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Biological treatment of ranitidine and cefixime trihydrate mixed solution by activated sludge in sequencing batch reactor

Soheil Aber[a,*], Leila Ghalamchi[a], Hossein Hosseinzadeh[a], Katayoun Nofouzi[b], Abdolhosein Naseri[c]

[a]*Research Laboratory of Environmental Protection Technology (RLEPT), Department of Applied Chemistry, Faculty of Chemistry, University of Tabriz, Tabriz, Iran, Tel. +98 41 3339 3153; emails: s_aber@tabrizu.ac.ir/soheil_aber@yahoo.com (S. Aber), ghalamchi_leila@yahoo.com (L. Ghalamchi), hosseinzadeh6911@yahoo.com (H. Hosseinzadeh)*
[b]*Department of Pathobiology, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran, email: nofouzikatayoon@gmail.com*
[c]*Department of Analytical Chemistry, Faculty of Chemistry, University of Tabriz, Tabriz, Iran, email: a_naseri@tabrizu.ac.ir*

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ABSTRACT

Hospital wastewater consists of various pharmaceutical compounds. If a proper treatment procedure is not considered, discharging these compounds into the environment can cause serious problems in the aqueous ecosystem, as well as those connected to humans wellbeing. This study aimed to remove ranitidine and cefixime trihydrate drugs simultaneously using adapted activated sludge in a sequencing batch reactor (SBR) and to monitor each drug degradation separately by means of statistical analysis using MATLAB 8.1 software. Total nitrogen, total phosphorus, and chemical oxygen demand analyses were conducted; furthermore, the spectrophotometric spectrum of the solution was recorded as well. The partial least squares analytical method using MATLAB 8.1 software was utilized to analyze the results with the obtained R^2 value of 0.99 for both ranitidine and cefixime. 71.67% of ranitidine and 59.67% of cefixime trihydrate were removed during the biological treatment in the SBR.

Keywords: Antibiotic; Drug; Partial least squares; Sequencing batch reactor; Wastewater

1. Introduction

Pharmaceutical wastewater is usually resistant to biodegradation due to the damage it causes to bacterial DNA or eukaryotic cells. The removal efficiency of pharmaceuticals is often low, depending on the sewage content and the process accomplished in the wastewater treatment plant [1,2]. Pharmaceutical compounds, for example, antibiotics are a source of emerging environmental contaminants. Industry, hospital, and household effluents are three major sources which discharge high amounts of the wastewaters containing various types of antibiotics to the environment [3]. In recent years, the occurrence and the fate of antibiotics in the environment have been an important issue for most researchers [4,5]. The environmental concentration of antibiotics in ng L^{-1} level is usually insignificant; however, they can be considered as emerging pollutants resulting in resistant bacteria and genes in the long term.

To date, lots of articles have been published on the removal of antibiotics in the effluent and sludge of wastewater treatment plants [6–10]. Compared to the physico-chemical elimination of organic compounds, biological treatment approaches, like activated sludge, are considered to have higher advantages, that is, they are economic, produce no toxic intermediates, and have an easy operation with no need for specific chemicals and equipment. In biodegradation processes, bacteria and other microorganisms decompose the carbon-containing substances into harmless or volatile compounds. Chemical treatment processes are sometimes suitable for low concentrations of contaminants,

* Corresponding author.

while biological processes, especially anaerobic ones, are better options for high concentrations [11–13]. The carbon sources for bacteria in the biological processes can be supplied via the oxidation of substrates (i.e., antibiotics or other drugs) [4,14,15]. Sequencing batch reactor (SBR) acts as a different process than the conventional activated sludge [16]. The importance of SBR, as compared with other biological processes, is due to its flexibility, use of a single tank for different treatment stages, and the effective removal of organic matter [17,18]. In the past, SBR technology was typically applied for high-strength wastewater treatment of small communities [19]. In modern technology, because of the higher flexibility and convenient control of SBR, in addition to its application in sewage treatment, it has proven to be effective in dealing with difficult-to-treat organic chemical decontamination [20–22]. Moreover, it is found that the SBR process can be successfully automated, thus saving more than 60% of operating costs for conventional activated sludge processes in a very short aeration time [19,21,23,24].

The partial least squares method (PLS) as a multivariate analysis is used to treat all experimental data. PLS is a linear inverse least squares regression model which has been used extensively in process monitoring and modeling of many noisy and collinear variables [25,26].

PLS analysis as a multivariate calibration method has been used in many applied science fields. Moreover, PLS provides a way for the preparation of inferential models when one has data on a large number of variables and these variables are extremely correlated with each other. PLS provides models with significant predictive capability, and it makes models that are very robust to sensor failure [27]. By not overfitting the data, PLS provides models with good predictive power, and through its highly efficient handling of missing data, it provides inferential models that are extremely robust to sensor failure.

Zwiener and Frimmel [2] reported the monitoring of biological degradation of mixed drugs including clofibric acid, ibuprofen, and diclofenac by GC-MS analysis. Yang et al. [3] represented the adsorption and biodegradation of three mixed sulfonamide antibiothoxazole, sulfadimethoxine, and sulfamonomethoxine in very low concentrations in an SBR using liquid chromatography tandem-mass spectrometry for concentration measurements. Contrary to the mentioned works, the advantage of the present study was the biodegradation of high concentrations of the mixed drugs via the cost-effective spectrophotometric method joint with PLS analysis.

One of the main objectives of this work was to evaluate the removal of mixed drugs from a contaminated solution during the SBR process. Cefixime trihydrate (CEF), as an antibiotic, and ranitidine (RAN), as H2-antagonists, were selected for this purpose. As the second main objective, the PLS technique was used for the multivariate calibration of drug contaminants, and for the easy and simultaneous attainment of the concentration of each drug by spectrophotometric analysis. In order to evaluate the effect of the pharmaceutical contaminants on sludge texture, the identification of bacteria, fungi, and multicellular organisms was performed. Kinetic studies on the removal of each of CEF and RAN in the mixed drug solution were conducted as well. In the present work, a simple and economic spectrophotometrical analysis procedure based on the PLS method was used for the simultaneous measurement of the concentration of each of RAN and CEF, which to the best of our knowledge, is novel and has not been studied yet. In the literature, the total concentration of pollutants is measured, however in the present work, the removal of each drug was separately investigated. Therefore, the ability of activated sludge to degrade each drug can be studied and related to the toxicity and structural properties of the drugs.

2. Materials and methods

2.1. Chemicals and procedures

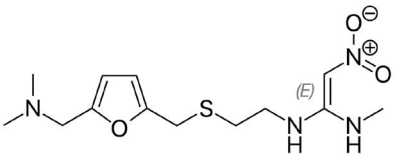
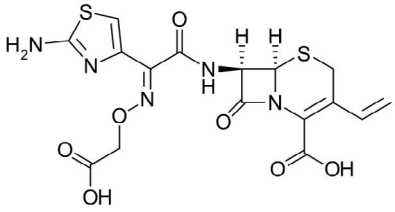
All experiments were performed with the analytical-reagent-grade chemicals. Cefixime trihydrate and ranitidine were purchased from Dana Pharmaceutical Company (Iran). Their respective structures are present in Table 1 [28,29]. A macronutrient solution consisted of glucose, urea, and K_2HPO_4 with the ratio of 100:5:1, and a micronutrient solution composed of several salts, that is, $KHCO_3$ (0.95), $CaCl_2{\cdot}2H_2O$ (0.39), $(NH_4)_2SO_4$ (5.19), ethylenediaminetetraacetic acid (EDTA) (100), $FeSO_4{\cdot}7H_2O$ (9.98), $ZnSO_4{\cdot}7H_2O$ (4.4), $CoCl_2{\cdot}6H_2O$ (3.22), $MnCl_2{\cdot}4H_2O$ (10.12), $CuSO_4{\cdot}5H_2O$ (3.14), $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (3.02) were prepared. All the micronutrient concentrations were expressed as mg L^{-1} in brackets. Chemical oxygen demand (COD) 5220D, total phosphorus (TP) 4500-P, total nitrogen (TN) 4500-N_{org}B, mixed liquor suspended solids (MLSS) 2540D, and mixed liquor volatile suspended solids (MLVSS) 2540E were analyzed using the standard method [30]. The drug removal was followed by a UV-Vis spectrophotometer (T80+, UK) in the range of 190–400 nm.

2.2. Sequencing batch reactor

The removal of CEF and RAN mixed drugs was investigated using the aerated activated sludge in a laboratory-scale SBR. Fig. 1 shows the schematic diagram of the used SBR. The SBR system consisted of a glass container with a volume of 1,000 mL as the reactor, a peristaltic pump, a mechanical stirrer, and an aeration pump. Aeration was provided during the filling and reacting phases using an aeration pump and a porous air diffuser so as to maintain an oxygen concentration level of 2.5 mg O_2 L^{-1}. The peristaltic pump was used to feed the reactor and to recycle the sludge. The mechanical stirrer was employed to keep the wastewater completely homogenous. The temperature was kept constant at approximately 23°C.

The activated sludge from a dairy wastewater treatment plant was used as inoculum and acclimatized with CEF and RAN in batch mode. The adaptation process was performed in five consecutive steps each lasting ca. 48 h. Synthetic wastewater (530.053 mL) composed of macro and micronutrients, CEF, and RAN was mixed with 140 mL of the activated sludge. At the acclimatization steps, the activated sludge volume (140 mL) and the micronutrient solution volume (0.053 mL) were constant, however, the volumes of the macronutrient solution and mixed drug solution (30 mg L^{-1} of each drug) were altered. At each step, the wastewater was aerated for 48 h, then the activated sludge was settled for

Table 1
Chemical structures and properties of RAN and CEF [28,29]

Drug name	Molecular formula	λ_{max} (nm)	M_w (g mol^{-1})	Chemical structure
RAN	$C_{13}H_{22}N_4O_3S$	317	314.4	
CEF	$C_{16}H_{15}N_5O_7S_2$	285	453.444	

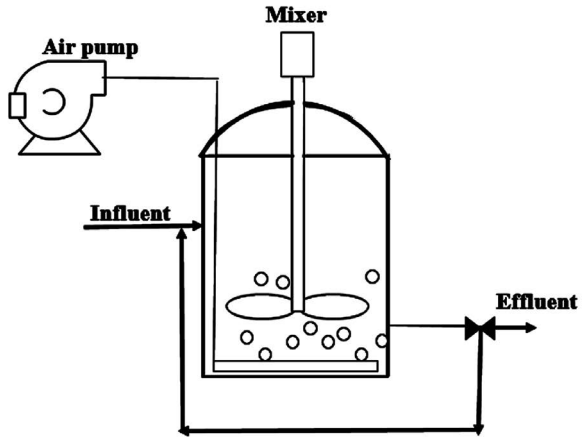


Fig. 1. Schematic of the laboratory SBR for the simultaneous removal of CEF and RAN by activated sludge.

30 min and the clear solution was wasted. At the first acclimatization step, 140 mL of activated sludge, 0.053 mL of the micronutrient solution, 424 mL of the macronutrient solution, and 106 mL of mixed drug solution were mixed in the SBR for the experiment. 318 mL + 212 mL, 212 mL + 318 mL, and 106 mL + 424 mL of macronutrient and mixed drug solutions, respectively were added during the second to fourth steps. In the fifth step, 530 mL of the mixed drug solution and 0.053 mL of the micronutrient solution were poured into the bioreactor without any macronutrient. After the fifth step, the acclimatization process was completed and the system was ready for the mixed drug treatment experiments. In the treatment experiments, the mixed drug solution (530 mL) with the concentration of 30 mg L^{-1} of each drug, was mixed with 0.53 mL of the micronutrient solution and then filled in the SBR system. The biodegradation of the drug solution was monitored for 10 cycles of SBR, each consisting of five consecutive steps of filling (30 min), reacting (660 min), settling (30 min), drawing (30 min), and idling. At the end of each cycle, the effluent was stored and used as the influent at the next cycle. At 12 h intervals, 5 mL of the wastewater was sampled and centrifuged at the rate of 2,500 rpm to remove the remaining suspended solids for COD and spectrophotometrical analyses. Before and after the treatment process, TN, TP, MLSS, MLVSS analyses were examined.

2.3. Microbiological characterization

2.3.1. Isolation of microorganisms

Sludge samples (1 g) were added to 100 mL of enriched culture media, sterile nutrient broth (N.B.) or sabouraud's glucose broth (S.B.), in Erlenmeyer flasks. The flasks were kept on a rotary shaker (Thermo Scientific, USA) at 100 rpm and at room temperature for 24 h. One loopful enriched sample from the N.B. flask was streaked on nutrient agar (N.A.) plates, and one loopful enriched sample from S.B. was streaked on sabouraud's agar (S.A.) plates. N.A. and S.A. plates were incubated for 24 h and 3 d, respectively at room temperature. Plating was done in triplicate for each medium. Isolated colonies were further studied by Gram's staining. Colonies on S.A. (fungi isolate) were separated on the basis of monochrome staining [31].

2.3.2. Gram's staining

The smear was prepared, heat-fixed, and then treated with crystal violet for 1 min. Then a Gram's iodine solution (iodine and potassium iodide) was added to form a complex between the crystal violet and iodine for 1 min. The smear was then washed with water and treated with 95% ethyl alcohol for 10–15 s. It was washed with water again and treated with safranin for 30 s; then it was washed, dried and examined [32].

2.3.3. Analysis of protozoa

Sample drops were microscopically analyzed. Activated sludge wet mounts and smears were prepared in triplicate.

Wet mounts were analyzed at 100× magnification using a compound microscope to determine the morphological characteristics of protozoans and metazoans.

2.4. Statistical analysis

PLS is one of the well-known prediction methods applied to statistically significant orthogonal factors in order to build regression models and determine the linear relationship between the dependent and independent variables [33]. PLS is a linear inverse least squares regression model [34,35] that takes into account changes in the independent variables, only. In general, PLS is an inverse calibration method. PLS is a factor analysis method based on calibration and prediction steps; in the first step a mathematical model is applied by using component concentrations and spectral data from a set of references; this is followed by a prediction step in which the model is evaluated to calculate the concentrations of the unknown samples. The advantage of the use of PLS on spectral data is the high-speed processing of both absorbance and concentration values and magnification of the small differences between the spectral curves to improve the resolution of the spectral overlap [36]. Fig. 2 illustrates the spectra of CEF, RAN, and their mixture without the presence of the activated sludge. Furthermore, by the deletion of baseline drifts, it could minimize the nonspecific matrix interferences [37–39]. Brestrich et al. [32] applied the PLS modeling for the determination of different protein species. They reported that PLS modeling is a promising option for in-line monitoring and control of future chromatography steps at a large scale. In another study, Gómez et al. [33] assessed the Fourier-transform infrared photoacoustic spectroscopy coupled with PLS analysis to predict ash content from the mines of Colombia.

2.4.1. Design of calibration and prediction sets in PLS using full factorial design

The full factorial design (FFD) investigates all possible combinations of the selected factor levels. This technique is very useful for preliminary studies to determine the factor levels, or in primary steps of optimization. FFD fulfills the orthogonality of the columns of the concentration matrix on each other [40]. The factorial design supports evaluating effects caused by independent factors and the interaction between them. The number of experiments is calculated according to the *LK* equation, where *K* is the number of factors and *L* is the factor levels [41]. In common calibration for making a relationship between concentration and absorbance values, several standard solutions with known concentrations (x) are prepared and their absorbance values (y) are recorded; consequently, the mathematical relationship between (x) and (y) is calculated. In this case, there are only one species in the solution. When two or more species are present in the aqueous solution, especially, when their maximum absorbance wavelengths are close to each other, a calibration and a prediction set of data should be introduced to the PLS technique [42]. Here at first, a full factorial design (FFD) (Table 2) of experiments was performed for the two factors (RAN and CEF drugs) and five levels (6, 12, 16, 24, and 30 mg L^{-1}) introducing

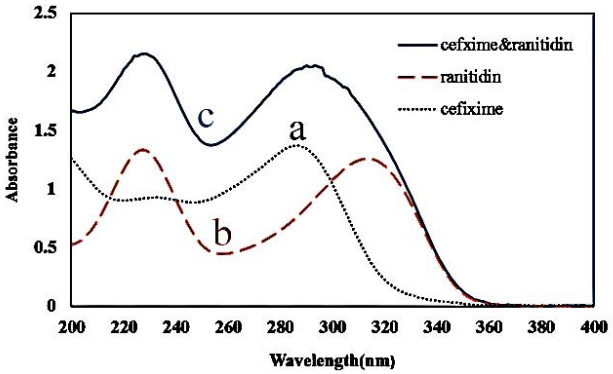


Fig. 2. The absorbance spectra of aqueous (a) CEF solution (30 mg L^{-1}), (b) RAN solution (30 mg L^{-1}), and (c) mixture of CEF and RAN (30 mg L^{-1} each).

25 experiments (52). In each run, the two drugs (CEF and RAN) were mixed and the UV-Vis absorbance of the solution was monitored. In order to investigate the interaction between the drugs, their concentrations were measured simultaneously in the calibration set using the PLS technique. In the present study, the responses of the designed experiments were the absorbance spectrum data. PLS analysis provided a mathematical relationship between response (absorbance) and the corresponding independent factor (drug concentration) to determine the concentration of each drug in the unknown complex matrix of activated sludge. A total of 25 experiments (proposed by FFD) were performed by recording the UV-Vis spectra of the solutions containing different concentrations of the mixed drugs. 20 (80%) of them were used in the calibration set and 5 (20%) in the prediction set of data. These data matrices were entered into the PLS toolbox in MATLAB 8.1 software. For the prediction set, other UV-Vis spectrophotometric spectra were recorded and the accuracy of the results was checked. Finally, the matrix of the absorbance values of the mixed drug solutions with unknown concentrations was used to calculate the concentration of each drug. Therefore, determining the concentration of each drug at different times of the biological treatment process was conveniently done using the PLS method. The root mean square error of prediction (RMSEP) expresses the prediction error (Eq. (1)), and R^2 (Eq. (2)) is an index of the quality of fitting.

$$\mathrm{RMSEP} = \sqrt{\frac{\sum_{i=1}^{n}(\hat{C} - C_i)^2}{n}} \tag{1}$$

$$R^2 = \frac{\sum_{i=1}^{n}(\bar{C} - C_i)^2}{\sum_{i=1}^{n}(C_i - \bar{C})^2} \tag{2}$$

where $\hat{C}$, C_i, $\bar{C}$ and n are the predicted concentration in the sample, the observed value of the concentration in the sample, the mean of the true concentrations in the

prediction set, and the number of samples in the prediction set, respectively [36].

3. Results and discussion

3.1. Reactor performance

In the present study, the removal of two drugs was investigated. In general, there are three main routes to remove organic contaminants by the activated sludge process, that is, volatilization, adsorption, and biodegradation [43]. High molecular weight, the presence of several polar functional groups, and the closed reactor could result in an insignificant removal of the mixed drugs due to volatilization. Sorption experiments using the autoclaved sludge proved that a negligible amount of COD removal was related to the adsorption. As can be seen in Fig. 3, after 12 h, only 4.2% of COD removal was seen. According to Fig. 4, after 12 h only 4.1% of RAN and 3.9% of CEF were removed. Each drug is readily degraded through the hydrolytic cleavage, and they are ultimately mineralized by microorganisms to CO_2 and H_2O [44]. The elimination of organic compounds by the activated sludge process depends on the activity of the complex population of heterotrophic organisms utilizing either oxygen or nitrate as the electron acceptor during their metabolic reactions. Bacteria in activated sludge degrade the organic substrates into low molecular-weight and small intermediates by extracellular hydrolysis. Bacteria assimilate the intermediates by their cells and use them as the source of carbon and energy. In fact, the bacteria in the mixed liquor can utilize pharmaceutical contaminants as the sole source of carbon and nitrogen [45,46]. The concentrations of CEF and RAN started to be nearly constant after 60 h of SBR operation, however, because of the toxic nature of CEF, its removal efficiency (59.67%) after 108 h was lower than that of ranitidine (68.3%) after the same contact time of 108 h. The results of MLSS, MLVSS, SVI, TN, and TP measurements in the mixed drug solution before and after biodegradation are shown in Table 3. As can be seen in Table 3, the incomplete removal of nutrients (TN and TP) may be due to the unavailability of a suitable condition for denitrification and phosphorus removal. The increase of MLSS and MLVSS can be due to the compatibility of the microorganisms with the wastewater. The obtained results confirmed the potential of the SBR system in the removal of pharmaceutical contaminants, which is in agreement with the previous studies. For example, Drillia et al. [47] investigated the sulfamethoxazole antibiotic in an SBR system. They succeeded in the complete removal of 20 mg L^{-1} of sulfamethoxazole in the SBR system with MLSS of 4,200 mg L^{-1} after 5 d. Lefebvre et al. [48] reported that SBR can treat a low organic loading rate of wastewater generated by an antibiotics company. Londoño and Peñuela [49] represented the ibuprofen and methylparaben removal in SBR. They reported that the ibuprofen removal was lower than 51% while a methylparaben removal of more than 97% was witnessed.

3.2. PLS analysis

Table 4 presents the RMSEP and R^2 values of CEF and RAN for the validation of the PLS multivariate calibration method. According to the R^2 value, a good agreement between experimental and predicted data was achieved. Fig. 5 shows the graphical RMSEP by means of an elliptical joint confidence region calculated to evaluate the slope and intercept of the reference regression and to predict values at a 95% confidence, residual values, and root mean square. This figure confirms the validity of the selected PLS model. In this figure, the predicted results from the 5 prediction series experiments were considered. In Fig. $5a_1$, the predicted concentrations against the real ones have been drawn. The badges were fitted with a straight line with a slope of 1 and an intercept of 0 which represents the validity of the model. In Fig. $5a_2$, the presence of the "+" sign in the confidence ellipse indicates the meaningful slope and non-meaningful intercept which proves the model validation. According to Fig. $5a_3$, two latent variables are detected by the model. Moreover, the random distribution of residual values against sample numbers is another proof of model validation (Fig. $5a_4$).

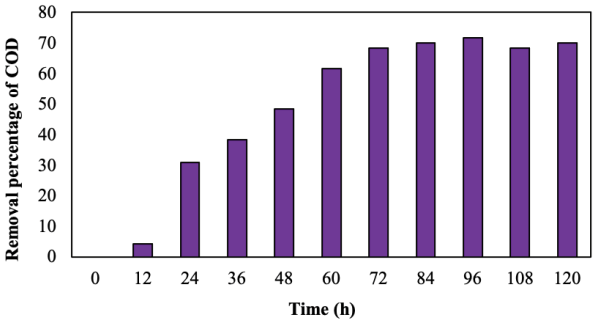


Fig. 3. COD removal in a mixed drug solution containing CEF and RAN by activated sludge in an SBR; drug concentration of 30 mg L^{-1} each, pH_0 = 7.17, $MLSS_0$ = 1,540 mg L^{-1}, DO = 2.5 mg L^{-1}.

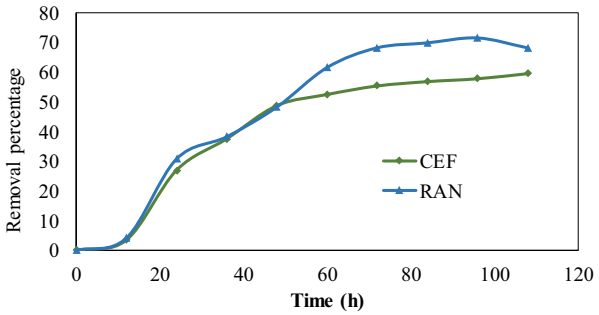


Fig. 4. The removal percentage of CEF and RAN via activated sludge in an SBR; initial concentration of each drug = 30 mg L^{-1}, pH_0 = 7.17, $MLSS_0$ = 1,540 mg L^{-1}, DO = 2.5 mg L^{-1}.

3.3. Microbial community

An important factor in biological processes is the nature of the microbial population. The activated sludge contains a variety of microorganisms, for example, viruses,

Table 2
Full factorial design of CEF and RAN concentrations in solutions for the preparation of multivariate calibration and prediction sets of data for PLS. The PLS applied for the measurement of CEF and RAN concentrations in an SBR used activated sludge for their simultaneous removal

Row	CEF concentration (mg L^{-1})	RAN concentration (mg L^{-1})	Application step in the PLS method
1	6	6	Calibration set
2	12	6	Calibration set
3	16	6	Prediction set
4	24	6	Calibration set
5	30	6	Calibration set
6	6	12	Calibration set
7	12	12	Prediction set sample
8	16	12	Calibration set
9	24	12	Calibration set
10	30	12	Calibration set
11	6	16	Prediction set
12	12	16	Calibration set
13	16	16	Calibration set
14	24	16	Calibration set
15	30	16	Calibration set
16	6	24	Calibration set
17	12	24	Calibration set
18	16	24	Calibration set
19	24	24	Calibration set
20	30	24	Calibration set
21	6	30	Calibration set
22	12	30	Prediction set
23	16	30	Calibration set
24	24	30	Prediction set
25	30	30	Calibration set

bacteria, fungi, algae, protozoa, and worms; so it is a rich source of organic matter [50]. Fungi, bacteria and protozoa isolates were obtained from the sludge. Gram type of the bacteria was determined and microscopic observation and other characterizations of sludge isolates were carried out (Table 5). Figs. 6 and 7 show the presence of both gram-positive and gram-negative bacteria, fungi, and rotifers in the activated sludge before and after adaptation to the drugs. The results confirmed that despite the presence of antibacterial agents and the toxic contents in the wastewater, multi-cellular microorganisms, fungi, and bacteria were present after the adaptation stage, showing its important role in developing the useful and effective microorganism [51]. The presence of rotifers in the activated sludge (Figs. 7c and d), after the adaptation stage, indicated the sustainability of the activated sludge [52]. According to the literature, the presence of rotifers causes the reduction of filamentous bacteria and thus, avoiding balking phenomena. So a better clarification of activated sludge occurs [52]. Due to the complex matrix of the activated sludge, it

Table 3
Conditions of the mixed liquor containing CEF and RAN (30 mg L^{-1} each) in an SBR system before and after their treatment by activated sludge

Parameters	Time = 0 h	Time = 108 h
pH	7.17	7.50
Temperature (°C)	26	26
SVI (mL g^{-1})	50	75
MLSS (mg L^{-1})	1,540.0 (±12.4)	2,000.0 (±23.7)
MLVSS (mg L^{-1})	640.0 (±20.1)	1,040.0 (±13.7)
DO (mg L^{-1})	2.5	2.5
TP (mg L^{-1})	0.069 (±0.007)	0.012 (±0.010)
TN (mg L^{-1})	3.50 (±0.06)	2.30 (±0.04)
EC (mS)	1.33	3.33

Table 4
Validation of multivariate calibration by PLS method for the simultaneous removal of CEF and RAN (30 mg L^{-1} each) in an SBR using activated sludge; pH_0 = 7.17, $MLSS_0$ = 1,540 mg L^{-1}, DO = 2.5 mg L^{-1}

Drug	Calibration data set		Validation data set	
	RMSEP (mg L^{-1})	R^2	RMSEP (mg L^{-1})	R^2
CEF	0.2523	0.9957	0.2476	0.9498
RAN	0.2583	0.9955	0.1251	0.9993

was not exactly clear which microorganisms removed CEF and RAN, and therefore, the separate contribution of each microorganism remained unknown. Fungal biomass can be used for the removal of organic materials in the sludge. Fungi can be applied for simultaneous bioflocculation, solid and pathogen reduction, and the removal and degradation of toxic compounds [50]. However, the alkaline solution decreases fungi growth and as a result, the fungal treatment. As observed in this study (Table 3) at the end of the experiments the solution pH increases resulting in a decline in the role of fungi in the treatment of drug solution.

3.4. Kinetics of biodegradation

In the present work, pseudo-zero, first, and second-order models were applied to investigate the kinetics of drug removal during the SBR process (Eqs. (3)–(8)).

$$\text{Pseudo-zero-order kinetics: } \frac{dc}{dt} = -k_0 \tag{3}$$

$$\text{Integrating} \rightarrow c_t = c_0 - k_0 t \tag{4}$$

$$\text{Pseudo-first-order kinetics: } \frac{dc}{dt} = -k_1 c \tag{5}$$

$$\text{Integrating} \rightarrow c_t = c_0 e^{-k_1 t} \tag{6}$$

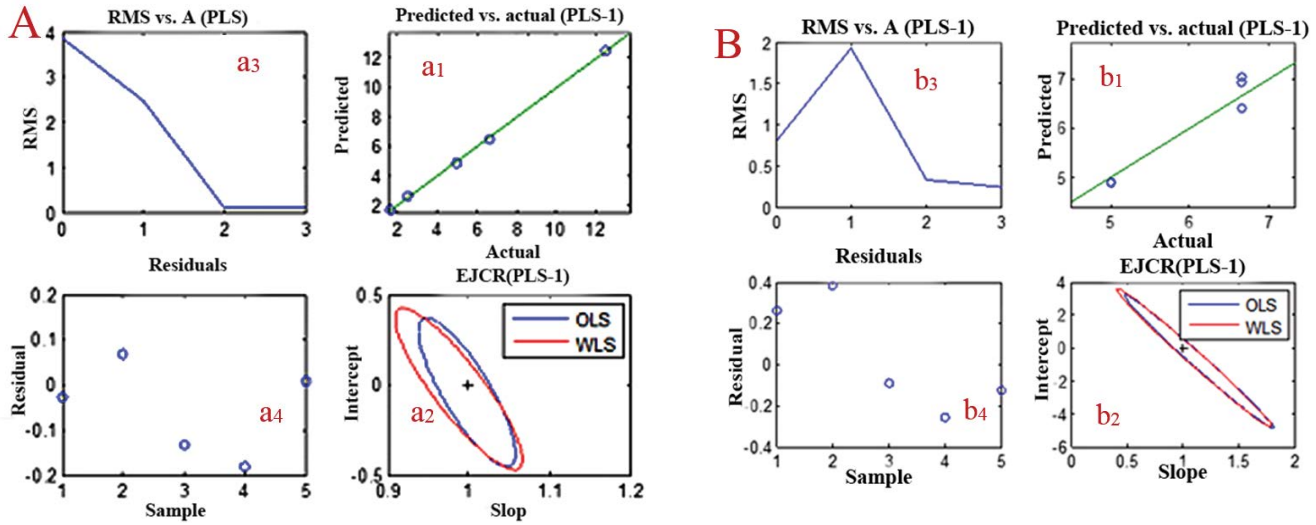


Fig. 5. Graphical form of RMSEP validation data for the monitoring of simultaneous removal of RAN (A) and CEF (B) (30 mg L^{-1} each) by activated sludge in an SBR.

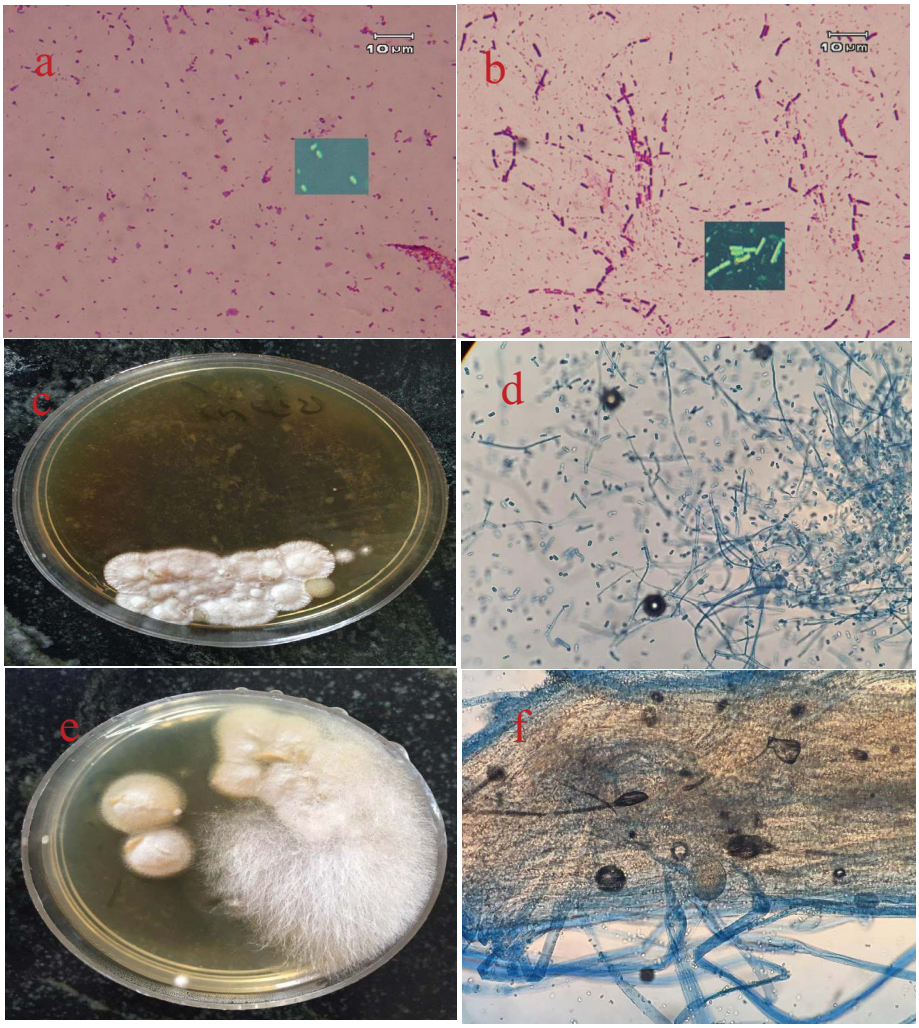


Fig. 6. Bacteria and fungi detection in activated sludge after adaptation with mixed CEF and RAN: (a and b) bacteria, (c–f) fungi in SBR.

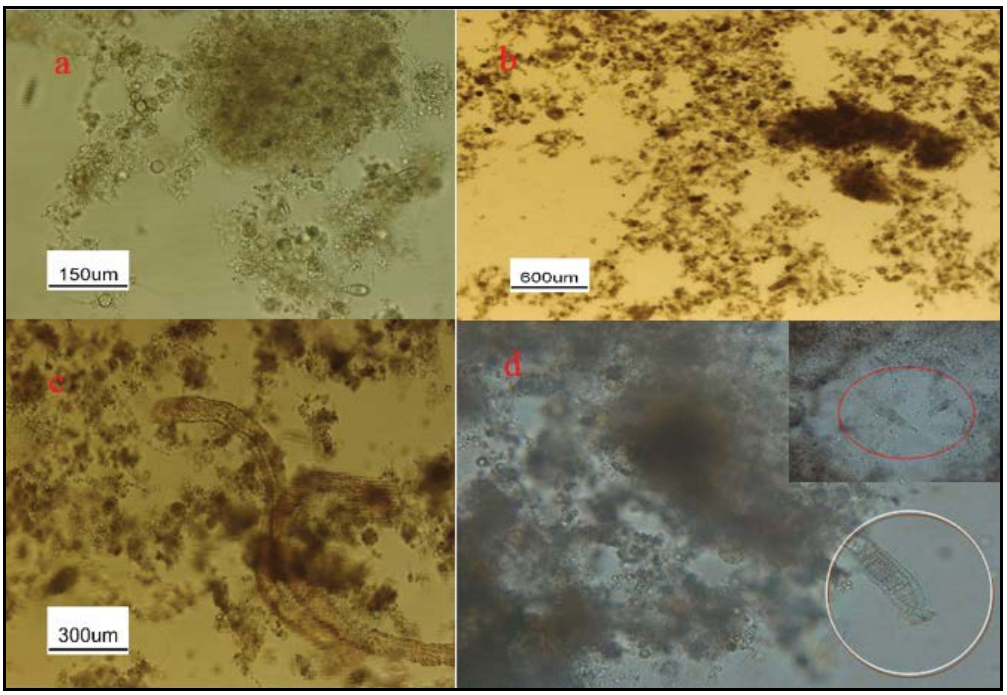


Fig. 7. Microscopic images of activated sludge: (a and b) before, (c and d) after adaptation with CEF and RAN.

Table 5
An outline of the microorganisms isolated from the activated sludge used for the simultaneous removal of CEF and RAN (30 mg L^{-1} each) in an SBR; pH_0 = 7.17, $MLSS_0$ = 1,540 mg L^{-1}, DO = 2.5 mg L^{-1}

Type of microorganism	Bacteria	Fungi	Protozoa
Escherichia coli	+		
Bacillus cereus	+		
Geotrichum		+	
Mucur		+	
Rotifer			+
Cryptosporidium			+

Pseudo-second-order kinetics: $\frac{dc}{dt} = -k_2 c^2$ (7)

Integrating $\rightarrow \frac{1}{c_t} = \frac{1}{c_0} + k_2 t$ (8)

where c is the concentration of the drug, t is the time, k_0, k_1, and k_2 represent the apparent rate constants of pseudo-zero, first, and second-order reaction kinetics, respectively [53,54]. Table 6 illustrates the regression analysis results based on the kinetic study of the removal of mixed CEF and RAN in the SBR. R^2 values showed that the removal of both CEF and RAN followed the pseudo-second-order kinetics with 0.9417 and 0.9582, respectively; significantly more than the other R^2 values obtained for the pseudo-zero and first-order models. Ong et al. [55] represented that decreasing the COD of Orange II in SBR obeyed the first-order kinetics. Also, Durai et al. [56] proved that the kinetics of biodegradation of tannery wastewater in SBR was first-order.

Table 6
Kinetic study results of the simultaneous removal of CEF and RAN in a solution containing 30 mg L^{-1} of each drug by activated sludge in an SBR; pH_0 = 7.17, $MLSS_0$ = 1,540 mg L^{-1}, DO = 2.5 mg L^{-1}

Pollutants	R^2			k		
	Zero-order	First-order	Second-order	Zero-order	First-order	Second-order
CEF	0.8374	0.8968	0.9417	0.170	0.009	0.0005
RAN	0.8714	0.9283	0.9582	0.231	0.016	0.0013

4. Conclusion

According to the obtained results, acclimated activated sludge in an SBR is able to successfully degrade mixed CEF and RAN drugs. The PLS analysis has the potential of determining the concentration of each of the drugs in the complex matrix of the mixed liquor used in the activated sludge process. Despite the antibacterial property and the toxicity of CEF, some protozoa, fungi, and bacteria have the ability to acclimatize themselves to the drug and continue thriving. The acclimatized activated sludge removes, rather unselectively, the studied drugs with a pseudo-second-order reaction model.

Nomenqular

ASP	—	Activated Sludge Process
CAS	—	Conventional Activated Sludge
CEF	—	Cefixime trihydrate
COD	—	Chemical Oxygen Demand
EJCR	—	Elliptical Joint Confidence Region
FFD	—	Full Factorial Design
MLSS	—	Mixed Liquor Suspended Solids
MLVSS	—	Mixed Liquor Volatile Suspended Solids
NA	—	Nutrient Agar
NB	—	Nutrient Broth
PLS	—	Partial Least Squares
RAN	—	Ranitidine
RMSEP	—	Root Mean Square Error of Prediction
SA	—	Sabouraud's Agar
SB	—	Sabouraud's Glucose Broth
SBR	—	Sequencing Batch Reactor
SVI	—	Sludge Volume Index
TN	—	Total Nitrogen
TP	—	Total Phosphorus

Symbols

C_i	—	Observed value of the concentration in the sample
$\hat{C}$	—	Predicted concentration
$\overline{C}$	—	Mean of the true concentrations in the prediction set
n	—	Number of prediction samples
R^2	—	Regression coefficients
k_0	—	Pseudo-zero-order reaction kinetics
k_1	—	Pseudo-first-order reaction kinetics
k_2	—	Pseudo-second-order reaction kinetics

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