-	-	-	-	-

[1] CM—Coated Monolith filter. X3-PDMS—Hempasil X3+, a polydimethylsiloxane (PDMS) based paint. PU—Hempel F0038 polyurethane-based paint. E—Econea. EM—Isocyanate-functional Econea derivative. BCCPCA—Degradation compound of Econea biocide. Samples with a minus/plus superscript were analyzed in the negative and positive modes. [2] For these samples, no significant compound amount was detected in the respective leaching waters.

From the data collected by the UHPLC-MS/MS analyses in an ESI negative mode, it was possible to quantify the total Econea biocide released from the coated filters (Table 3), which showed a substantially higher biocide release, 22.96 wt.% of the total Econea content in the coating, from the CM/X3-PDMS-E-coated filter, containing incorporated Econea, when compared with the CM/X3-PDMS-EM filter containing grafted Econea with a biocide release as high as 3.32 wt.%. These results show the effectiveness of the biocide grafting in the CM/X3-PDMS-EM filter, achieving a 9.22-fold reduction in the biocide leaching (mg/L), resulting in a decrease in the average leaching rate of 2.67 µg/day within 45 days, when compared to the average leaching rate of 24.59 µg/day obtained for the CM/X3-PDMS-E filter.

Table 3. Detected compound by UHPLC-MS/MS analyses in 45 days of leaching waters obtained from submerged monolithic-coated filters with biocidal polydimethylsiloxane (PDMS) and polyurethane (PU)-based coatings, prepared by direct incorporation of the biocide, CM/X3-PDMS-E and CM/PU-E, or by biocide grafting, CM/X3-PDMS-EM and CM/PU-EM.

Coated Monolithic Filters [1]	Biocide Content (mg)	Concentration in Leaching Waters (45 days)		Econea Biocide Leached from Coated Filters (wt.%)
		BCCPCA mg/L	Econea [2] mg/L	
CM/X3-PDMS	-	-	-	-
CM/X3-PDMS-E	4.82	4.12	4.43	22.96
CM/X3-PDMS-EM	3.62	0.45	0.48	3.32
CM/PU	-	-	-	-
CM/PU-E	15.04	0.88	0.95	1.57
CM/PU-EM	13.79	0	0	0

[1] CM—Cordierite Monolithic filter. X3-PDMS—Hempasil X3+, a polydimethylsiloxane (PDMS) based paint. PU—Hempel F0038 polyurethane-based paint. E—Econea. EM—Isocyanate-functional Econea derivative. [2] Econea concentration calculated considering the average amount of its degradation by-product (BCCPCA) in the leaching waters.

Moreover, from the filter coated with PU-based coating containing incorporated Econea (CM/PU-E), only 1.57 wt.% of Econea was leached from the coating. Improvements upon the biocide grafting strategy were still possible since, for the CM/PU-EM, no leached biocidal compound derivative was detected. This successful immobilization is associated with the isocyanate function of the Econea derivative. This function is compatible with polyurethane systems as they already contain isocyanate-based compounds, usually in the curing component of paint formulations, and which, together with a polyol-based component, regulates the formation of the polyurethane polymer matrix. The composition of these components typically contains additional additives to ensure their final function, such as the adhesion, wettability, or the color of paints. However, their final composition is not fully disclosed by suppliers for confidentiality reasons.

3.3. Potential of Non-Biocide-Release-Coated Filters for Waterborne Bio-Decontamination

3.3.1. Antimicrobial Susceptibility of Coated Monolithic Filters

For the potential antifouling purpose application, it is crucial to study the bioactivity of the coatings when applied to the ceramic materials in close to real conditions. For this purpose, the antimicrobial activity of coated filters, against one of the most aggressive resistant bacteria, MRSA, was evaluated as a function of time through a time-kill methodology.

The monoliths coated with biocidal polydimethylsiloxane (PDMS) coatings containing incorporated (CM/X3-PDMS-E) and grafted (CM/X3-PDMS-EM) Econea showed a high inhibition of bacterial growth when compared to the growth of free bacteria or even with the filter coated with the pristine PDMS coating (CM/X3-PDMS) (Figure 3), revealing an auspicious antimicrobial activity for the monolithic filters coated with X3-PDMS-based coatings, containing either incorporated (CM/X3-PDMS-E) or grafted Econea (CM/X3-PDMS-EM) (Figure 3). A slight increase in bacterial growth was also observed for the filter coated with pristine PDMS coating (CM/X3-PDMS) relative to the free bacteria growth curve. This behavior confirms the high susceptibility of bacteria to physically adsorb on PDMS-based surfaces [44,45], which is an effect that is clearly overcome by the biocidal coated filters. Furthermore, prior work has also confirmed that the bacteriostatic properties intrinsic to the Econea biocide and its derivative for the tested bacteria are also inferred on coatings, upon their effective immobilization [35], thus, promoting the minimization of the selective pressure on bacteria to develop resistance to treatment.
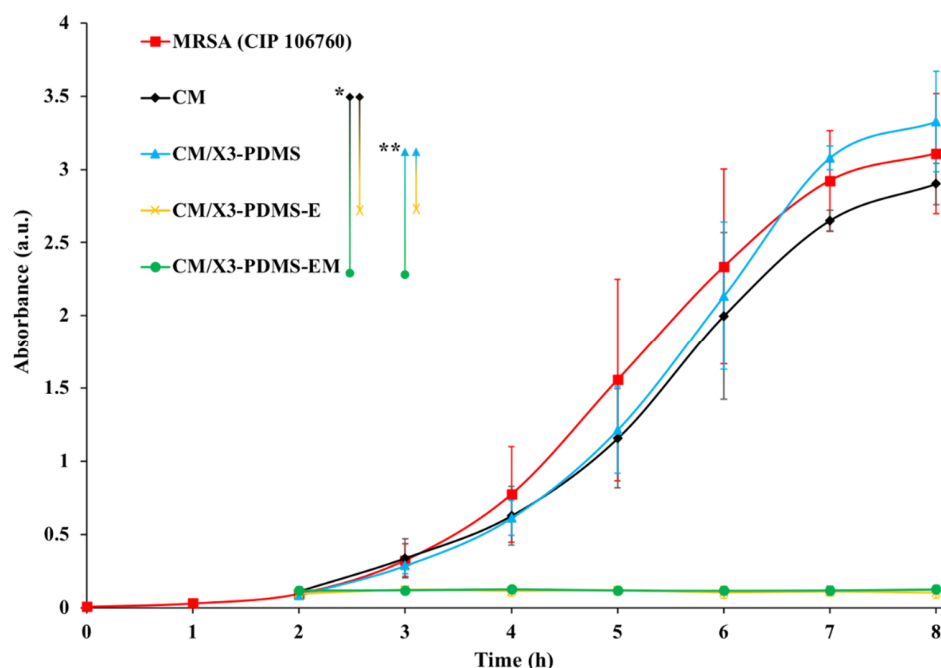
Figure 3. Antimicrobial activities against the bacteria methicillin-resistant *S. aureus* (MRSA), expressed by the optical density (OD$_{620\,nm}$), obtained for uncoated (CM) and coated monolithic filters (200 cpsi, 1.5 cm × 1.5 cm × 2 cm) with polydimethylsiloxane-based coating: containing 0.56 wt.% of incorporated Econea (CM/X3-PDMS-E) or grafted Econea (CM/X3-PDMS-EM), and free of biocide (CM/X3-PDMS, control). The means ± standard deviations (SDs) for three independent tests are presented. Statistical analysis was performed by the Friedman test and the differences are indicated with * $p < 0.05$.

For the filters coated with biocidal polyurethane (PU)-based coatings containing incorporated Econea (CM/PU-E) and grafted Econea (CM/PU-EM), Figure 4, a higher inhibition of the MRSA growth was attained when compared to the free cultured bacteria (MRSA) or even with the filter coated with the pristine PU coating (CM/PU). Moreover,

the differences between the coated filters containing Econea (incorporated or grafted) were evident, showing an expressed delay and subsequent MRSA growth decrease for the monolith coated with PU-based coating containing incorporated Econea (CM/PU-E) when compared with the one containing grafted Econea (CM/PU-EM), revealing that, for the grafting strategy, the delay and reduction in the bacterial growth are not as effective as for the incorporation strategy, which can be associated with the biocide immobilization strategy itself. The leaching strategy (CM/PU-E) released biocide into the culture media (Tables 2 and 3), and, under submersion conditions, a higher concentration of the biocide is expected at the outer coating film, and, under these conditions, more effective contact between the biocide and the bacteria was clearly promoted, thus, providing a higher antimicrobial efficacy. Nevertheless, a grafting strategy is still the most desirable approach to avoid the harmful effects associated with a release strategy and, most likely, an increase in the biocide content of the coating formulations would allow obtaining a similar antimicrobial effect for the grafting strategy, but, and to maintain its original properties, it will also require adjustments in the pristine commercial coating formulation and these adjustments go beyond the goal of this work.
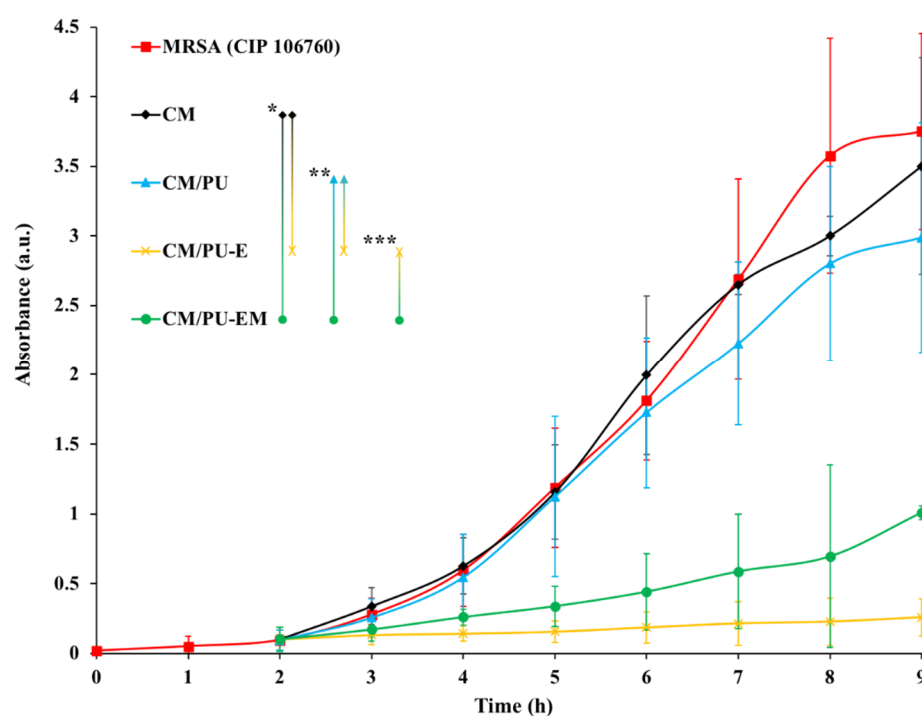

Figure 4. Effects of growth inhibition against the methicillin-resistant *S. aureus* (MRSA) expressed by the optical density ($OD_{620\,nm}$), obtained for uncoated (CM) and coated monolithic filters with pristine polyurethane (PU)-based coating (CM/PU) and containing 2 wt.% of incorporated Econea (CM/PU-E) or grafted Econea (CM/PU-EM). The means ± standard deviations (SDs) for three independent tests are presented. Statistical analysis was performed by the Friedman test and the differences are indicated with ** $p < 0.01$.

3.3.2. Biofilm Formation on Coated Substrates under Dynamic Conditions

A dynamic biofilm formation assay was performed with *E. faecalis* with the aim of evaluating the antifouling activity of polyurethane coating formulations when exposed to shear forces typically found in water-based industrial systems (Figure 5). Both PU-modified coatings (PU-E and PU-EM) reduced biofilm formation compared to the pristine PU under flow conditions ($p < 0.01$). The number of biofilm cells expressed as CFU per cm², was reduced on average by 60% after 48 h, with the PU containing incorporated Econea (PU-E) being the most effective surface in reducing bacterial adhesion, with 72% inhibition when compared to PU.
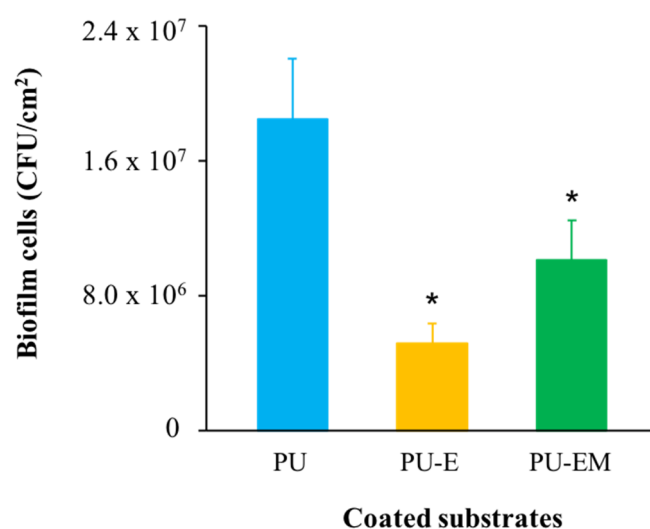
Figure 5. Anti-biofilm performance of pristine polyurethane (PU)-based coating (control), and PU coatings containing incorporated (PU-E) and grafted Econea biocide (PU-EM) against *Enterococcus faecalis* under dynamic conditions. The means $\pm$ standard deviations (SDs) for two independent experiments are presented. Statistical differences ($p < 0.01$) are pointed as * when each PU modified coating (PU-E or PU-EM) is different from the control.

Similar to the quasi-static tests of coated filters, the acrylic surfaces coated with biocidal polyurethane-based coatings also showed to be promising in preventing biofilm formation under dynamic conditions (Figure 5), regardless of how the Econea biocide was associated with the pristine coating (incorporated or grafted). However, the PU coating with incorporated Econea (PU-E) was more effective in reducing the biofilm CFUs than the PU coating with grafted Econea (PU-EM), which is in accordance with the results of antimicrobial susceptibility of PU-coated filters (Figure 4), and this may be related to the biocide immobilization strategy that was followed, as previously discussed.

Furthermore, to assess the contribution of the wettability properties (hydrophilicity/hydrophobicity) of the antimicrobial coatings, measurements of the water contact angle on the coatings are provided in Supplementary Figure S7. The PDMS-based coatings did not reveal significant differences in their wettability, despite their peculiar switchable behavior that changed from a highly hydrophobic (100°) into hydrophilic (50°) surface, associated with the intrinsic properties of the Hempasil X3+ commercial coating [46]. This coating is a hydrogel-based antifouling coating able to act in a dual-action mode, providing a fouling releasing effect (hydrophobicity), inferred by its low surface energy and relatively higher elasticity (low Young's modulus), and a hydrophilic character inferred by the hydrogel-polydimethylsiloxane matrix.

The PU-based formulations revealed stable hydrophobic behavior with contact angles ranging from 75° to 85°, and without significant differences between the formulations with or without biocide, even for higher biocide contents (3 wt.%). These results suggest that the wettability of the coating formulations is not significantly affected by the presence of biocides for the tested contents, confirming that an antimicrobial improvement on those can be achieved upon biocide immobilization and with minimal interference on the original properties of the coatings, which agrees with previous work [24].

4. Conclusions

In this work, monolithic ceramic filters were coated, for the first time, with polydimethylsiloxane (PDMS) and polyurethane (PU)-based marine coatings containing grafted Econea, using an innovative non-biocide release strategy. The effectiveness of the grafted approach was demonstrated through the detection and quantification of the biocide released from the coated filters when submerged for periods of 45 days in water. A 9.22-fold reduction in the leaching of the biocide from PDMS-based coated filters containing 0.56 wt.%

grafted Econea was attained, and no detectable biocide or derivatives were identified in the leaching waters obtained from the PU-based coated filters containing 2 wt.% of grafted Econea, suggesting complete immobilization of the biocide in the PU-based filters.

The coated filters also showed auspicious antimicrobial and bacteriostatic behaviors, providing complete growth inhibition on the biocidal PDMS-based filters, and inhibition effects as low as 66% on PU-based filters containing grafted Econea against the bacterium methicillin-resistant *Staphylococcus aureus*. Dynamic tests that simulate real aquatic conditions also showed promising results for the biocidal polyurethane (PU)-based coatings, with a 48% and 72% biofilm CFU reduction for the PU coating containing grafted and incorporated Econea, respectively, when compared to the PU reference coating.

These results are in line with those obtained in previous real field tests [24] that have already demonstrated the efficacy of both biocide-based coating matrices against marine biofouling, becoming a potential alternative antifouling strategy for water biodecontamination in aquatic systems, such as ballast water on ships, while minimizing the release of biocidal agents into the aquatic environment, and, therefore, contributing to the global search for sustainable and environmentally-friendly antifouling solutions to overcome the challenges of biofouling on industrial surfaces.

Supplementary Materials: The following are available online at https://www.mdpi.com/2079-6412/11/3/323/s1. Figure S1: Eco-friendly strategy to generate the isocyanate derivative of Econea biocide and promote its grafting in polymeric matrices; Figure S2: Antimicrobial activity of Econea biocide (b) and its isocyanate functionalized derivative (EM) (c) against MRSA (ATCC 33591) (a); Figure S3: Minimum bactericidal concentration (MBC) assay against methicillin-resistant Staphylococcus aureus (MRSA) for Econea biocide (E) (a), ranging from 500 µg/mL (E1) and 0.49 µg/mL (E11), and for the isocyanate functional derivative (EM) (b), ranging from 500 µg/mL (EM1) and 7.81 µg/mL (EM7). MRSA is a free bacterial culture assay without any established contact with the biocides; Figure S4: Antimicrobial susceptibility against MRSA and E. faecalis (Ef) bacteria of (a) pristine polyurethane (PU)-based coating (control) and PU-based coating containing grafted Econea biocide at different contents, 2 wt.% (b) and 3 wt.% (c); Figure S5: Base peak chromatograms from the analyses of leaching waters from monoliths (CM) coated with polyurethane (PU) and polydimethylsiloxane (PDMS)-based coatings with incorporated Econea (CM/X3-PDMS-E and CM/PU-E), grafted Econea (CM/X3-PDMS-EM and CM/PU-EM), and biocide-free (CM/X3-PDMS and CM/PU): ESI (-) (a) and ESI (+) (b); Figure S6: Chemical structure of 4-bromo−2-(4-chlorophenyl)−5-(trifluoromethyl)−1H pyrrole−3-carbonitrile (Econea) (a) and 3-bromo−5-(4-chlorophenyl)−4-cyano−1H-pyrrole−3-carboxylic acid (BCCPCA) (b); Figure S7: Contact angle measurements of water on polydimethylsiloxane (PDMS) and polyurethane (PU)-based marine coatings.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data is contained within the article or Supplementary Materials.

Conflicts of Interest: The authors declare no conflict of interest.

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