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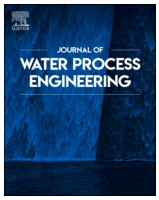

Investigation of biologically active zeolite: Role of colonization in the removal of ^{14}C-labelled sulfamethoxazole in wastewater

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ABSTRACT

Up-to-date approaches to remove micropollutants in wastewater treatment are based on adsorbing materials like activated carbon. These fossil-based materials can also provide a surface for microbial colonization, which could further improve the removal of MPs. As zeolite filters have shown interesting performance in the removal of MPs in previous works, this study aimed to investigate the effect of microbial colonization on such filters on the elimination of ^{14}C-labelled sulfamethoxazole (SMX), an antibiotic from the class of sulfonamides. Lab scale removal tests were set in 100 mL reactors and monitored for 150 days at room temperature. Taxa known to be linked to organic pollutant degradation (*Caulobacterales, Rhizobiales, Burkholderiales*) were found among the microbial community attached to the zeolite. Bacterial colonization of zeolite filters improved the removal of ^{14}C-sulfamethoxazole by 35 % compared to the control. An analysis of the microbial community dynamics over time revealed the increased abundance of the *Vicinamibacterales* taxon after 50 days of contact with SMX. This order abundance, linked to degradation of sulfonamides, went from 0 to 17 %; and Shannon diversity ranged from 1.51 to 1.99. Data showed that zeolite filters as adsorbing material in wastewater treatment plants can improve MPs removal by supporting bacterial colonization, making it an interesting support that could synergize with biological activated carbon.

1. Introduction

Micropollutants (MPs) represent a group of chemical compounds that are found in wastewater and surface waters at extremely low concentrations, often measured in the range of micrograms or nanograms per liter [1]. MP are chemically very diverse and originate from various sources, including domestic wastewater, industrial discharges, agricultural runoff, and improper disposal of chemicals [2]. Even at low concentrations, they could pose a risk because they could affect the growth, development, and reproduction of organisms in aquatic ecosystems [3]. These pollutants comprise many categories, such as pharmaceuticals, personal care products, industrial chemicals, endocrine disruptors, pesticides and more. Both their detection and removal from water are challenging because of their minute concentration levels, often escaping conventional wastewater treatment processes [4].

Adsorption is a commonly advanced treatment for removing micropollutants from wastewater. In this process, adsorbent materials are used to capture and retain the micropollutants on their surface through different chemical interactions. Adsorbents materials shall ideally have high surface areas and specific affinity towards the target compounds to allow for effective removal [5]. Activated carbons (ACs) and zeolites are among the most studied materials. Activated carbon is a highly porous material with an extensive surface area, making it an effective adsorbent for various micropollutants. It is commonly used in wastewater treatment plants (WWTPs) but once exhausted it must be removed and its production costs can be high [6]. Zeolites are crystalline

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aluminosilicates with a regular, porous structure that provides a high specific surface area for adsorption. They are mostly known for their good efficiency in water softening and removal of heavy metals [7]. Moreover, they can selectively remove certain micropollutants based on their molecular size and charge. The selectivity can be tuned by adjusting the Si/Al ratio of their chemical composition: the higher the ratio, the higher is the affinity for hydrophobic organic pollutants [8]. Such synthetic zeolites are often reported in the literature, as they can be very effective in removing different organic pollutants, such as ketoprofen, atenolol and hydrochlorothiazide [9]. Although synthetic zeolites have a better affinity for organic micropollutants, they are generally more expensive than natural zeolites. Natural zeolites were recently engineered into a filter form to remove micropollutants in water alone and in combination with powdered activated carbon (PAC), contributing to enhancing total treatment removal for eleven chemicals (initial concentration = 1 μg/L), mostly pharmaceuticals [10].

In a WWTP, therefore, adsorbent materials can become an adhesion surface for a biofilm, which could synergistically improve the MP removal efficiency of the adsorbent material. Biofilm can play an important role in wastewater treatment, helping in contaminants removal, nutrient consumption (nitrogen, phosphorous) and water quality improvement and several biofilm-based processes are implemented in WWTPs, like sequencing batch reactors or moving bed biofilm reactors [11,12]. When activated carbon (in granulated form, GAC) is colonized, in the presence of organic matter, it is defined as biological activated carbon (BAC). Here, microorganisms can attach to the porous carbon surface and develop a biofilm [13]. The combination of adsorption and biodegradation in BAC results in a synergistic removal mechanism for micropollutants: MPs are adsorbed onto the activated carbon surface, where they become accessible to the microbial community residing within the biofilm. Microorganisms can then metabolize and biodegrade the adsorbed pollutants, further reducing their concentration [14]. A big advantage of this process is that biofilm can decrease the organic load on GAC over time, maintaining its adsorptive properties [15,16]. Literature is therefore rich in works discussing the underlying mechanisms and the MP removal performance of biological activated carbons (see also Ibn Abdul Hamid et al., Korotta-Gamage et al.), yet far less focus has been put on the combinations of zeolites and microbial colonization [17,18]. For example, Gorodylova et al. investigated the removal performance of two pesticides by a zeolite-based biocomposite observing a faster degradation compared to the natural environment performance in soil, while Bai et al. used natural zeolite as microporous carrier for two specific pyridine and quinoline degrading bacteria in both synthetic and industrial wastewater, reaching over 98 % degradation for both chemicals [19,20].

Antibiotics are some of the most studied MPs, particularly due to their latent threat linked to antibiotic resistance in bacteria [21,22]. Sulfamethoxazole (SMX), a common antibiotic from the class of sulfonamides, is used to treat various infections and often found in WWTP effluent water [23]. In addition, sulfamethoxazole is also present on the European Union substances Watch List, as it can pose a risk to or via the aquatic environment, but for which monitoring data is insufficient to come to a conclusion on the actual risk posed [24]. Both physical and chemical processes have been applied to remove SMX, but biological removal is deemed desirable as it tends to generate fewer degradation byproducts. For example, Alvarino et al. studied the fate of SMX in aerobic conditions with hetero- and autotrophic bacteria, showing that in aerobiosis, the antibiotic was mostly biotransformed in the heterotrophic conditions and only a small percentage was adsorbed or mineralized [25]. Also, Bouju et al. studied the mineralization of radiolabeled sulfamethoxazole, using different strains isolated from a membrane bioreactor (MBR) acclimatized to different chemicals, revealing that five of six isolates could mineralize SMX, as 24 to 44 % of the total radioactivity could be detected as $^{14}CO_2$ [26]. Therefore, sulfamethoxazole is an MP of great interest, and it was the focus of the present study.

In previous research, it was investigated how 3D-printed natural zeolite filters could be used and implemented in a wastewater carbon-based tertiary treatment step to understand the pure physical adsorption efficiency of several MPs (including SMX) [10,27]. Since literature is scarce on the combination of a biofilm and natural zeolites in pollutant elimination, and the pure physical adsorption was not contributing enough to MP removal, the contribution of microbial colonization was investigated. The aim of the study was to: 1) offer evidence of microbial colonization in the experimental time frame of this engineered zeolite filter in a real wastewater situation; 2) evaluate the effect of the microbial colonization on the role of SMX removal. Moreover, it was decided to monitor the fate of radioactivity stemming from ^{14}C-SMX to better understand whether it could be mineralized to $^{14}CO_2$ or adsorbed. This work, therefore, could offer a first insight on the contribution of a colonized zeolite filter as tertiary treatment of a WWTP in terms of micropollutant removal.

2. Materials and methods

2.1. Zeolite filters

Zeolite filters were manufactured as described by König et al. and Cuomo et al. [10,27]. Alumina substrates, previously 3D printed and sintered up to 1600 °C, were then coated with a zeolite-based slurry, made by dispersing zeolite powder in distilled water with the addition of a dispersant (Dolapix PC 67, a polycarboxylic acid) and several binders (PVA KB2046, a polyvinyl alcohol, Optapix 170 VAF NEU and Optapix KG 50, two polymers). The filter stability was measured after 2 h of operation with a water flow rate of 240 L h^{-1}. Filters were stable and kept their weight constant during the test (i.e. mass loss <0.1 %). Zeolites were obtained from Zeocem (Micro 50, Zeocem, Bystrè, Slovakia) and were characterized by a particle size of 50 μm with 66.97 % SiO_2 and 10.61 % Al_2O_3 [28]. The filters had a porosity of 89 % and a specific surface area of 545 m^2/m^3. The zeolite coating thickness was 75 ± 25 μm and the coating to support mass ratio was 50.

2.2. Zeolite filter microbial colonization

Microbial colonization was promoted on zeolite filters (net zeolite weight: 0.934 g, cv ± 15 %) by submerging the filters in supernatant of settled sludge sampled from the MBR of the inner WWTP in FHNW (FachHochschule Nord West Schweiz, Muttenz, Switzerland) (see Table 1). The supernatant was used as a source of microorganisms and fresh supernatant was pumped into the vessel for a continuous flow. A peristaltic pump (ISMATEC MCP, IDEX Corp., USA) set with a 0.1 mL flow was used to let the sludge flow in a 250 mL Pyrex beaker where zeolite filters were placed. A volume of 800 mL of sludge was poured in a 1 L reservoir inflow glass bottle and let it settle to allow the sedimentation of sludge flocks, to avoid blockages of the pump (see Fig. 1). An initial volume of 100 mL of settled sludge was added to the vessel containing the filters to fully cover them. The setup was built to have a constant flow and to ensure the filters were always fully covered by sludge, with a hydraulic retention time of 84 h and kept on for 12 weeks.

2.3. Microscopy analyses

Epifluorescence microscopy observation was run with an Olympus CKX53 equipped with a pE-300 LED excitation module and an EP50 camera (Olympus Europa SE & Co. KG, Germany). Samples were cut from the colonized zeolite filters (0.123 ± 0.013 g) and then stained by incubating them with SYBR Green (SG) and Propidium Iodide (PI) (both 0.1 % *v*/v in Phosphate Buffer Saline, PBS 1×) for 20 min [29]. Colonization of zeolite filters was observed after 12 weeks of MBR sludge treatment (see previous paragraph).

Table 1
Characterization of MBR sludge.

Parameter	COD	$N\text{-}NH_4^+$	$N\text{-}NO_2^-$	$N\text{-}NO_3^-$	N_T	P_T	TSS	pH
Unit	[mg O_2/L]	[mg/L]	[mg/L]	[mg/L]	[mg/L]	[mg/L]	g/L	
Mean ($n = 15$)	20.67	0.68*	0.12	26.26	28.62	8.19*	4.91	20.67
Standard deviation	4.19	0.8	0.09	12.76	10.6	6.28	1.09	4.19

COD: Chemical Oxygen Demand. $N\text{-}NH_4^+$: Ammonium. $N\text{-}NO_2^-$: Nitrites. $N\text{-}NO_3^-$: Nitrates. N_T: Total nitrogen. P_T: Total phosphorous. TSS: Total suspended solids.
* $n = 30$.

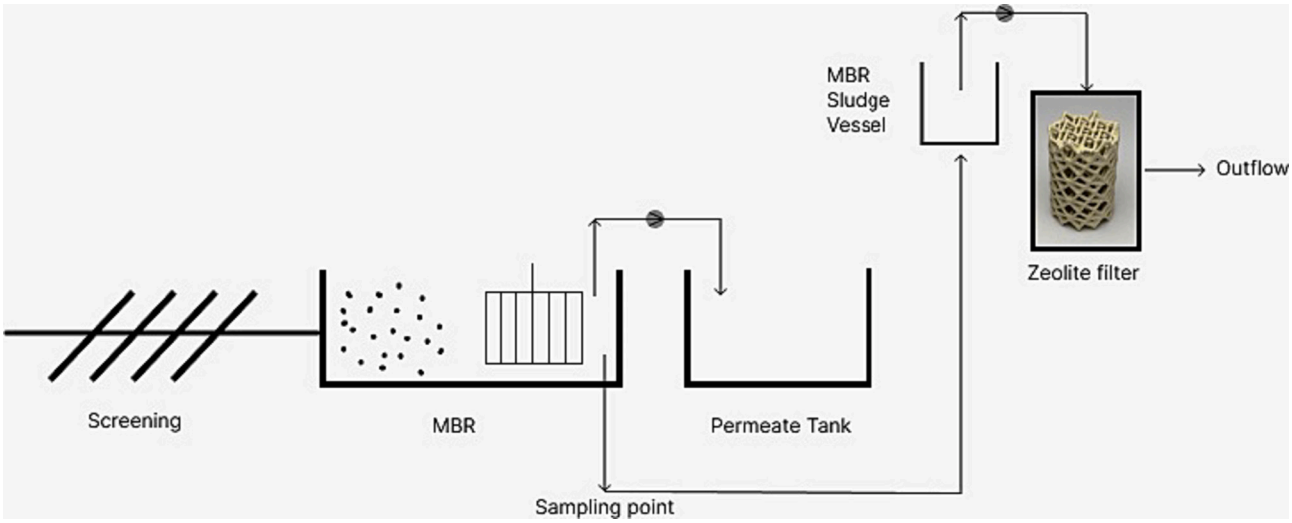

Fig. 1. WWTP scheme from where MBR sludge was sampled (left) and colonization setup scheme (right).

2.4. ^{14}C-SMX batch removal tests

The experimental design consisted of a main test (named SCZF – [aniline ring-^{14}C(U)] SMX and colonized zeolite filter, Fig. 2) where the colonized zeolite filter was studied and different controls: "Control" had only the MBR sludge, "S" had the labelled SMX and "SZF" had the labelled SMX and a non-colonized zeolite filter (see Table 1). The experiments were run for 150 days at room temperature, except for SZF which was kept running for a shorter time (100 days).

All tests were run with settled MBR sludge (50 mL) sampled from the same WWTP (FHNW Muttenz, CH) inside 100 mL glass bottles. ^{14}C radiolabeled SMX was added to the samples to reach a final concentration of 3 μg/L and an adsorbent material according to the experimental plan (see Table 2). Radiolabeled SMX (uniformly ring-labelled, specific activity of 2.22 GBq/mmol; see Table SI1) was purchased from ARC (American Radiolabeled Chemicals, USA, order number 3653-250 U). SZF and SZCF were fit with a NaOH trap to capture any $^{14}CO_2$ formation during the experiment. The trap was installed by gluing a copper wire on the bottom side of the bottle cap holding a 2 mL Eppendorf ® tube filled with 1 mL of 1 M NaOH. Before and after sampling, each bottle was manually shaken for 30 s. For the time of the incubation, bottles were kept under a chemical hood with no direct light applied.

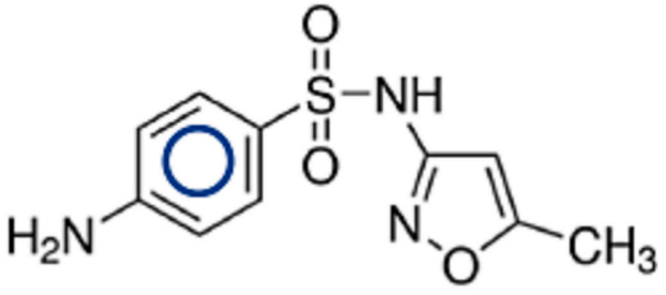

Fig. 2. Chemical structure of radio-labelled sulfamethoxazole. The ^{14}C labeling is indicated by the blue circle [30]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Scheme of the SMX removal tests ($n = 3$).

Test ID	MBR sludge	^{14}C-SMX	Zeolite filter	NaOH trap (1 M)
Control	Yes	No	No	No
S	Yes	Yes	No	No
SZF	Yes	Yes	Yes	Yes
SCZF	Yes	Yes	Yes, colonized	Yes

2.5. Liquid scintillation counting (LSC) - radioactivity monitoring

1 mL samples were taken from the supernatant and from the NaOH trap of each replicate at different times during the whole duration of the testing, namely 2, 4, 6 h and then on days 1, 2, 9, 14, 17, 35, 42, 49 and 150. Then, 5 mL (15 mL for NaOH trap samples) of Supersolve ®- X (Zinsser Analytics, Germany) were added to each sample before analysis in the LSC machine (Hidex 600 SL, Hidex, Finland). The output values of radioactivity were expressed in DPM (disintegration per minute). Radioactivity was evaluated with the software MikroWin 600 SL, version 5.04 (Hidex, Finland). At the end of the experiments, the whole filters were also analyzed to measure the residue radioactivity by oxidizing them with an Oxidizer 600 OX (Hidex, Finland). Here, organic carbon in the samples gets combusted to form CO_2, which is added to 15 mL of LSC cocktail Oxidizer 600 OX LSC Radiocarbon (Hidex, Finland) before LSC analysis. Trends of radioactivity over time were reported as partitioning of ^{14}C, meaning that each sampling point was expressed as a percentage calculated compared to the initial radioactivity value found (100 %) (see Fig. 3).

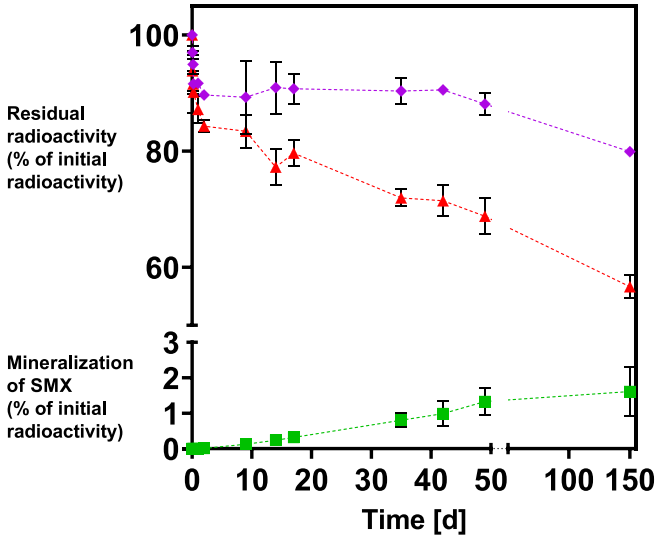

Fig. 3. Evolution of residual radioactivity of tests S (purple, no zeolite filter), SZCF (red, colonized zeolite filter) and mineralization of SMX (green)($n = 3$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.6. DNA extraction and sequencing

Samples of the colonized filters (named A, B and C) (0.123 ± 0.013 g) were obtained shortly by removing the filter from its 100 mL bottle cutting a small piece from the upper part. Samples were taken at 0, 17, 50 and 150 days and immediately stored at −20 °C. DNA was extracted using the ZymoBIOMICS DNA Miniprep Kit (ZymoResearch Corp., USA) by following the manufacturer's instructions and quantified through spectrophotometry (see Tables SI2, SI3) (Nanodrop ND-1000 spectrophotometer, ThermoFisher). Sequencing libraries were made amplifying the V4 region of 16S bacterial rDNA. Amplification protocol foresaw 35 cycles made by an initialization step at 95° for 10 min, a denaturation step at 95° for 30 s, an annealing step at 55° for 30 s and an elongation step at 72° for 3 min. This library was then sequenced with a 16S™ NGS Library Prep Kit (V4) (ZymoResearch Corp., USA) using an Illumina MiSeq (Illumina, USA). Bacterial community analyses sequencing and data analyses were carried out as described elsewhere [31]. Barcode primers and DNA quantification data are available in the supplementary information.

2.7. Statistical analyses

Each SMX batch removal experiment was run in triplicates and a single LSC run was carried out for every replicate. Analysis of variance (ANOVA) was carried out to assess statistically significant differences among the samples. Data from radioactivity monitoring experiments and from microbial community analyses were processed with MS Excel (Microsoft Corp., Redmond, USA) and GraphPad 10 (GraphPad Software, San Diego, USA).

3. Results and discussion

3.1. ^{14}C-SMX batch removal tests

One set of SMX removal experiments was composed by Control, S and SCZF, while SZF was tested separately to also study the removal efficiency of a clean zeolite filter. The fate of the radiolabeled chemical was monitored over time using LSC, and the radioactivity in the supernatant is calculated as residual radioactivity over time: in Fig. 3, the residual radioactivity of tests S and SCZF are reported in the upper part of Y axis, while the production of $^{14}CO_2$ is shown on the bottom of the Y axis.

While the residual radioactivity in test S (no colonized filter present) is overall stable over time, after a small initial drop of radioactivity in the supernatant, the residual radioactivity in test SZCF samples was constantly decreasing over time. In contrast, radioactivity in the supernatant of SZF with non-colonized zeolite remained constant over time (Fig. SI1) with approximately 90 % lasting after 100 days, suggesting that the contribution of the abiotic zeolite filter to the removal of SMX is less pronounced than that of the biological component. Hence, the removal in the samples with colonized filters cannot be solely explained by sorption to the zeolite. Also, it is important to notice that in S, SZF and SZCF, an initial steep decrease within a few hours before intermediate stabilization at about 85–90 % of the initially applied radioactivity can be observed. This behavior could be explained by a sorption equilibrium in the system which is rapidly reached. The removal in the samples with a clean zeolite filter is also corroborated by a previous study: the same zeolite filters were tested in distilled water to evaluate their removal efficiency towards eleven different micropollutants: SMX (1 μg/L of SMX and a zeolite amount of 0.934 g) removal efficiency reached around 15 % [10].

In SZF and SZCF, 1 M NaOH traps were installed to monitor any $^{14}CO_2$. Production of radioactive carbon dioxide was monitored over time and a very low (see Fig. 4) formation of $^{14}CO_2$ was observed. This resulted in a cumulative production of 2 % of $^{14}CO_2$ relative to the initially applied radioactivity, while NaOH traps installed in SZF showed no $^{14}CO_2$ production (see Fig. SI1). This implies that the microbial community grown over time was capable of fully degrading and mineralizing only a limited amount of sulfamethoxazole. A very similar behavior was described by Betsholz et al., where the fate of radiolabeled SMX was monitored in the presence of colonized granular activated carbon, showing that transformation of sulfamethoxazole could not be detected via the formation of $^{14}CO_2$ [30].

3.2. Imaging, DNA extraction and sequencing

Molecular biology analyses were carried out to monitor changes in the microbial community over time. Samples of colonized zeolite from the radioactive SMX monitoring experiments were taken at time 0 and after 17, 50 and 150 days. To demonstrate the colonization of zeolite, epifluorescence microscopy imaging was performed on colonized zeolite filter samples after the biofilm growth setup was run for 12 weeks. In Fig. 4, live cells stained by SYBR Green are fluorescing green, while dead cells colored by PI are fluorescing red. The presence of green colored cells shows that a microbial colonization was obtained on the zeolite filters. Moreover, it is possible to notice the morphology of filamentous bacteria, that can be found in the sludge of wastewater treatment plants when a carrier is added: Song et al., for example, promoted the growth of filamentous bacteria in activated sludge by adding string carriers [28].

The sample at time 0 was taken from colonized filters when they were still altogether at the end of the colonization step (see paragraph 2.3), therefore it was collected into a single sample "Time 0" (see Fig. 5). Sequences were analyzed and organized into a total of 6 phyla, 7 classes and 14 orders. The data in Fig. 5 shows the composition and relative abundance of the 14 orders observed in every sample. The core taxa (on order level) were composed of *Caulobacterales*, *Rhizobiales*, *Burkholderiales*, *Pseudomonadales, Corynebacteriales, Gemmatimonadales* and *Sphingomonadales*. The dominant orders were *Caulobacterales*, *Rhizobiales*, *Burkholderiales*, *Pseudomonadales* and *Vicinamibacterales*, with abundances ranging from 5 to 56 %, 13 to 56 %, 3 to 38 %, 2 to 27 %, 0 to 17 % respectively. These orders were all present for the whole experiment at different abundances, apart from the order of *Vicinamibacterales,* which was observed only in the last sample. Biodiversity was observed by calculating Shannon diversity indexes (SDI) for each sampling at every different time (namely 0, 17, 50 and 150 days) (see Fig. 5, Table SI4) [32]. After 50 days of testing, the taxa richness, measured as SDI, returned to the initial values (absence of labelled SMX), suggesting

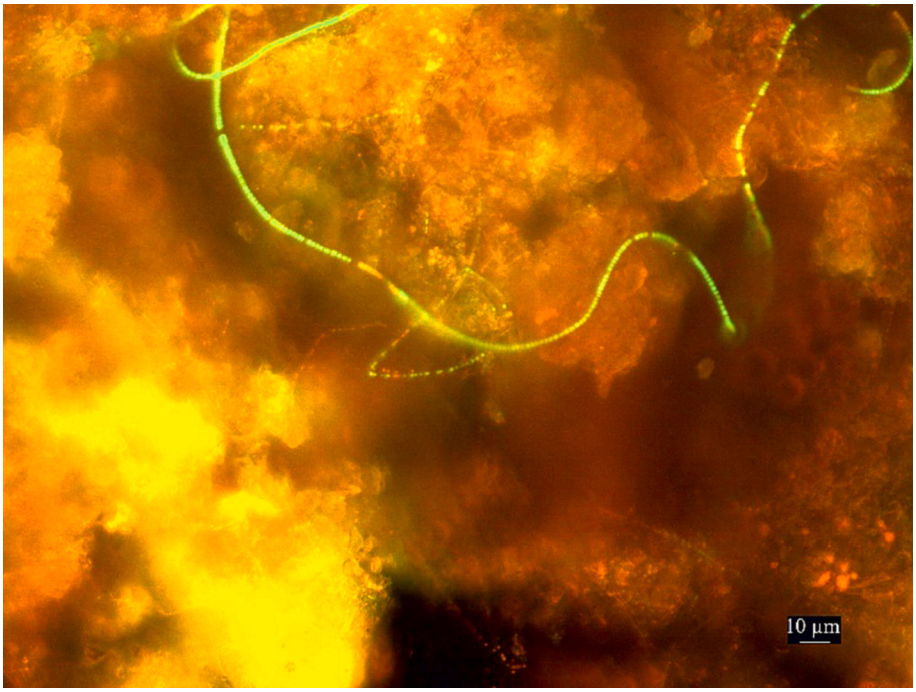

Fig. 4. Epifluorescence image of the colonized zeolite filter (Magnification 100×, Olympus CKX53).

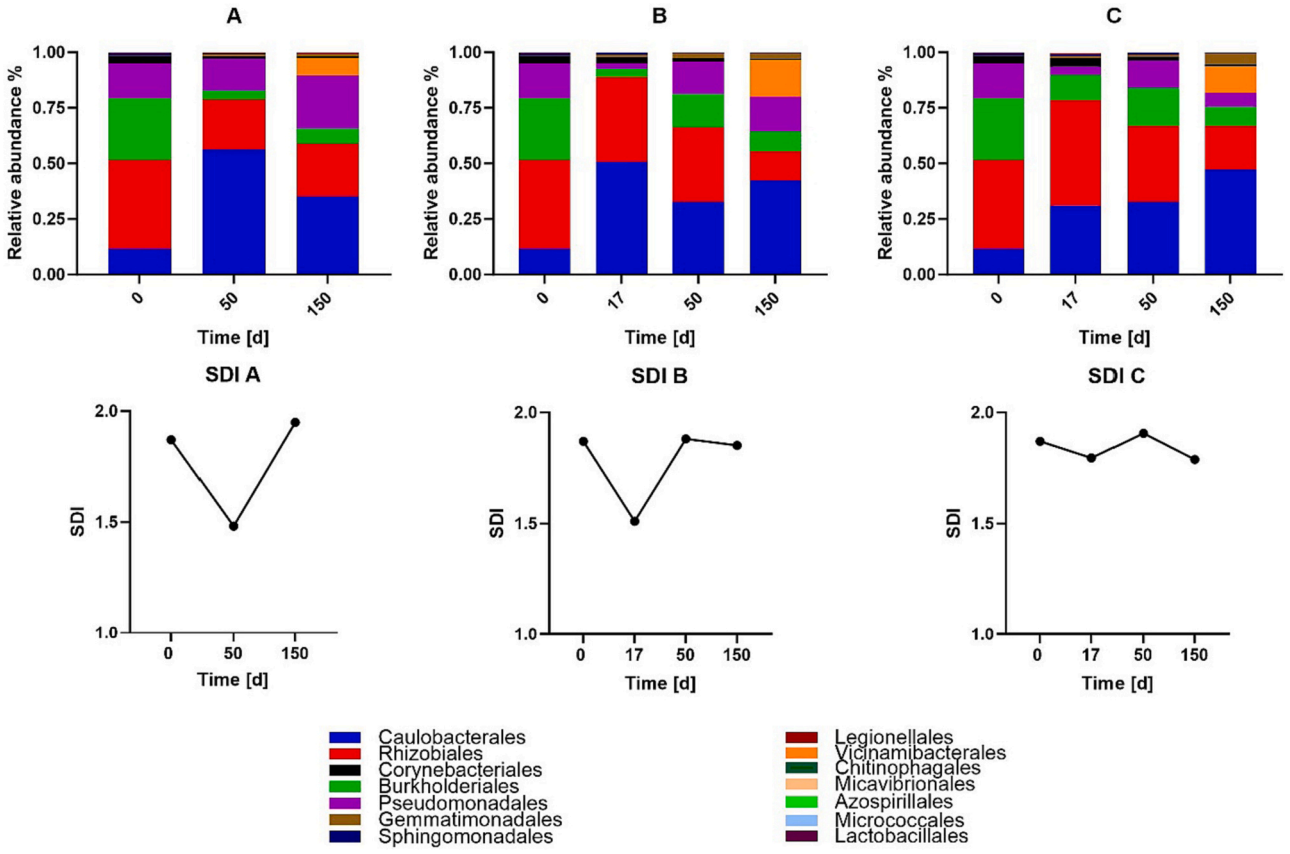

Fig. 5. Upper part: Microbial community composition of colonized zeolite filters A, B and C on order level expressed as relative abundance (RA%). Orders with RA > 3 % in at least one sample are included in the plot. Lower part: SDI evolution for filters A, B and C.

an increase in adaptation for taxa that became more abundant (i.e. *Vicinamibacterales*).

3.3. Fate of radiolabeled SMX

The evolution of residual radioactivity in Fig. 3 shows that the residual radiation in both control and test dropped by 10 % over the first three days, suggesting that both systems have reached a first equilibrium point. After the third day of testing, the S radioactivity remains stable until the end of the experiment (except for a small drop occurring in the very last 50 to 150 days), while the radioactivity in SZCF keeps decreasing over time until the remaining radioactivity left is about 55 % of the initial value. Since SZF, ran with a clean zeolite filter, did not show a decreasing trend over time, we can infer that the major contribution in SMX removal from the supernatant in SZCF is due to microbial colonization. This colonization could have either adsorbed or partially biotransformed SMX, possibly to intermediates other than full mineralization, given that only 2 % of the SMX was fully degraded to $^{14}CO_2$. This phenomenon has also been observed in Betsholz et al., where the monitored removal of ^{14}C-SMX was promoted by different materials with grown biofilms (MBBR carriers, sand filter and GAC): sand filters and MBBR carriers could remove up to 10 % and 30 % of the applied radioactivity, respectively; while biofilm + GAC reached higher percentages, also depending on the concentration of activated carbon used [30].

Several of the identified bacteria found in microbial community analyses are commonly found in wastewaters: Fan et al. reported the presence of *Rhizobiales* and *Burkholderiales* while monitoring a full scale WWTP, while Sanchez Zurano et al. studied a pilot-scale reactor for microalgae wastewater treatment, finding *Pseudomonadales* as one of the most dominant orders [33,34]. Also, *Burkholderiales*, found with an initial relative abundance (RA) of 27 % and decreased over time to 10 % after 150 days of testing, were found in a SMX degradation work in the presence of different exogenous cofactors reaching a removal of over 94 % [35]. Interestingly, *Achromobacter denitrificans* PR1, belonging to *Burkholderiales* order, was also found effective in SMX and other sulfonamides' removal [36]. *Rhizobiales* were detected at time 0 with a RA of 40 % and decreased over time down to 20 % when it was detected at 150 days of testing. Also this order is involved in SMX degradation: a pure culture of *Ochrobactrum* sp. SMX-PM1-SA1 was found able to remove up to 45 % of SMX [23]. *Pseudomonadales* were found with an initial RA of 15 % and their abundance remained stable around this value until the end of testing. Bacteria belonging to this order were found linked to SMX degradation: Jiang et al. studied the removal of SMX by a pure culture of *Pseudomonas psychrophila* HA-4, reaching a maximum removal performance of 34 %; also *Acinetobacter* sp. was able to degrade SMX up to 98 % [37,38].

Vicinamibacterales order was detected (up to 17 %) in all samples and in the period from 50 to 150 days of SMX monitoring, where the radioactivity decreased by 15 % in the supernatant. For example, the family *Vicinamibacteraceae* (belonging to the *Vicinamibacterales* order), was identified in agricultural soil samples irrigated with swine wastewater as a degrader of another sulfonamide, sulfamethazine (SMZ), with a chemical structure very similar to SMX [39]. The observation of this order after 50 days of testing could also explain why we observe a decrease of radioactivity not only in SZCF but also in S (see Fig. 4), as *Vicinamibacterales* presence could also be inferred in the sludge and not only on the zeolite filter. Biofilms, thanks to a 3D structure, provide a microenvironment suitable for different metabolic needs and protective properties for microorganisms [40]. Therefore, it can help the taxa potentially involved in the MP removal, to resist environmental changes. In fact, after 150 days, the Shannon biodiversity index increased back to the initial values, showing the development of a complex microbial community where *Vicinamibacterales* can have a role in SMX degradation. Future investigation would compare the removal performance in tests focusing on different growth mode (biofilm versus suspended) of the most interesting taxa reported in the present paper.

Therefore, the presence of heterotrophic bacteria could explain the behavior of the residue radioactivity measured in SZCF in combination with bioadsorption processes: for example, Yang et al. demonstrated the adsorption of SMX by a composite of chemically modified biochar and wastewater sludge [41]. Additionally, a radioactivity balance was carried out by oxidizing (see Chapter 2.5) both colonized and non-colonized zeolite filters at the end of testing: results showed that the residual radioactivity in the colonized filters is two times the radioactivity found in the non- colonized filters. This further confirms that the presence of a biofilm had a positive effect on SMX removal, especially considering that byproducts deriving from SMX partial biodegradation could have a higher hydrophobicity and therefore, higher affinity for the biofilm. Reis et al. listed over 10 main byproducts of SMX deriving from bacterial degradation in both aerobic and anaerobic conditions, while Hu et al. analyzed over 20 SMX metabolites in a degradation study carried out with two bacterial strains isolated from pig slurry [36,42]. For example, 4-Nitrophenol, 4-Nitro-sulfamethoxazole, Acetanilide, Phenylacetic acid and Benzoic acid (SMX metabolites) have log K_{ow} values of 1.91, 1.22, 1.16, 1.41 and 1.87 respectively. All of these are higher than the log K_{ow} of SMX: its theoretical log K_{ow} is 0.9, but since the experimental pH is 7, SMX is present in its dissociated form and could have an even lower log K_{ow}. (Data retrieved from Pubchem). Lastly, the possibility to promote a colonization with a positive effect on MP removal on zeolite filters, could open the way for these supports in real wastewater applications, just like activated carbon is used and colonized over time in WWTPs. For example, Sbardella et al. demonstrated that a BAC plant, combined with ultra filtration, could remove up to 78 % of antibiotics over a 1-year time span, while in the present work, the zeolite/biofilm system was capable to uptake up to 35 % of radiolabelled SMX during 150 days, making it interesting for a real scale application [43]. In a recently published work by our group, we demonstrated how the integration of zeolite filters along with activated carbon could be interesting for WWTPs in both economics and performance. In addition to these findings, the results of the current work, in the hypothesis of a real scale situation where activated carbon and zeolite are used, could therefore make the implementation of zeolite filters even more interesting thanks to the biofilm related pollutant removal contribution.

4. Conclusions

In this work, the occurrence and activity of a microbial colonization on an engineered zeolite-based filter using MBR sludge was tested in the degradation of a radiolabeled micropollutant (sulfamethoxazole). The role of microbial biomass present on the zeolite filter was shown: the main finding was the removal of up to 35 % of the radioactivity of sulfamethoxazole when compared to the control. Microbial community analyses showed that three main orders (*Caulobacterales, Rhizobiales, Burkholderiales*), related to degradation of organic pollutants, changed in their respective relative abundance over time, while *Vicinamibacterales* order became abundant after 50 days of SMX presence. The present paper reports that the overall removal of sulfamethoxazole can be explained by a combined effect of bacterial activity and adsorption, both on biomass and, to a limited extent, on the zeolite. The findings here reported propose the colonized zeolite filters as an interesting solution for MP removal in WWTPs in combination with biological activated carbon. Future interest lies in the exploration of regeneration processes of the biological zeolite system to observe changes in MP removal efficiency.

CRediT authorship contribution statement

M. Cuomo: Investigation. **R. König:** Conceptualization. **E. Zanardini:** Supervision. **A. Di Guardo:** Writing – review & editing. **E. Terzaghi:** Data curation. **B.A. Kolvenbach:** Supervision. **F. Demaria:**

Investigation. **P.F.X. Corvini:** Writing – review & editing. **P. Principi:** Supervision.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Roger Koenig reports financial support was provided by Federal Office for the Environment. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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