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Research Article

Treatment and Toxicity of Some Pharmaceuticals Such as Oxytetracycline and Sulfamethoxazole in Advanced Membrane Processes

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Abstract

In this study, the toxicity of a pharmaceutical industry wastewater was investigated by using six different trophic levels (four bacteria, a yeast, a mold, an algae a crustacean and a fish). The bacteria were *Escherichia coli, Bacillus cereus, Vibrio fischeri*, and Methane Archae Bacteria. The algae was *Chlorella sp*, the yeast was *Candida sp*, the fungi was Aspergillus, the crustacean was Daphnia magna and the fish was Lepistes sp.. Furthermore biodegradability and bioaccumulation tests were performed with two parmaceutical pollutants. The toxicity of this wastewater originated from its high sulfamethoxazole and oxytetracycline concentrations. This wastewater was treated by a reverse osmosis membrane reactor. The effects of increasing pressure on the rejections and recoveries of sulfamethoxazole and oxytetracycline was studied. Furthermore ,the effects of pH and temperature variations on the permeate yield was studied.The reverse osmosis reactor stability was not affected by pH, temperature and pressure increase. The toxicity of pharmaceutical industry wastewater decreased completely in the permeate, the biodegradability of the wastewater increased and it's bioaccumulative properties disappered.

INTRODUCTION

Pharmaceuticals are a class of emerging environmental contaminants that are extensively being used in human and veterinary medicine [1]. These chemicals are designed to have a specific mode of action, and many of them for some persistence in the body. The emissions of the pharmaceuticals to be evaluated for potential effects on aquatic flora and fauna in the ecocystem [2]. The drug residues in treated wastewater and surface water are very widespread. In contrast, only little is known about ecotoxicological effects of pharmaceuticals on aquatic and terrestrial organisms and wildlife, and a comprehensive review on ecotoxicological effects is lacking [3]. Aquatic organisms are particularly important targets, as they are exposed via wastewater residues over their whole life. Standard acute ecotoxicity data have been reported for a number of pharmaceuticals, however, such data alone may not be suitable for specifically addressing the question of environmental effects, and subsequently in the hazard and risk assessment [4]. The low volatility of pharmaceuticals indicates that distribution in the environment will occur primarily through aqueous transport, but also via food chain dispersal. In wastewater treatment, two elimination processes are generally important: adsorption to suspended solids (sewage sludge), and biodegradation. Adsorption is dependent on both hydrophobic and electrostatic interactions of the pharmaceutical with particulates and microorganisms. Acidic pharmaceutical such as the ibuprofen, fenoprofen, ketoprofen, naproxen, diclofenac and indomethacin having pKa values ranging from 4.9 to 4.1, as well as clofibric acid, bezafibrate (pKa 3.6) and gemfibrozil occur as ion at neutral pH, and have little tendency of adsorption to the sludge [5,6]. But adsorption increases with lower pH. At neutral pH, these negatively charged pharmaceuticals therefore occur mainly in the dissolved phase in the wastewater. For these compounds and the antitumor agent ifosfamide sorption by non-specific interactions seems not to be relevant. In general, sorption of acidic pharmaceuticals to sludge is suggested to be not very important for the elimination of pharmaceuticals from wastewater and surface water [7]. Therefore, levels of pharmaceuticals in digested sludge and sediments are suggested to be relatively low, as was demonstrated in several monitoring studies [8] However, basic pharmaceuticals and zwitterions can adsorb to sludge to a significant extent, as has been shown for fluoroquinolone antibiotics [8,9]. For the hydrophobic antibiotics (logKow 4.0) sorption to sludge is likely to play a role in the removal from wastewater [10,11]. Degradation in sludge seems not significant [10]. The estradiol occurs in digested sludge, where concentrations of 17 ng/g were reported [11,12]. In case a pharmaceutical is occurring mainly in the dissolved phase, biodegradation is suggested to be the most important elimination process in wastewater treatment. It can occur either in aerobic (and anaerobic), zones in activated sludge treatment, or anaerobically in sewage sludge digestion [13].

Sulfonamides have been developed as the first antibiotics to systemically treat infectious diseases of humans and animals. Sulfonamides contamination has been frequently found in

groundwater, surface water, wastewater, and soil. Adverse ecological effects and related human health issues have been demonstrated because of the accumulation properties and toxicity of sulfonamides. Sulfamethoxazole is the most frequently found sulfonamide in environment with a concentration range of 10 mg /l to 231 mg /l, respectively, in wastewaters [14]. Antibiotic medicine sulfamethoxazole features classic PPCPs, with very low removal ratio in water treatment and high frequency to be detected. In recent decades, although the consume of sulfamethoxazole has been reduced, it is the most popular germifuga in animal food production [15]. It is reported that SMX applied in veterinary directly discharges into the aquatic environment, which has high toxicity [16]. Therefore, there have been large amount of studies on sulfamethoxazole. However, most attention has been focused on identification, fate, and distribution of PPCPs in municipal wastewater treatment plants [17,18]. It is significant to develop treatment method to remove SMX. The commonly used treatment methods include advanced oxidation process, adsorption, and membrane technology [11-13]. Bioflocculation ,absorption method has several advantages over other methods, such as going green, being environmentally protective, no second pollution, and being biodegradable [14,19]. What is more, bioflocculation has been proved to be highly effective and wildly applied, and yet there is no published research on bioflocculation removal of PPCPs. Thus, it is meaningful to study the removal of PPCPs by bioflocculation. Bioflocculant MFX is a metabolized production with good flocculant activity, generated and secreted by *Klebsiella* sp. into the extracellular environment [15,16, 20]. SMX can persist in the environment for long periods of time because of its low biodegradability, which may result in various, direct and indirect, toxicological effects on the environment and on human health. In the study performed by Dirany et al. (2011), 89% inhibition was detected for 200 mg/l SMX using the bioluminescence Microtox® method, based on the inhibition of luminescence of marine bacteria *Vibrio fischeri* [21]. In the study performed by Dantas et al. (2021), bioluminescence inhibition decreaed only by % 27 after ozonation and electrophentone [22]. In this study, high inhibition was detected for Sulfamethoxazole abatement by means of ozonation. High inhibiton was detected (87%) for SMX in the acute toxicity tests performd by freshwater Microalga- *Raphidocelis subcapitata.*

The primary and secondary treatments of residual antibiotic wastewater in wastewater treatment plants is not sufficient to remove 100% of antibiotics; thus, the advanced and tertiary treatment of such antibiotic pollutants is much needed as the pollution increases with increased antibiotic consumption [23]. Advanced oxidation process such as photolysis, ozonation, Fenton, and photo-Fenton processes, and the oxidation of antibiotics in the presence of ozone/UV/hydrogen peroxide mainly involve transformation and release of oxidized products with complete removal efficiency [24]. The vital technique to remove various kinds of pollutants is adsorption, and the main advantage of this process is the application of low-cost adsorbent with less toxicity [25]. Adsorption and advanced treatment processes are the two most widely as well as accepted techniques for the tertiary treatment of wastewater in wastewater treatment plants compared to other technologies such as membrane filtration and reverse osmosis which have high production and operational cost [26] .

Micropollutants removal by membrane separation processes is a commonly researched topic, especially when reverse osmosis (RO) and nanofiltration (NF) are applied, which are membranes capable of retaining particles bigger than 10 and 100 Da, respectively [27,28]. Such application is possible since the molecular sizes of most micropollutants range from 200 to 400 Da [29]. A common phenomenon in filtration processes with RO membranes is the concentration polarisation, which is influenced by flux and concentration conditions of the feed stream. With elevated feed flows due to high operational pressures, the retained species tend to accumulate close to the membrane surface, which decreases the permeate flux [24,25]. Another phenomenon that can occur and that results in the decrease of permeate flux over time is fouling and it is usually associated with a decrease in solute rejection [26,30]. The chemical adsorption, modifies the internal structure of membranes by filling their intermolecular spaces with hydrophobic components, thus reducing the diffusive effect of water and facilitating the passage of compounds with high Log K_{ow} values [27]. Many studies have observed this phenomenon in the evaluation of rejection efficiency of micropollutants by RO membranes [28,31].

With the rapid development of membrane technology, membrane separation process has been gaining attention for antibiotic wastewater treatment. The reverse osmosis (RO) process, nanofiltration (NF) process and ultrafiltration (UF) process have been studied to remove tetracycline antibiotics from wastewater [32]. The rejection of examined antibiotics by some RO/NF memmbranes could achieve 98.5% [33]. More importantly, the tetracycline antibiotics in the RO or UF retentate can be recovered through conventional crystallization [34]. These advantages, RO has been used for the treatment of municipal wastewater, oily wastewater and trace organic compounds in water [35]. Furthermore, to produce fresh water and regenerate draw solution, RO could be combined with other membrane processes, such as RO and membrane distillation [36].

The aim of this study was to treat a pharmaceutical wastewater containing high oxytetracyclne and sulphametazasol antibiotic concentrations using a BW30-reverse osmosis membrane. The effects of increasing membrane pressures on the product-permeate flow rates, on the rejections of oxytetracyclne and sulphametazasol, on the solute permeate, on the recoveries of of oxytetracyclne and sulphametazasol were investigated. Furthermore, the effects of temperature and pH on the removals of the aforementioned two antibiotics were investigated in permetate samples of reverse osmosis. The toxicity of the pharmaceutical wastewater was investigated using six different trophic levels (four bacteria, a yeast, a mold, an algae a crustacean and a fish). The bacteria used in the acute tests were *Escherichia coli*, *Bacillus cereus*, *Vibrio fischeri*, and *Methane Archae* Bacteria. The algae algae used in the acute toxicity tests was *Chlorella* sp, the yeast was *Candida* sp, the fungi was *Aspergillus*, the crustacean was Daphnia magna ana the fish was *Lepistes sp*. Furthermore biodegradability and bioaccumulation tests were performed with two parmaceutical pollutants.The acute toxicity test results performed after reverse osmosis treatment were evaluated for toxicity removals.

MATERIAL AND METHODS

Reverse osmosis reactor configuration and membrane properties

Bench-scale reverse osmosis unit used is a dead-end type containing a stainless steel, flat-sheet cell with an effective separation area of 86.5 cm^2. Reverse osmosis membrane (BW30-4040, DowFilmetec®) used was polyamide type. The membrane used in this study was TW30 and is made of a thin-film composite synthetised by interfacial polymerisation of a aromatic polyamide on a polysulfone. The properties of the membrane and some operational parameters were shown in Table 1.

The membrane's pure water permeability was determined using a stainless-steel dead-end membrane system . PWP provides an indication of the maximum flux that can be achieved with the evaluated membrane. It corresponds to the slope of the average flux of ultrapure water through the effective surface area of the membrane (86.5 cm^2), as a function of feed pressures (5, 10, 15 and 20 bar).

The zeta potential analyses the surface electrical charge of the membrane according to the pH of the medium to which it is exposed and it was measured using a Zeta Plus analyser (Anton Paar), and the software. Membrane's contact angle is an indication of surface's hydrophilicity or hydrophobicity. A contact angle of less than 90° indicates hydrophilicity whereas a contact angle above 90° indicates hydrophobicity. The static contact angle of dry membrane samples was measured in triplicate with ultrapure water using a goniometer analyser wth a software of SCA21.

The culture of bacteria [*Escherichia coli* - ATCC 3509 (RSHM NO: 5010) and *Bacillus cereus* - RSKK 11015 (NTC 9946)] , yeast (*Candida albicans* ATCC 628) and mold (*Aspergillus niger*), were purchased from Turkey Public Healthı Institutions Algae - *Chlorella sp*. was isolated from Golcük Ödemiş Lake and was cultivated. Bioluminescent bacteria - Vibrio fischeri were purchased from Hach-Lange Company as a lyphilized culture. Water flea - *Daphnia magna* and fish - *Poecillia reticulata* was purchased from an aquarium maker. Pharmaceutical industry wastewater samples were diluted with a ringer trace metal solution and inoculations were made on the nutrient agar pales for bacterial toxicities. After 24 and 48 hours incubationa at 21^{0}c temperature the colony counts were correlated with control sapmles containing no pharmaceutical wastewater. Increased concentration of the sulphametazasol and oxytetracycline were contacted with yeast and fungi ana they incubated at yeast ana czapex agar at 21^{o}C for 5 days. Percent inhibitions were compared to the control group. In the Microthox bacteria acute toxicity tests the bioluminescent bacteria were diluted at ratios varying between 1/1 ;1:6 ; 1/8 ; 1/16 ; 1/32 and were put to microthoc cells containing bacteria and 1.5 ml NaCl. Luminesans values were measured after 5, 15 ana 30 minutes incubations time. The Inhibitions and . EC 50 values were calculated after incubation period using a LUMIS soft ware program. Anaerobic toxicity assasy (ATA) was performd at 35°C in in amber bottles with a volüme of 150 ml. Vanderbilt Mineral Medium, 3000 mg/1 glucose-COD, sodium thiogylcollate (to maintain the anaerobic environment), $NaHCO_3$ (to keep neutral pH) was added into sterile 5-Liter flask. Pharmaceutical wastewater was diluted and they were added into the amber bottles. 5 liters of the mixture were distributed into each vial having a liquid volüme of 75 ml and they were stirred in a sonicator for 1 hour. 40 mg/l of the anaerobic sludge was added and the mouths of the bottles were sealed with rubber stoppers. After 24 and 48 hours incubation period the methane gas was measured with Dragger automotic gas meter. For the Algae (*Chlorella sp*.) acute toxicity tests were performed in the same manner and they were incubated for 24, 48 and 72 hours. The inhibition of formula was as follows : [(1-N/N 0) X 100] (N: is the number of organism cells exposed to pharmaceutical test, N0 is the number of organism cells at the beginning of the test). Biodegradability test was conducted in 2-liter glass beaker.1, 10 and 100 mg/1 initial COD was adjusted using glucose (For 10 mg/l-COD: 0.1 g ; for 100 mg/l-COD: 0.5 g glucose was added). The pH of the water was 7 ± 0.2 while the dissolved oxygen was maintained between 4 and 6 mg/1 by using an air pump. During 28 days the of decreasing COD values were noted. Bioaccumulation test is performed in two stages. In the first stage; the uptake of chemical compound by fish was monitored in a 28- days incubation period. Than the release of the chemical to the water was monitored during 28 days and the bioaccumulative factor BCF was calculated.

Table 1: Properties of the membrane and some operational.

Parameters	Parametric value
Flux (L h^{-1} m^{-2})	20-120
Salt rejection (%)	97-99
Zeta potential (mV)	−45
Contact angle (°)	65,4
Effective surface area (cm^2)	112
Pure water permeability (L h^{-1} m^{-2} bar^{-1})	3,25 -9,36
Pressure applied	1,4-80
Surface nature	Hydrophilic
Maximum operating pressure (bar)	80
pH range	2–11

Analytical procedure for oxytetracycline sulphaemetazasol measurements

Antibiotics were extracted from refuse samples by adding 0.2 g Na_2EDTA and 5 mL acetonitrile-phosphoric acid buffer (pH 3.0) to 1 g of sample in a 15 mL polypropylene centrifuge tube. EDTA complexes divalent cations and has been found to increase antibiotics recovery from refuse samples. Samples were vortex mixed for 2 min and sonicated for 30 min. After mixing, samples were centrifuged for 10 min at 3500 rpm. The supernate was then transferred to a 20 mL clean brown borosilicate glass vial. The extraction was repeated 3 times and the supernate from each replicate was combined. Finally, 1 mL of the extraction was filtered through a 0.2 µm hydrophobic PTFE membrane (Jinteng, China) into a brown borosilicate glass vial and stored (less than 1 week) at 4°C until LC–MS/MS analysis.

Antibiotics were quantified via liquid chromatography–tandem mass spectrometry (LC–MS/MS) using a Shimadzu LC-20A (Shimadzu, Japan) and AB Sciex API4000$^+$ triple-quadrupole

mass spectrometer (AB Sciex, USA) with a Phenomenex C18 column (50 × 2.1 mm, 2.6 µm) run at a column temperature of 30°C. Gradient separation was performed using a 0.1% formic acid/ultrapure water (> 18 MΩ) solution and acetonitrile. The injection volume was 3.0 µL. Detection was achieved using electrospray ionization while running in positive ion mode for all compounds. Data acquisition was performed in the multiple reaction monitoring (MRM) mode. The limits of detection (LOD) (signal-to-noise (S/N) of 3:1) for SMX, TC, and OTC were 0.04, 0.05, and 0.05 ng/g, respectively. The limits of quantification (LOQ) (S/N of 10:1) for SMX, TC, and OTC were 0.99, 0.46, and 0.44 µg/g, respectively.

RESULTS AND DICUSSION

Toxicity test results in the raw pharmaceutical wastewater

Bacterial toxicity test results in raw and in diluted pharmaceutical wastewater: The acute bacterial toxiity test results showed that the raw pharmaceutical wastewater were toxic to all bcteria types studied. In the direct raw pharmaceutical wastewater containing 5000 µg/l oxytetracycline and in relevant diluted wasewater containing 3500, 2500, 1500, 100 and 500 µg/l oxytetracyline (Table 2). High inhibitions percentages were detectedfor oxytetracyline. The pharmaceutical wastewater was found to be very toxic to *Vibrio fisheri* and methane bacteria with high inhibitions compared to the other two bacteria namely *E.coli* and *B subtilis*. The toxicity in other words the inhibitions decreased in diluted pharmaceutical wastewater containing low oxytetracycline concentrations. The EC 50 values, in other words the toxicant concentrations affecting the half of the bacteria were low in the high inhibitions detected Microtox and and methane bacteria acute toxicity test. These bacteria cn be classified as more sensitive bacteria to oxytetracycline thant the other two bacteria.

The results of algae, fungi and yeast toxicity test results also showed that pharmaceutical wastewater containing oxytetracycline were acute toxic to the aforementioned organisms. However these type of oranisms exhibited low inhibitions compared to bacteria since their trophic level is high, they are more developed, they are eutrophic and exhibited resistance to the oxytetracycline compared to the bactera which are prokaryots (Table 3).

The biodegradability and bioaccumulation studies showed that pharmaceutical wastewater containing high oxytetracycline concentrations exhibited low biodegdarabilities and high cumulative properties. Bacterial toxicity test results performed in raw diluted pharmaceutical wastewater exhibited lowe inhibitions with more high EC 50 values. Biodegradability and bioacumulation test results in raw diluted water water showed that the biodegradation percentages increased while the bioaccumulative properties of the the pharmaceutical wastewater decreased (Table 4 and Table 5).

Table 2: Bacterial toxicity test results in raw diluted pharmaceutical wastewater for oxytetracycline.

Oxytetracycline concentration in raw pharmaceutical wastewater (mg/l)	İnhibitions in *E.coli* (%) compared to control without pharmaceutical wastewater	İnhibitions in B.subtilis (%) compared to control without pharmaceutical wastewater	İnhibitions in *Vibrio fisheri* in Microthox test (%) compared to control without pharmaceutical wastewater	İnhibitions in Anaerobic methane (%) compared to control without pharmaceutical wastewater
5000	100	99	100	100
3500	70	83	85	81
2500	50	54	65	63
1500	40	38	48	43
1000	30	29	45	40
500	0	0	0,2	0,1
EC 50 VALUE(mg/l)	980	798	340	299

Table 3: Algae-Fungi and yeast toxicity test results in raw diluted pharmaceutical wastewater.

Oxytetracycline concentration in raw pharmaceutical wastewater (µg/l)	İnhibitions in Chlorella –algae (%) compared to control without pharmaceutical wastewater	İnhibitions in *Penicllium sp.fungi* (%) compared to control without pharmaceutical wastewater	İnhibitions in Candida –yeast (%) compared to control without pharmaceutical wastewater	İnhibitions in *Pseudokirinella* subcapitata (%) compared to control without pharmaceutical wastewater
5000	89	86	98	84
3500	56	58	54	54
2500	34	39	32	48
1500	20	28	30	33
1000	19	20	29	29
500	1,2	0	0	0
EC 50 VALUE(µg/l)	1250	1045	340	1299

The acute toxicity test results with sulphametazasole also exhibited inhibitions to all bacteria. The bacteria types exhibited more toxicity to sulphametazasole than that oxyteracycline with compared the inhibition percentages and more low EC 50 values.

The results of algae, fungi and yeast toxicity test results also showed that pharmaceutical wastewater containing oxytetracycline were acute toxic to the aforementioned organisms. However these type of oranisms exhibited low inhibitions compared to bacteria since their trophic level is high , they are more developed, they are eutrophic and exhibited resistance to the oxytetracycline compared to the bactera which are prokaryots (Table 6).

The biodegradability and bioaccumulation studies performed with sulphametazasole showed that this chemical is sligtly lower biodegradable than that oxytetracycline. Sliglty lower biodegdrabability percentages accompanied with high bioaccumulative properties compared to oxytetracycline (Table 7).

Treatment of pharmaceutical wastewater with RO

Effect of the feed water pressure on the product flow rate in RO: As shown on Figure 1 at a feed water temperature of 21^{0}C and at a salinity of 6000 mg/l the product flow rate increases as the feed pressure increases. The productivity is expressed as a percentage of the nominal value of the unit. The product flow rate increases from 100 to 1100 m 3 /day as the feed pressure increased from from 10 bar to 70 bar respectively. The relationship is almost linear between the feed pressure and the product flow rate. The increase of product flow rate is expected with increasing feed pressure. In orher words, as the pressure was increased the RO membrane pushes more water through the pore.

Effect of water pressure on the products oxytetracycline and sulphatametazole concentrations and their rejection versus time in RO: As the feed water pressure was increased the oxytetracycline and the sulphametazasole coconcentrations decreased versus operation time in RO (Table 8). As the feed

Table 4: Biodegradability and Bioaccumulation test results.

Oxytetracycline concentration in raw pharmaceutical wastewater (μg/l)	Biodegradability percentages (%) after 28 days incubations	Bioaccumulation BCF factor after 28 days incubations
5000	9	9,56
3500	23	7,34
2500	45	4,89
1500	59	2,78
1000	61	1,56
500	86	0,05

Table 5: Bacterial toxicity test results in raw diluted pharmaceutical wastewater for sulphametazasole.

Sulphametazasole concentration in raw pharmaceutical wastewater (μg/l)	İnhibitions in *E.coli* (%) compared to control without pharmaceutical wastewater	İnhibitions in *B.subtilis* (%) compared to control without pharmaceutical wastewater	İnhibitions in *Vibrio fisheri* in Microthox test (%) compared to control without pharmaceutical wastewater	İnhibitions in Anaerobic methane (%) compared to control without pharmaceutical wastewater
9000	99	97	100	100
5000	68	80	86	89
2500	45	50	69	68
1500	39	38	56	43
1000	30	29	49	40
700	0	0	0,2	0,1
EC 50 VALUE(μg/l)	645	698	240	234

Table 6: Algae-Fungi and yeast toxicity test results in raw diluted pharmaceutical wastewater.

Sulphametazasole concentration in raw pharmaceutical wastewater (μg/l)	İnhibitions in Chlorella -algae(%) compared to control without pharmaceutical wastewater	İnhibitions in Penicllium sp.fungi (%) compared to control without pharmaceutical wastewater	İnhibitions in Candida –yeast (%) compared to control without pharmaceutical wastewater	İnhibitions in Pseudokirinella subcapitata (%) compared to control without pharmaceutical wastewater
9000	89	86	98	84
5000	56	58	54	54
2500	34	39	32	48
1500	20	28	30	33
1000	19	20	29	29
700	1,2	0	0	0
EC 50 VALUE(μg/l)	1250	1045	340	1299

Table 7: Biodegradability and Bioaccumulation test results with Sulphametazasole.

Sulphametazasole concentration in raw pharmaceutical wastewater (μg/l)	Biodegradability percentages(%) after 28 days incubations	Bioaccumulation BCF factor after 28 days incubations
9000	6	12,56
5000	20	10,34
2500	40	6,89
1500	50	4,50
1000	60	1,89
700	86	0,05

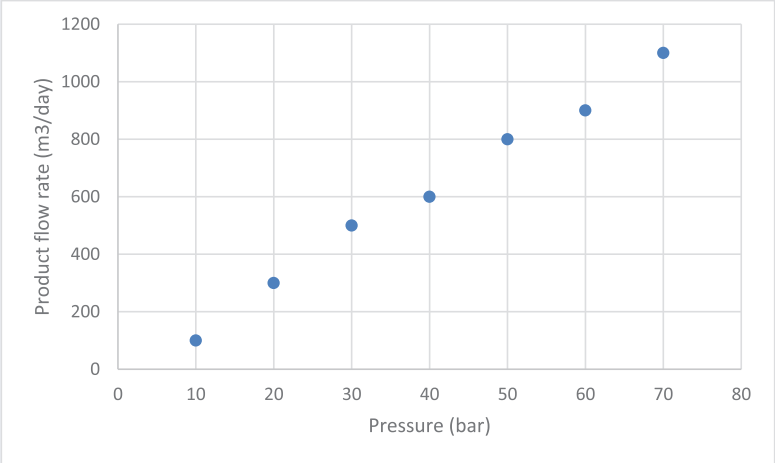

Figure 1 Effect of the feed water pressure on the product flow rate containing oxytetracycline and sulphatametazole in RO membrane reactor.

pressure was at high concentrations the oxytetracycline concentration decreased quikly from 500 μg/l to 40 μg/l after 10 minutes RO operation. In other words the majority of the oxytetracycline (460 μg/l oxytetracycline; 80% of the initial oxytetracycline), removed witheen 10 minutes. The decrease in oxytetracycline and sulphametazasole concentrations after 30 min RO operation were fund to slow. After 50 minutes of operation the oxytetracyline aconcentration decresaed to 0,05 mg/l in the permeate of RO. The decrease in concentrations for both pollutants are rapid when the feed pressure decreases down to 60-70 bars. However, At larger feed pressures (60-70 bar), the decrease in the product is much slower. Sulphametazasol and Oxytetracycline rejections increases with feed water pressure up to an upper limit in their rejection curves.

It was found that RO membranes are imperfect barriers to oxytetracycline ana sulphatametazol in feed water. Increasing feed water pressure sligtly increases the passage of two pollutants, but water is pushed through the membrane at a faster rate thanthe forementioned two chemicals can be transported.

As the feed water pressure was increased the oxytetracycline and the sulphametazasole coconcentrations decreased versus operation time in RO. As the feed pressure was at high concentrations the oxytetracycline concentration decreased quikly from 500 μg/l to 40μg/l after 10 minutes RO operation. In other words the majority of the oxytetracycline (460 μg/l oxytetracycline; 80% of the initial oxytetracycline) removed witheen 10 minutes. The decrease in oxytetracycline and sulphametazasole concentrations after 30 min RO operation were fund to slow. After 50 minutes of operation the oxytetracyline aconcentration decreaed to 0.05 mg/l in the permeate of RO. The decrease in concentrations for both pollutants are rapid when the feed pressure decreases down to 60-70 bars. However, at larger feed pressures (60-70 bar), the decrease in the product is much slower. Sulphametazasol ana Oxytetracycline rejections increases with feed water pressure up to an upper limit in their rejection curves.

It was found that RO membranes are imperfect barriers to oxytetracycline ana sulphatametazol in feed water. Increasing feed water pressure sligtly increases the passage of two pollutants , but water is pushed through the membrane at a faster rate than the forementioned two chemicals can be transported.

Effect of pressure and flux on the solute permeability coefficients and recoveries of oxytetracycline and sulphametazasole: Table 9 depicted the effects of pressure and flux on the permeability of sulphametazasole and oxytetracycline. The pressures in RO was increased from 20 bar up to 80 bar RO unit. The effect of water flux on recoveries and on permeability coefficients of both pollutants versus applied pressure was determined. As the water flux and water pressure was increased the the water permeability coefficients (***Aw***) and solute rejection efficiencies (R) of sulphametazol and oxytetracycline increased up to a pressure of 60 bar and a water flux of 60 L/m2/h,

Table 8: Effect of water pressure on the products oxytetracycline and sulphatametazole concentrations and their rejection versus time.

Feed water pressure (bar)	Time(min)	product oxytetracycline concentration in the permeate of RO (μg/l)	product sulphametazasole concentration in the permeate of RO (μg/l)	Oxytetracycline rejection percentage (%)	sulphametazasole rejection percentage (%)
30	5	500	789	59	69
40	10	40	100	89	79
50	30	30	40	90	87
60	40	20	10	99	99,90
70	50	0,05	0,05	99,99	99,99
80	60	0,05	0,05	99,99	99,99
90	70	0,05	0,05	99,99	99,99
100	80	0,05	0,05	99,99	99,99
110	100	0,05	0,05	99,99	99,99
120	120	0,05	0,05	99,99	99,99

Table 9: Effect of pressure and flux on the solute permeability coefficients and recoveries of oxytetracycline and sulphametazasole.

Pressure (Bar)	Water flux (L / m2.h)	Recoveries of sulphametazasole	Recoveries of oxytetracycline.	Sulphametazasole permeability	Oxytetracycline permeability
20	40	87	88	30	28
30	50	89	89	50	46
40	55	90	90	60	58
50	60	99	99	80	80
60	70	99,99	99,99	97	96
70	90	99,99	99,99	97	96

respectively, and remained constant. Further increae of pressure and flux did not change the recoveries and permeabilities of both pollutants. As s result, it can be concluded that the solute permeability coefficients of pollutants varied with pressure and correlated with water flux. This implies that solute the sulphametazol and oxytetracycline permeabilites ($\boldsymbol{Bs}$) in solute is governed by the solvent flux ($\boldsymbol{Jw}$).

Effect of increasing temperature on permeate flux and sulphamerazin and oxytetracycline rejections: As plotted in Figure 2, the feed temperature had opposite effects on permeate flux and rejection . The permeate flux increased linearly with temperature, while the rejection was not declined slightly and remained as in low temperature at high temperatures. The rising trend of permeate flux is similar to previous studies, with a 60% increase in the permeate flux when the feed temperature increased from 18 to 50°C in the study performed by Xie et al. (2012) [37]. The high temperature increased the pore size of the membrane because of thermal expansion, allowing more water to pass through surface pore of the RO membrane. With the increment of temperature, the solubility of the solute also was not increased, and a higher diffusion rate of solute through the membrane is possible were not detected. This phenomenon did not cause to decrease of the rejection at high temperatures contrarily to the studies performed by Kosutic et al. [38]. According to the data, the optimal temperature for optiumum RO membrane continous operation was found to be as 45°C. At all temperatures maximum rejections (99%) were detected for both pollutants.

Effect of pH on THE RO membrane reactor performance: Figure 3 exhibited the effect of feed pH on membrane performance. The results showed that the effect of pH on the permeate flux and rejection percentages of sulphamerazine and oxytetracycline were not significant. The variation of pH from 3 up to 14 did not change the permeate flux and the rejections ercentages of both pollutants. The rejection percentages remainedaround 99% . However, the variation trend of permeate flux and rejection is similar to the study performed by Koyuncu et al. [39]. The optimum operating condition of pH was as pH= 6,0 since the pH of the pharmaceutical wastewater was sligtly acidic. As a result, no pH adjustment should be made. This cause decreasing of the tratment cost of the RO operation. Under this pH conditions the maximum value of permeate flux and rejections were 98 L/m2.h and 98%,respectively. According to the experimental results above, the optimal RO operating conditions for high removals of sulphamerazin and oxytetracyclin (99%) were; an operating pressure of 50 bar, at a feed temperature of 21°C at an original pharmaceutical pH of 6.00 and at a water flux of 50 L/m2.h The permeate flux and rejection results also proved that the RO membrane was capable to treat the pharmaceutical wastewater containing toxic pollutants such as oxytetracycline and sulpamerazine.

Toxicity studies after RO treatment

Tables 10, 11 and 12 exhibit the toxicity test results performed after RO treatment in the permeate for oxytetracycline at all studied organisms. The results showed that the toxicity

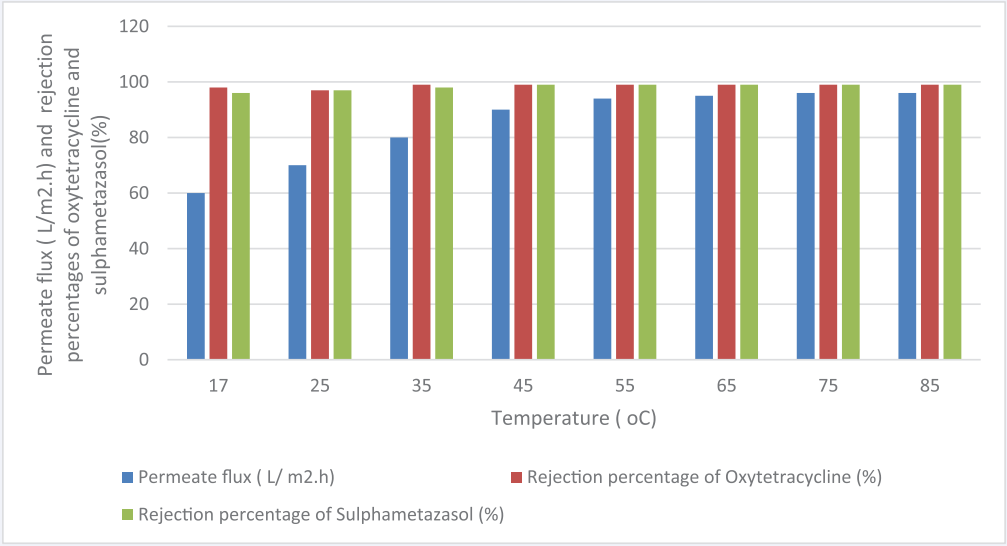

Figure 2 Effect of increasing temperature on permeate flux and sulphamerazın and oxytetracycline rejections.

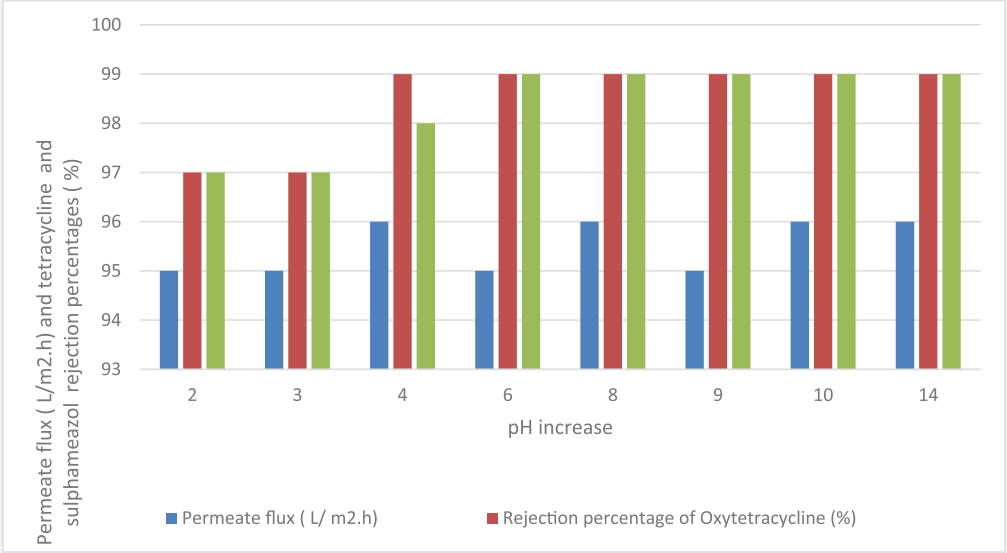

Figure 3 Effect of pH on THE RO membrane reactor performance.

originated from oxytetracycline removed completely in the RO treatment. No toxicity was found in the permeate samples of the RO after treatment.

Tables 13, 14 and 15 exhibit the toxicity test results performed after RO treatment in the permeate for sulphametazasole at all toxicity tests. Similar to the oxytetracycline, the toxicity originated from sulphametazasol removed completely in the RO treatment. No toxicity was found in the permeate samples of the RO after treatment.

CONCLUSIONS

In this work, the oxytetraycline and sulphametazasole antibiotics in a pharmaceutical wastewater was effectively removed with a reverse osmosis membrane process. The optimization experiments on operating parameters proved that the RO membrane was capable of wastewater treatment. The product flow rate increased with increasing feed pressure. Sulphametazasol and Oxytetracycline rejections increases with feed water pressure up to an upper limit. Increasing feed water pressure sligtly increases the passage of Sulphametazasol and Oxytetracycline pollutants, but water is pushed through the RO membrane at a faster rate than the these two antibiotics transported. As the water flux and water pressure was increased the water permeability coefficients and solute rejection efficiencies of increased up to a pressure of both antibiotics 80 bar and a water flux of 60 L/m2/h, respectively. Further increae of pressure did not change the recoveries ana permeabilities of both antibiotics.

The optimal conditions for RO membrane operation were as folows: Feed flow rate is 35L/·m2/h; operating pressure is 60 bar;

Table 10: Bacterial toxicity test results in the permeate of pharmaceutical wastewater for oxytetracycline.

Oxytetracycline concentration in the permeate of pharmaceutical wastewater (μg/l)	İnhibitions *in E.coli* (%) compared to control without pharmaceutical wastewater	İnhibitions in *B.subtilis* (%) compared to control without pharmaceutical wastewater	İnhibitions in *Vibrio* fisheri in Microthox test (%) compared to control without pharmaceutical wastewater	İnhibitions in Anaerobic methane (%) compared to control without pharmaceutical wastewater
500	1	2	1	1
200	0	0	0	0
1	0	0	0	0
EC 50 VALUE(μg/l)	Not detected	Not detected	Not detected	Not detected

Table 11: Algae-Fungi and yeast toxicity test results in the permeate of pharmaceutical wastewater for oxytetracycline.

Oxytetracycline concentration in the permeate of pharmaceutical wastewater (μg/l)	İnhibitions in Chlorella –algae (%) compared to control without pharmaceutical wastewater	İnhibitions in Penicllium sp.fungi (%) compared to control without pharmaceutical wastewater	İnhibitions in Candida –yeast (%) compared to control without pharmaceutical wastewater	İnhibitions in Pseudokirinella subcapitata (%) compared to control without pharmaceutical wastewater
500	0	0	0	0
50	0	0	0	0
1	0			
EC 50 VALUE(μg/l)	Not detected	Not detected	Not detected	Not detected

Table 12: Biodegradability and accumulation test results in the permeate of pharmaceutical wastewater for oxytetracycline.

Oxytetracycline concentration in the permeate of pharmaceutical wastewater (μg/l)	Biodegradability percentages (%) after 28 days incubations	Bioaccumulation BCF factor after 28 days incubations
500	86	0,05
50	98	0,02
1	99	0,01

Table 13: Bacterial toxicity test results in the permeate of pharmaceutical wastewater for Sulphametazasole.

Sulphametazasole concentration in the permeate of pharmaceutical wastewater (μg/l)	İnhibitions in E.coli (%) compared to control without pharmaceutical wastewater	İnhibitions in B.subtilis (%) compared to control without pharmaceutical wastewater	İnhibitions in *Vibrio* fisheri in Microthox test (%) compared to control without pharmaceutical wastewater	İnhibitions in Anaerobic methane (%) compared to control without pharmaceutical wastewater
700	2	2	3	3
300	0	0	0	0
1	0	0	0	0
EC 50 VALUE(μg/l)	Not detected	Not detected	Not detected	Not detected

Table 14: Algae-Fungi and yeast toxicity test results in the permeate of pharmaceutical wastewater for Sulphametazasole.

Sulphametazasole concentration in the permeate of pharmaceutical wastewater (μg/l)	İnhibitions in Chlorella –algae (%) compared to control without pharmaceutical wastewater	İnhibitions in Penicllium sp.fungi (%) compared to control without pharmaceutical wastewater	İnhibitions in *Candida* –yeast (%) compared to control without pharmaceutical wastewater	İnhibitions in Pseudokirinella subcapitata (%) compared to control without pharmaceutical wastewater
700	1	1	1	1
300	0	0	0	0
1	0	0	0	0
EC 50 VALUE(μg/l)	Not detected	Not detected	Not detected	Not detected

Table 15: Biodegradability and accumulation test results in in the permeate of pharmaceutical wastewater for Sulphametazasole.

Sulphametazasole concentration in the permeate of pharmaceutical wastewater (μg/l)	Biodegradability percentages (%) after 28 days incubations	Bioaccumulation BCF factor after 28 days incubations
700	86	0,05
300	98	0,02
1	99	0,01

The permeate flux recovered 89-99% of the reduced value within 10 min of cleaning. High treatment effects were maintained as the rejection values were high (95.00–97.00%). Although the pharmaceutical wastewater was found to be toxic no toxicity was detected after RO treatment in the permeate of RO. This study indicated that the RO membrane treatment system is a capable wastewater treatment process at a laboratory scale.

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