Fate of antibiotics in activated sludge followed by ultrafiltration (CAS-UF) and in a membrane bioreactor (MBR)

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Abstract

The fates of several macrolide, sulphonamide, and trimethoprim antibiotics contained in the raw sewage of the Tel-Aviv wastewater treatment plant (WWTP) were investigated after the sewage was treated using either a full-scale conventional activated sludge (CAS) system coupled with a subsequent ultrafiltration (UF) step or a pilot membrane bioreactor (MBR) system. Antibiotics removal in the MBR system, once it achieved stable operation, was 15–42% higher than that of the CAS system. This advantage was reduced to a maximum of 20% when a UF was added to the CAS. It was hypothesized that the contribution of membrane separation (in both systems) to antibiotics removal was due either to sorption to biomass (rather than improvement in biodegradation) or to enmeshment in the membrane biofilm (since UF membrane pores are significantly larger than the contaminant molecules). Batch experiments with MBR biomass showed a markedly high potential for sorption of the tested antibiotics onto the biomass. Moreover, methanol extraction of MBR biomass released significant amounts of sorbed antibiotics. This finding implies that more attention must be devoted to the management of excess sludge.

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1. Introduction

Organic micropollutants (OMPs) have become a major health issue in terms of sewage treatment quality due to their potentially harmful impacts on the internal systems of a variety of organisms (Damstra et al., 2002; Khan et al., 2004). The current strong interest in OMPs has developed in reaction to: 1) natural population growth and an increased standard of living, both of which have contributed to overall increases in quantities of sewage, the most concentrated source of OMPs (Heberer et al., 2002; Kreuzinger et al., 2004; Paxeus, 2004); 2) the increased frequency of indirect potable wastewater reuse, which enhances the transport of the relatively stable OMP molecules from the municipal effluents to the ground water, from where they can become a health threat to biological systems, including those of humans (Daughton and Ternes, 2001).
The main processes effective in OMP removal include advanced oxidation processes, adsorption to activated carbon, biological treatments comprising conventional activated sludge (CAS) and membrane bioreactor (MBR), the latter of which incorporates microfiltration or ultrafiltration (UF), and high pressure separation technologies, such as nanofiltration and reverse osmosis (RO) (Ternes and Joss, 2006). Previous studies comparing the removal efficiencies of several micro-pollutants by MBR and CAS produced diverse data that showed no definite advantage of one method over the other. For example, Clara et al. (2004) reported almost identical removal efficiencies for ethinylestradiol (60–70%), ibuprofen (90–95%), and galaxolide (60–80%) and showed that the CAS performed 20% better than the MBR for diclofenac (30% and 50% removal rates, respectively). Ternes (1998) found that the CAS removed 69% of diclofenac while Zwinner and Frimmel (2000) observed only 1–5% removal levels for the same drug. Gobel et al. (2007), who tested macrolide and sulfonamide antibiotics removal using MBR and CAS, also obtained diverse results. They measured similar removal rates in the range of 95–100% for erythromycin (ERY), clarithromycin (CLA), and trimethoprim (TMP) in both systems. Removal rates for roxithromycin (ROX), however, were slightly better in the CAS (80% as opposed to 65% by the MBR), while the MBR removed sulfamethoxazole (SMX) significantly better than the CAS (80% as opposed to 65% by the MBR), the latter of which incorporates microfiltration or ultrafiltration (UF), and high pressure separation technologies, such as nanofiltration and reverse osmosis (RO) (Ternes and Joss, 2006). Previous studies comparing the removal efficiencies of several micro-pollutants by MBR and CAS produced diverse data that showed no definite advantage of one method over the other. For example, Clara et al. (2004) reported almost identical removal efficiencies for ethinylestradiol (60–70%), ibuprofen (90–95%), and galaxolide (60–80%) and showed that the CAS performed 20% better than the MBR for diclofenac (30% and 50% removal rates, respectively). Ternes (1998) found that the CAS removed 69% of diclofenac while Zwinner and Frimmel (2000) observed only 1–5% removal levels for the same drug. Gobel et al. (2007), who tested macrolide and sulfonamide antibiotics removal using MBR and CAS, also obtained diverse results. They measured similar removal rates in the range of 95–100% for erythromycin (ERY), clarithromycin (CLA), and trimethoprim (TMP) in both systems. Removal rates for roxithromycin (ROX), however, were slightly better in the CAS (80% as opposed to 65% by the MBR), while the MBR removed sulfamethoxazole (SMX) significantly better than the CAS (80% vs. 65%, respectively). Gobel et al. (2007) concluded that for the MBR, only a small portion of the removal was caused by sorption (5–10%) while biodegradation, which was strongly influenced by the sludge retention time (SRT), played a major role. Le-Minh et al. (2010) stated that the similarities of SRT and hydraulic retention time (HRT) between CAS and MBR leads to comparable antibiotic removal levels. The papers mentioned above lead to the conclusion that the variance of micro-pollutant removal efficiencies is based on two factors: the first is related to the environmental conditions, including temperature, mixed liquor suspended solids (MLSS) concentration, SRT, raw sewage content, and the microbial community. The second factor is related to the characteristics of each compound, such as contaminant concentration and hydrophobic/hydrophilic nature of the molecule, among others. These operational variables must be adequately defined to obtain results that will lend themselves to comparison (Le-Minh et al., 2010).

This paper compares the OMP removal rates obtained for a full-scale CAS process coupled with a UF pilot with those of a pilot MBR system. The aim of the research was to estimate the antibiotics removal efficiencies of the two pilots and to determine the fates and removal mechanisms of selected antibiotics by running batch adsorption tests using the MBR biomass. Both the MBR and CAS-UF systems are located in the Shafdan wastewater treatment plant (WWTP) in Tel-Aviv, Israel, treating the same raw sewage.

### Table 1 – Operational parameters in CAS/UF and MBR.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CAS/UF</th>
<th>UF</th>
<th>MBR</th>
<th>Stable operation</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRT [h]</td>
<td>14–16</td>
<td>N.R.</td>
<td>9–12</td>
<td>9</td>
</tr>
<tr>
<td>MLSS [g/L]</td>
<td>2–3</td>
<td>N.R.</td>
<td>3.8–10.4</td>
<td>9–11</td>
</tr>
<tr>
<td>SRT [d]</td>
<td>2–4</td>
<td>N.R.</td>
<td>&gt;70</td>
<td>40</td>
</tr>
<tr>
<td>Capacity [m³/h]</td>
<td>N.R.</td>
<td>45–47</td>
<td>0.02–0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Filtration/BW [min]</td>
<td>N.R.</td>
<td>50/1</td>
<td>5/0.5</td>
<td>5/0.5</td>
</tr>
<tr>
<td>Filtration/BW [m³/h]</td>
<td>N.R.</td>
<td>45/45</td>
<td>(0.04–0.02)/(0.022–0.044)</td>
<td>0.04/0.044</td>
</tr>
</tbody>
</table>

N.R. – Not Relevant; BW – Back-wash.
the outlet of the anoxic zone to the inlet of the anaerobic zone and 4Q from the membrane tank to the inlet of the anoxic zone.

2.2. Analytical procedures

A comparison of the operational parameters of the CAS/UF with those of the MBR shows that MLSS concentration and SRT were markedly higher in the MBR (Table 1). We expected this finding to enhance significantly OMP biodegradation due to higher variety of bacteria species attributed to increased SRT. In addition, the CAS capacity was more than 1000 times higher than that of the MBR, which, however, was run at a much higher SRT. The relatively high SRT (>70 d) needed to build up sufficient biomass in the MBR was reduced to 40 d once stable operation conditions were achieved.

In addition to the standard test parameters (Table 2) that were evaluated according to standard methods (Standard Methods 2540D and 2540E), two kinds of antibiotics were concentrated by solid-phase extraction (SPE) and analyzed by LC/MS/MS in both Germany (TUB) and Israel (Gush Katif Lab) according to the protocol described by Asmin et al. (2006). The types of antibiotics collected included: macrolides (ERY, ROX, and CLA), sulfonamides [SMX, sulfamethazine (SMZ)], and TMP. The higher macrolide concentrations in the raw sewage of Israel relative to most sulfonamides (except SMX) can be attributed to the higher consumption of the former, which is estimated by the European Surveillance of Antimicrobial Consumption (ESAC) to be three times higher than that for sulfonamides (3 vs. 1 defined daily dose per 1000 inhabitants per day – ESAC, 2005). The correlation between a compound’s concentration and its removal potential depends on the background solution content as described by Yangali et al. (2009). A sludge background, as opposed to a distilled water background, contains a variety of compounds at different concentrations and with different properties. This situation can lead to a matrix effect, which, in turn, has an influence on OMP removal (Ternes and Joss, 2006). The increase in biomass concentration changes the background characteristics constantly, and therefore, it is also expected to change the removal ratio.

Each sampling campaign for antibiotics analysis lasted 24 h, during which a composite sample was established by an automatic sampler set to pump 150 ml once an hour. The purpose of the composite sampling is to avoid the daily fluctuations in the antibiotics concentration. In addition, the samplings were taken only during mid-week (Monday to Wednesday) due to the significant changes in antibiotics concentrations on weekends, as reported by Ternes and Joss (2006). Excess sludge from the MBR was disposed of once a day in the morning. No excess sludge was discarded during the 24-h sampling campaign.

The laboratory analysis results showed that the limit of detection (LOD) for sulfonamides and macrolides were 5 and 10 ng/L, respectively. The limit of quantification (LOQ) for sulfonamides and macrolides were 25 and 50 ng/L, respectively. Analytical reproducibility of duplicates was up to 18.7%.

3. Results and discussion

3.1. Research approach

This paper is divided into two parts: the first, done to evaluate the antibiotics removal efficiencies of the two pilots, covers MBR and CAS/UF testing as ‘black boxes’ without mass balance by testing the influent and effluent for antibiotics (Section 3.5). It should be noted that the accuracies of mass balance assessments of solutions with low antibiotics concentrations and in which the biomass contains the target compounds is limited. The second part of the paper includes tests with MBR biomass to distinguish between biodegradation and sorption removal mechanisms by applying certain conditions (Sections 3.6–3.7). In this case, mass balance was preserved by increasing the antibiotics concentration significantly (10 μg/L instead of the tens or few hundreds of ng/L typically found in the raw sewage) and by providing conditions that favored sorption to the stabilized MBR biomass over biodegradation. As can be seen in Table 5, desorption of antibiotics during the batch experiment would have had a negligible effect on the results.

3.2. Removal of organic compounds and nutrients

Both wastewater treatment systems exhibited high rates of organic compound removal and complete nitrification (Table 2). Only partial denitrification and a relatively low rate of

Table 2 – Removals of organic compound and nutrients in the CAS/UF and MBR systems (n = 98, 62, 36 for CAS/UF, biomass buildup and stable operation, respectively).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CAS/UF</th>
<th>MBR</th>
<th>Stable operation</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD</td>
<td>925 ± 57</td>
<td>27.7 ± 2.5</td>
<td>96.8 ± 1.5</td>
</tr>
<tr>
<td>NH₄-N</td>
<td>42.3 ± 4.8</td>
<td>2.9 ± 0.7</td>
<td>93.5 ± 3.3</td>
</tr>
<tr>
<td>NO₃-N</td>
<td>–</td>
<td>1.2 ± 0.9</td>
<td>–</td>
</tr>
<tr>
<td>NO₂-N</td>
<td>–</td>
<td>0.4 ± 0.4</td>
<td>–</td>
</tr>
<tr>
<td>PO₄-P</td>
<td>10.1 ± 1.8</td>
<td>1.1 ± 0.4</td>
<td>85.1 ± 7.9</td>
</tr>
</tbody>
</table>

*Represents the range of concentrations and removal rates.
Table 3 – Characteristics of the tested macrolides, sulfonamides, and trimethoprim (Ternes and Joss, 2006).

<table>
<thead>
<tr>
<th>Macrolides</th>
<th>Roxithromycin (ROX)</th>
<th>Clarythromycin (CLA)</th>
<th>Erythromycin (ERY)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structure</td>
<td><img src="image1" alt="Structure ROX" /></td>
<td><img src="image2" alt="Structure CLA" /></td>
<td><img src="image3" alt="Structure ERY" /></td>
</tr>
<tr>
<td>MW, g</td>
<td>747.953</td>
<td>837.047</td>
<td>733.93</td>
</tr>
<tr>
<td>Log K&lt;sub&gt;ow&lt;/sub&gt;</td>
<td>2.75</td>
<td>3.16</td>
<td>3.06</td>
</tr>
<tr>
<td>pKa</td>
<td>8.8</td>
<td>8.9</td>
<td>8.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sulfonamides and trimethoprim</th>
<th>Sulfamethazine (SMZ)</th>
<th>Sulfamethoxazole (SMX)</th>
<th>Trimethoprim (TMP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structure</td>
<td><img src="image4" alt="Structure SMZ" /></td>
<td><img src="image5" alt="Structure SMX" /></td>
<td><img src="image6" alt="Structure TMP" /></td>
</tr>
<tr>
<td>MW, g</td>
<td>290.32</td>
<td>253.279</td>
<td>278.328</td>
</tr>
<tr>
<td>Log K&lt;sub&gt;ow&lt;/sub&gt;</td>
<td>0.9</td>
<td>0.89</td>
<td>0.91</td>
</tr>
<tr>
<td>pKa</td>
<td>7.4</td>
<td>5.6</td>
<td>7.2</td>
</tr>
</tbody>
</table>
phosphorus removal were observed in the MBR due to difficulty to preserve anaerobic and anoxic conditions during biomass buildup. Complete denitrification and high phosphorus removal were achieved in the MBR once stable operation conditions were established. The improvement in phosphorus removal is explained by the increase in excess sludge amount (0.5–6 L and 6–8 L during the biomass buildup stage and the stable operation, respectively). The results shown in Table 2 are slightly different from those presented in Sahar et al. (2010), since more up-to-date data is included in the current table.

### 3.3. Expected removal mechanism based on antibiotics characteristics

The main removal mechanisms in biological treatment are biodegradation and sorption, both of which depend on bacteria and on the characteristics of the OMPs (Ternes and Joss, 2006; Le-Minh et al., 2010). Although an increase in biomass concentration may enhance OMP biodegradation, it does not necessarily enhance sorption, due to the dynamic equilibrium in sorption/desorption conditions and the limited number of newly formed binding sites. Another factor that can significantly affect OMP removal is the SRT (which is directly related to biomass concentration), since high SRTs favor the development of diverse bacterial populations, some of which are capable of bioaccumulating and biodegrading more complex organic molecules (Clara et al., 2004; Le-Minh et al., 2010). An increase in SRT causes a reduction in the food to microorganism (F/M) ratio, thereby reducing the bacteria population growth rate (and excess amounts of sludge). As a result, the concentration of renewable binding sites is decreasing, and therefore, theoretically, the MBR should be less effective at removing OMPs by sorption to sludge. Practically speaking, the process is not that straightforward, and environmental factors such as background fluid content and the contaminant concentration may stimulate significant sorption, as will be explained in Section 3.7.

Although all the antibiotics used in the study are originally targeted to destroy bacteria, sulfonamides and macrolides possess different structural characteristics (Table 3) and mechanisms of action. While the macrolides each have one ring with side chains or sugars, every sulfonamide has two relatively small rings connected by a sulfa atom and nitrogen bonds. Their structural differences are expressed in the sulfonamides’ more polar and hydrophilic nature, relative to macrolide characteristics (log $K_{ow} < 1$ and log $K_{ow} > 3$, respectively). Ternes and Joss (2006) stated that compounds with log $K_{ow} < 1$ are considered potentially bioaccumulative, and therefore, the main macrolide removal mechanism was hypothesized to be sorption to sludge (bioaccumulation should be more dominant for ERY and CLA, with log $K_{ow}$ of 3.06 and 3.16, respectively, and less for ROX since its log $K_{ow}$ is 2.75). In contrast, we expected sulfonamides to be readily biodegraded due to their hydrophilic nature and relatively low log $K_{ow}$. Our conjectures about antibiotic removal mechanisms are supported by the different resistance mechanisms bacteria possess against antibiotics. To prevent macrolides from binding and to thwart their lethal effects, bacteria must be able to methylate their ribosomes and to operate an active protein pump to eject the unwanted molecules from the bacteria cell (Tenson et al., 2003). Sulfonamides, on the other hand, can be degraded by enzymatic activity (Bajpai et al., 2000).

### 3.4. Effect of MLSS concentration on antibiotics removal in the MBR

Biomass buildup is an inherent stage in starting or restarting of an MBR. Therefore, it is important to identify MBR capability in removing OMPs during that stage, which can last a few days up to several weeks. The acclimation period required by the MBR to raise its biomass concentration enabled us to characterize the effect of MLSS concentration on removal efficiency. During acclimation, MLSS concentration was increased from

![Table 4](image-url) MBRs’ permeate concentrations and improvement in antibiotics removal after stabilizing the operation conditions at MLSS of 10 g/L ($n = 6$).

![Table 5](image-url) Average antibiotics concentrations in the raw sewage and after desorption from MBR MLSS by methanol ($n = 3$).
3.8 to 10.4 g/L. At the biomass buildup stage, each sampling campaign was set after a week of stable operation at the desired MLSS concentration. On the one hand, extending the duration at each sampling point would have probably affected the antibiotics removal, due to the changes in the biomass characteristics, but on the other hand, a biomass buildup stage is short, as mentioned before, and hence, the results can indicate the actual removal of the antibiotics in MBR during that stage. Due to the relatively short biomass buildup time, the data can also be referred to as an average (as presented in Fig. 3).

Throughout the entire acclimation period, TMP was removed completely. As opposed to that, the concentration of SMX in the MBR effluent gradually decreased from 157 to 71 ng/L (54% improvement), with the increase of MLSS concentration (Fig. 1). The feed concentrations of SMX during the biomass buildup varied between 184 and 251 ng/L. SMZ was not included in Fig. 1, since its concentrations during the biomass buildup stage were below the LOQ.

In contrast to the SMX, the macrolides showed no correlation between the biomass concentration and the antibiotics removal rate (Fig. 2). One can assume that an increase in biomass concentration should increase the OMPs removal, either by biodegradation or by sorption, since more bacteria mass is involved. Nevertheless, it is deemed during the unstable conditions of biomass buildup in the MBR that adsorption and desorption rates change significantly according to the compound’s characteristics, its concentration, and the background fluid content and conditions (matrix), especially in cases of low compound concentrations relative to background compound concentrations. Therefore, the fluctuations in Fig. 2 support the assumption that macrolide

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**Fig. 1** – Sulfamethoxazole and trimethoprim concentrations and removal rates in MBR treatment during MLSS buildup.

**Fig. 2** – Macrolide concentrations and removal rates in MBR treatment.
antibiotics concentrations. Due to the variation in the feed concentrations of macrolides (ERY: 327–674 ng/L, ROX: 682–2764 ng/L, CLA: 442–2235 ng/L), the results in Fig. 2 are presented as normalized concentration (C/C0). Despite those variations, the permeate concentration range was relatively low (up to 250 ng/L for ERY and CLA and up to 350 ng/L for ROX). Therefore, it can be hypothesized that in relatively high biomass concentrations (MLSS > 4000 mg/L), OMP removal is quite effective and does not change much with further increases of biomass concentration. An additional step was taken to distinguish between sorption and biodegradation as described in Section 3.6.

3.5. Comparison between CAS/UF and MBR

The discussion about the MBR is divided according to its main periods of operation: acclimation (biomass growth) and stable operation, the latter occurring when the MLSS concentration remains at approximately 10 g/L. During acclimation, the average MBR removal rate of SMX was 18% better relative to the same rate in the CAS/UF (Fig. 3). For TMP, that advantage was even higher (54%). This can be explained by the relatively high SRT in the MBR, which allows for the enrichment of different bacteria types capable of more efficient antibiotics removal, as claimed by several studies (Clara et al., 2004; Kreuzinger et al., 2004; Ternes and Joss, 2006). SMZ results are not included since in most cases, its concentration was below LOQ. The macrolides, on the other hand, were removed slightly better in the CAS/UF (10–12%), indicating that both SRT and MLSS concentrations did not markedly affect their removal mechanism. It is important to indicate that the production rate of binding sites in MBR and CAS is different by definition (due to different SRT, MLSS concentration, and F/M ratio). In this study, the differences might have been even higher since the COD concentration at the influent to the MBR was half that of the feed point to the CAS (due to the 800 micron filter in the MBR — see Table 2). A relatively low binding site production rate could explain the limited macrolide removal in the MBR during biomass buildup.

After the MBR had stabilized and operated steadily for a month at a high MLSS level, the improvement in antibiotics removal was remarkable, as can be seen in Table 4 (from 30% for CLA to around 80% for ROX and almost 90% for TMP). This improvement may be related to the reduction in SRT, which increased the production of new binding sites and enabled the rate of sorption to increase. In addition, the well developed and stable bacteria colonies could have increased biodegradation of the antibiotics. The improvement during the stable operation is also noticeable when the change in feed concentration is eliminated by presenting the normalized concentration (Table 4, columns 5, 6). The stabilized MBR demonstrated a better removal rate of 15–42% relative to the CAS for all of the antibiotics tested (Fig. 4). Each sampling repetition was done after an additional month of stable operation. As mentioned before, MBR nutrient removal capacity also increased dramatically, indicating that the treatment process had stabilized. These results may lead to the conclusion that the higher MLSS (or SRT), which is theoretically associated with the development of a more diverse population of bacteria, caused the improvement in MBR performance compared to CAS.

The addition of UF to the CAS system, however, significantly increased (by up to 28%) the removal of all tested antibiotics (Fig. 5). UF filtration of synthetic mixtures containing various OMPs has shown that alone, it is not a significant barrier to these compounds since the UF membrane pore sizes are 100 times larger than the micro-pollutant molecules (Radjenovic et al., 2007). The addition of UF for the secondary effluents had a negligible effect on the MLSS concentration, SRT, and the bacterial composition in the CAS system. Therefore, it is hypothesized that the OMP removal mechanisms enhanced by UF addition were due to the action of the biofilm that formed on the UF membrane surface, which incidentally made the “bio-membrane” a tighter physical (enmeshment) and chemical (sorption) barrier (Yangali et al., 2009; Sahar et al., 2010).

UF operation comprised 50 minutes filtration followed by 1 min back-wash (as opposed to 5 min filtration followed by 30 s back-wash in the MBR). This means that biofilm formation (bio-fouling) is a result of the long filtration periods inherent in the daily operation of the UF system. The addition of UF to the CAS system, therefore, reduced the relative advantage the MBR had over the CAS (+UF) to a maximum of 20%. In fact, the two systems exhibited the same removal rate for CLA (94%). Thus, we theorize that the ability of an MBR to remove macrolides and sulfonamides is only slightly better than that of a CAS/UF system. Such a conclusion also indicates that biodegradation is probably not the main removal mechanism in these two systems.

3.6. Batch adsorption experiments with MBR biomass

A series of batch adsorption experiments were carried out to determine which of the two competing mechanisms, biodegradation or adsorption, was dominant. Preliminary tests using heat (105 °C) or toxin (sodium azide) to inhibit the
biological activity (as was done by Andersen et al., 2005) conferred upon the biomass characteristics of a passive sorbent (comparable to activated carbon). Therefore, it was decided to use the MBR biomass without further intervention and to favor adsorption over biodegradation by limiting contact time. Once stable operation was achieved, biomass (at a concentration of 10 g/L) was taken from the MBR system. The biomass was divided between four 1-L beakers and diluted with MBR permeate to the following concentrations: 10, 8, 6, and 4 g/L MLSS. The reason for using MBR permeate instead of synthetic effluent, as described by Nyholm et al. (1996) and Andersen et al. (2005), was to minimize the effect of ion concentrations on the bacteria cells. Each beaker was spiked with a synthetic solution of six different antibiotics, each at a concentration of 10 μg/L. The experiments were run for a relatively short time (1 h), during which the beakers were mixed at 100 rpm.

The batch experiment results indicate that sorption, and not biodegradation, was probably the main removal mechanism for both the sulfonamides and for the macrolide antibiotics (Fig. 6), in contrast to studies mentioned by Le-Minh et al. (2010). With the exception of SMX in an MLSS concentration of 4 g/L (64% removal), macrolides and the sulfonamides were removed at efficiencies of >82% at all MLSS levels. No correlation was found between the MLSS concentration and the macrolide removal rate, as was stated in Section 3.4.

3.7. Desorption experiments with methanol

To verify the sorption hypothesis and identify desorption potential, methanol was used as a solvent to extract sorbed antibiotics from biomass samples taken out from the MBR during steady state operation. Mixed liquor (1 L, 10 g/L MLSS)
was centrifuged and separated into its solid and liquid phases. The solid phase was washed three times with tap water (0.1 L each time) to insure that the only source of desorbed antibiotics would be the solid phase, as described by Andersen et al. (2005). The solid phase was added to a 1-L beaker and was filled with methanol which was selected for this task due to its dual hydrophobic–hydrophilic nature and its efficient performance in SPE procedures to desorb organic contaminants, such as the selected antibiotics, from cartridge polymers (Ternes and Joss, 2006). Solvent/biomass suspensions were mixed at 100 rpm for 1 h followed by a second solid–liquid phase separation by centrifuge. The liquid was analyzed for antibiotics as described in Section 2.2 above. The results in Table 5 demonstrate release of large amounts of desorbed macrolides (83.7–95.2 ng/g MLSS), a medium amount of desorbed SMX (49.3 ng/g MLSS), and low amounts of desorbed SMZ and TMP (2.4–3.9 ng/g MLSS). There is a noticeable correlation between the desorbed compounds and their original concentrations in the raw sewage (except for TMP, which had the lowest desorption rate but a higher concentration than the SMZ in the raw water). These results indicate that sludge is an efficient sorbent for antibiotics, and strengthen the proposed hypothesis regarding the significance of this mechanism in Activated Sludge systems.

### 3.8. Effect of compound characteristics on antibiotics removal

It is common to estimate a removal rate according to several molecular characteristics such as M.W., pKa, and log \( K_{ow} \). Such studies were done by Yangali et al. (2009) and Comerton et al. (2007). In these papers, the experiments were performed for each compound separately using a distilled water solution under markedly divergent conditions to those in the current study in which pilot plants treated raw sewage. The similarity between macrolides and sulfonamides in terms of M.W. and log \( K_{ow} \) (see Table 3) encouraged us to look for a linear correlation. From Table 6, it seems that as the SRT value and MLSS concentration increased, the linear correlation between the molecular characteristics and antibiotics removal decreased. Although the CAS/UF exhibited moderate correlations (0.55–0.56) for M.W. and log \( K_{ow} \) (see Table 3) encouraged us to look for a linear correlation. From Table 6, it seems that as the SRT value and MLSS concentration increased, the linear correlation between the molecular characteristics and antibiotics removal decreased. Although the CAS/UF exhibited moderate correlations (0.55–0.56) for M.W. and log \( K_{ow} \), the MBR correlations were significantly lower (0.1).

### 4. Conclusions

The efficiency and mechanisms of antibiotics removal from wastewater were tested in CAS/UF and MBR pilot plants treating the same raw sewage of the Tel-Aviv region in Israel. It was found that under stable operation the MBR demonstrated higher removal efficiency over the CAS for all the tested antibiotics. The incorporation of UF after CAS improved significantly the antibiotics removal, thereby reducing the difference between the removal efficiencies of the two systems. It is therefore hypothesized that the biofilm formed on the membrane (in both MBR and UF system) makes it’s separation characteritic tighter related to organic compounds and thus contributes to an enhanced removal of the antibiotics. This may explain the findings indicating better removals of various OMPs in MBR systems compared to CAS systems. However, it means also that the MBR advantage is probably due to the bio-membrane removal mechanism rather then to superior biodegradation.

### Table 6 – Linear correlation between molecular characteristics of sulfonamides and macrolides and their removal rates.

<table>
<thead>
<tr>
<th>Molecular characteristics</th>
<th>Linear correlation value (( R^2 ))</th>
<th>CAS/UF</th>
<th>MBR</th>
</tr>
</thead>
<tbody>
<tr>
<td>pKa</td>
<td>0.64</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>M.W.</td>
<td>0.55</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>log ( K_{ow} )</td>
<td>0.56</td>
<td>0.1</td>
<td></td>
</tr>
</tbody>
</table>

RR = Removal rate.
Batch experiments with MBR biomass showed that the biomass possessed an extremely high sorption potential for all the antibiotics (>82% for sulfonamides and >92% for macrolides at an MLSS concentration of 4, 6, 8, or 10 g/L). This was further demonstrated by methanol extraction, of solid-phase biomass taken out of the MBR, which released significant amounts of antibiotics into the liquid phase. It is therefore theorized that sorption mechanism (to both suspended and membrane-attached biomass) is also a significant removal mechanism. These results indicate that greater attention should be devoted to excess sludge management due to its antibiotics accumulating potential.

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References


Standard Methods 2540E current ed., Fixed and Volatile Solids Ignited at 550 °C.


