

Ampicillin Removal by Polyvinylidene Difluoride (PVDF), Polyethersulfone (PES) and Nylon for Membrane Bioreactor Application

Ruby S. Labinghisa and Analiza P. Rollon

Abstract—Micropollutants comprising of pharmaceutically active compounds (PhACs) are usually not degraded or removed in conventional wastewater treatment systems and are persistent in aquatic environment. This study determined the rejection of ampicillin by hydrophobic and hydrophilic membranes, the effects of operational parameters such as flow rate (1.4 and 2.2 mL/min), influent concentration (40, 70 and 100 ppb) and the extent of biodegradation and adsorption of ampicillin in batch membrane bioreactor with and without nitrification. Ampicillin (AMP) removal was higher in the bioreactor where nitrification occurred and at lower concentration and flow rates. The results showed that membrane bioreactor (MBR), with combined biological degradation and membrane filtration is a viable system for ampicillin removal. Besides biodegradation in the bioreactor, the cake layer deposited over the membrane surfaces played an important role in AMP rejection. A big part of the removal by the membrane system was attributed to the sieving and adsorption onto the cake layer.

Index Terms—Biodegradation, COD, micropollutant, pharmaceutical, rejection.

I. INTRODUCTION

Antibiotics are drugs specifically designed to treat or prevent infective diseases in human or animal body. Its use has become indispensable in human life and the global market consumption increase steadily every year. Human environments, medical wastes, farms, pharmaceutical and hospital sewage residues may contain various antibiotics and antibiotic resistance genes that can contaminate natural environments. Its exposure in aquatic environment may increase the number of antibiotic resistant bacteria, posing a serious threat to public health in that more and more infections may no longer be treatable with known antibiotics.

Ampicillin (AMP) is one of the most widely used antibiotics. Though quite expensive, this wastewater must be treated properly prior to the release into environment. The present studies to treat the chemical synthesis-based pharmaceutical wastewaters mainly focus on physical and chemical treatment, such as UV/ZnO photo-catalytic process, photo-Fenton process, ultrasonic process, advanced

oxidation processes (AOPs) and adsorption. Though high drug and COD removal rates were achieved in these bench-scale experiments, they are not suitable for full-scale wastewater treatment plant (WWTP) due to their high cost. Pharmaceutical wastewater may contain diverse refractory organic materials that cannot be readily degraded. Biological treatment is still a viable choice for treatment in addition to physicochemical processes [1]. Existing wastewater-treatment facilities should be upgraded and implementation of new technologies is envisaged as the next step in improvement of wastewater treatment. Interest in membrane bioreactor (MBR) technology for wastewater is rapidly increasing worldwide [2]. Although efficient, common drawbacks of membrane filtration for wastewater treatment are the high operating costs and membrane fouling. Many researchers have recently devoted their efforts in significantly reducing the operation cost for membrane systems. However, fouling problem remains to be a major obstacle to membrane technology [3]. Factors considered to be related to fouling and efficiency of MBRs that this study aims to examine are the hydrophobicity of the pollutant as well as the membrane used and several operational parameters such as flow rate and influent concentration. The main removal mechanisms for micropollutants such as endocrine disrupting compounds (EDCs) and pharmaceutically active compounds (PhACs) are through biological degradation and sorption to particles [4]. Knowledge on factors affecting PhAC removal is important as wastewater treatment plants (WWTPs) are not specifically designed to reduce them. Commonly cited factors are hydrophobicity or hydrophilicity of pollutant and membrane materials, membrane characteristics, biodegradability of pollutant, operational parameters (e.g. sludge retention time, hydraulic retention time, pollutant influent concentrations, pH and temperature) as mentioned in the studies conducted by Paetkau. Biological degradation particularly in nitrifying conditions was recommended for these compounds. As suggested by Gaulke, current trend on EDC such as estrogen degradation highlights the need for nitrification to achieve high removal. Their study showed that chemical reaction through the transformation of estrogen with ammonia oxidizing bacteria is seen as estrogen degradation in wastewater treatment. As sorption and biodegradation are considered significant removal mechanism, hydrophobicity of both pollutant and membrane are important [5]. Discussed in the study by Schuman, hydrophobic character of a compound can be indicated by K_{ow} value [6]. This is the partition coefficient between octanol and water for a given compound. Gaulke also

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mentioned that sorption to biosolids is dependent on solid-water distribution coefficient, K_d [5]. Study on solute rejection during membrane filtration of activated sludge, showed that hydrophobic membrane always have greater solute rejection than that of hydrophilic membrane [7]. Thus, the effects of different membranes and their hydrophobicity is included in this study.

This study aims to determine the applicability of aerobic membrane bioreactors in removing ampicillin (AMP) in wastewater. Specifically, this study aimed to determine: (1) the effect of nitrification on biological ampicillin removal, (2) the effects of influent flow and AMP concentration on AMP removal, (3) the AMP removal behavior by different membrane materials as characterized by their rejection and their hydrophilicity or hydrophobicity, and (4) the removal mechanism of AMP in the system, either by biodegradation or adsorption to cake sludge and to membrane.

II. MATERIALS AND METHODS

A. Overview

The system was first acclimatized by feeding the synthetic influent comprising of the macro and micronutrients until stable mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), and consistent COD removal were achieved. The minimal incubation medium used in the batch tests was based on Stanier medium as also used by De Gussemé [8].

Once nitrification was achieved as shown by regular ammonia-nitrogen and nitrate-nitrogen monitoring, target pollutant (ampicillin) was spiked in both systems without (system A) and with (system B) nitrification. Ampicillin (AMP) was monitored every 3-4 days. When the systems were already acclimatized as shown by AMP removal, batch mode of experiment was started to determine the effect on AMP removal by nitrification, varying the influent concentration, flowrate, and membranes (hydrophobic polyethersulfone and polyvinylidene difluoride, and hydrophilic nylon). Also removal mechanism was studied by measuring the biodegraded AMP in the influent before passing the membranes and the AMP removed by measuring the effluent after passing the wastewater through the membranes (sorption). Kinetics of AMP removal was also determined at various influent concentrations, flowrates and membranes.

B. Experimental Set-up

A 4 L Erlenmeyer flask reactor was aerated using air diffuser stones and the silicon tubing was used since plastics could affect the compounds. The system was operated in batch mode. It was mixed during feeding and sampling of MLSS using magnetic stirrer regulated at 180 rpm based on Chang *et al.* [7].

The syringe filters used had the same specifications, Whatman Puradisc 0.45 μm pore size, 25 mm diameters and filter areas of 4.2 cm^2 . Only the membrane materials differ using hydrophilic Nylon, slightly hydrophobic (60%) Polyethersulfone (PES) and hydrophobic Polyvinylidene difluoride (PVDF).

The permeate flux was manually measured in relation to membrane fouling. The mass of the effluent that passed

through the membrane filter in a time interval was determined by measuring the weight of the effluent receiving flask before and after the time interval. Volume was also measured using 10 and 100mL graduated cylinders.

C. Reactor Operation and Monitoring

To start up, the reactor was seeded with a sludge taken from an aerobic wastewater treatment system of a shopping mall. At the start, these were fed with sodium acetate (400 mg TCOD/L) as the organic substrate for 2 weeks until a stable sludge was achieved at around 3.5 – 4 g/L. Presence and extent of nitrification was also monitored. Sample (200 mL) was taken from the reactor every 3-4 days for the analysis of ammonium-nitrogen ($\text{NH}_3\text{-N}$) and nitrate-nitrogen ($\text{NO}_3\text{-N}$). MLSS, MLVSS, $\text{NH}_3\text{-N}$, $\text{NO}_3\text{-N}$ was measured every 3-4 days, TCOD, SCOD every 2 days, and pH was monitored everyday using standard procedure. The target pollutant, AMP was spiked starting 1 $\mu\text{g/L}$ until 100 $\mu\text{g/L}$.

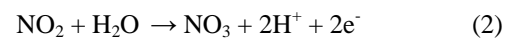
D. Analytical Methods

The measurements of MLSS, MLVSS, TCOD, SCOD, $\text{NH}_3\text{-N}$, $\text{NO}_3\text{-N}$ were based on standard procedure. TCOD and SCOD was measured using dichromate digestion high range. Hach colorimeter D-790 was used in measuring. AMP used was Sodium ampicillin and was measured using spectrophotometric method according to Khan *et al.* on Shimadzu UV-VIS [9].

III. RESULTS

A. Bioreactor Performance during Acclimatization without Filter

During acclimatization of the system with nitrification, from the 9th day of the run, conversion of ammonia to nitrate began to occur as indicated by a decrease in $\text{NH}_3\text{-N}$ and increases in $\text{NO}_3\text{-N}$. To maintain the pH in the range 7.5-8.0, sodium hydroxide was added. Nitrification generates H^+ as shown in (1) and (2).



The pH decrease accompanying the conversion of ammonia to nitrate supports the occurrence of nitrification and signifies growth of nitrifying bacteria in the bioreactor. Systems A (without nitrification) and B (with nitrification) were acclimatized. The systems began stabilizing in terms of conversion rates around day 19. From this day, the MLSS and MLVSS levels were 3.5 - 4 g/L MLSS and 1-2 g/L MLVSS. At this time, the ammonium-nitrogen ($\text{NH}_3\text{-N}$) and nitrate-nitrogen ($\text{NO}_3\text{-N}$) was then monitored at System B to determine if nitrification was taking place. Sodium acetate was fed as organic substrate (400 mg COD/L) to maintain a stable sludge concentration at the range 3,000-4,000 mg/L MLSS, which is a typically applied value for membrane bioreactors [10]. As shown in Fig. 1, TCOD values in succeeding batch gradually decreased starting from 505.33 mg/L TCOD and 339 mg/L SCOD, the value dropped to 26.5 mg/L TCOD and 47.33 mg/L SCOD at the end of acclimatization period.

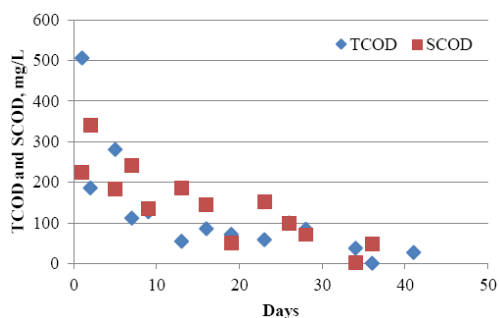


Fig. 1. TCOD and SCOD levels in the system with nitrification (B) during acclimatization at sequencing batch mode.

These decreases in TCOD and SCOD showed that microorganisms present in the system were able to feed on acetate, the organic substrate. AMP was then spiked to the bioreactor beginning at 1 $\mu\text{g/L}$ and AMP concentration was gradually increased to a final concentration of 100 $\mu\text{g/L}$ (or 100 ppb). The AMP levels during sequencing batch runs in systems without (A) or with nitrification (B) decreased from 100 ppb at the beginning of each new batch to below 10 $\mu\text{g/L}$ (Fig. 2). This AMP decrease indicates that both systems were able to degrade the added AMP in the one-month duration of acclimatization.

B. Effect of Nitrification on AMP Removal

Simultaneous sequencing MBR runs with and without nitrification were done for two weeks. The TCOD and SCOD removal values without nitrification (system A) were 37.32% and 27.89%, respectively (Fig. 3). The TCOD and SCOD removal values were higher with nitrification (system B), i.e., 59.97% and 60.04%, respectively. The systems had a stable sludge concentration throughout the runs, i.e., in the range 3,000-4,000 mg/L, which is typically maintained in MBRs [10].

For AMP removal, the average was 78.13% and 87.60% in systems A and B, respectively. Ampicillin and COD removal was greater in the system with nitrification than without nitrification. The nitrifiers could have initially degraded AMP into intermediates that subsequently serve as a substrate for heterotrophic organisms.

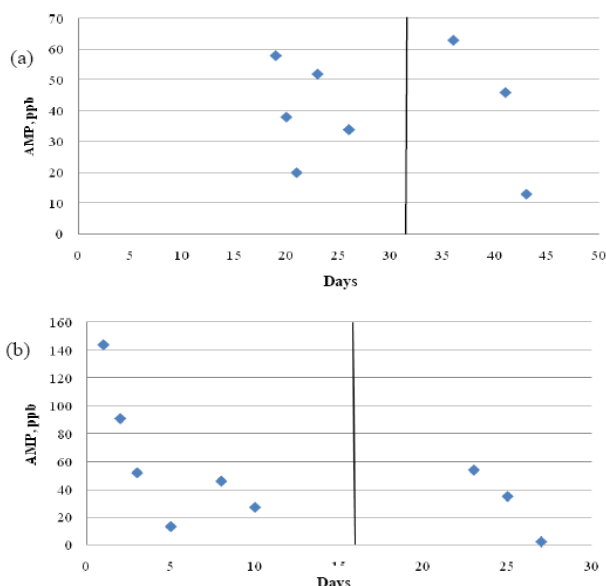


Fig. 2. Ampicillin level in system A without nitrification (a) and system B with nitrification (b). Vertical lines denote the time of feeding the reactor with fresh influent.

There were previous studies that explored ways to enhance the removal, degradation or both of micropollutants such as endocrine disrupting compounds (EDCs). De Gusseme *et al.* on using nitrifier enriched culture (NEC) for 17 α -ethinylestradiol (EE2) concluded that nitrifying MBR offers opportunities as a promising add-on technology for WWTP effluent polishing [8]. The study by Clara *et al.* demonstrated degradation of EE2 by a nitrifying sludge with a high ammonium (NH_4^+) oxidizing activity [11]. These authors brought forward the concept that nitrifiers initially degrade EE2 into intermediates that subsequently serve as a substrate for heterotrophic microorganisms. On the other hand, De Gusseme *et al.* suggested that the primary mechanism for EE2 degradation is more likely linked to the activity of heterotrophic bacteria [8]. They suggested that the EE2 removal by axenic cultures of ammonia-oxidizing bacteria (AOB) is only due to the abiotic nitration reaction with EE2, which is governed by the high $\text{NO}_2\text{-N}$ levels after oxidation of the high $\text{NH}_4\text{-N}$ concentrations in batch tests. Besides the application of nitrifiers in EDC removal, studies on PhACs mainly focused on AOPs and some are coupled with biological treatment. In this study nitrification was found to enhance ampicillin removal.

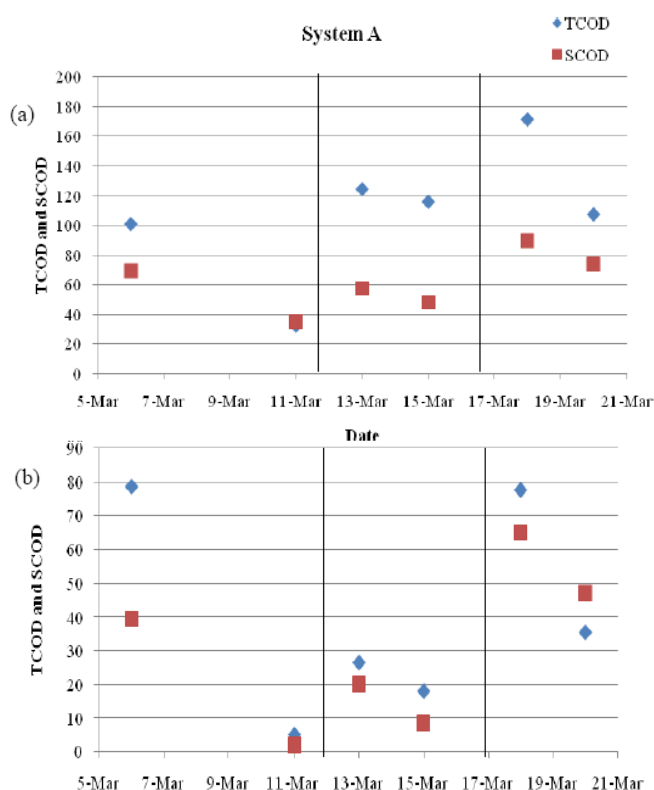


Fig. 3. TCOD and SCOD levels in the MBR without nitrification (A) and system with nitrification (B). Vertical lines denote the time of feeding the reactor with fresh influent.

C. Ampicillin (AMP) Removal via Membrane Filtration

1) Effects of influent AMP concentrations on AMP removal for PES, nylon and PVDF membranes

In general, the percent AMP removal at the same duration of run (250 min) increased as the influent concentration decreased from 100 ppb to 40 ppb. The total amounts of AMP removed within 250 min were similar at different initial AMP concentrations. The AMP removal rates were the same for a given membrane regardless of initial

concentration. Hence, the concentration of AMP after 250 minutes decreased as initial concentration decreased (Fig. 4).

Among membranes the removal rates were 35-45, 30-40 and 35-60 $\mu\text{g/L}/250\text{ min}$ for PES, Nylon and PVDF membranes, respectively. It is interesting to note that PES and PVDF are hydrophobic while the Nylon membrane used was hydrophilic. The latter membranes are suitable for use with a wide range of biological preparations and can be used where other membranes are unsuitable or difficult to use due to its characteristic.

The cake sludge formed through time on the membrane probably aided in removing AMP as the reactor broth passed through the membrane filter.

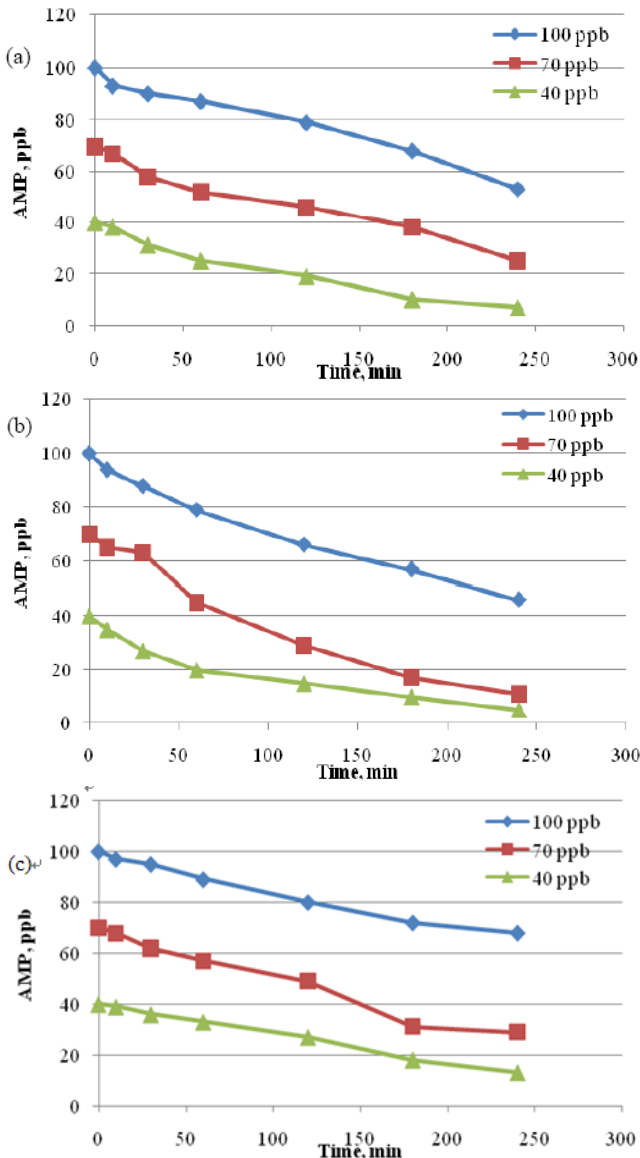


Fig. 4. AMP in MBR at 2.2 mL/min flow through (a) PES, (b) PVDF and (c) nylon membrane (top to bottom).

At 4 hours duration of each runs, the membrane filter and probably also the cake sludge formed on the membranes were only able to almost completely remove the AMP at lower influent concentration. If the run duration is extended to several days or weeks, the microorganisms that attached, retained and developed on the cake sludge could have adapted to the prevailing influent AMP. The results of the above MBR runs using different membrane materials and at

different influent AMP concentration study could not give indications on whether AMP has inhibitory or limiting effects on the microorganisms present in the MBR system.

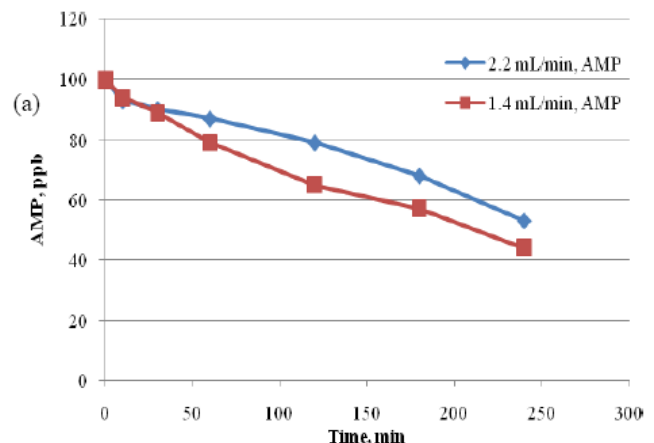
2) Effects of flow rate

The rates of decrease in AMP concentration with time were higher at lower flow rate (1.4 mL/min) than those at higher flow rate (2.2 mL/min) as shown in Fig. 5. The removal rates among the three membranes were higher using PES and PVDF membrane materials (Fig. 6). The mass flow rate for Nylon is the highest among the other membranes, considering that the same filter area was used in all runs. This was probably because Nylon is hydrophilic, allowing higher permeates across its membrane. The higher AMP removal at lower flow rate was probably due to the corresponding longer hydraulic retention time (HRT), i.e., longer time that the wastewater remained in the bioreactor prior to its flow through the membrane filter. Paetkau suggested that longer HRTs (greater than 10 hours) are associated with high micropollutant levels [4]. At longer HRT, the microorganisms present in the system have greater time to grow and consume or degrade the pollutant (substrate) and become better adapted in degrading the micropollutants. Moreover, lower flow rate probably enabled enough time for formation of sludge cake on the filter, thereby aiding the removal of the pollutant (AMP in this study). As corroborated by the mass permeate data for each flow rate, the lower flow rate would also enable AMP to be adsorbed to the sludge/filter membrane.

3) Effects of membrane materials

As earlier mentioned, the highest AMP removal (thus, lowest effluent AMP effluent concentration) was that by hydrophobic PVDF, followed by slightly (60% based on hydrophobic PES, and then that of hydrophilic Nylon [2].

The mass permeate of each membrane, which was highest for Nylon and lowest for PES, had an effect on the AMP removal. The effect was probably due to sludge cake formation on the membrane. Maximous *et al.* [2] suggested that the relatively higher cake resistance (a factor of permeate flux) of the membranes rationalize the increased solute rejection in the hydrophobic membrane. This means that the deposited cake layer plays an important role in solute rejection. Choi and Ng determined the effect of membrane types and material on performance of submerged membrane bioreactor [12].



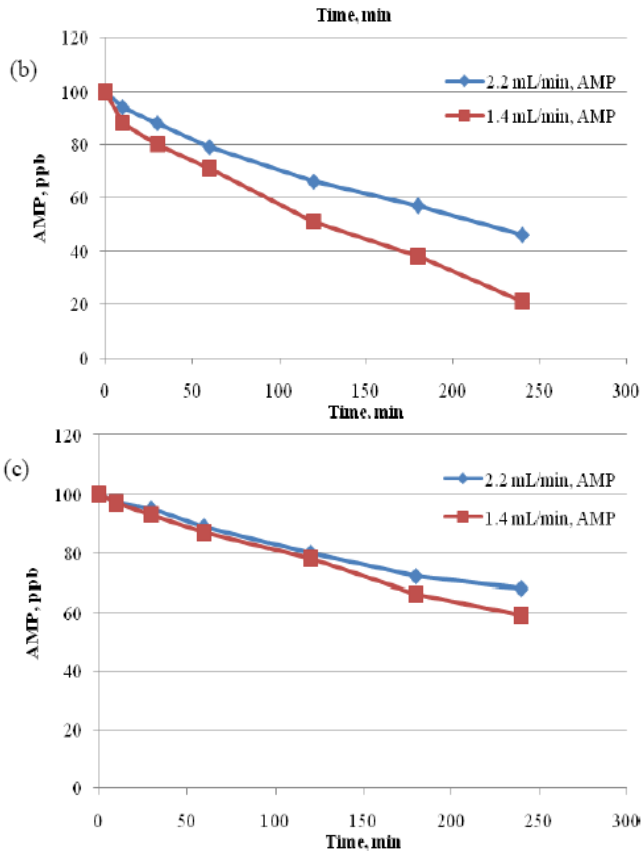


Fig. 5. Effect of flow rate on AMP removal in MBR using PES, PVDF and nylon membrane (top to bottom).

They found a lower Total Organic Carbon (TOC) level in the permeate compared to the supernatant and they attributed this to a possible combination of biodegradation by the biofilm (cake layer) developed on the membrane surface and further filtration by cake layer and narrowed pores. Hydrophobic membranes tend to have lower permeate as discussed in previous section, thus cake sludge is formed resulting in higher cake sludge resistance and higher rejection of micropollutant.

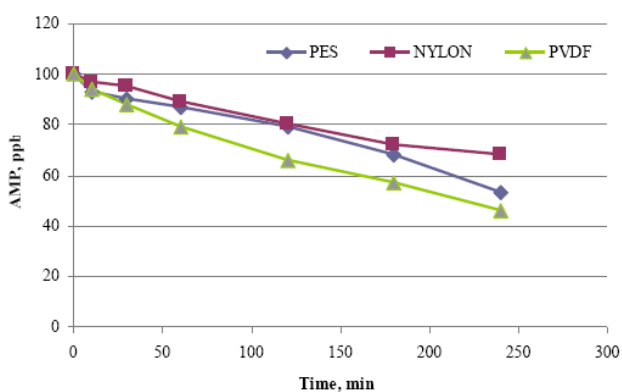


Fig. 6. AMP effluent of different membrane materials at 100 ppb and 2.2 mL/min.

4) AMP removal mechanism: biodegradation and adsorption

For PES membrane, the cumulative removal of AMP was monitored and for this membrane material, the mass permeate became constant after nine hours. The cumulative AMP removal (as indicated by the decreasing AMP concentration in the reactor and prior to the membrane) increased in time suggesting that biodegradation was taking place inside the bioreactor.

The removal across the membrane in the first hour was 7 ppb, and as the cake sludge formed, the AMP removed increased until the removal reached 84 ppb in 8 hours. Hence, besides filtration, the adsorbed colloids and sludge on the membrane enhanced the AMP rejection over time. Espinasse *et al.* suggested that besides the obvious rejection of pollutant, the formation of a deposit on the membrane surface generally changes its properties, which would later affect membrane fouling and solute rejection [13]. This is an important problem for applications that are very sensitive to the surface properties, as in food and pharmaceutical industries. Fouling happens when a natural dispersion is ultra-filtered. In this study the colloidal range was only in macrofiltration level. Fouling is often the consequence of the concentration of colloids (macromolecules or sub-micronic particle). However, these adsorption of suspended particles on the membrane also lessens the permeate flux and can cause fouling.

The cumulative removal in the influent of the reactor was 6 ppb AMP for the first hour until it reached 47 ppb at the end of the run. Thus, while there was a removal after filtration, biodegradation was also taking place, and aided removal even before membrane filtration. For nylon, the run was done for 13 hours. Across the membrane filters, removals were 9 ppb for the first hour, and continued to increase at the succeeding time intervals. This increase was probably mainly due to possible formation of sludge cake on the membrane surface. After 13 hours, the cumulative AMP removal across nylon was 72 ppb.

In PES, the removal across the membrane in the first hour was 7 ppb, and as the cake sludge formed, the AMP removed increased until the removal reached 84 ppb in 8 h.

For PVDF, the run was done for 14 hours. The cumulative AMP removal across the PVDF membrane started with 7 ppb until 19 ppb for the first hour. After 14 hours, the cumulative AMP removal across PVDF was 85 ppb.

Summarizing the results, the effect of varying operational parameters such as flow rate and influent concentration had a significant effect on the AMP removal. Lower flow rates resulted in higher AMP removal due to longer time for the sludge cake to be formed and for solutes to be adsorbed on the sludge cake and membrane layers. The study also found that higher influent concentration resulted in higher AMP effluent concentrations. This study however was not able to determine if the higher AMP concentration had a limiting effect on the substrate removal and growth of microorganisms present in the reactor.

The results of this study also suggest that cake layer and fouling resistances of hydrophobic membranes such as PVDF and that of slightly hydrophobic PES are always higher than those for hydrophilic membranes such as nylon. Since cumulative AMP removal through time was increasing, adsorption to cake sludge played an important role in AMP removal mechanism. AMP is removed partly via adsorption onto the membranes and through membrane pores.

IV. CONCLUSIONS

This study has found that MBRs using suitable membrane

materials is a promising option for AMP removal from effluents of treatment systems treating domestic or municipal sewage. This study has shown the effects of the presence of nitrification, the different operational parameters and type of membrane materials on ampicillin (AMP) removal in membrane bioreactor application. It has also determined the type of kinetic equation that describes AMP removal via biodegradation and via combined biodegradation and membrane filtration system.

A. Effect of Nitrification

The occurrence of nitrification or the presence of nitrifiers in MBR enhances AMP removal via biodegradation. AMP degradation takes place with or without nitrification but its rate and extent are higher when nitrification is present. The average AMP removal was 78.13% and 87.60% in systems A (without nitrification) and B (with nitrification), respectively.

B. Effect of Operational Parameters at Different Membrane Materials

The percent AMP removal was lower at higher influent concentration. However, the amounts of AMP removed were the same regardless of initial influent concentration. That is, the AMP removal rates were the same for a given membrane material regardless of initial AMP concentration. Higher AMP removal rates were achieved at lower flow rates. Among the three membranes evaluated in this study, the PES and PVDF membranes, which are hydrophobic and slightly hydrophobic achieved higher AMP removal at 47% and 54%, respectively. Nylon, which is hydrophilic, achieved lower AMP removal of 32%. Hydrophobic membranes (PES and PVDF) showed greater solute and AMP rejection than hydrophilic membrane (Nylon).

C. AMP Removal Mechanism

In the MBR system, AMP is removed via biodegradation, filtration by the membrane material and the sludge cake formed. The predominant removals were attributed to the sieving and/or adsorption onto the cakes as higher removal was observed until constant permeate flux (PES was 9.33 µg/L-hr, Nylon was 5.54 µg/L-hr and PVDF was 6.07 µg/L-hr). Some parts of the pollutant were adsorbed into the membrane pores and surfaces. In time, as the latter thickens, the cake layer resistance slows down AMP removal.

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