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Removal of antimicrobials using advanced wastewater treatment

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ABSTRACT

Removal of numerous classes of pharmaceuticals from the municipal and industrial wastewater, using conventional wastewater treatment, is incomplete and several studies suggested that improvement of this situation would require the application of advanced treatment techniques. This is particularly important for the treatment of industrial effluents, released from pharmaceutical industries, which can contain rather high concentrations of antimicrobials. The aim of this work was to evaluate membrane bioreactors (MBRs), nanofiltration, reverse osmosis and ozonation, as well as their combinations, for the removal of antimicrobials from a synthetic wastewater which simulated highly contaminated industrial effluents. The study was performed using a mixture of four important classes of antimicrobials, including sulfonamides (SA), fluoroquinolones (FQ), macrolides (MAC) and trimethoprim (TMP). Performance of two different types of MBRs, Kubota and Zenon, was evaluated under different regimes regarding hydraulic retention time, total organic load and total nitrogen load. It was shown that elimination of SA in MBR treatment was very efficient, while the elimination of MAC, FQ, and TMP was incomplete. A mass balance of these contaminants in MBR suggested that microbial transformation represented the main mechanism, while only a small percentage was eliminated from the aqueous phase by adsorption onto sludge particles. Nanofiltration and reverse osmosis achieved high elimination rates however produced highly contaminated concentrate. High removal was achieved using ozonation, but further research is needed to characterize formed ozonation products.

1. Introduction

Pharmaceutical compounds belong to the most intensively studied categories of emerging contaminants [1]. Consequently, there is an increasing body of evidence, showing their significant potential to harm the environment [2]. Among different harmful effects, a special attention has been paid to the assessment of environmental risks associated with the widespread occurrence of antimicrobials in the aquatic environment [3]. One of the key issues is the possible importance of the aquatic route for the spreading of antibiotic resistance [4]. It was assumed that the chronic exposure to antimicrobials, occurring in municipal and industrial wastewater effluents, may give a raise to the emergence of antimicrobial-resistant organisms [5]. One strategy to limit proliferation of resistant bacteria is to reduce the exposure to antimicrobials by improving their removal from wastewater.

It was shown that the removal of pharmaceuticals during conventional activated sludge treatment (CAS) is incomplete [6] and different alternative approaches for their elimination have been proposed, including membrane biological reactors (MBRs), membrane filtration technologies and advanced oxidation processes [7–9]. Among the most promising new technologies to achieve this goal are membrane bioreactors, which showed improved removal efficiencies of xenobiotic contaminants compared to CAS treatment [10–12]. As discussed in a recent review by Sipma et al. [13], combination of high solid retention times and high biomass concentration can improve biodiversity of microbial population in the reactor and promote selection of special microorganism strains capable of transforming refractory pollutants such as pharmaceuticals [14,15]. Further advantages of MBRs are complete retention of the suspended solids and reduced excess sludge production [16]. Consequently, emissions of some adsorbable contaminants via treated effluents can be significantly reduced [14,17]. The main disadvantages of the MBR technology, higher costs and energy demands, have been significantly reduced in the past years [18]. Nevertheless, the expected benefits of MBR systems over the CAS treatment for the removal of pharmaceuticals have not yet been fully demonstrated in real systems. In fact, the results from different studies dealing with the comparison of these two technologies are rather variable and sometimes conflicting [13].

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A special attention in the last few years was paid to the advanced filtration and oxidation techniques [8,9,19,20]. Unlike ultrafiltration, nanofiltration (NF) and reverse osmosis (RO) are rather efficient in rejecting most of the pharmaceutical compounds from the wastewater effluents [8]. High removal rates can also be achieved by ozonation [20,21], which is comparatively less expensive than filtration techniques, but it is still relatively energy-demanding. The main problem associated with the application of this technique is the formation of numerous by-products, whose ecotoxic properties are often completely unknown.

It should be stressed that most of the reports on antibiotic behavior during wastewater treatment were strongly focused on municipal wastewaters, which are characterized by the presence of complex mixtures of different pharmaceuticals, occurring in relatively low concentration [6]. However, recent studies by Larsson et al. [22] indicated that pharmaceuticals can reach the aquatic environment at much higher concentrations as a result of the wastewater discharges from the pharmaceutical production facilities. Fluoroquinolone ciprofloxacin was detected in receiving ambient waters at extremely high concentration, reaching into mg per liter range [22]. Similar problem was reported in Croatia in a small water course that received combined wastewater effluents from the baker's yeast factory and production of macrolide antibiotic azithromycin [23]. To the best of our knowledge, the treatment of such wastewaters, containing high levels of organic carbon and antibiotics, using MBR treatment was never reported in the literature.

The aim of this work was therefore to assess the application of advanced treatment techniques, including MBR, nanofiltration, reverse osmosis and ozonation for the removal of four important classes of antimicrobials, at the concentrations characteristic for industrial wastewater effluents. Performance of two different types of MBRs, Kubota and Zenon, has been evaluated under different regimes regarding hydraulic retention time, total organic load and total nitrogen load.

2. Experimental

2.1. Chemical and reagents

Sulfadiazine (SDZ), sulfathiazole (STZ), sulfapyridine (SPY), sulfamethazine (SMZ), sulfamethoxazole (SMX), trimethoprim (TMP), erythromycin (ERY) and roxithromycin (ROX) were purchased from Sigma-Aldrich (Steinheim, Germany). Ciprofloxacin (CIP) and azithromycin (AZI) were obtained from Fluka (Buchs, Switzerland). *N*-acetyl sulfamethoxazole (*N*-acetyl SMX) and [$^2H_5$] *N*-acetyl sulfamethoxazole (d^5-*N*-acetyl SMX) were purchased from Toronto Research Chemicals (North York, ON, Canada). Clarithromycin (CLA) was kindly supplied by Pliva (Zagreb, Croatia).

All chemicals used for the preparation of the MBR feed (Table 1) were of p.a. grade. Yeast extract and peptone were purchased from Biolife (Milano, Italy). Inorganic salts ($FeCl_3{\cdot}H_2O$, KH_2PO_4, $MnCl_2{\cdot}H_2O$ and NH_4Cl) were obtained from Kemika (Zagreb, Croatia). All HPLC solvents (gradient grade) and additives were purchased from Merck (Darmstadt, Germany). Ultrapure water used as eluent for HPLC separations was prepared using Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Experimental setup of MBR treatment

All experiments were performed with synthetic wastewater, which was prepared by diluting the corresponding ingredients in the tap water having alkalinity of 300 mg L^{-1} $CaCO_3$, total hardness of 375 mg L^{-1} $CaCO_3$ and 5 mg L^{-1} of nitrate (NO_3–N). The synthetic wastewater contained glucose (114 mg L^{-1}), peptone (86 mg L^{-1}) and yeast extract (1.2 mg L^{-1}) as the main organic carbon and nitrogen sources, as well as several inorganic salts that provided trace elements, and inorganic nitrogen and phosphorus (Table 1). The synthetic wastewater was spiked with selected antimicrobials belonging to four different classes. These included sulfonamides (sulfadiazine, sulfathiazole, sulfapyridine, sulfamethazine and sulfamethoxazole), fluoroquinolones (norfloxacin and ciprofloxacin), macrolides (azithromycin, erythromycin, clarithromycin and roxitromycin) and trimethoprim. Each antimicrobial compound was added to the synthetic wastewater in the concentration of 100 μg L^{-1}, resulting in the total antimicrobial concentration of 1.1 mg L^{-1}. Such enhanced concentration levels, applied in the present study, simulate the situation in wastewater discharges from the pharmaceutical industry [23]. All antimicrobial additions were made from the stock solutions (concentration between 0.67 mg mL^{-1} and 10 mg mL^{-1}) prepared in methanol. As a consequence, the final feed medium contained 0.4 mL of methanol per liter. The default total organic carbon (TOC) concentration was 220 mg L^{-1}, while the ratio between chemical oxygen demand (COD) and TOC was around 3.

Table 1
Characteristics and default composition of synthetic wastewater for the experiments with membrane bioreactors.

Parameter	Concentration/value
COD (mg L^{-1} O_2)	690
BOD (mg L^{-1} O_2)	400
TOC (mg L^{-1})	220
pH	6.5
Conductivity (μm cm^{-1})	600
Total nitrogen (mg L^{-1})	25
Total phosphorus (mg L^{-1})	3
NH_4Cl (mg L^{-1})	43
KH_2PO_4 (mg L^{-1})	18
$MnCl_2{\cdot}4H_2O$ (mg L^{-1})	2
$FeCl_3{\cdot}6H_2O$ (mg L^{-1})	1
Peptone (mg L^{-1})	86
Glucose (mg L^{-1})	114
Yeast extract (mg L^{-1})	1.2
Methanol (mL L^{-1})	0.397
Antimicrobials (mg L^{-1})	1.1

Two different MBR membranes were examined in this study. The first one was a Zenon MBR, equipped with Zenon ZeeWee 10 hollow fiber membrane (Zenon, Canada), while the second one was a Kubota MBR equipped with plate&frame membrane XJ3 (Kubota, Japan). The corresponding membrane areas were 0.93 m^2 and 0.33 m^2 for Zenon and Kubota MBRs, respectively, while the pore size was 0.4 μm for both systems. The membranes were submerged in the bioreactor vessels having useful volumes of 44 L and 15 L for Zenon and Kubota MBRs, respectively. In order to provide dissolved oxygen, necessary for the biological treatment, as well as to reduce membrane fouling, membranes were scourged by air flow (3.4 m^3 h^{-1}) through diffusers placed at the bottom of the bioreactors. The permeate suction for both MBRs was facilitated by a laboratory vacuum pump. The flow rate of feed water was 2.7, 5.2 and 10.6 L h^{-1} for the Zenon MBR and 1.0, 1.8 and 3.6 L h^{-1} for the Kubota MBR, which resulted in permeate flux of 2.8, 5.7 and 11.3 L m^{-2} h^{-1}, respectively.

The study was performed in two separate experiments, including a detailed study of the factors affecting removal efficiency of MBR treatment, and the study which examined the efficiency of the MBR treatment in combination with membrane filtration and ozonation. The main MBR treatment experiment was carried out over a period of four months. The main aim of the experiment was to determine the influence of the changes in the treatment conditions, including hydraulic retention time (HRT) and carbon and nitrogen loads, on the removal efficiency. The examined conditions included even a period of complete starvation, when feed supply to

Table 2
Variations of selected process parameters during the main experiment with membrane bioreactors.

Day	Abbreviation	TOC (mg L^{-1})	Total N (other than NO_3) (mg L^{-1})	HRT (h)
0–21	16 h	220	25	16
22–29	8 h	220	25	8
30–39	4 h	220	25	4
40–51	16 h	220	25	16
52–63	16 h/low C	165	25	16
64–70	16 h/low N	220	5	16
71–74	16 h/no N	180	0	16
75–78	–	–	–	–
79–87	16 h/low C	140	25	16
88–92	–	–	–	–
93–98	8 h	220	25	8
99–105	8 h/high N	220	80	8
106–110	8 h/low N, low C	120	12	8

TOC—total organic carbon; HRT—hydraulic retention time.

the bioreactors was interrupted for few days. The overview of the implemented changes of treatment conditions is given in Table 2.

MBRs were inoculated with activated sludge from the full-scale municipal wastewater treatment plant of the city of Velika Gorica, which has been shown to be chronically exposed to low concentrations of antimicrobials [24]. During the whole experiment there was no wastage of sludge from both MBRs, except minute amounts used as samples for analyses, which resulted in a very long sludge age. Both MBRs were supplied with the same feed from a 350 L tank, containing synthetic wastewater. The feed medium was prepared daily and the actual concentrations of all relevant parameters, including common indicators of the carbon and nitrogen loading as well as concentration of antimicrobials, were determined in subsamples taken directly from the 350 L-tank, containing daily portion of the synthetic wastewater feed. The effluents from MBRs were collected in plastic containers of 1.5 L, from which subsamples were taken for further analysis. The common wastewater parameters, including suspended solids (MLSS), ammonia, nitrite, nitrate, COD, and TOC were determined using standard methods [25]. The same parameters, except MLSS, were determined daily in the composite samples of the MBR treated effluents.

In the second series of experiments, the effluents from MBRs were additionally treated using filtration techniques (reverse osmosis and nanofiltration), as well as by ozonation (Fig. S1 in Supplementary material). This time, the MBR experiment itself lasted for 16 days and involved only the Kubota MBR system. Furthermore, hydraulic retention time (16 h) and feed water composition were identical to those employed in the period 0–21 day in the first experiment (Table 2) and were kept constant during the entire experiment.

Nanofiltration was carried out using a pilot plant, which consisted of pressure pump, pressure vessel with a Filmtec nanofiltration membrane NF 200B (FilmTec Corporation, Minneapolis, USA.) and associated pipelines, valves, pressure gauges and flow meters. A similar pilot plant with a spiral wound Filmtec BW 4040 membrane was used for the reverse osmosis experiments.

Ozonation was performed using an Ozotech ozone generator (Ozotech Inc., Yreka, USA). Ozone gas was dispersed into a 2 L glass cylinder through a diffuser placed at its bottom. In a preliminary experiment the ozone dose was determined by the standard iodometric titration method in 2 L of ozonated potassium iodide solution with samples being taken at 1 min intervals. The ozone dose was determined to be 0.81 mg L^{-1} h^{-1}. The ozonation time in the preliminary experiment varied between 2 and 24 h. The second ozonation experiment was carried out at a much higher ozone dose (1.31 mg L^{-1} h^{-1}), while the applied ozonation times were much shorter (15, 45 and 120 min). Besides for the treatment of MBR effluent, ozonation was also applied for the treatment of RO concentrate using a prolonged ozonation time of 22 h.

2.3. Determination of antimicrobials

Our previously described multiresidue method [26] based on liquid chromatography–tandem mass spectrometry (LC-MS/MS) was used for the quantitative determination of selected antimicrobials. The original method was slightly modified in order to include a major metabolite of SMX, *N*-acetylsulfamethoxazole. The target analytes were separated by reversed-phase chromatography on a C_{18} column using gradient elution with water and methanol both acidified with 0.1% of formic acid. The mass spectrometric analyses were performed on a TSQ Quantum triple quadrupole instrument (Thermo Electron, San Jose, USA), using electrospray ionization in positive mode. Detection and quantification of all analytes were performed using selected reaction monitoring (SRM). Instrumental detection limits of individual antimicrobials varied from 2 pg for AZI to 12 pg for TMP, corresponding to 0.2–1.2 μg L^{-1}, which allowed measurements of antimicrobials by directly injecting filtered synthetic wastewater samples to monitor daily variations of their concentrations. For a more detailed analysis, including the identification of sulfonamide metabolites, samples (50 mL) were enriched using solid phase extraction (SPE) on Oasis HLB cartridges (Waters, Milford, MA, USA). The method detection limits, based on 50 mL samples, varied from 20 to 100 ng L^{-1}. Absolute recoveries of individual antimicrobials were between 49% and 119% with RSD between 1% and 18% [26]. The analysis of activated sludge samples was performed using accelerated solvent extraction, followed by subsequent extract cleanup on Oasis HLB columns [23]. Absolute recoveries of individual antimicrobials were from 35 to 65%, which was fully compensated by surrogate standards, while the repeatabilities varied from 1 to 12%.

3. Results and discussion

3.1. Factors affecting removal of antimicrobials in MBR treatment

The changes of the key parameters, showing performance of the Zenon and Kubota MBRs, are presented in Fig. 1. Both MBRs proved capable of removing over 90% of organic load (98% of TOC and 95% of COD) after a very short adaptation period of 3 days (Fig. 1A). Gradually increasing activity of nitrifying bacteria was observed during the experiment in both MBRs as microbial mixed culture adapted and a sufficient number of nitrifying bacteria developed in the bioreactor as a consequence of a prolonged sludge age. The complete disappearance of nitrite was achieved after 40 days and complete and undisturbed nitrification continued throughout the experiment (Fig. 1B). The nitrate fluctuations in the effluent reflected the variations in the activity of the nitrifying microorganisms as well as the variability of the nitrogen load in the feed. The nitrate concentrations in Zenon MBR were consistently lower than those in the Kubota MBR, which was probably caused by the existence of poorly aerated zones at the bottom of that MBR, giving raise to denitrification. This is in agreement with a previous report on the mixing characteristics of the two MBRs by Curlin et al. [27].

The active biomass concentration, expressed as MLSS concentration, showed significant fluctuations (Fig. 2A), which was caused by the large changes in the organic load in the feed, employed in different phases of the experiment. MLSS concentrations, reaching up to 15 g L^{-1} and 12 g L^{-1} for Kubota and Zenon MBRs, respectively, were achieved. Although several disturbances were induced to the biomass during the experiment, some of which lead to its starvation and cell lysis, the MLSS concentration remained always above 5 g L^{-1}. The MLSS development and nitrification performance

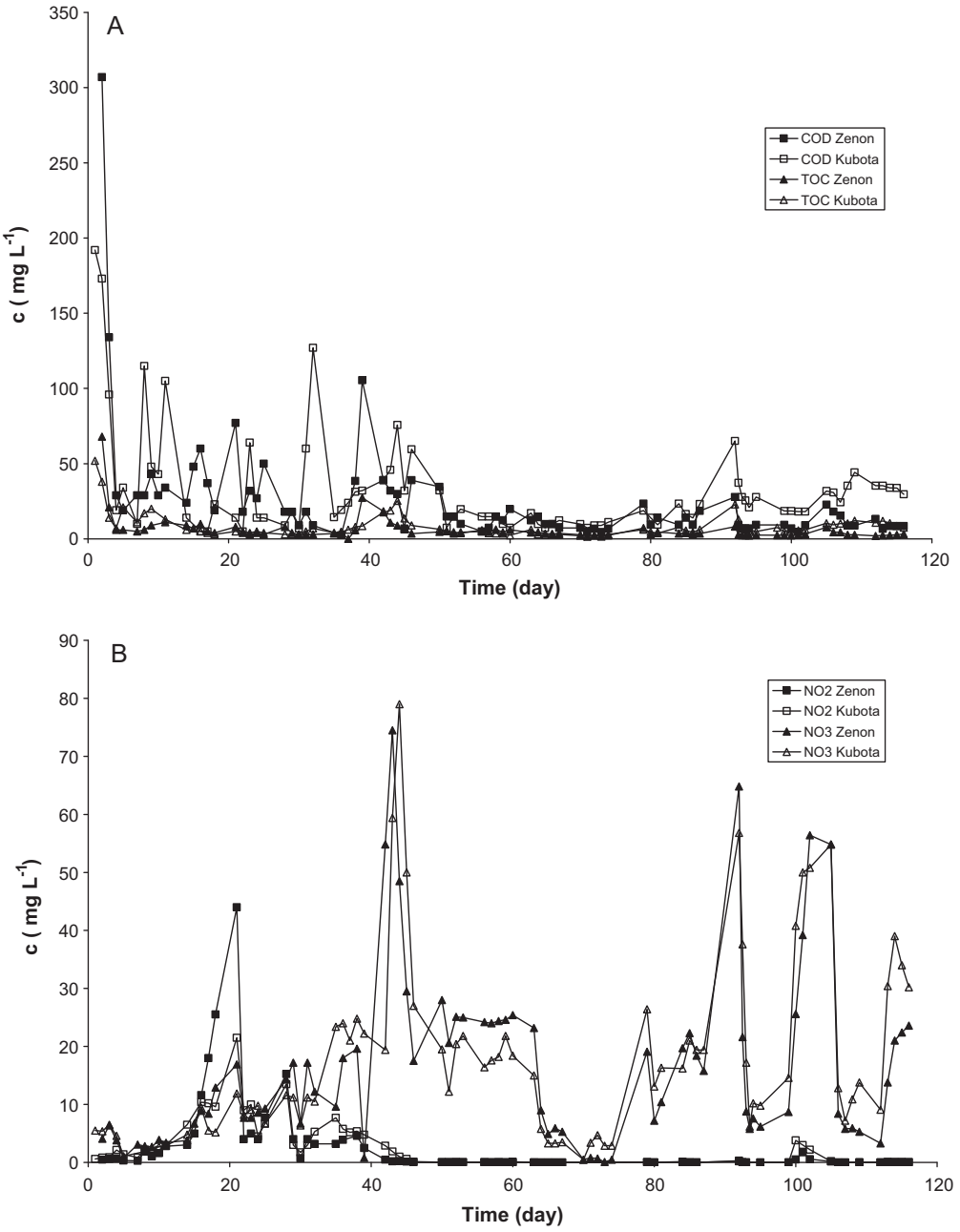

Fig. 1. Dynamics of chemical oxygen demand (COD) and total organic carbon (TOC) (A) and nitrite and nitrate concentrations (B) during the main experiment with Zenon and Kubota MBRs.

followed similar pattern in both MBRs during the whole experiment, which is not surprising since the original inocculum, feed loading rates and oxygen concentrations were almost the same for both bioreactors. Regarding the filtration performance, both membranes showed a significant loss of permeability during the first 5 to 6 days of filtration, but afterwards maintained quite stable filtration throughout the experiment (Fig. 2B). Chemical cleaning with hypochlorite solution was necessary in approximately two-month intervals.

The removal efficiencies of individual antimicrobials, including sulfonamide SMX, fluoroquinolone NOR, macrolide AZI and trimethoprim (TMP), are presented in Figs. 3 and 4. Only one representative for each type of antimicrobial compound is shown, while additional data, showing removal of all antimicrobials studied, can be found in Supplementary information to this article. Moreover, as both MBRs showed similar elimination rates for most of the investigated compounds, only data for the Zenon MBR are presented, while the results for the Kubota MBR can be found in Supplementary information.

Except at the beginning of the experiment, removal efficiencies for all investigated sulfonamides were very high. Elimination of SMX in Zenon MBR is showed in Fig. 3A. As can be seen, after a short adaptation period that lasted about 2 weeks, elimination rate of SMX exceeded 95% and remained high throughout the experiment. For most of the sampling days elimination rate was even higher than 99%. The achieved removal efficiency was significantly higher than the removal rates in some previous reports [17,28–30]. The imposed changes in the selected process parameters (Table 2), including variations in organic and nitrogen load, did not significantly influence the overall elimination efficiency. The only exception was a short period between the days 88 and 92 when the feed interruption was applied for 5 days, resulting in

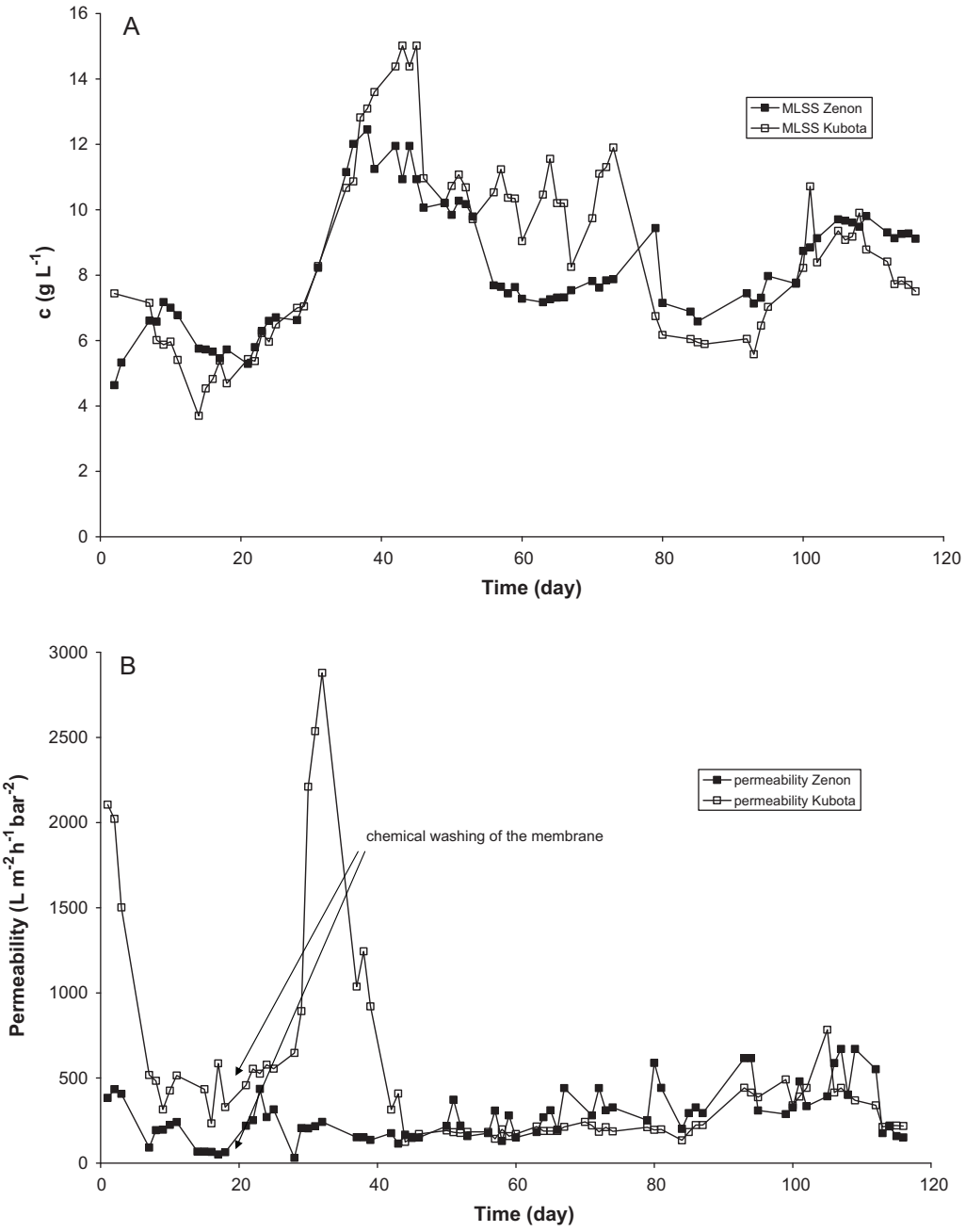

Fig. 2. Changes of mixed liquor suspended solids (MLSS) (A) and permeability of the membranes (B) during the main experiment with Zenon and Kubota MBRs.

a slight decrease of removal efficiencies for sulfonamides in both Zenon and Kubota MBRs (93% and 95%, respectively).

The elimination dynamics of fluoroquinolones during the experiment was rather different from that of sulfonamides as illustrated in Fig. 3B for their representative compound NOR. In both MBRs, the highest removal of NOR was achieved at the beginning of the experiment (>60%), before fully nitrifying conditions were established. However, removal efficiency decreased significantly two weeks after the beginning of the experiment and remained relatively low (20–50%) throughout the experiment. Nevertheless, some variations were observed in relation to the changes of the process parameters, in particular hydraulic retention time. The removal of fluoroquinolones at HRT of 16 h was significantly higher ($39 \pm 14\%$) than at HRT of 4 h ($20 \pm 8\%$). It is interesting to note that the removal of NOR increased significantly when the feed stoppage was applied for 5 days (day 88 to day 92), but decreased again after few days when the feed supply was re-established. It is difficult to explain this observation by a change of biotransformation efficiency in a system, which was changing towards less nitrifying conditions. Some literature reports suggested that physico-chemical partitioning might have a significant influence on the overall removal efficiency of fluoroquinolones [31], however this mechanism does not seem to be very likely since the biomass concentration in that period was fairly constant. An alternative explanation could be that changing sludge characteristics rather than its concentration contributed to the enhanced partitioning onto sludge, but this assumption warrants an additional experimental verification.

The removal dynamics of the macrolide representative AZI in Zenon MBR is presented in Fig. 4A. Removal of macrolide antibiotics was strongly affected by the hydraulic retention time. At the beginning of the experiment, when HRT was 16 h, high

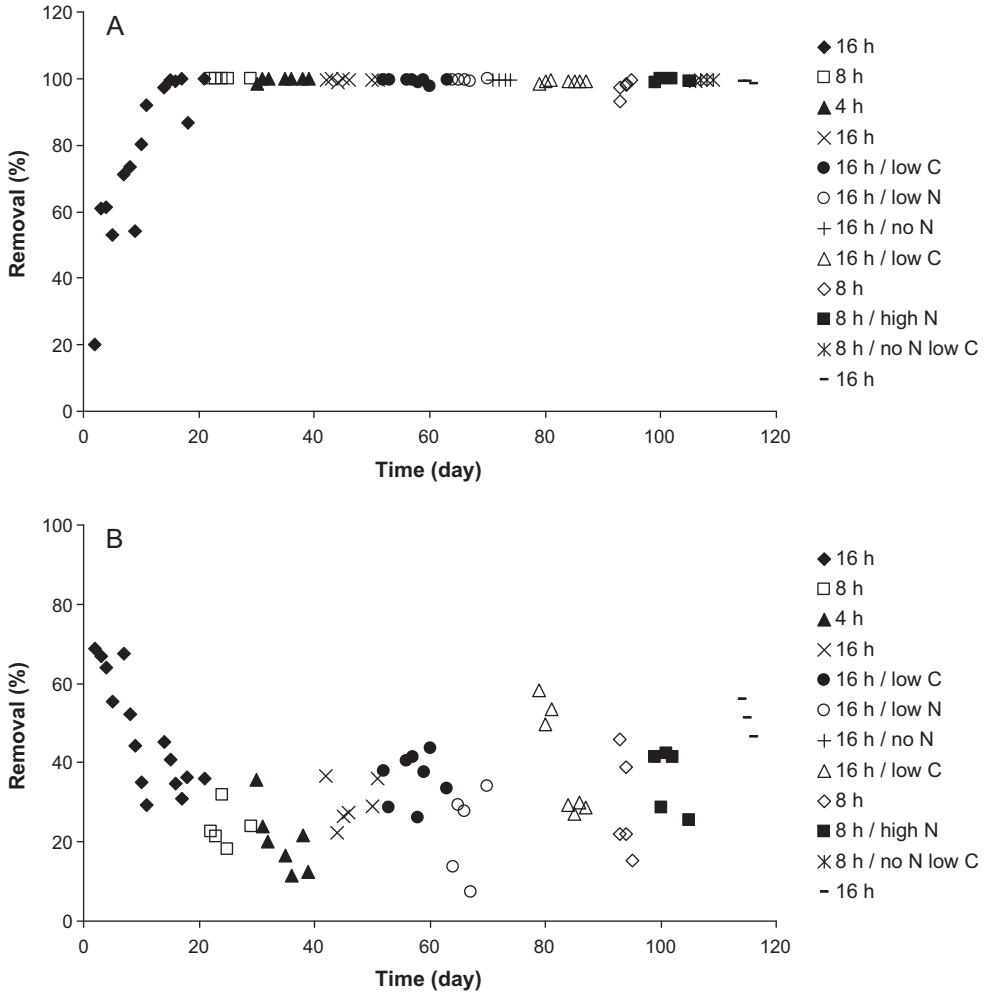

Fig. 3. Removal of sulfamethoxazole (A) and norfloxacin (B) during the main experiment with Zenon MBR.

elimination rates of macrolides were achieved in both MBRs (70–80%). The removal efficiency decreased significantly when HRT was shortened to 8 h and 4 h (elimination rates of about 40–50% and 10–20%, respectively). Regarding the comparison of the elimination rates of different macrolides, it should be pointed out that the elimination of erythromycin was significantly lower than those of azithromycin and roxithromycin. The determination of ERY was based on a complete conversion of ERY into ERY–H_2O before LC/MS/MS measurement [26] and therefore the removal figures for ERY represent the total removal, including ERY and ERY–H_2O. As a consequence, the applied method does not allow us to speculate whether abiotic hydrolysis had any effect on the efficiency of microbial degradation of ERY. Removal efficiencies obtained in this work were generally comparable to those reported by Göbel et al. [15], with the exception of azithromycin, whose elimination was significantly higher in our experiment, but only at the HRTs longer than 8 h. Apart from the predominant impact of HRT, the impact of other parameters on removal efficiency was not that pronounced. High elimination efficiencies were obtained at different compositions of synthetic wastewater, with drastically changing carbon and nitrogen loads. However, it should be pointed out that the highest removal for the Zenon MBR was obtained after the day 40, i.e. after establishment of full nitrification. This is in agreement with the report by Suarez et al. [30], who clearly demonstrated that removal in aerobic nitrifying conditions was more efficient than that in anoxic (denitrifying) conditions.

The lowest removal rates in MBR treatment were observed for trimethoprim (Fig. 4B). Although some authors [32] claimed that activated sludge with high nitrogen content significantly improved elimination of this compound, it could not be confirmed in our experiments. In fact, under all applied experimental conditions, including the period of full nitrification, removal of trimethoprim rarely exceeded 20% in both MBRs, which is comparable to the elimination rates of 36% reported for this compound by Reif et al. [17], but significantly lower than the removal rates reported in some other papers [7,11,15,29].

3.2. Assessment of removal mechanisms

There are two key processes that have to be considered when assessing the removal efficiency of MBR treatment: biological transformation and adsorption onto MLSS. Volatilization, which can play a significant role in the removal of volatile compounds from aerated systems, is not likely to be pronounced for antimicrobial compounds due to their very low Henry constants [30]. Moreover, since there was no washout of the MLSS from the MBRs and the concentration of antimicrobials in the feed was kept constant during the experiment, the general model describing mass balance of antimicrobials in the wastewater treatment can be greatly simplified. The total flow of antimicrobials in the MBR treatment is a sum of their corresponding mass flows in the liquid and solid phases. Moreover, it could be assumed that, few days after

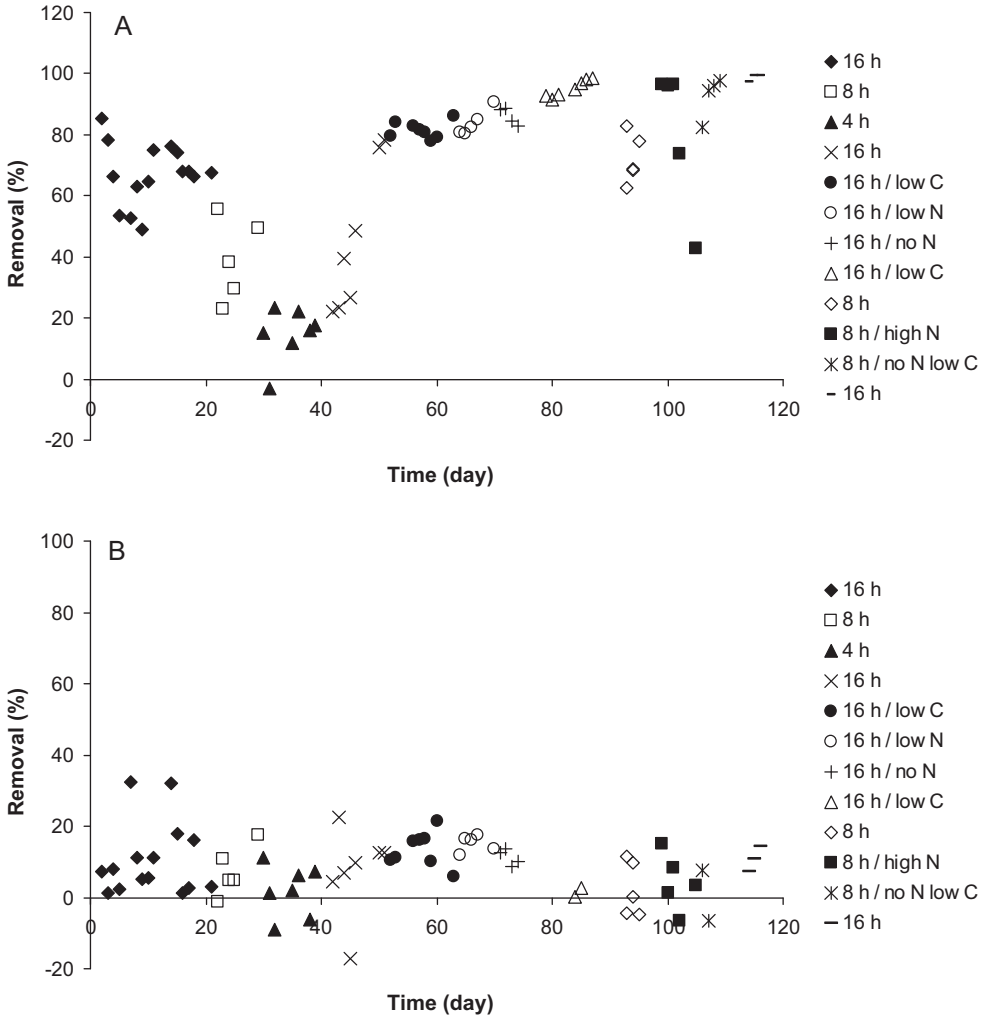

Fig. 4. Removal of azithromycin (A) and trimethoprim (B) during the main experiment with Zenon MBR.

continuous supply of antimicrobials at a virtually constant loading rate, equilibrium was established between the antimicrobials in the dissolved phase and those adsorbed on MLSS. The percentage of the removal due to the biotransformation for the entire experiment can therefore be calculated from the following equation:

$$\text{Removal}(\%) = \frac{\sum C_{\text{feed}} V_{\text{feed}} - \sum C_{\text{eff}} V_{\text{eff}} - C_{\text{feed}} V_{\text{R}}\, C_{\text{ads}}}{\sum C_{\text{feed}} V_{\text{feed}}} \times 100$$

where C_{feed} is the daily measured actual concentration of antimicrobials in the feed, C_{eff} daily measured concentration in the MBR effluent and C_{ads} concentration adsorbed on the sludge, determined at the end of the experiment. V_{feed} and V_{eff} are the total volumes of the feed and effluent, respectively, passing through MBR in one day. V_{R} is the volume of the reactor vessel and C_{MLSS} concentration of the sludge. The data presented in Table 3 show the estimated total amount of antimicrobials accumulated in the sludge during the experiment and the percentage of the removal due to the biotransformation and adsorption onto the sludge. As can be seen, the adsorbed amounts varied in a wide range, but the estimated contribution of the adsorbed antimicrobials remained relatively low for all antimicrobials. This is, in part, a result of the unique feature of MBRs, which, unlike CAS treatment, can be operated at high sludge age and with very little or no excess sludge production [33,34]. In the conventional systems, which produce large amounts of excess sludge, a significant percentage of the total removal of pharmaceuticals having high distribution coefficients between sludge and aqueous phase (K_{d}), such as fluoroquinolones, can be attributed to physico-chemical partitioning [15,23,31]. Sorption of individual antimicrobials classes onto the sludge was not correlated with their K_{ow} values, indicating that hydrophobic interactions were not the predominant mechanism. For example, although fluoroquinolones have the lowest $\log K_{\text{ow}}$ values (around −1.0), their adsorption onto the sludge was found to be significantly higher than the adsorption of other investigated compounds, including macrolides ($\log K_{\text{ow}}$ values between 1.8 and 4.0). Consequently, the dominant mechanism of adsorption of fluoroquinolones must have been associated with the electrostatic interactions, very probably with the cell membranes of the microorganisms, as suggested by Xu et al. [35]. Comparison of the contribution of physico-chemical partitioning with the total removal efficiency (determined by comparing the concentrations in the feed and MBR effluents) clearly reveals biological transformation as the strongly predominant removal mechanism for easily (sulfonamides) and moderately degradable (macrolides and fluoroquinolones) antimicrobials. For the most biorefractory compound in our experiment, trimethoprim, the relative contribution of adsorption was slightly higher (Table 3), but the biotransformation remained predominant.

In order to further assess removal of antimicrobials, the MBR effluents were analyzed for some biodegradation products. The dynamics of the well-known metabolite of SMX, *N*-acetyl SMX during the first 18 days of the experiment is shown in Fig. 7. It shows

Table 3
Assessment of mechanisms of antimicrobial removal in MBR treatmant (the presented balance represents the situation on the day 73).

Compound	Antimicrobials accumulated on sludge (mg)		Removal due to the biotransformation (%)		Removal due to the adsorption on sludge (%)	
	Zenon MBR	Kubota MBR	Zenon MBR	Kubota MBR	Zenon MBR	Kubota MBR
SDZ	0.008	0.010	95.4	96.4	0.005	0.005
STZ	0.012	0.014	96.2	97.5	0.007	0.008
SPY	0.053	0.029	95.2	97.4	0.010	0.016
SMZ	0.040	0.015	95.9	97.2	0.008	0.008
SMX	0.019	0.006	95.3	96.1	0.004	0.003
TMP	1.80	0.568	6.65	5.45	0.35	0.32
NOR	5.42	3.80	28.0	30.1	1.06	2.09
CIP	5.53	3.55	26.0	27.1	1.08	1.95
AZI	1.70	0.597	43.8	48.4	0.33	0.33
ERY	3.64	0.699	26.7	34.0	0.71	0.38
ROX	1.86	0.230	53.5	56.4	0.36	0.13

that *N*-acetyl SMX was formed indeed during the MBR treatment with the maximal formation of *N*-acetyl SMX observed in the first 3 weeks of the experiment, which is in accordance with the dynamics of the elimination of the parent compound. After this initial period, during the phase of fully nitrifying conditions, the concentrations of *N*-acetyl SMX were negligible, which suggested either further transformation or lack of formation of this metabolite under nitrifying conditions prevailing in the experiment. The extracts were screened for possible less-known sulfonamide metabolites using UPLC-QTOF-MS technique but we were not able to detect any additional sulfonamide-related compounds. Further research is needed to assess possible metabolites of macrolides, fluoroquinolones and trimethoprim.

3.3. Combination of MBR treatment with filtration techniques and ozonation

In our second experiment, the effluent from the Kubota MBR was further treated using filtration techniques (reverse osmosis and nanofiltration) as well as ozonation. The removal efficiencies achieved in the MBR treatment step were up to 70% for sulfonamides and macrolides, up to 60% for fluoroquinolones, and up to 20% for trimethoprim, which is somewhat lower than the efficiencies obtained in the first MBR experiment. This lower removal can be explained by the fact that second MBR experiment lasted only for 16 days, and that time period was probably too short to achieve the maximum removal efficiencies. Since all investigated antimicrobials were incompletely removed, their presence in MBR effluent allowed us to evaluate efficiencies of some alternative techniques for their further elimination.

Removal efficiencies of selected antimicrobials from the MBR effluents, achieved by filtration techniques, are shown in Fig. 5. The experiments confirmed that molecular size was an important parameter for the removal of antimicrobials. For nanofiltration, the highest removal rates (>95%) were obtained for macrolides, which are relatively large molecules with molecular masses above 700 Da. The elimination efficiencies of sulfonamides, fluoroquinolones and trimethoprim, having molecular masses in the range from 250 to 332 Da, were between 85% and 95%. No target analytes could be detected in RO permeates, which meant that the removal efficiency achieved by this technique was practically 100% (Fig. 5). This is in agreement with some other reports [36], which investigated the possible application of reverse osmosis as the key step of water reuse for potable water supply. Reverse osmosis proved to be clearly superior to nanofiltration with respect to removal efficiencies of all investigated antimicrobials. Nevertheless, due to the relatively high flux and low operational pressure, nanofiltration showed some clear practical advantages, which can be very useful when dealing with larger volumes of wastewater effluents such as municipal wastewater, typically containing high organic load and relatively low levels of antimicrobials [3]. However, since the elimination of investigated antimicrobials by nanofiltration is incomplete, this issue could become critical in case of industrial wastewater effluents from the pharmaceutical production facilities, which contain enhanced concentration of pharmaceuticals [22,23]. In such cases additional treatment by reverse osmosis might be necessary to reduce the concentration in the final effluents to the ng L^{-1} range.

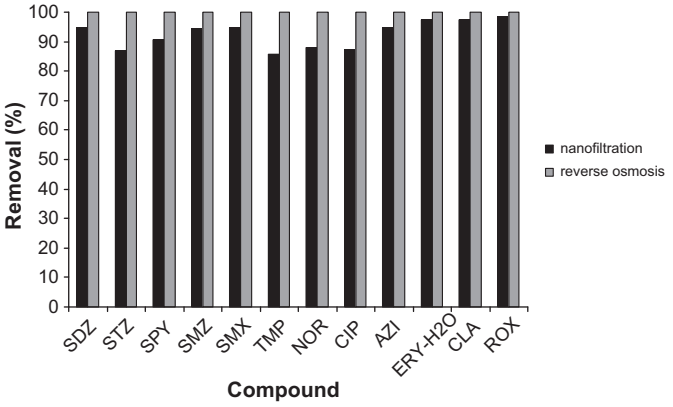

Fig. 5. Removal of antimicrobials from the effluent of the Kubota MBR by filtration techniques.

Although filtration techniques proved to be promising methods for the reduction of investigated antimicrobials in the filtrate, it should be stressed that the filtration techniques do not provide any degradation of the removed compounds, i.e. all antimicrobials end up in the concentrate stream, which needs further treatment.

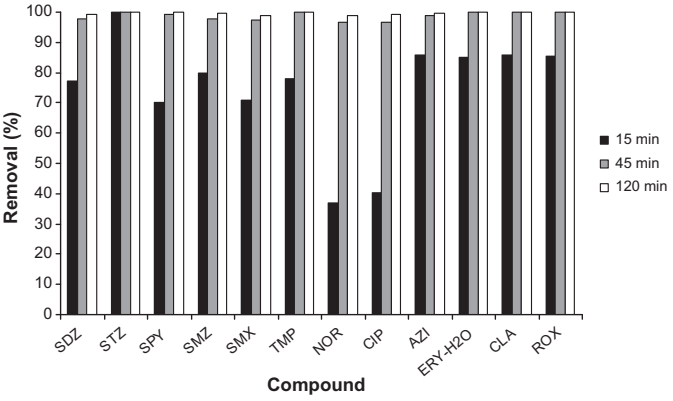

Fig. 6. Removal of antimicrobials from the effluent of the Kubota MBR by ozonation (ozone dose 1.31 mg L^{-1} h^{-1}).

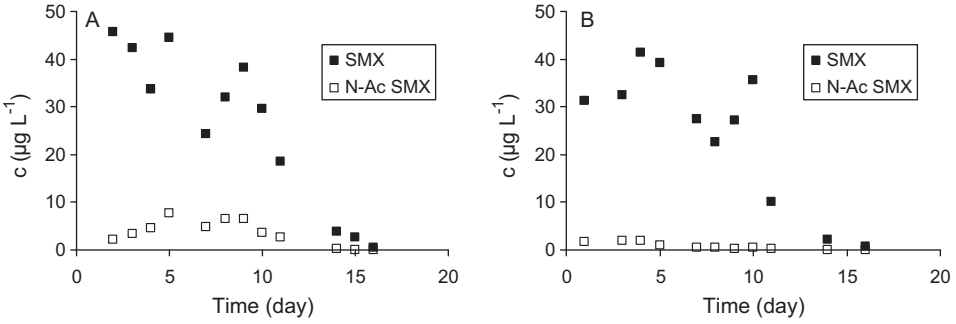

Fig. 7. Dynamics of sulfamethoxazole (SMX) and its major metabolite *N*-acetyl sulfamethoxazole (*N*-Ac SMX) in Zenon MBR (A) and Kubota MBR (B).

To address this problem, MBR effluent as well as RO concentrate were subjected to an additional ozonation step. In the first ozonation experiment, MBR effluent was ozonated for 2, 6 and 24 h using ozone dosing of 0.81 mg L^{-1} h^{-1}. All investigated antimicrobials degraded completely after 2 h, with the exception of fluoroquinolones, for which removal rates were about 90% (data not shown). Their complete removal (>99%) was obtained only after 6 h.

The results of the MBR effluent ozonation at a higher ozone dose (1.31 mg L^{-1} h^{-1}) are presented in Fig. 6. As can be seen, only sulfathiazole was completely degraded within 15 min, while the elimination rates for the majority of the tested compounds were between 70% and 85% and for fluoroquinolones even lower (about 40%). After 45 min of ozonation, removal rates for all the compounds, including fluoroquinolones, exceeded 97%, while complete removal (>99%) was reached after 2 h.

Ozonation of RO concentrate at the identical ozone dose of 1.31 mg L^{-1} h^{-1} also resulted in a complete removal of the target antimicrobials provided that longer ozonation times were applied. However, there are some issues that need to be pointed out regarding ozonation. Since the TOC concentration of the samples during ozonation did not change significantly, it was concluded that mineralization of organic matter in the treated samples did not occur to a measurable extent. Generally, ozonation changes the chemical structure of a compound, mainly by increasing the number of polar functional groups. Detailed studies on clarithromycin [37], ciprofloxacin [38], roxithromycin and trimethoprim [39] showed that ozonation typically leads to a number of different products. It can be assumed that this affects biological activity of the original compounds, including the reduction of antibiotic activity [20]. However, a comprehensive evaluation of the ozonation as an alternative technique for the removal of antimicrobials warrants additional information regarding the biological activity of ozonation products, which is currently largely unknown.

4. Conclusions

Our model experiments with selected antimicrobials showed that their elimination using MBRs was rather variable. MBRs proved to be very efficient for the removal of sulfonamides, while elimination of fluoroquinolones, macrolides and especially trimethoprim was incomplete. Although several disturbances were induced to the biomass regarding carbon and nitrogen loads, these changes did not show a dramatic impact on the overall treatment efficiency. This observation may be of importance for the treatment plants with irregular wastewater inflow, such as industrial effluents.

Filtration techniques, especially reverse osmosis, proved to be very efficient for the removal of antimicrobials. However, the main issue concerning the application of these techniques is the fact that all removed antimicrobials end up in the concentrate stream, which requires additional treatment. Ozonation was found to be very effective for their removal from MBR effluent, as well as from RO concentrates, but further research is needed to address the issue of ozonation products.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2011.05.021.

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