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Original Article

Moving Bed Sequenced Batch Reactor System in Tetracycline Antibiotic Removal from Real Hospital Wastewater

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Abstract
Background: Water contamination by synthetic organic chemicals like antibiotics is a major environmental issue. Tetracycline (TC), an antibiotic in a wide family, is notable. A moving bed sequenced batch reactor (MBSBR) is tested for treating hospital raw wastewater containing TC.
Methods: A 35-L pilot system was constructed, with 30 L usable. PVC suspended carriers (Kaldnes K3) with 584.3 m^2/m^3 specific surface area made about 70% of the functional volume. The independent variables in this study were hydraulic retention duration (1, 1.5, 2, and 2.5 hours) and starting TC concentration (5, 10, and 15 mg/L).
Results: The findings of the study demonstrated satisfactory performance under the conditions of an initial TC concentration of 5 mg/L and an organic load of 350 mg/L. The overall removal efficiencies for TC, chemical oxygen demand (COD), and biological oxygen demand (BOD_5) were 72.8%, 83%, and 93.9%, respectively. The optimal performance of the system was primarily observed during the initial phase, characterized by a TC concentration of 5 mg/L and a hydraulic retention time (HRT) of 2.5 hours. The experimental results also indicated that the maximum removal efficiency was 1.8 kg COD/m^2.day, as determined by a fitted surface loading rate (SLR). Furthermore, the food-to-microorganism (F/M) ratio decreased from 0.101 to 0.038 as the HRT increased from 1 to 2.5 hours.
Conclusion: The results of the study indicate that the MBSBR exhibits a high level of efficiency in removing TC from hospital wastewater.
Keywords: Moving bed sequenced batch reactor, Hospital wastewater, Tetracycline

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Introduction
The occurrence of pharmaceutical compounds, such as antibiotics and their metabolites, in water sources has become an imperative concern due to their potential impact on both the environment and human health. Hospital wastewaters are a significant source of pollution, including heavy metals, pathogenic microorganisms, disinfectants, and pharmaceutical compounds, especially antibiotics. These agents exhibit resistance to biodegradation in aquatic environments and ecosystems.[1] Antibiotics are among the most essential pharmaceuticals and are widely employed in the treatment and prevention of bacterial infections in humans, animals, and plants. They are also utilized as growth promoters in animal husbandry. The extensive use of antibiotics can contribute to the proliferation and dissemination of antibiotic-resistant bacteria. Furthermore, resistant bacteria can enter aquatic environments from various sources.[2]

Recent studies have primarily focused on characterizing the sources of effluent, often neglecting their impact on the wastewater treatment process. The release of antibiotics into receiving environments has significant public health implications, as highlighted in the following sentences. When resistant bacteria carry transmissible genes, they can transfer them to other bacterial communities, making infections caused by these bacteria difficult to treat and reducing the effectiveness of antibiotics for bacterial infections. These organisms may serve as vectors or reservoirs of resistant genes.[3] Additionally, the prevalence of nosocomial infections is likely to increase, leading to higher treatment costs and hospitalization rates. The excessive and inappropriate use of antimicrobial agents contributes to the spread of antibiotic resistance in the environment, posing a major public health concern.[4]

One of the major families of antibiotics is tetracyclines (TCs). TCs are broad-spectrum antibiotics commonly used to treat various bacterial infections, including urinary tract infections, acne, gonorrhea, chlamydia, and others. However, the extensive use of TCs and their derivatives has been associated with a range of side effects, such as photosensitivity, nail discoloration, and onycholysis.[5] TCs have been detected in water, wastewater, and soil near wastewater treatment plants (WWTPs) in different countries. For instance, concentrations of TCs have been reported in Sweden (ranging from 0.064 to 2.480 µg/L), Luxembourg (ranging from 1.0 to 85.0 µg/L), and Canada (ranging from 0.038 to 0.977 µg/L).[6]

Large quantities of hospital wastewaters are typically discharged into public sewage systems, thereby significantly contributing to the overall pharmaceutical compound load in the influent of $WWTP_s$.[7] $WWTP_s$ are not specifically designed to effectively remove pharmaceuticals such as antibiotics. This is because these compounds fall into the category of resistant organic compounds (ROCs), which pose challenges for conventional treatment processes.[8] Certain WWTPs employ disinfection methods such as chlorination and UV radiation to treat effluent before it is discharged into the environment. However, the incomplete degradation of ROCs during the disinfection process can result in the formation of potentially hazardous disinfection by-products such as trihalomethanes, haloacetic acids, N-nitrosodimethylamine, and others.[9] The most commonly employed strategies for treating hospital wastewater include chemical pre-treatment, advanced oxidation processes, and specific biological treatment methods such as membrane bioreactor, up-flow anaerobic sludge blanket, moving bed biological reactor (MBBR), and integrated fixed active sludge,[10] Among which biological treatments are considered the most cost-effective, reliable, and efficient process for reducing pharmaceutical compounds in wastewater effluent.[11]

One of the innovative biological processes for wastewater treatment is the moving bed sequenced batch reactor (MBSBR).[12] In recent decades, there has been a growing focus on hybrid systems that combine the advantages of suspended growth and attached growth biofilm systems. Among these, the MBBR has emerged as a popular hybrid system. The MBBR is particularly favored because it allows for a higher biomass concentration in the reactor, leading to improved treatment efficiency and stability. This is achieved by utilizing carrier elements of various types and nature.[13] Another highly successful biological treatment option widely studied and used is the sequencing batch reactor (SBR). The SBR offers several significant advantages, including a smaller footprint, ease of adjusting operational conditions, and operational flexibility.[14] Recently, MBBRs have been operated in a sequencing batch mode to capitalize on the benefits of both processes. The MBSBR has gained considerable attention due to its ability to combine the advantages of MBBR and SBR. MBSBR exhibits enhanced biomass concentration, resulting in higher specific removal efficiencies, greater volumetric loads, and increased process stability against shock loading.[15] It is a continuously operating, non-clogging biofilm process that does not require backwashing, has low head loss, and provides a high specific biofilm surface area. The attached biofilm grows on small carrier elements that are suspended in constant motion throughout the entire volume of the reactor.[16]

Based on the information provided, it appears that the study was conducted to evaluate the removal of TC antibiotics from real-life hospital wastewater using the MBSBR process. This study represents the first investigation into the removal of TC antibiotics using the MBSBR process. Additionally, the study aims to assess the efficiency of the MBSBR process in treating the wastewater from Imam Khomeini Hospital in Tehran and propose improvements to the existing conditions.

Materials and methods

Study Design and Samples Collection

This experimental study aimed to evaluate the effectiveness of the MBSBR process in treating wastewater at Imam Khomeini hospital in Tehran, Iran. The hospital currently utilizes an extended aeration activated sludge system for wastewater treatment, but it fails to meet the required standards for sewage discharge into the sewer network. Additionally, various concentrations of antibiotics have been detected in the effluent. The hospital generates a total volume of 10-15 m^3/day of wastewater. During the study, a total of 72 wastewater samples were collected from the entrance of the WWTP, specifically after the primary unit and before entering the biological unit. The collection and preservation of the samples followed the recommended Method No. 1060 from the standard methods for the examination of water and wastewater (23rd edition).[17] The wastewater samples were analyzed for physicochemical characteristics and quality based on standard methods outlined in the examination of water and wastewater.[18] The physicochemical characteristics of the wastewater have been presented in Table 1.

Experimental Set-up and Operating Conditions

For the pilot-scale MBSBR process (Figure 1), the reactor chamber was constructed using Plexiglass and had a total volume of 35 L, with a useful volume of 30 L. The dimensions of the reactor were 25.5 × 25.5 × 54 cm (length, width, and height). The reactor was filled with approximately 70% of its volume with Kaldnes K3 media, which is composed of high-density polyethylene. The media had an internal diameter of 25 mm and a height of 10 mm. The specific surface area of the media was 584.3 m^2/m^3. The working volume of the reactor was determined as the volume occupied by the media. To supply the required air for the process, a piston aquarium pump (HAILEA model = AC328-China) with an output air capacity of 80 L/min was used. The reactor was fed using a peristaltic pump.

Table 1. Physicochemical characteristics of the wastewater

	Factors								
	COD	BOD	TSS	TDS	DO	pH	Free chlorine	TC	Temperature
Unit	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	-	(mg/L)	(mg/L)	(°C)
Inlet	362	254	186	950	0.57	6.5-8	0	5	20-22
Outlet	35	18	46	800	3.8	7.8	0	1.2	20-22

TSS, Total suspended solids; TDS, Total dissolved solids; COD, chemical oxygen demand; BOD, biological oxygen demand; TC, tetracycline; DO, dissolved oxygen.

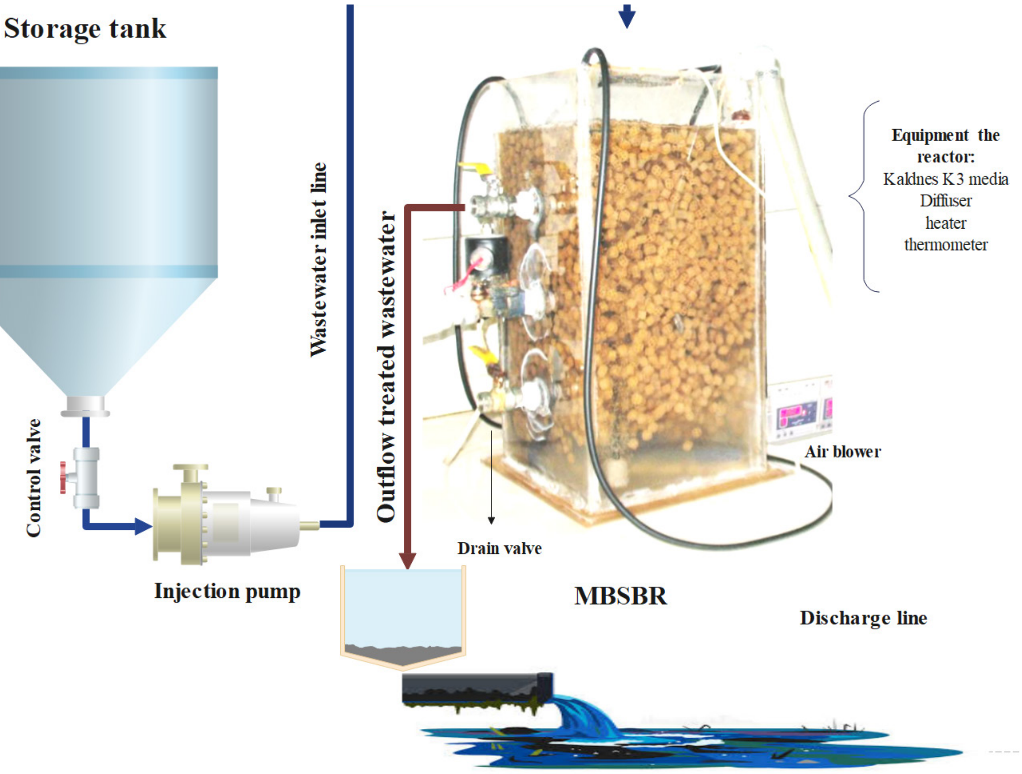

Figure 1. Schematic diagram of the MBSBR system

Pilot Start-up

In the initial phase of the pilot-scale MBSBR process, sludge seeding was obtained from the recycle line of Imam Khomeini hospital WWTP, and half of the reactor's volume was filled with it. During this stage, manual feeding to the pilot was performed for one week to allow the microorganisms to adapt to the system. The reactor was operated in batch mode during this period. Synthetic wastewater with a carbon-to-nitrogen-to-phosphorus (C:N:P) ratio of 100:5:1 was dosed into the system using a dosing pump. The planned time cycles for the pilot-scale MBSBR process included the following stages: wastewater feeding (30 min), aeration (120 min), sedimentation (180 min), discharge, and relaxing time (60 min). The physicochemical parameters of the wastewater, including temperature (°C) and pH, were measured daily using a thermometer (HACH-USA) and a portable pH meter (Ecomet-USA). The daily dissolved oxygen (DO) demand in mg/L was determined during the aeration phase. The concentrations of mixed liquor suspended solids, mixed liquor volatile suspended solids (MLVSS), surface loading rate (SLR), and food-to-microorganism (F/M) ratio were also determined. All hydraulic experiments were conducted within a temperature range of 21 to 29 °C. In the first phase, the optimum hydraulic retention time (HRT), TC concentration, and flow rate were measured. In the second phase, the TC concentration was altered to 5, 10, and 15 mg/L. For each TC concentration, the HRT, MLVSS, and F/M ratio were determined. In the final step, four different alterations were made to the HRT, and TC and chemical oxygen demand (COD) variations were recorded and analyzed.

TC, BOD_5, and COD Tests

To construct the TC standard curve, gradient dilutions of the standard stock solutions were prepared. The standard solutions of TC were diluted in water to concentrations of 1, 2, 3, 5, 10, and 15 mg/L for calibration purposes. Quantification of TC was carried out using high-performance liquid chromatography (HPLC) equipment from Agilent Technologies Co. Ltd., USA. The HPLC system consisted of a Shimadzu LC-20 AB pump, a Shim-Pack VP-ODS-C18 column (4.6 mm × 250 mm × 5 µm), and a UV detector (Shimadzu UV-1600 spectrophotometer). The mobile phase used for the HPLC analysis was a mixture of methanol and water (50:50 v/v, HPLC grade, Merck). The flow rate was set to 1 mL/min, and the temperature was maintained at 25 °C. A 20-

μL solution of TC, prepared according to the standard curve dilutions, was injected into the HPLC column. The retention time for TC was determined to be 3.6 min, and the compound was detected at a wavelength of 359 nm, as detailed in a previous work.[19] For HPLC analysis of wastewater samples, several steps were followed to remove interfering and suspended substances. First, the sample was filtered using a 0.45 μm filter to eliminate suspended solids and other particulate matter. Then, the mixture was centrifuged at high speed for several min to separate the organic layer from the aqueous layer. Finally, the solution was transferred into a clean vial and injected into the HPLC system. The COD analysis was performed using the closed reflux method (No. 5220 D, colorimetric method) outlined in the Standard Methods for the Examination of Water and Wastewater.[20] Also, biological oxygen demand (BOD_5) was measured by a respirometric method using the BOD_5 track system (No. 5210 D, HACH-USA).[21] The samples from reactor were passed through 0.41 μm Whatman filter paper.

Results and Discussion

In this study, the reactor filled with 70% media and a 350 mg/L organic load rate (OLR) was set up for four HRT values (1, 1.5, 2, and 2.5 hours). TC in different concentrations was added to the reactor to study the removal efficiencies of the system. The outlet flow rates were 4, 5, 6.6, and 10 L/h. The TC removal efficiency by means of the designed MBSBR reactor at different HRTs and TC concentrations has been presented in Figure 2.

It was observed that the maximum TC removal efficiency (72.8%) occurred at an HRT of 2.5 hours, initial TC concentration of 5 mg/L, and solid retention time (SRT) of 26 days. The dissolved solids (DO) concentration was monitored daily and measured within the range of 3.5 to 4.6 mg/L. Increasing the HRT at lower TC concentrations provides microorganisms with the opportunity to degrade antibiotics. HRT is a crucial parameter in designing and operating wastewater treatment systems. In the MBSBR process, HRT plays a vital role in determining pollutant removal efficiency. The MBSBR process operates in cycles, each consisting of stages such as filling, reaction, settling, and decanting. During the reaction stage, pollutants are removed by microorganisms attached to the media. HRT in the MBSBR process is defined as the time required for one complete cycle, and it can be adjusted by changing the duration of each stage or the volume of wastewater treated per cycle. The effect of HRT on pollutant removal depends on several factors, including influent characteristics, microbial activity, and media properties. At low HRTs, there may not be enough time for microorganisms to completely degrade pollutants, resulting in lower removal efficiencies and higher effluent concentrations. Conversely, at high HRTs, excess biomass growth can lead to clogging and reduced treatment efficiency.[22] In conclusion, HRT is a crucial parameter for determining pollutant removal efficiency in MBSBR processes. Optimal HRTs should be selected based on influent characteristics and system design to achieve high treatment efficiencies while avoiding operational issues such as clogging. Based on previous studies, some biological processes used for TC removal from water and wastewater include those conducted by Topal and co-workers, where 39.4 ± 1.9% of TC was removed in a municipal biological WWTP in Turkey.[23] Chen and co-workers implemented an anaerobic/aerobic moving-bed biofilm reactor system for TC degradation, achieving a removal efficiency of 41.49% under optimum conditions.[24] BOD_5 and COD removal has been represented in Figures 3 and 4, respectively.

The maximum BOD_5 and COD removal occurred under operating conditions with 70% packing, an HRT of 2.5 hours, and a TC concentration of 5 mg/L. The process demonstrated high performance, achieving 93.9% BOD_5 removal and 83% COD removal. According to the literature, the MBSBR process generally exhibits high removal efficiency for organic matter in wastewater.[25] The results indicate that increasing the HRT enhances

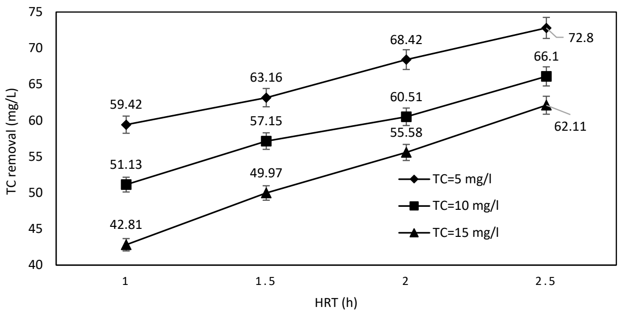

Figure 2. TC Removal Efficiency in Different HRTs (media filling = 70%, OLR = 350 mg/L, TC = 5-15 mg/L, and flow rate = 4, 5, 6.6 and 10 L/h)

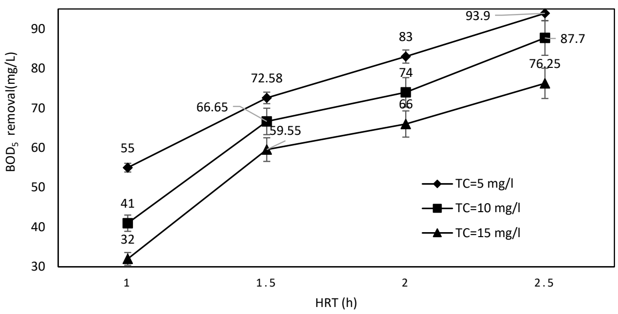

Figure 3. BOD_5 Removal in Different HRTs and TC Concentrations (media filling = 70%, OLR = 350 mg/L, TC = 5-15 mg/L, flow rate = 4, 5, 6.6 and 10 L/h)

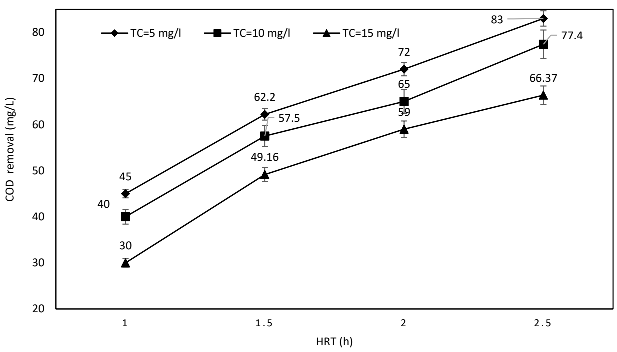

Figure 4. COD Removal in Different HRT and TC Concentrations (media filling = 70%, OLR = 350 mg/L, TC = 5-15 mg/L, flow rate = 4, 5, 6.6 and 10 L/h)

TC, COD, and BOD_5 removal. In a previous study, Bay and co-workers applied a sequencing batch moving-bed biofilm reactor to treat wastewater containing the cefixime antibiotic. In their work, COD removal under optimum conditions was 74.81%. However, as the concentration of cefixime increased, the efficiency of COD removal decreased to 23.2%.[26] Similarly, in Wang and colleagues' study investigating the MBSBR system for nitrification and denitrification, the average initial COD concentration was reported as 256.67 mg/L, and under optimum conditions, 92% of the initial COD was removed.[22] Figure 5, illustrated TC, COD, and BOD_5 removal in different HRTs.

A possible reason for these results is the longer HRT, which strongly influenced the growth of the bacterial community in the system. This, in turn, led to a higher level of TC, BOD_5, and COD removal. With a longer HRT, the bacterial community, acting as an oxidation mechanism, has sufficient time for the degradation of ROS_s, such as TC. In Li and co-workers' study, an HRT of 20 hours was reported as optimum for the degradation of ROPs.[27] Figure 6 described the relationship between F/M ratio and TC removal efficiency in 5 mg/L TC at different HRTs.

The results indicated that an increase in HRT during the process led to an increase in TC removal and a decrease in the F/M ratio. This observation suggests the dominance of microorganisms in the system, actively consuming organic matter. The F/M ratio is a crucial parameter in designing and operating biological treatment systems, including MBSBRs. It represents the amount of organic matter added to the reactor per unit mass of microorganisms in the system. In MBSBRs, the efficiency of pollutant removal is directly correlated with the F/M ratio. At low F/M ratios, fewer microorganisms are present to consume organic matter, resulting in lower removal efficiencies. Conversely, high F/M ratios lead to an excess of microorganisms, causing reduced removal efficiencies due to incomplete degradation and accumulation of intermediates. Maintaining an optimal F/M ratio is crucial for efficient pollutant removal in MBSBRs, and this optimal ratio depends on the specific wastewater characteristics and operating conditions. Generally, an F/M ratio between 0.1 and 0.5 g COD/g VSS/day has proven effective for most wastewater treatment applications using MBSBR.[28] In Faridnasr and colleagues' study, a suitable F/M ratio of 0.65 ± 0.03 was reported for the MBSBR system in sugar-industry wastewater treatment.[29]

The relationship between SLR and TC removal is illustrated in Fig. 7, with an SRT of 26 days. SLR represents the amount of wastewater flow per unit area of the reactor surface. In MBSBRs, SLR is a critical factor influencing the removal efficiency of pollutants from wastewater. As SLR increases, removal efficiency also improves up to a certain threshold. This is attributed to higher SLRs providing more surface area for microbial growth and activity, resulting in enhanced pollutant removal. However, beyond a certain point, further increases in SLR may lead to reduced removal efficiency due to insufficient contact time between wastewater and biomass. Consequently, there exists an optimum SLR range for achieving maximum removal efficiency in MBSBR. This optimal range varies based on factors such as wastewater characteristics, reactor design, and operating conditions. In summary, there is a direct relationship between SLR and removal efficiency in MBSBR up to an optimum range, beyond which increasing SLR may result in reduced removal efficiency.[30]

As observed and anticipated, the optimum SLR rate was determined to be 1.8 kg COD/m^2.day for a TC concentration of 5 mg/L and an SRT of 26 days. In a study conducted by Shaha and colleagues, SLR was identified as a crucial parameter. The reported optimum SLR in that study was 1.2 kg COD/m^2.day.[31]

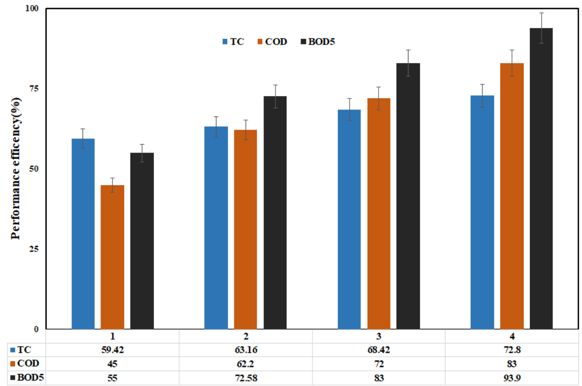

Figure 5. Compression of TC, COD, and BOD_5 Removal in Different HRTs (media filling = 70%, OLR = 350 mg/L)

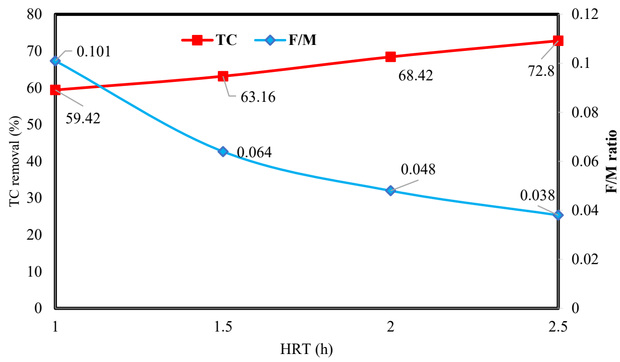

Figure 6. F/M Ratio, and TC Removal in HRTs (Media filling = 70%, OLR = 350 mg/L)

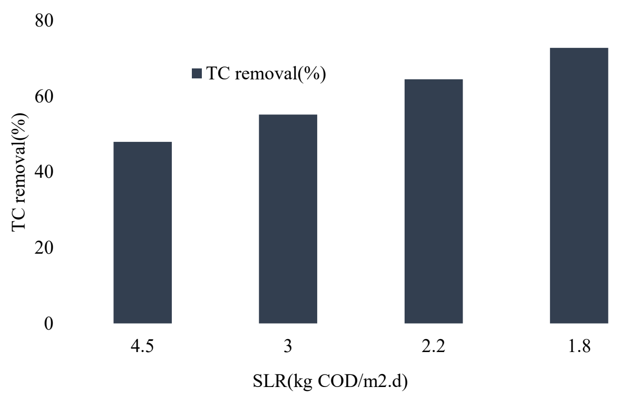

Figure 7. Relationship Between SLR and TC Removal in 5 mg/L TC Concentration (media filling = 70% and OLR = 350 mg/L)

Conclusion

In this experimental study, the TC removal efficiency in real hospital wastewater in the MBSBR system was investigated. Based on the results in Table 1, the TC concentration in hospital wastewater was 5 mg/L, and we added another concentration (10 and 15 mg/L) to the MBSBR system. The results show that the MBSBR system is suitable and efficient for the removal of TC, COD, and BOD_5 from hospital wastewater. The overall removal efficiency of TC, COD, and BOD_5 was 72.8%, 83%, and 93.9%, respectively. Moreover, due to the antimicrobial effect of TC, it was challenging to operate the reactor with high TC concentration, and at a concentration of 15 mg/L TC, only 42.8% of the initial TC concentration was removed. The optimum response of the MBSBR reactor was primarily achieved in the first phase when TC concentration was 5 mg/L, and HRT was 2.5 h. The experimental results indicated that the maximum removal efficiency was 1.8 kg COD/m^2.day as a fitted SLR. The F/M ratio at different HRTs decreased from 0.101 to 0.038 when the HRT increased from 1 to 2.5 hours.

Authors' Contribution

Conceptualization: Roya Mafigholami, Jamal Mehralipour.
Data curation: Majid Jahanshiri.
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Funding acquisition: Roya Mafigholami, Majid Jahanshiri.
Investigation: Majid Jahanshiri, Jamal Mehralipour.
Methodology: Majid Jahanshiri, Jamal Mehralipour, Roya Mafigholami.
Resources: Roya Mafigholami.
Software: Jamal Mehralipour, Mona Hosseini.
Supervision: Roya Mafigholami.
Validation: Jamal Mehralipour.
Visualization: Roya Mafigholami, Jamal Mehralipour.
Writing–original draft: Mona Hosseini.
Writing–review editing: Jamal Mehralipour.

Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical Approval

Not applicable.

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