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Carbamazepine and oxcarbazepine removal in pharmaceutical wastewater treatment plant using a mass balance approach: A case study

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Abstract−The manufacturing of the antiepileptics, carbamazepine (CBZ) and oxcarbazepine (oxCBZ), results in generation of wastewater containing these micropollutants which exhibit toxicity even at trace levels. Therefore, we focused on monitoring their fate and removal in various units of a full-scale wastewater treatment plant (WWTP) using mass balance approach. An apparent CBZ removal of 50±3% was observed by conventional activated sludge process in the biological treatment unit, whereas oxCBZ still persisted after the biological treatment and showed negative mass balance. However, reverse osmosis resulted in 91% oxCBZ removal, whereas CBZ still continued to persist as a result of lower solubility of CBZ as compared to oxCBZ. Only 3% CBZ exhibited sorption onto the suspended solids and sludge, which was negligible for oxCBZ, thus demonstrating their tendency to remain in aqueous phase. Additionally, we attempted to understand the fundamental mechanism behind the removal of these pharmaceuticals and it was apparently the collective effect of sorption, mineralization, biotransformation, biodegradation, phototransformation/photodegradation, etc. Thus, the integrative data presented in the present study on productivity of these pharmaceuticals, their mass loading in influent and effluents allied with their removal efficiency will be significantly constructive in benchmarking the operational effectiveness through operational optimization and design improvement of the current conventional treatment plant.

Keywords: Carbamazepine, Effluent Treatment Plant, Mass Balance, OxcarBazepine, Removal Efficiency, Sorption

INTRODUCTION

Carbamazepine (CBZ), 5H-dibenzo[b,f] azepine-5-carboxamide (Supp. Fig. S1(a)) and its structural derivative oxcarbazepine (oxCBZ), 10,11-dihydro-10-oxo-5H-dibenzo[b,f] azepine-5-carboxamide (Supp. Fig. S1(b)) are well-known antiepileptic psychiatric drugs used worldwide for treatment of mood related disorders and have broad pharmaceutical applications. These are pharmaceutical and personal care products (PPCP's) which are categorized as endocrine disrupting pollutants and are toxic even in trace amounts [1]. The bulk manufacturing of these drugs, owing to their increased consumption and their perplexing degradation nature, have led to their increased occurrence in industrial, hospital and municipal wastewaters, surface and drinking water streams of several countries [2]. Furthermore, these compounds easily pass through the conventional wastewater treatment plants (WWTP), and enter the environment through discharge of effluents or biosolids, thus contaminating both surface and groundwater and contributing to environmental pollution which affects the local ecosystem [3,4]. These circumstances have forced the taking of preventive measures for their production, release and degradation.

Most WWTPs employ conventional activated sludge processes owing to their moderate operating and maintenance costs. These processes either mineralize these micropollutants or degrade them to suitable/tolerable form [5]. Besides the activated sludge process, granulated activated carbon columns, membrane bioreactors and sequential batch reactors have also been employed for the removal of these micropollutants to some extent [6,7]. Nonetheless, the investigation into the removal efficiency of CBZ by WWTPs found that CBZ removal efficiency is less than 10%, which indicates its persistence due to recalcitrant nature [8]. However, most of the previous studies on CBZ removal have been conducted mostly in sewage treatment plants which consist of treated effluents from various plants and other sources such as pharmaceutical run-off via human excretions [9-12]. On the contrary, the pharmaceutical manufacturing and formulation facilities generate huge amount of wastewater during different stages of CBZ and oxCBZ manufacturing process, which is comprised of these wash out pharmaceuticals in significant concentrations. These pharmaceutical manufacturing facilities still remain an under-investigated source and contribute >20% of the pharmaceutical WWTP flow [13]. An effective treatment for the removal of these micropollutants at the point source itself will aid in reducing the economic and ecological load on subsequent treatment plants and prevent deleterious effects on the environment. Additionally, the rigorous regulation regime regarding the industrial discharge compels meticulous monitoring of the effluent discharge and overall removal efficiency of the pharmaceutical active constituents from the effluent [14]. However, there are limited studies to the best of our knowledge, pertaining to the on-site monitoring

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of the fate of these micropollutants in the pharmaceutical manufacturing industry, which is the major point source for dissemination of contamination and pollution of these pharmaceuticals.

Therefore, in the present case study, the fate and removal of CBZ and oxCBZ was examined from an industry manufacturing these pharmaceuticals using a mass balance approach in the biological treatment plant (BTP) unit of an industrial WWTP. The mass balance approach used in the present study along with the insights into removal mechanism might aid in standardizing and/or validating the operational design and optimization for benchmarking the operational effectiveness of WWTP. Furthermore, the recommendations furnished pertaining to the enhancement of removal efficiency will aid in combating the release of these micropollutants in the ecosystem.

MATERIALS AND METHODS

1. Chemicals and Reagents

CBZ and oxCBZ (≥99% pure) from Sigma Aldrich (U.S.A.) were used for HPLC standard and calibration analysis. Acetonitrile, methanol, isopropanol, KH_2PO_4, potassium biphthalate, etc. were of HPLC grade and were procured from Fischer Scientific, Mumbai, India. Standards and the buffer solutions were prepared by dissolving the compounds in deionized water (18 MΩ cm, Millipore, USA). All the other reagents were of analytical grade.

2. WWTP Description and Sampling

The selected WWTP is located in India and is being operated by a pharmaceutical industry manufacturing a wide range of drugs. The aspects of water consumption and wastewater generation for manufacturing of these drugs are detailed in Table 1. The selected WWTP is designed for mean flow capacity of 260 m^3/day and has a handling flow of 240 m^3/day, which is 92% of its design capacity. The WWTP is comprised of two-stage multi-effect evaporator (MEE), a biological treatment plant (BTP) unit (influent 240 m^3/day),

Table 1. Water consumption and wastewater generation during CBZ and oxCBZ production process

Drug	Production capacity (kg/day)	Water consumption for washing (m^3/day)	Total wastewater generation (m^3/day)
CBZ	930±9.37	30.81±3.0	52.32±4.5
oxCBZ	350±2.71	58.34±5.0	66.82±6.1

multimedia filtration unit (MMF) and a reverse osmosis (RO) system for tertiary treatment of wastewater. RO reject has a flow of 40 m^3/day while RO permeate is having a flow of 190 m^3/day, respectively. The schematic representation of the full-scale WWTP is depicted in Fig. 1 while the operating parameters of the BTP unit are presented in Table 2.

The wastewater entering the WWTP was comprised of effluents from production plants and wash out from pilot plants. The effluents were first subjected to scum removal followed by neutralization in an effluent neutralization tank, and coagulation in reactivator clarifiers. This was followed by a two-stage MEE treatment to recover the solvents and remove TDS and major compounds. The MEE concentrate was subjected to agitated thin film dryer (ATFD) and the condensate resulting from ATFD was fed to MEE condensate stream, while the dried cakes were incinerated. The MEE condensate was further subjected to biological treatment and entered the BTP, which consists of the aeration tanks (AT-1 and AT-2) entailing an activated sludge system and clarifiers (Clarifier-1 and Clarifier-2). The AT-1 was supplemented with viable microbial consortia through wet seeding. A detailed schematic of BTP unit is presented in Fig. 2. Some part of the waste activated sludge (WAS) was returned back to AT-2 and is indicated as return activated sludge (RAS) in Fig. 2. The effluent from BTP was further subjected to MMF and subsequently entered the RO system for tertiary treatment to generate clean water which was then used either in cool-

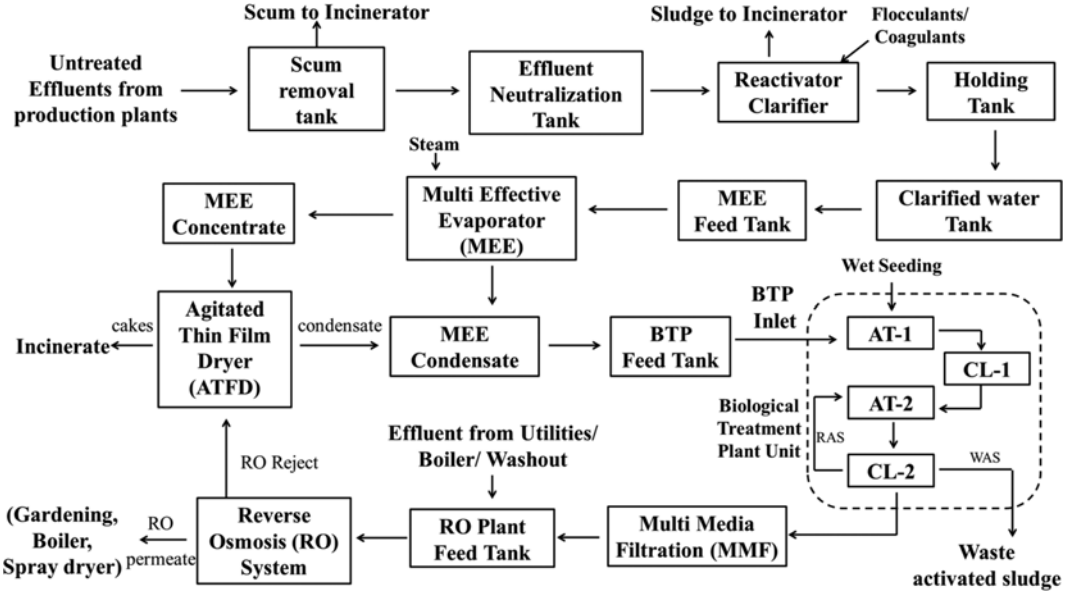

Fig. 1. Design of full scale WWTP.

Table 2. Parameters evaluated and measured during the activated sludge process

Treatment units	Flow (m^3/day)		HRT (days)		pH	TDS (mg/L)		COD (mg/L)		TOC (mg/L)		MLSS (mg/L)		CBZ (mg/L)		oxCBZ (mg/L)		CBZ adsorbed (g/kg dry sludge)	
	Mean ±S.D	RSD (%)	Mean ±S.D	RSD (%)	Range	Mean ±S.D	RSD (%)	Mean ±S.D	RSD (%)	Mean ±S.D	RSD (%)	Mean ±S.D	RSD (%)	Mean ±S.D	RSD (%)	Mean ±S.D	RSD (%)	Mean ±S.D	RSD (%)
BTP inlet	240± 12.75	5.31	-	-	8.1-8.4	1720± 206.52	12.01	5328± 1714.5	32.18	1837± 389.16	21.19	36± 12.8	35.57	2.21± 0.1	4.59	5.54± 0.19	3.48	-	-
Aeration Tank-1	240± 12.02	5.01	3.54± 0.18	5.02	8.1-8.7	1851.6± 163.57	8.83	3128± 279.59	8.94	NA	NA	687± 271.86	39.57	1.7± 0.14	8.33	5.12± 0.13	2.46	0.059± 0.036	60.8
Clarifier-1	239.6± 14.55	6.07	0.16± 0.01	6.3	8.2-8.4	2168.2± 118.62	5.47	2368± 377.78	15.95	418.4± 52.31	12.50	150.4± 56.69	37.69	1.69± 0.14	8.23	5.07± 0.11	2.27	-	-
Aeration Tank-2	239.6± 4.67	1.95	1.46± 0.03	1.96	8.4-8.9	1795.6± 202.18	11.26	2332± 150.57	6.46	NA	NA	813± 244.41	30.06	1.21± 0.08	6.59	7.38± 0.18	2.46	0.044± 0.013	29.3
Clarifier-2	229.8± 7.82	3.40	0.17± 0.01	3.4	8.4-8.8	2031.2± 105.13	5.18	2240± 753.45	33.64	510± 33.26	6.52	187± 90.77	48.54	1.15± 0.08	7.25	7.17± 0.128	1.79	-	-
MMF outlet	230.4± 11.54	5.01	-	-	8.6-8.7	1808± 171.81	9.50	2096± 521.54	24.88	468.3± 27.66	5.90	160± 18.05	11.28	1.1± 0.16	14.49	6.53± 0.1	1.58	-	-
RO permeate	190.6± 19.13	10.03	-	-	6.8-7.6	161.3± 15.96	9.89	160± 48.48	30.3	NA	NA	14.7± 5.36	36.19	0.07± 0.01	10.15	0.03± 0.01	26.06	-	-

BTP: Biological Treatment Plant, RO: Reverse Osmosis, HRT: Hydraulic Retention Time, TDS: Total Dissolved Solids, COD: Chemical Oxygen Demand, TOC: Total Organic Carbon, MLSS: Mixed Liquor Suspended Solids, CBZ: Carbamazepine, oxCBZ: Oxcarbazepine

S.D: Standard Deviation, RSD: Relative Standard Deviation

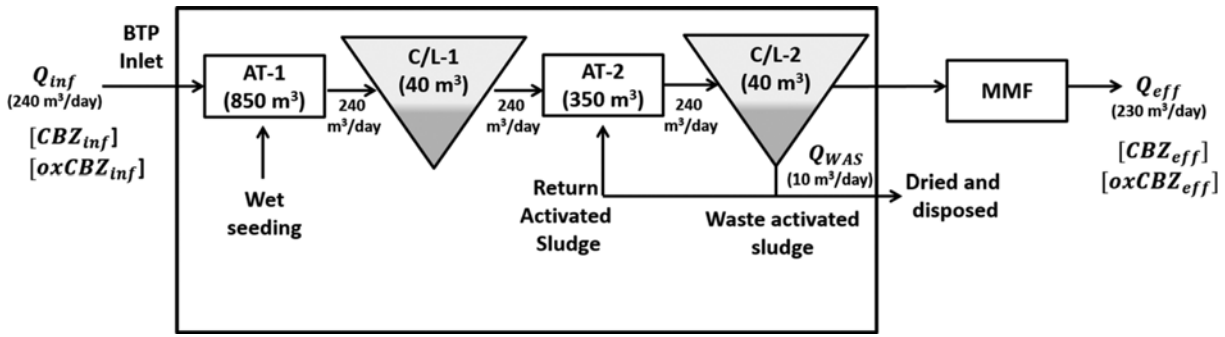

Fig. 2. Schematic of BTP unit indicating flow balance.

ing towers or for gardening purposes, among other uses.

The samples were collected daily, in triplicate, by manual grab method from MEE condensate, BTP unit, aeration tanks, clarifiers, MMF unit and RO unit at 3h interval from 8 a.m. to 8 p.m. for 10 consecutive days in April 2015. The grab samples of biosolids were also collected. The sampling was done under dry weather conditions and no rain occurred during the sampling period. The samples were collected in triplicate (1 L each) in sampling bottles from the locations mentioned above along with their inlets and outlets and immediately carried to laboratory, filtered and stored at 4 °C until further analysis which was usually carried out within 48 h. The distribution of sample in each unit of WWTP was examined through MINITAB 16.0 (PA, USA) software and is represented graphically along with the central tendency and degree of variability in the samples.

3. Analytical Methods

The samples collected from different stages of WWTP were analyzed for pH, chemical oxygen demand (COD), total organic carbon (TOC), total dissolved solids (TDS) and mixed liquor suspended solids (MLSS) along with CBZ and oxCBZ concentrations. All the experimental analysis was at CSIR-National Environmental Engineering Research Institute (CSIR-NEERI), Nagpur, India.

The pH of all the samples was measured using a pH meter (Cyberscan Eutech 510, U.S.A.). TOC analysis was performed using Shimadzu TOC-L instrument equipped with an ASI-V autosampler. CBZ and oxCBZ were analyzed by HPLC (High performance liquid chromatograph, Waters) equipped with a photodiode array (PDA) detector (Waters 2998) at λ=285 nm for CBZ and λ=256 nm for oxCBZ, using a high strength silica C-18 column (4.6 mm× 250 mm, 5 μm, Waters, U.S.A.). The eluent mixture for CBZ consisted of methanol:water (70 : 30) and 0.1% formic acid and was used at a flow rate of 1.5 mL/min. The mobile phase for oxCBZ was comprised of isopropanol : acetonitrile : water in ratio 3 : 12 : 85 along with 1.2 g/L KH_2PO_4 for buffering. The column was maintained at 25 °C throughout the analysis and injection volume was 20 μL. The limit of detection (LOD) for CBZ and oxCBZ was 0.004 mg/L and 0.005 mg/L, while the limit of quantification (LOQ) for CBZ and oxCBZ was 0.013 mg/L and 0.016 mg/L, respectively, for the method developed for quantification according to the ICH guidelines.

For quality assurance of concentration measurements, a calibration curve was prepared prior to the analysis by using standard solutions of CBZ and oxCBZ. The standard solutions were prepared by dissolving accurately weighed CBZ and oxCBZ standards (Sigma Aldrich, USA) in analytical grade methanol in separate volumetric flasks to obtain a solution having known concentration. The desired range of standard concentrations was prepared by diluting the stock solution using pure methanol. The calibration curve obtained through HPLC quantification (Supp. Fig. S2(a), S2(b)) was used to estimate the concentration in test solution and other samples collected during the present study.

Further, a test solution of CBZ and oxCBZ was prepared by accurately weighing these compounds using an ISO certified weighing balance in volumetric flasks and diluted as per the desired concentration of the test solution The test solution with X concentration was spiked to 10X with known standard, while on the other hand the X concentration was also diluted to 0.1X concentration. All these test solutions (0.1X, X and 10X) were analyzed using HPLC, and the responses were measured by recording the major peaks and their corresponding peak area. The test solutions were prepared in triplicate and the % recovery was calculated based on the average values of each test solution. The % recovery obtained is given hereunder:

CBZ: 83.55±0.78% (0.1X), 81.01±0.2% (1X) and 99.31±0.26% (10X)

oxCBZ: 91.56±1.46% (0.1X), 102.06±2.18% (1X) and 100.08± 0.16% (10X)

4. Mass Balance Evaluation

Mass balance approach was used to evaluate the occurrence, distribution and fate of CBZ and oxCBZ in the aqueous and suspended solids of WWTP. However, the mass balancing of any treatment plant is challenging in terms of representative sample collection and accurate quantification. In addition, it requires meticulous observations of the operating parameters along with the details of the plant design. To accomplish this, the samples were collected as indicated in previous section and analyzed for CBZ and oxCBZ concentrations, COD, MLSS etc., at different stages of the treatment process since these represent the main conversions in a WWTP. These balances were made for the individual unit operations of the WWTP. However, only measurable flows were calculated in order to close the balance by direct measurement.

A simple mass balance scheme is shown in the following equations

Overall flow balance

$$Q_{Inf} - Q_{eff} - Q_{WAS} = 0 \quad (1)$$

where Q_{inf} is influent flow, Q_{eff} is effluent flow and Q_{WAS} is flow (m^3/day) of waste activated sludge.

Similarly, a mass balance was also carried out to examine the fate of CBZ and oxCBZ on the basis of mass load in the influent and effluent along with the suspended solids and biosolids. The total mass loading of CBZ and oxCBZ in the influent was calculated by multiplying the respective influent concentration with the corresponding flow rate at the sampling time.

For aqueous phase,

$$M_{CBZ,\,aq} = C_{CBZ,\,aq} * Q \quad (2)$$

$$M_{oxCBZ,\,aq} = C_{oxCBZ,\,aq} * Q \quad (3)$$

where $M_{CBZ,\,aq}$ and $M_{oxCBZ,\,aq}$ are the daily input or output of CBZ and oxCBZ or mass loading in the aqueous phase, mg/day; $C_{CBZ,\,aq}$ and $C_{oxCBZ,\,aq}$ is the concentration of CBZ/oxCBZ (mg/L) in the aqueous phase and Q is the flow rate (L/day).

For suspended solid phase,

$$M_{CBZ,\,aorb} = C_{CBZ,\,sorb} * Q * S \quad (4)$$

where $M_{CBZ,\,sorb}$ and $M_{oxCBZ,\,sorb}$ are the mass of CBZ and oxCBZ sorbed to the suspended solids, respectively, mg/day; $C_{CBZ,\,sorb}$ and $C_{oxCBZ,\,sorb}$ are the concentration of CBZ/oxCBZ (mg sorbed per mg of suspended solids) in the solid phase and Q is the flow rate (L/day) and S is the suspended solids in the corresponding stream (mg/L).

The aqueous phase removal efficiency (φ) was calculated based

on the concentration of the drugs. Thus, mass balance was represented in simple terms as,

$$M_{enter}=M_{exit}+M_{lost} \quad (5)$$

where M_{enter} is the mass of CBZ/oxCBZ entering the WWTP, M_{exit} is the mass of CBZ/oxCBZ exiting the plant while M_{lost} is the mass of CBZ/oxCBZ degraded and/or adsorbed. Eqs. (6) and (7) can be used collectively for the mass balance of complete WWTP. The equation is as given hereunder.

$$M_{CBZ/inf/diss}+M_{CBZ/inf/sorb}=M_{CBZ/eff/diss}+M_{CBZ/eff/sorb}+M_{CBZ/sludge/sorb}+M_{CBZ/lost} \quad (6)$$

$$M_{oxCBZ/inf/diss}+M_{oxCBZ/inf/sorb}=M_{oxCBZ/eff/diss}+M_{oxCBZ/eff/sorb}+M_{oxCBZ/sludge/sorb}+M_{oxCBZ/lost} \quad (7)$$

5. Sludge Retention Time (SRT)

One of the most vital parameters of an activated sludge system is the sludge retention time (SRT), i.e., the residence time of microorganisms, which dictates the process performance and sludge production. SRT thus influences the enrichment of microbiocoenosis which in turn governs the significance and degree of adsorption and biodegradation [15]. The treatment performance was calculated on the basis of chemical oxygen demand (COD) and SRT.

The SRT was calculated by using a classical method of measurement of mixed liquor suspended solids (MLSS) in an activated sludge system [14]. Eqs. (8) and (9) represent the classical SRT calculations in aeration tanks 1 and 2, respectively

$$SRT_{AT1}=\frac{V_{reactor\_AT1}\times MLSS_{reactor\_AT1}}{(Q_{WAS\_AT1}\times MLSS_{WAS\_AT1})+(Q_{eff\_AT1}\times MLSS_{eff\_AT1})} \quad (8)$$

$$SRT_{AT2}=\frac{V_{reactor\_AT2}\times MLSS_{reactor\_AT2}}{(Q_{WAS\_AT2}\times MLSS_{WAS\_AT2})+(Q_{eff\_AT2}\times MLSS_{eff\_AT2})} \quad (9)$$

where, SRT_{AT1} and SRT_{AT2} are the sludge retention times corresponding to AT-1 and AT-2; $V_{reactor\_AT1}$ and $V_{reactor\_AT2}$ represents the volume capacity in tanks AT-1 and AT-2; $MLSS_{reactor\_AT1}$, $MLSS_{reactor\_AT2}$, $MLSS_{WAS\_AT1}$, $MLSS_{WAS\_AT2}$ $MLSS_{eff\_AT1}$ $MLSS_{eff\_AT2}$ are the mixed liquor suspended solids present in AT-1, AT-2, waste activated sludge of AT-1 and AT-2 and effluents of AT-1 and AT-2, respectively; Q_{WAS_AT1} and Q_{WAS_AT2} correspond to the flow rates of waste activated sludge from AT-1 and AT-2, respectively.

6. Mechanism for Removal of CBZ and oxCBZ

Mass balancing approach based on monitoring the fate of a target compound during a WWTP is challenging in terms of the heterogeneous behavior of biomass for different compounds. Biodegradation and adsorption are simultaneous processes occurring during an activated sludge process and difficult to distinguish. The amount of pharmaceutical constituents adsorbed by the suspended solids and waste sludge was determined through probe sonication-assisted extraction. To accomplish this, the pharmaceuticals bound to the solids were subjected to a hybrid extraction process constituting solid-liquid extraction and probe-sonication-assisted extraction. The corresponding samples were centrifuged followed by probe-based sonication in with frequency of 20 kHz. The samples from aeration tanks 1 and 2, were quantified using HPLC for both CBZ and oxCBZ, pre- and post-sonication assisted extraction (Vibra Cell Sonics) [16]. To determine the efficacy of sonication-assisted extraction, a known concentration of CBZ was spiked to the wastewater matrix from aeration tank and was aerated at 37 °C for a contact time of 48 h. This was followed by solid-liquid separation and probe-assisted sonication of the solid fraction suspended in equal volume of methanol. The resulting supernatant was syringe-filtered and quantified for CBZ using HPLC. The % CBZ recovery was obtained to be 70.76±0.87%.

RESULTS AND DISCUSSION

1. Occurrence and Fate of Organics in the WWTP

All the parameters evaluated and measured for the WWTP are presented in Table 2. The values in Table 2 are the mean of all the daily samples collected and analyzed during the sampling period and are denoted along with their standard deviation and relative standard deviation. Further, the degree of sample variability is reflected through the Box and Whisker plots obtained for each parameter and are depicted in Fig. 3(a)-(g). Fig. 3(d) represents the chemical oxygen demand (COD, mg/L) in the different units of the treatment plant. It can be observed that the BTP inlet showed a higher variability in terms of COD and the COD decreased progressively in different units of WWTP. The BTP inlet with a flow rate of 240 m^3/day had an initial COD load of 1,278.7 kg/day. A COD removal of 41.29% was observed in AT-1, while 24.29%

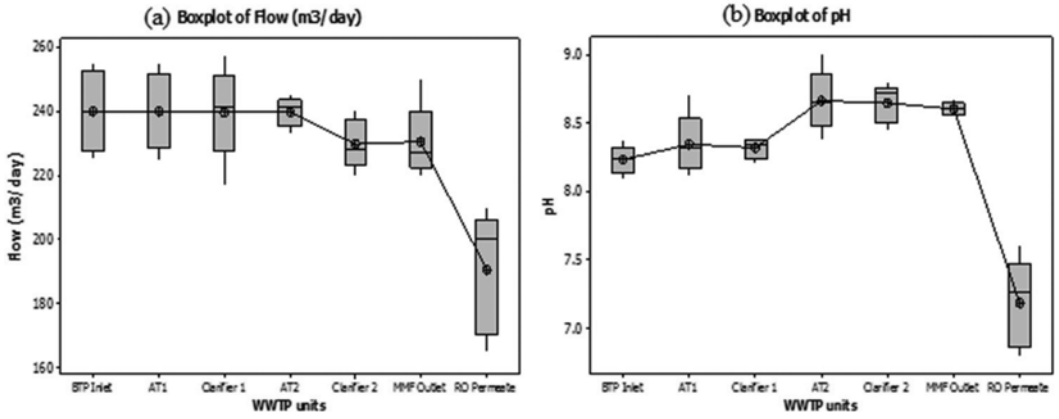

Fig. 3. Box and Whisker plots for (a) Flow, (b) pH, (c) TDS, (d) COD, (e) MLSS, (f) CBZ concentration and (g) oxCBZ concentration.

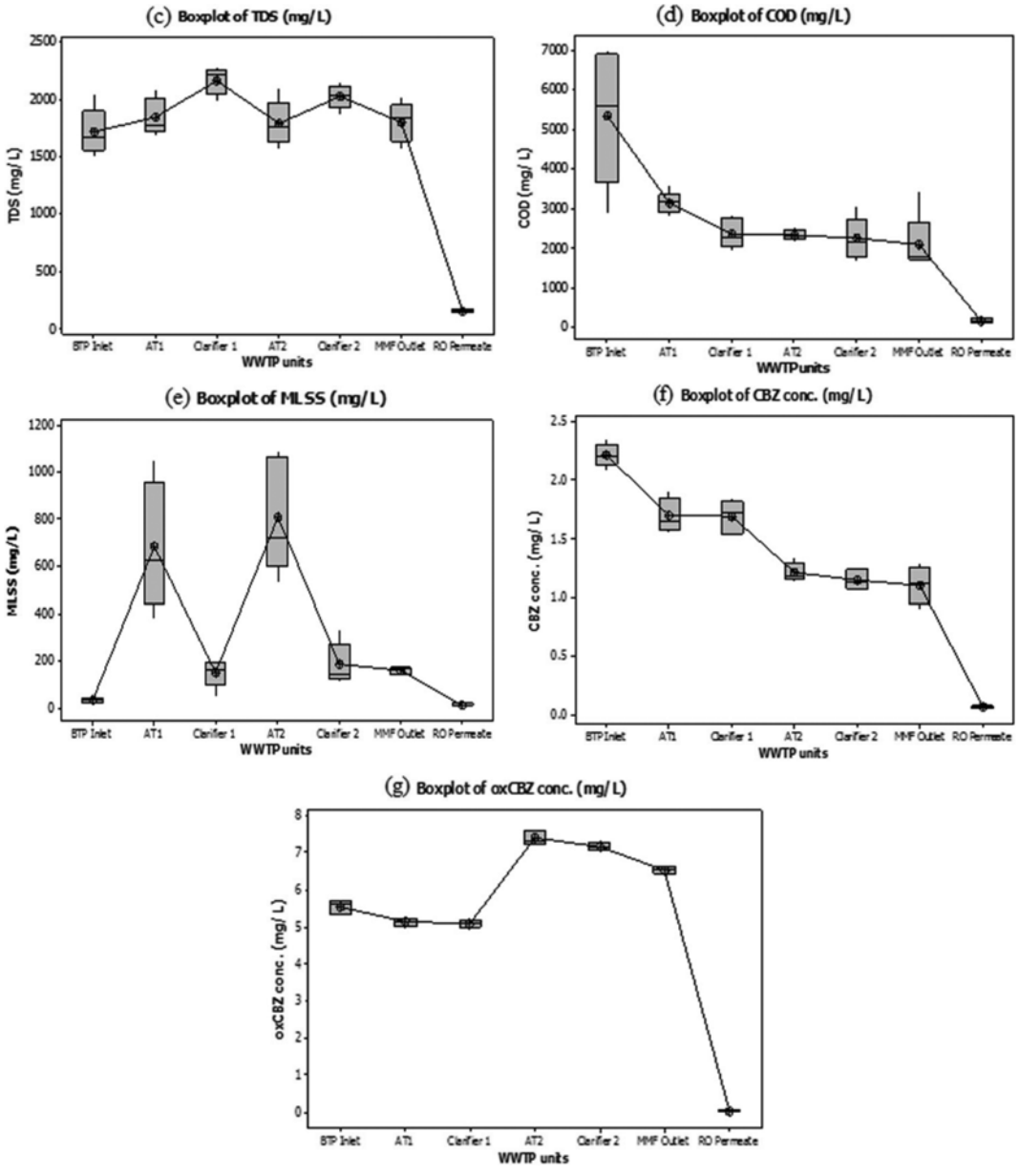

Fig. 3. Continued.

COD removal occurred in clarifier-1. A combined COD removal of 9.3% was observed in AT-2 and clarifier-2, which was comparatively less as compared to AT-1 and clarifier-1. The decrease in COD removal in AT-2 as compared to AT-1 is likely due to the degradation of most of the biodegradable material in AT-1 as a result of which lesser biodegradable material was left for degradation in AT-2. A COD removal of 796.64 kg/day was acquired in MMF outlet based on the initial COD of 1278.7 kg/day, resulting in % COD removal of 62.3% after MMF stage. The MMF was followed by RO which ensued in a total COD removal of 76.69% in the WWTP. COD (kg/day) and %COD removal in each unit of WWTP is depicted graphically in Fig. 4(a). The biological treatment and the successive MMF thus resulted in an effective COD removal, which reflected the removal of organic matter and presumably the removal of organic pharmaceuticals: CBZ and oxCBZ. To confirm this, the concentration of CBZ and oxCBZ was measured at various stages of WWTP in parallel to ascertain the actual aqueous phase drug removal and correspondence of COD removal with CBZ and oxCBZ removal. The temperature of the activated sludge system at any time throughout the monitoring of wastewater treatment plant was between 25-30 °C.

The variability in the concentrations of CBZ and oxCBZ in aqueous phase was monitored in all the units of WWTP, i.e., MEE, BTP, MMF and RO system, for tertiary treatment of wastewater and is depicted in Fig. 3(f) and (g). Generally, a higher degree of variability was observed in CBZ concentration as compared to oxCBZ. However, the daily mass loading of CBZ and oxCBZ did not vary considerably; similarly, a minimum variability was observed for the concentration of these pharmaceuticals in RO permeate. The BTP inlet consisted of 2.22 mg/L CBZ and 5.54 mg/L oxCBZ. The con-

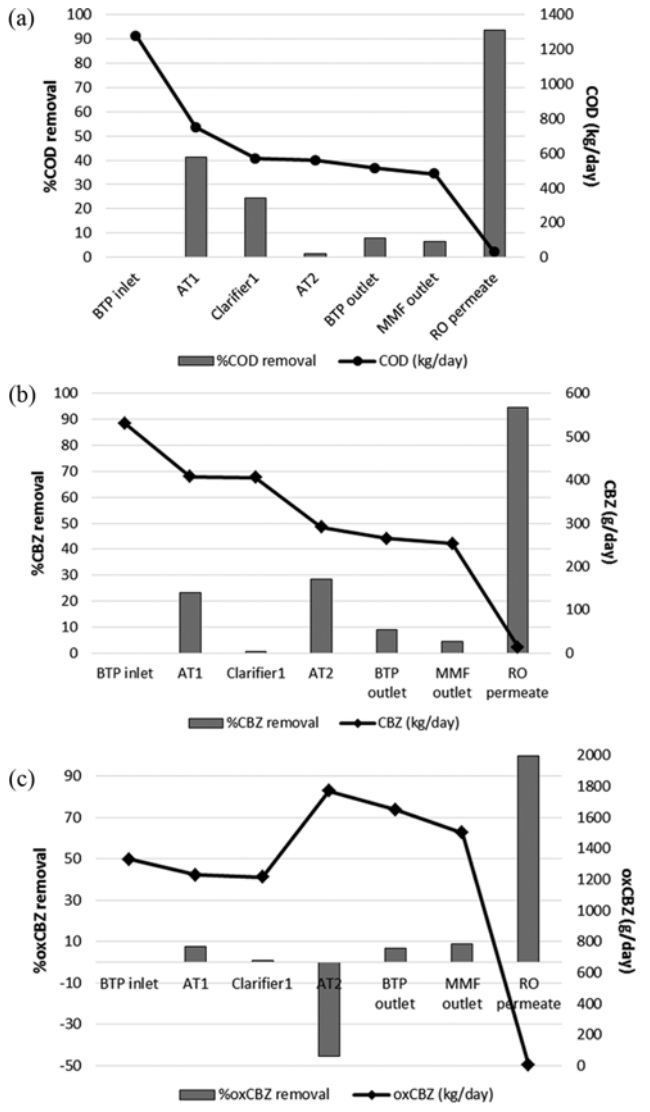

Fig. 4. (a) COD and % COD removal in different units of WWTP; (b) CBZ and % CBZ removal in different units of WWTP; (c) oxCBZ and % oxCBZ removal in different units of WWTP.

centrations of the drugs reported in the present study were higher since the studies were carried out in the WWTP at the manufacturing site itself. The distribution of concentrations of CBZ and oxCBZ in the studied WWTP indicated the persistence of CBZ and oxCBZ in the WWTP. The concentration of CBZ dropped from 2.215 mg/L in the inlet of BTP to 1.104 mg/L in the outlet of MMF (Fig. 3(f)). On the other hand, oxCBZ concentration increased from 5.54 mg/L in the BTP inlet to 6.53 mg/L in the MMF outlet (Fig. 3(g)). This indicated the higher persistence of oxCBZ as compared to CBZ in aqueous phase, which is attributed to the three times higher solubility of oxCBZ compared to CBZ [17]. These results are in agreement with the previous studies reported earlier by other researchers where a negative removal of oxCBZ (−73.2%) was reported in sewage treatment plants (STPs) by Gurke et al., while CBZ removal of 7% and 23.1% was reported by Bernhard et al. and Behera et al. [12,18,19]. The concentration of oxCBZ in AT-2 was higher than that in AT-1 by 44.14% as presented in Table 2, while 23.07% and 28.82% decline in CBZ concentration was observed in the AT-1 and AT-2, respectively (calculated on the basis of CBZ concentration in the preceding units). Further, an overall CBZ removal of 50.22% was observed in the aqueous phase after the MMF stage. Most of the studies have indicated the persistence of CBZ with its removal efficiency of below 10% [8]. Studies by Miao et al. and Behera et al. have shown CBZ removal of 29% and 23.1% in the aqueous phase. respectively [9,19]. However, similar CBZ removal efficiency to that mentioned in this study has been stated by Paxéus who reported a removal efficiency of 53% in the liquid phase [20].

Contrarily, an increase in the concentration of oxCBZ was observed in AT-2. This may be attributed to the systematic underestimation of the influent load since the desired analyte may enter the WWTP influent in small number of wastewater packets in unpredictable amounts at different time intervals, resulting in daily concentration fluctuations [21]. This can also be corroborated from Fig. 3(a), which illustrates the variability in flow observed during the sampling period. The larger boxes in the Box and Whisker plot indicate significant variability in the flow in each unit of WWTP, although the average flow did not vary considerably from BTP inlet to AT-2. Furthermore, the transformation products formed during the treatment process in the plant may switch back to the parent compound [22]. However, as observed from Fig. 3(g), the mass loading of oxCBZ in AT-2 is higher than the influent. Similar higher mass loads of oxCBZ in effluent as compared to influent have been observed by Gurke et al. [12]. It may be contemplated that oxCBZ might have been present in the conjugated form as glucuronide in the influent stream, which during the course of treatment process was eventually deconjugated to oxCBZ, thereby leading to its enhanced concentration in AT-2 [12]. Nonetheless, this may further be confirmed through LC-MS/MS analysis for the presence of glucuronides which needs further investigation. Therefore, sampling variation as a result of flow deviation, deconjugation and retransformation might have collectively led to increased oxCBZ concentration in the effluent [23].

CBZ and oxCBZ adsorbed onto the suspended solids and WAS were also estimated in order to get a comprehensive estimate of the total removal of pharmaceutical constituents. It was deemed that the expansion and compression cycles caused by the ultrasonic waves while traveling through the sample present in excess water create a negative pressure and cavities/bubbles in the liquid. The further collapse of these bubbles generated shockwaves which aided in releasing the adsorbed pharmaceuticals, which were further quantified by HPLC [16]. 7.73 g CBZ was found to be adsorbed onto the sludge per day. oxCBZ did not adsorb onto the sludge, indicating its tendency to remain in aqueous phase owing to its higher solubility. The tendency of both the pharmaceuticals to remain in the aqueous phase can be attributed to their low water-sludge distribution coefficient (1.2 L/kg), which is far off from that required (500 L/kg) for significant sorption onto the sludge [24]. Moreover, the low potential of these drugs for adsorption onto sludge is also evident from their low log K_{OW} values (<2.5) (log K_{OW} of CBZ is 2.25; log K_{OW} of oxCBZ is 1.11) [9].

SRT strongly influences the establishment of diverse microbiocoenosis in the activated sludge processes which subsequently play

a significant role in mineralization and transformation of micropollutants. SRT of AT-1 and AT-2 was calculated from Eqs. (8) and (9) and was found to be 3.54 and 1.46 days, respectively. Although it has been reported that higher SRTs result in higher removal efficiency of pharmaceutical constituents due to the enrichment of slow growing bacteria and establishment of diverse biocoenosis, this is not applicable to CBZ and oxCBZ removal efficiency, which is independent of the SRT as reported from the studies of Bernhard et al. [18]. The possible explanation for the same can be attributed to the complex aromatic structure of CBZ and oxCBZ (Suppl. Fig. 1(a), (b)), which is stabilized by resonance, thereby rendering the microorganisms to utilize them as carbon source and hence, SRT did not affect the biodegradation and sorption of drugs onto the sludge either. This is also evident from the studies carried out in the SRT ranging from 3-275 days [8,21,25].

2. Mechanism of Removal

The removal of an analyte refers to the conversion of the parent compound into intermediate compounds, i.e., other than the analyte. This conversion or loss takes into account losses occurring through diverse mechanisms such as biodegradation, air stripping, biotransformation, mineralization, sorption and phototransformation. It is reported that a Henry coefficient of 3×10^{-3} Pa·m^3/mol is required to strip in a fine bubble column. However, a Henry coefficient of 1.09×10^{-5} Pa·m^3/mol for CBZ limits the use of air stripping for CBZ removal [26]. The water-sludge distribution coefficient is lower as compared to the required distribution coefficient for sorption onto sludge as discussed earlier. Thus, it can be seen that sorption of these micropollutants is less (as also evident from the present study) and the drugs tend to remain associated with the aqueous phase. This was further corroborated from the degree of sorption of pharmaceuticals as indicated in section 3.1. Another important mechanism of drug removal from WWTP is phototransformation and biotransformation. Phototransformation usually occurs since the WWTP operates in an open environment which is exposed to sunlight. Although the sunlight is blocked due to the turbidity of wastewater, water in the upper layer and clarifier comes in contact with the sunlight, which may result in phototransformation of CBZ. CBZ has a relatively low rate of photolysis when irradiated with sunlight. Although many studies are directed towards CBZ photodegradation, yet there is a lack of information on its photochemical nature and fate during phototransformation in WWTPs. It is, however, reported that the absorption of sunlight by humic substances in wastewater allow the formation of singlet oxygen or hydrogen peroxide and may be a contributing factor for CBZ degradation [10]. Further, overlapping of the intermediates or metabolites generated during photochemical degradation and biotransformation does not allow the determination of exact process responsible for their generation [27]. CBZ and oxCBZ which are hydrophilic and are resistant to degradation; however, physical or chemical transformations may also be one of the mechanisms involved in removal efficiency of these drugs due to which the compounds may be transformed or conjugated and are not detected and is indicated as their removal [5,28]. The transformation products and the pathway adopted for their formation during the biotransformation of CBZ and oxCBZ during the biological process in WWTP have been elucidated by Kaiser et al., 2014. Moreover, the reactions such as oxidation, hydroxylation, intramolecular ring closure, α-ketol rearrangement, etc. involved in transformation have also been reported [29].

3. Evaluation of Mass Balance

Mass balance of a plant is instructive in examining the fate of a particular compound and gives an estimate of its removal efficiency. The overall in- and outflow measurements were performed to close the overall flow balance (Fig. 2). The measurements presented are an average of periodic sampling from the respective units. Standard deviations were measured to evaluate the flow consistency of the influent and variability of the measurements and the values represented are an average of all the measurements undertaken (Table 2, Fig. 3).

The parameters such as mass loading and removal efficiency were calculated on the basis of Eqs. (2)-(7). Table 2 details the concentration profile of CBZ and oxCBZ in the aqueous phase of wastewater at different stages of treatment along with mass balance related data such as flow rates and mixed liquor suspended solids (MLSS). The results of mass balance are presented in Table 3. It was comprehended that major portion of these drugs existed in aqueous phase of wastewater and only 2.94% CBZ contributed in suspended solids and sludge. A CBZ adsorption of 0.059 g/kg of dry sludge and 0.044 g/kg of dry sludge was observed in AT-1 and AT-2 units, respectively (Table 2); however, no adsorption was observed in case of oxCBZ. The daily mass loading of CBZ in influent was 0.53 kg/day, while that in effluent was 0.254 kg/day. Simi-

Table 3. Mass balance for the daily input and output of CBZ and oxCBZ in aqueous phase, suspended solids and sludge of wastewater in the existing WWTP

Micro-pollutant	Input					Output							% Removal based on aqueous phase
	Aqueous phase (g/day)		Suspended solids (g/day)		Total Input (g/day)	Aqueous phase (g/day)		Suspended solids (g/day)		Sludge (g/day)		Total output (g/day)	
	Mean ±S.D*	RSD	Mean ±S.D	RSD**	Mean ±S.D	Mean ±S.D	RSD	Mean ±S.D	RSD	Mean ±S.D	RSD	Mean ±S.D	
CBZ	531.67 ±39.47	7.42	8.07 ±2.5	30.98	539.74 ±41.97	254.33 ±45.3	17.81	8.15 ±2.28	27.98	7.73 ±2.46	31.82	270.21 ±50.04	49.93%
oxCBZ	1329.84 ±89.46	6.73	-	-	1329.84 ±89.46	1504.51 ±79.3	5.27	-	-	-	-	1504.51 ±79.3	−13.13%

*S.D: standard deviation, **RSD: relative standard deviation

larly, the daily mass loading for oxCBZ in influent was 1.33 kg/day while that in effluent was 1.5 kg/day. This fate of oxCBZ ensued in a negative mass balance, indicated by a net increase in the mass while taking its course through the WWTP. This was indicative of absence of sorption occurring onto the sludge, thereby allowing the passage of the micropollutants through the WWTP. The lack of sorption onto sludge is due to the hydrophilic nature of these drugs [28]. The reasons for the increase in oxCBZ concentration and eventually mass load were already discussed in section 3.1. Similar negative removals have been observed in some earlier studies [12]. However, the mass balance data is representative of the samples collected at 24 h interval and the biosolid grab samples. It is also envisaged that the mass of CBZ lost (i.e., which is absent from the liquid phase as well as sludge) during the treatment should be taken into consideration, and this loss might also be a consequence of formation of other compounds in the plant due to conjugation of derivatives leading to formation of glucuronides as a result of biotransformation [28]. However, this can be taken into consideration through further examination of effluents for the presence of intermediate metabolites, conjugated compounds, such as glucuronides, transformation products, hydroxyl and carboxy derivatives, through advanced sophisticated analytical techniques that would require further studies. Biotransformation of CBZ into other derivatives also contributes to the mass of CBZ lost during its passage through the WWTP.

The final step of RO was found to be the most effective in terms of COD, TDS and pharmaceutical removal as can also be observed from Fig. 4(a), Fig. 3(c), Fig. 4(b) and (c) respectively. Further, RO resulted in maximum removal of organic matter as demonstrated in terms of COD (93.69% COD removal calculated based on the concentration in the preceding unit) (Fig. 3(d), 4(a)). Moreover, RO resulted in effective removal of TDS as can be deduced from Fig. 3(c). Similarly, CBZ and oxCBZ removal was be 94.74% and 99.66% after RO (Fig. 3(f), (g); Fig. 4(b), (c)). The efficiency of any WWTP is mostly measured in terms of removal of the key parameters such as COD, TDS and organic pharmaceuticals. The results obtained in the present study, thus, indicate the efficiency of RO in removing the organic matter, TDS and pharmaceutical removal to a higher degree.

4. Recommendations

Wastewater characteristics mainly depend on the type of raw materials used, products formed, operational parameters and also on existing preventive measures used to control the pollution. Therefore, to carry out the mass balancing for removal of a particular micropollutant in an activated sludge process, it is crucial to accurately measure the flow rate, sludge formation (MLSS concentration), and concentration of micropollutants in the activated sludge (biomass) and in the influent and effluent streams.

Further, as indicated in our findings, the MLSS of the biological reactor system is low as compared to MLSS commonly observed in case of an activated sludge process for pharmaceutical wastewater. This necessitates the increment and improvement in the quality of activated sludge by introducing and cultivating the right sludge or bacteria type. Further, the recycle and reuse of the waste activated sludge (WAS) for second stage biological treatment should always be based on the quality and viability of the sludge produced so as to achieve effective treatment of the wastewater containing high COD and micropollutants.

The biomass concentration can be improved by adopting a membrane bioreactor (MBR) system which would prevent the biomass to escape from the reactor system; hence the reactor can be operated at a higher MLSS leading to improved efficiency of treatment plant. Further, the advent of novel membrane materials and evolution of membrane-based technologies will lead to an improved footprint of the technology making the entire process more feasible, cost-effective and energy-efficient.

The micropollutant (CBZ and oxCBZ) concentrations were observed to be slightly higher than the quantitation limit after the final step of reverse osmosis. However, the extent of removal might further be enhanced by incorporating appropriate pretreatment methods such as advanced oxidation processes (AOPs): ozonation, Fenton treatment, UV-H_2O_2, etc. [30-32]. This will lead to the fragmentation of complex compounds into less toxic and simpler molecules which are amenable to biodegradation. Furthermore, a proper integration of suitable AOPs with the conventional biological treatment may not only aid in removal of micropollutant but will also help in establishing a cost-effective treatment process.

Furthermore, it can be derived from Table 1 that the CBZ plant produces 930 kg CBZ/day generating 32.88 ton liquid waste/day while oxCBZ plant produces 350 kg oxcBZ/day generating 48.3 ton liquid waste/day. This combined liquid waste is treated in WWTP that has an input loading of 0.53 kg/day CBZ and 1.33 kg/day oxCBZ, as indicated in Table 3. Thus, the data on the ratio of waste: production of this WWTP can be applied to the production data of other WWTPs operating under similar operating conditions, and it may thus be a significant parameter to characterize the source of environmental contamination and its relative contribution. This will aid in designing an appropriate treatment method for enhancing the removal of these pharmaceuticals. Additionally, a suitable treatment of wastewater released from the pharmaceutical manufacturing plant at the manufacturing site itself will prevent discharge of these pharmaceuticals in the environment, which will not only aid in maintaining the discharge regulations but will also shield the deleterious impacts on environment and human health. It is also recommended to have stricter discharge regulations so that the pharmaceutical run-off into surface water or ground water through effluent discharge and to agricultural lands through the discharged biosolids is prevented at the manufacturing site itself.

CONCLUSION

Recapitulating the present research work provides a comprehensive assessment of the fate and removal of the pharmaceuticals, CBZ and oxCBZ, in a pharmaceutical industry manufacturing these drugs. The biological treatment plant (BTP) was monitored exclusively among the other units of WWTP. The study was carried out at the point source itself in order to prevent pharmaceutical discharge into the ecosystem. An overall COD balance revealed a COD removal of 76.69% corresponding to 971.83 kg/day COD removal. The mass balance of BTP showed 50.17±3% CBZ removal, while oxCBZ showed negative mass balance in the aqueous phase of BTP. Studies on sorption onto sludge indicated a low sorption

of CBZ and negligible sorption of oxCBZ due to the low water-sludge distribution coefficient and low log K_{ow} values; as a consequence, both the pharmaceuticals prevailed in the aqueous phase. Reverse osmosis removed 91.4% oxCBZ, while the CBZ and oxCBZ concentrations were approximately equal to the quantitation limit. The integrative data presented in this study on productivity of these pharmaceuticals, their mass loading in influent and effluents allied with their removal efficiency will aid in standardizing the operational effectiveness and design improvement of the current conventional treatment plant. Furthermore, policy changes for stringent discharge regimes are recommended so that the entry of these pharmaceuticals in the ecosystem through run-off is prevented on the manufacturing site itself.

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SUPPORTING INFORMATION

Additional information as noted in the text. This information is available via the Internet at http://www.springer.com/chemistry/journal/11814.

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