

*Original Research*

# Biodegradation of Wastewater Containing High Concentration of Sulfamethoxazole by Antibiotic Adopted Biofilm in Attached Growth Bioreactor

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## Abstract

Pharmaceutical wastewater treatment is a complicated process due to the presence of various kinds of toxic chemicals and antibiotics that are harmful to any living organisms. In this study we employed a pilot-scale net-like rotating biological contactor (NRBC) with three compartments and total volume of 78.75 L to eliminate high concentrations of sulfamethoxazole (SMX) from a synthetic wastewater. In this system, 16 plastic discs in each compartment were fixed on a horizontal shaft, rotating with constant speed (4 rpm). Activated sludge collected from a local hospital wastewater treatment plant was used as inoculum and the system was working in batch condition in order to develop adapted living organisms as biofilm on the rotation discs. Then the NRBC was successfully operated at continuous mode for 10 months at various organic loading rates (OLR) (0 to 21.3 gCOD/L.d), hydraulic retention times (HRT) (12 to 72 h), and concentrations of sulfamethoxazole (5 to 120 mg/L). It was found that by increasing in OLR and HRT, SMX removal efficiency was increased. Also, the obtained results indicated that increasing COD concentration had a positive impact on SMX removal efficiency, which was most probably due to the utilization of SMX as a nitrogen source. Finally, it was revealed that most SMX and organic matter removal occurred in the first compartment of the NRBC, and antibiotic concentration was negligible in the outlet stream.

**Keywords:** COD removal, NRBC, pharmaceutical wastewater, sulfamethoxazole degradation

## Introduction

Pharmaceutical wastewater is known as highly polluting toxic effluent that includes large amounts of suspended solids and soluble organics [1]. The composition of these pollutants depends on the type of drug produced

and the chemicals involved in downstream processing [2]. The existence of pharmaceutical and personal care products (PPCPs) in wastewater and water environment have been recognized all over the world [3-4].

Concerned about their potential risk, monitoring studies on their existence in the environment have been reported in literature [5], but few reports have discussed the exact fate of PPCPs in surface water and wastewater [6]. Experimental studies conducted in the last few decades

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have clearly revealed antibiotic contamination in aquatic environments [2]. This fact has increased public awareness and therefore it has received considerable attention in recent years. For example, contaminants such as organic matter and PPCPs exist in 80% of 139 U.S. streams [7] and their chemical effect, microbial persistence, and synergistic agents are still unknown [8]. Pharmaceutical residue enters the environment from several sources such as hospitals, pharmaceutical production industries, wastewater treatment plants (WWTPs), and landfills [9].

Pharmaceutical industrial wastewaters contain organic matter, raw substances, catalysts, chemical additives, reactants, and active pharmaceutical ingredients (APIs) [10]. Among PPCPs, antibiotics are a major problem for the environment because of their high consumption value in human health and veterinary medicine as well as widespread use as growth promoters for therapeutic goals [11]. Many antibiotics are hydrophilic so that they can be detected in surface water, groundwater, influent, and effluent of WWTPs in the range of a few ng/L and µg/L [12]. Antibiotics mostly enter the environment via diffuse agricultural input, landfill depletion, pharmaceutical industrial wastewaters, domestic wastewater (DWW), and WWTP effluents [13-15].

Sulfamethoxazole ( $C_{10}H_{11}N_3O_3S$ ), a sulfonamide antibacterial, is a popular bacteriostatic antibiotic. Sulfamethoxazole is usually used in combination with trimethoprim at a ratio of 5:1 (as in co-trimoxazole, which is also known as Bactrim or Septra [11]). According to previous research, SMX is hardly biodegradable [16]. It has been reported that more than 50% of pharmaceutical wastewater is released without any treatment, and the existence of PPCPs in this wastewater lead to low efficiency in removal of chemical oxygen demand (COD) [17-18].

The most common and economical wastewater treatment approach is biological treatment [19]. The methods are frequently used in antibiotic removal consist of biological treatments such as conventional activate sludge (CAS), membrane bioreactor (MBR) in combination with methods like microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO), advanced oxidation processes (AOPs) and adsorption to activated carbon [20-24]. In several studies, MBR was considered a suitable alternative to CAS systems for antibiotic removal [1]. For example, similar efficiency in removal of erythromycin (ERY), clarithromycin (CLA), and trimethoprim (TMP) in both systems was reported. Although the removal of roxithromycin (ROX) is slightly desired in the CAS, MBR removal of sulfamethoxazole (SMX) is significantly better than by CAS. The removal of sulfamethoxazole in MBR and CAS were 95 and 60%, respectively [25].

In Sweden, wastewater plants found a high rate of sorption to sludge for fluoroquinolones while reporting a slight removal of sulfamethoxazole and trimethoprim [26]. In 2004 Carballa et al. reported about 67% removal efficiency in a Spanish WWTP for sulfamethoxazole, and the same results were achieved in sewage from different

Table 1. Removal efficiencies of sulfamethoxazole in different studies.

Type of treatment system	Removal efficiency of SMX (%)	Ref.
Biological treatment of municipal wastewater	50-90	[20]
Sewage treatment plants	40	[26]
Municipal sewage treatment plant	60	[27]
Sewage treatment plants	67	[28]

wastewater treatment parts [27-28]. Furthermore, trimethoprim displays a high resistance to biodegradation in activated sludge treatment compared to sulfonamides [26]. The obtained results from these studies are summarized in Table 1.

Rotating biological contactor (RBC) is a system that contains a series of circular discs that rotate with a central horizontal shaft, and almost 40-45% of the entire disc surface area is immersed in wastewater [29-30]. This system requires relatively little space and low capital cost for a treatment plant and disposal of sludge with easy construction, simple operation, low energy and maintenance costs, and resistance against sudden pollution load conditions [30]. Because of attached growth on the discs, a rotating biological contactor has high capacity to treat wastewater and decomposition of materials resistant to biodegradation. Furthermore, this system can tolerate hydraulic and organic load shocks [31].

In this study the biodegradation of SMX under aerobic conditions in a three-stage RBC system was investigated by an adapted enhanced culture of microorganisms. Also, the effect of HRT, OLR, and SMX concentration on RBC performance in removal of organic matter and antibiotics was assessed.

## Materials and Methods

### Chemicals

Sulfamethoxazole (analytical grade) was purchased from Chemidaru pharmaceutical company (Tehran, Iran) and all other chemicals were supplied by Merck (Darmstadt, Germany). The solid phase extraction cartridges (SPE) were purchased from Waters (HLB cartridges of 50 mg). Millipore (0.22 and 0.45 µm) and Whatman (0.7 µm) filters were used for filtration purposes. Acetonitrile, methanol, and water were HPLC-grade and purchased from Merck. Ultrapure water was prepared through a Milli-Q purification system (Millipore, Schwabach, Germany).

### Analytical Methods

The samples were collected from different compartments of NRBC plus inlet and outlet streams (Fig. 1). The

collected samples were settled before a COD test for 30 min. The COD analysis was carried out using dichromate reagent based on standard method using close reflux. The colorimetric method was developed at a wavelength of 600 nm by means of a spectrophotometer (2100 SERIES, UNICO, U.S.A.). Total suspended solids (TSS) were determined (APHA Standard Methods, 2005). pH was measured using a portable pH meter (HI 8224, Hanna, U.S.A.). SMX determination was carried out using HPLC (Smartline, Knauer, Germany) equipped with a UV/VIS detector, a four-channel pump, and an injection valve to which a 20  $\mu$ l sample loop was attached. Samples were injected using a 100  $\mu$ l syringe (Hamilton, Reno, Nevada, USA). The column was a ProntoSil (C18 H column) that was 250 mm in length and 4.6 mm in diameter. Before HPLC analysis, all samples were treated by solid phase extraction techniques (Waters HLB cartridges of 50 mg). Thus, the sample should be free of organic solvent and inorganic matter that may harm the HPLC column. The mobile phase was made up of 87% buffer solution (0.02M  $C_2H_3O_2NH_4$  in ultra-purified water at pH = 6) with 13% acetonitrile. The flow rate was set at 1 mL/min and the wavelength used for detection was 265 nm [11]. Additionally, all microorganism observations were done under a Nikon YS 100 microscope (Nikon, Japan).

#### NRBC Setup

A pilot-scale RBC in three compartments was made-up of 8 mm thick acrylic plastic sheets. Set-up dimensions were 75 cm long, 35 cm wide, and 30 cm deep. The

schematic diagram for the fabricated RBC is shown in Fig. 1.

The discs, which were made of blue plastic sheet with thickness of 3 mm, were fixed on a horizontal steel shaft. The shaft passed through the center of the discs and were rotated by an electric motor (NDRD Motor, Model SK 63/4, Germany) with constant rotational speed (4 rpm). The feed rate was controlled by a peristaltic pump (Etatron D.S., Model PDP-B-V, Italy) with changeable speed (0-150 ml/min).

#### Composition of Synthetic Wastewater

The feed was a synthetic wastewater consisting of sugar cane molasses ( $C_6H_{12}O_3NNaS$ ), commercial grade potassium hydrogen phosphate and potassium dihydrogen phosphate ( $K_2HPO_4$  and  $K_2HPO_4$ ), and urea ( $CH_4N_2O$ ) with a ratio of C:N:P at 100:5:1. Experiments were conducted with different concentrations of synthetic feed and sulfamethoxazole. Activated sludge for inoculum was collected from the wastewater treatment plant of Valiasr Hospital (Qaemshar, Iran). The consortium of bacteria in the activated sludge was adapted to the presence of sulfamethoxazole in the RBC.

#### Bioreactor Operation

The NRBC system was first operated in batch mode for a duration of 20 days. The COD concentration of synthetic wastewater was increased from 600 to 8,000 mg/l. At this period the system was run at HRT of

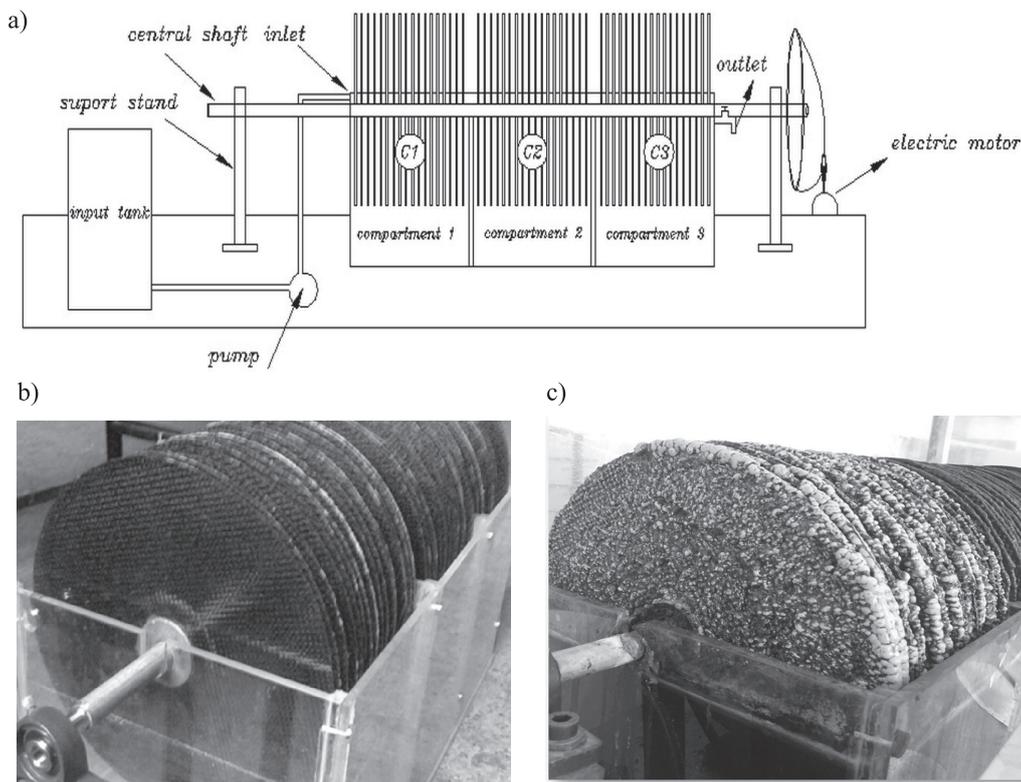


Fig. 1. a) Schematic diagram of the fabricated RBC, b) bare discs, and c) thick biofilm developed on the surface of discs.

24 h and the rotational speed of discs was fixed at 4 rpm. For the duration of the first week, considerable biofilm was developed on both sides of the discs and after 12 days the biofilm was fully developed (Fig. 1).

Then, the system was continuously operated for 10 months and in this period the NRBC was run at various HRT, COD, and SMX concentrations. Meanwhile, we investigated the effect of different influent CODs, SMXs, and HRTs, plus COD and SMX removal efficiencies on the NRBC system.

## Results and Discussions

### Continuous COD Removal in the NRBC Reactor

Before adding SMX, the NRBC was continuously operated at HRT of 12 to 60 h and the inlet COD concentrations were varied from 8,000 to 32,000 mg/L (OLR 2.7 to 21.3 g COD/L.d). The samples were collected in three stages of the NRBC system. The obtained results indicated that by increasing HRT, COD removal was gradually increased. However, for HRT greater than 36 h, there was no significant improvement in COD removal efficiency. The maximum COD removals using COD concentrations of 8,000 mg/L at various HRTs (12, 24, 36, 48, and 60 h) were 75, 86, 96, 96.6, and 97%, respectively. In NRBC, the optimal HRT for desired rate of COD removal was 36 h. So the optimal HRT for the desired rate of COD removal was considered to be 36 h.

### Continuous COD Removal and SMX in the NRBC Reactor

When the system reached a steady-state condition, antibiotic addition was performed in different concentrations of SMX and OLR.

Dosages of SMX in inlet flow were varied and in each dose the system was run for the duration, gradually reaching stable condition. The durations for each dose (1, 5, 15, 30, 60, and 120 mg/L) to reach constant COD removal efficiency were different (3, 7, 7, 14, 14, and 14 days). The fluctuations in COD removal efficiency in low SMX concentrations (1 to 15 mg/L) were negligible, and for this reason the required adaptation times were less than high concentrations of SMX (15 to 120 mg/L).

Inlet COD concentration was 8,000 mg/L, which is the usual average concentration in pharmaceutical industry wastewaters in Iran. In order to investigate the biodegradability of antibiotic as organic matter, the removal of SMX and COD were measured as SMX concentrations and HRTs were varied (Fig. 2). As expected, both COD and SMX concentrations were decreased by increasing HRTs. At optimal HRT (36 h) with different concentrations of SMX (from 5 to 120 mg/L), the percentages of antibiotic removal gradually dropped from 80 to 62% as antibiotic concentrations increased. The results demonstrate that SMX biodegradation occurred before HRT of

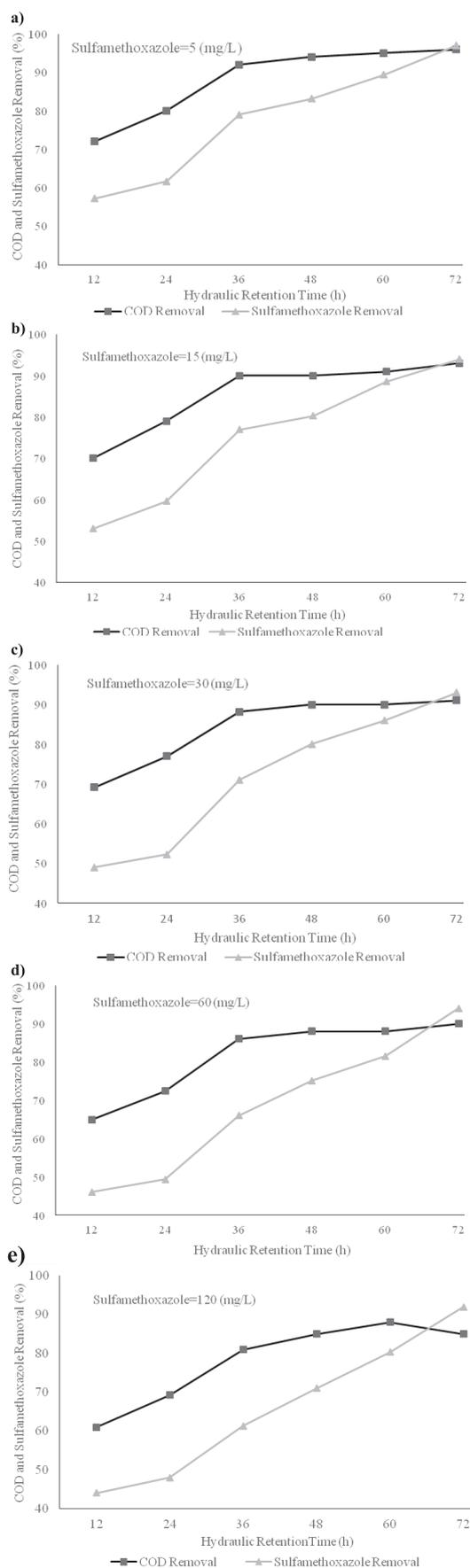


Fig. 2. Removal efficiencies for a) 5 mg/L, b) 15 mg/L, c) 30 mg/L, d) 60 mg/L, and e) 120 mg/L SMX and COD with respect to HRTs.

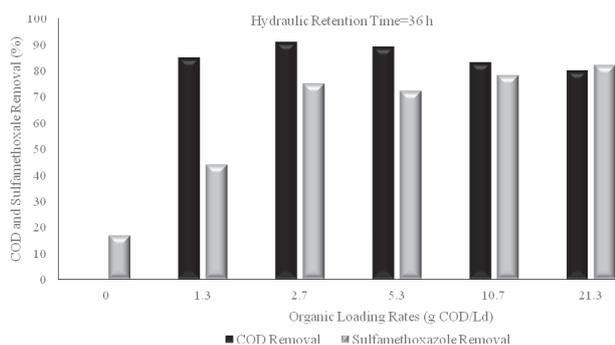


Fig. 3. Removal of SMX in various COD concentrations.

60 h. These results proved that microorganisms can gradually become adapted to new environmental and nutritional sources. Also, the figures exhibit that about 50% of SMX biodegradation was degraded in the first 12 h; however, the majority of SMX elimination occurred within 72 h. Moreover, as SMX concentration increased, antibiotic removal efficiency slightly decreased. For instance, when increasing SMX concentration from 5 to 120 mg/L at HRT of 60 h, SMX removal efficiency decreased from 89.4 to 80.3%.

In a similar research conducted on the treatment of domestic wastewater by an activated sludge system, SMX removal efficiency was reported to be 52%. However, this value increased to 60% for an activated sludge system followed by a horizontal subsurface flow bed. These results indicate that the NRBC system in removal of antibiotics was more efficient than the activated sludge system, which was traditionally used in wastewater treatment plants [15].

We observed that morphology and general appearance of the attached biofilm as well as quantity of fungi and bacteria were significantly changed during the antibiotic removal process. The main reason for this change is the chemical shock, which resulted from the addition of antibiotics so that the fungi species became dominant in the biological film (as shown in Figure 1c) due to their higher resistance to antibiotics [32]. The fungi were identified by their mycelia under a microscope as well as silky macroscopic structure.

Moreover, we observed that the type of species of microorganism and overall biomass quantity were changed significantly from stage to stage. The fungi species were dominant in stage 1 and the biofilm thickness in this stage was also greater than the other stages, which is definitely due to the fact that the nutrients and antibiotic concentration were relatively higher in stage 1 than the other stages. These observations confirmed the results obtained from the similar research conducted by Su et al. [33].

#### Impact of COD Concentration on SMX Removal

In order to investigate the effect of COD concentration on SMX removal efficiency, antibiotic biodegradation was analyzed at HRT = 36 h. To achieve this purpose the

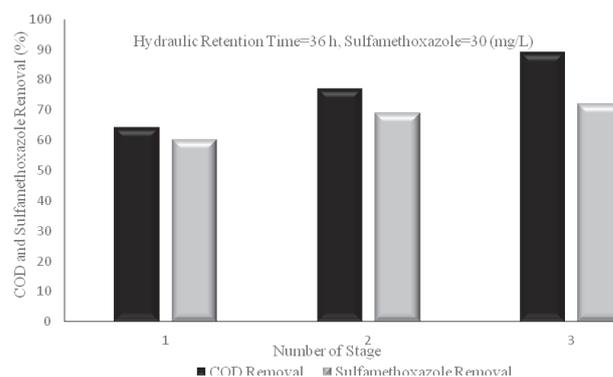


Fig. 4. SMX and COD removal efficiencies in each NRBC compartment.

inlet COD was changed from 0 to 32,000 mg/L in six steps, and SMX concentration was kept constant (30 mg per liter of inlet synthetic wastewater). Fig. 3 illustrates the obtained results, which proposed that SMX elimination was enhanced by increasing OLR. The SMX biodegradation trend was different with organic matter elimination. In other words, the higher COD concentrations led to more antibiotic removal efficiency. It seems that when there is willingness of degradable carbon sources, sulfamethoxazole served as a nitrogen source so that antibiotic removal efficiency enhanced with increasing COD concentrations.

#### SMX and COD Removal Efficiency in Each Compartment of NRBC

COD and SMX value in each compartment were analyzed separately for estimating the capability of the system in elimination of organic compounds, such as simultaneous antibiotic removal in each stage of NRBC at HRT = 36 h and SMX = 30 mg/L. As Fig. 4 depicts, most removal efficiency in decomposition of both COD and SMX takes place in compartment 1 because microbial activity in the first stage is expected to be high, which is due to exposure of this compartment to the high nutrients.

#### Conclusion

In the present work, an attached growth bioreactor was employed for the treatment of wastewater containing a high concentration of sulfamethoxazole, and its capability in antibiotic removal was evaluated at different inlet COD and HRTs. SMX and COD removal efficiency in each compartment of NRBC was investigated separately. The obtained results indicate that high SMX removal efficiency takes place at various HRTs, and even at high organic load rates. In other words, in NRBC systems, enhancement of organic load rates not only did not have a deleterious effect on antibiotic elimination but also improved removal efficiency. The obtained results show that at optimal HRT, the percentages of antibiotic removal were gradually dropped from 80 to 62% as concen-

trations of antibiotics increased. Also, by increasing SMX concentration from 5 to 120 mg/L at HRT of 60 h, SMX removal efficiency decreased from 89.4 to 80.3%. In addition, it was concluded that the most SMX removal efficiency occurred in the first compartment of the NRBC.

These results provide guidelines for employing the attached growth system instead of a traditionally used activated sludge system for the pharmaceutical industrial purpose; however, further studies are needed to investigate the performance of the RBC system in the elimination of other antibiotics.

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