

Removal of azithromycin from wastewater using advanced oxidation processes (UV/H₂O₂) and moving-bed biofilm reactor (MBBR) by the response surface methodology (RSM)

Rouhollah Shokri¹, Reza Jalilzadeh Yengejeh¹, Ali Akbar Babaei^{1,2,✉}, Ehsan Derikvand³, Ali Almasi⁴

1. Department of Environmental Engineering, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran
2. Department of Environmental Health Engineering, School of Public Health, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
3. Department of Water Science, Shoushtar Branch, Islamic Azad University, Shoushtar, Iran
4. Department of Environmental Health Engineering, School of Public Health, Social Development and Health Promotion Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran

Date of submission: 20 Oct 2019, *Date of acceptance:* 08 Dec 2019

ABSTRACT

Antibiotics are among the major concerns in terms of environmental control due to their cumulative properties, adverse health effects on humans, and development of drug resistance. The present study aimed to investigate the efficiency of the combination of UV/H₂O₂ and moving-bed biofilm reactor (MBBR) systems in the removal of azithromycin from aqueous solutions using the response surface methodology (RSM). In the UV/H₂O₂ process, a low-pressure mercury vapor lamp with the power of eight Watts, wavelength of 254 nanometers, and intensity of 1.02 mw/cm² was used to determine the effects of pH, azithromycin concentration, hydrogen peroxide concentration, and contact time on the removal efficiency of azithromycin. According to the obtained results, the highest removal efficiency in the UV/H₂O₂ process was obtained with the azithromycin concentration of 2 mg/l. Therefore, 2 mg/l of azithromycin was selected as the optimal concentration with the highest removal efficiency. Following that, the optimal concentration of azithromycin was injected into the MBBR reactor. In the combined process of UV/H₂O₂ and MBBR, the highest removal efficiency of azithromycin was 91.2%. Therefore, it could be concluded that the combined system of UV/H₂O₂ and MBBR had the highest efficiency in the removal of azithromycin from aqueous solutions.

Keywords: Advanced Oxidation Processes, Moving Bed Biofilm Reactor, Antibiotic Azithromycin, Response Surface Methodology

Introduction

Numerous antibiotics enter aqueous and soil environments when inappropriately disposed.¹ Antibiotics are not completely metabolized in the body, and 30-90% of these agents remain active when excreted.² According to the United States Environmental Protection Agency (USEPA), the acceptable standard level of antibiotics in wastewater is 1 mg/l.³ Many researchers have been searching for proper

methods for the treatment of the wastewater containing these medications.⁴

Physicochemical and biological methods are the main processes used for wastewater treatment. Biological processes are inexpensive, while they are not effective in the removal of resistant organic matters from wastewater.⁵ On the other hand, high efficiency and high quality of wastewater could be achieved using physicochemical processes although they are costly.⁶ Therefore, it is not possible to use physicochemical or biological methods alone for the treatment of high-concentration wastewater.

Several biofilm systems are available for the biological processes of wastewater treatment, including trickling filters, rotating

✉ Ali Akbar Babaei
aababaei52@gmail.com

Citation: Shokri R, Yengejeh J, Babaei AA, Derikvand E, Almasi A. Removal of azithromycin from wastewater using advanced oxidation processes (UV/H₂O₂) and moving-bed biofilm reactor (MBBR) by the response surface methodology (RSM). J Adv Environ Health Res 2019; 7(4): 249-259

biological contactors, fixed-bed submerged biofilters, granular bed biofilters, and fluidized bed reactors, all of which have certain advantages and disadvantages.⁷

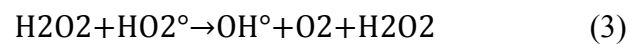
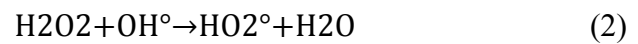
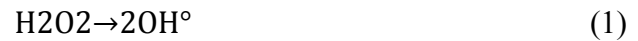
In Norway, the moving-bed biofilm reactor (MBBR) system was developed for wastewater treatment 30 years ago. In this system, the moving-bed reactor provides a surface for the growth of microorganisms.^{8, 9} The main advantage of MBBR is the ability to accumulate biomass and biofilm in the reactor, which allows the presence of a large number of microorganisms in the system.¹⁰

In general, advanced oxidation processes are the most effective technologies for the decomposition and removal of hazardous, resistant, and biologically non-degradable organic pollutants from aqueous environments. Such examples are ultraviolet (UV) activation with hydrogen peroxide,¹¹ photocatalysis with activated-carbon impregnated magnetite composite,^{12, 13} cane bagasse adsorption,¹⁴ photocatalytic degradation based on sulfate radicals,¹⁵ photo-Fenton oxidation of pharmaceutical wastewater,¹⁶ and the photo-electro-Fenton process.¹⁷

Some of the advantages of advanced oxidation processes include rapid reaction rates, small footprint, potential to reduce toxicity and possibly complete the mineralization of the treated organics, no concentrating of waste for further treatment with other methods (e.g., membranes, not producing materials that require further treatment (e.g., spent carbon from activated carbon absorption, and no sludge production as with physical, chemical, and biological processes (wasted biological sludge). On the other hand, the limitations of advanced oxidation processes include capital intensity, complex chemistry to be tailored to specific applications, and required quenching of excess peroxide for some applications.¹⁸

In the past decades, advanced oxidation processes have been widely used, achieving a dominant position in the treatment of water and wastewater. The main mechanism involved in these processes is the production of hydroxyl radicals, which are able to oxidize most organic compounds promptly and in a non-selective

manner.¹⁹ The most common mechanism for the photolysis of hydrogen peroxide is O=O bond degradation through the action of UV light and formation of two hydroxyl (OH⁰) radicals, as Eq. 1 to Eq. 4:



The occurrence of these reactions in an environment containing organic pollutants leads to the formation and degradation of the radicals.²⁰ For instance, the ozone-based oxidation process causes the bromide to be decomposed into bromate ions, which are suspected of carcinogenicity²¹ and require the purification of the exhaust gases and floatation of volatile organic carbon. However, the replacement of ozone with the UV rays in the UV/H₂O₂ process leads to the elimination of organic pollutants, resolving the issue.²² However, biological systems alone are not sufficient to remove resistant organic pollutants. As such, resistant organic compounds may remain intact when leaving the treatment plant, and combined physicochemical and biological methods are essential to the effective treatment.²³

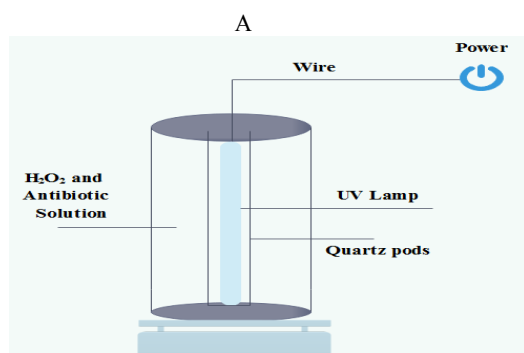
Response surface methodology (RSM) is known as an effective statistical method for the optimization of the parameters of various processes and assessment of the fitting of a model. In this study, we used the RSM to identify the independent variable and determine whether its interaction elicits the desired response.²⁴ The present study aimed to determine the efficiency of the combined systems of advanced oxidation process (UV/H₂O₂) and MBBR in the removal of azithromycin from aqueous solutions.

Materials and Methods

UV/H₂O₂ process

The experiments were conducted in a 500-cc Pyrex glass equipped with a low-pressure

mercury vapor lamp (Phillips, Germany) with the power of eight Watts, wavelength of 254 nanometers, and intensity of 1.02 mw/cm^2 . The pH of the system was adjusted using the caustic soda and 1N sulfuric acid.¹¹ Afterwards, sampling was performed from the UV/H₂O₂ reactor. To measure azithromycin concentration, the 10-cc sample received one cc of 0.01 M potassium permanganate solution and one cc of 0.1 M potassium carbonate solution. Following that, the mixture was properly stirred and brought to the required volume with distilled water. Finally, the absorbance was read via UV/Vis spectrophotometry (UV2100, Unico, US) at the wavelength of 547 nanometers. After determining the optimum conditions through the UV/H₂O₂ process, the optimum concentration of azithromycin was injected into the MBBR reactor.^{25, 26} Fig. 1-A depicts the pilot schematic.



MBBR on a pilot scale

In the present study, a 3.92-liter MBBR reactor was used on the laboratory scale. The reactor was composed of glass with an inner diameter of 10 centimeters, height of 50 centimeters, and effective volume of 3.14 liters. Air was required to provide the dissolved oxygen and flow the media in the entire reactor volume entering the reactor, which was performed using an air compressor with two air diffusers located on the floor. In order to prevent the media from exiting, a mesh sheet was installed at the reactor outlet. In addition, the polyethylene (PE) kaldnes media (K1) (Pakan Ghatreh, Iran) were used as a substrate with the specific gravity of 0.96 g/cm^3 and specific surface area of $500 \text{ m}^2/\text{m}^3$ in order to determine the efficacy of the MBBR reactor in the removal of azithromycin. Fig. 1-B shows the pilot schematic.

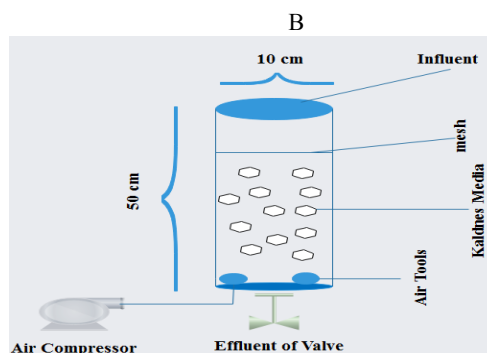


Fig. 1 Pilot schematic A) UV/H₂O₂ and B) MBBR reactor

MBBR reactor setup

For the initial commissioning of the reactor, 20% of the reactor volume was filled with the kaldnes media. Following that, more than one-third of the reactor volume was filled with the return sludge from the secondary sedimentation tank in the wastewater treatment plant at Valiasr Hospital in Khorramshahr, Iran; afterwards, the reactor was put into operation through the batch process. Meanwhile, the reactor was fed with glucose and major nutrients. In addition to providing the dissolved oxygen content (2-3 mg/l), the incoming air caused the media to rotate in the reactor, and the pH remained constant (7.2) throughout this stage. In the current research, the influential

factors in the activity of microorganisms were continuously evaluated, including temperature, dissolved oxygen, pH, and other parameters. Twelve weeks after commissioning the reactor, biofilm growth was observed on the kaldnes media, and the reactor was used through the batch process afterwards in order to assess the rate of azithromycin removal in different conditions. After determining the optimal removal conditions through the UV/H₂O₂ process, the experiments continued to evaluate the combined efficacy of the UV/H₂O₂ process and MBBR reactor.

To sample the MBBR reactor, the samples were initially passed through the Whatman filter paper and filtered using a 0.5- μm pipette tip

filter. Following that, the samples were centrifuged at 250 rpm for five minutes to be homogenized and to separate total suspended solids from the solution. Finally, the concentration of azithromycin was read via UV/Vis spectrophotometry (UV2100, Unico, US) at the wavelength of 547 nanometers. The obtained results were analyzed in the Design Expert software using the analysis of variance (ANOVA).²⁵

Experimental design

The effects of mixed liquor suspended solids (MLSS) concentrations, hydraulic retention time (HRT), and packing rate on the efficiency of MBBR in the removal of azithromycin were investigated and expressed in percentages using the Design Expert software (Table 1). The sample size of the study was calculated using the RSM method and central composition design (CC.D), and the experimental runs were designed using the Design Expert software (StatEase Inc., version 6.0). In addition, removal efficiency was selected as the response in the test setup of the UV/H₂O₂ process and MBBR reactors.

Table 1. Variables and study range based on response surface methodology (RSM)

Coded level				
Factors	Symbol	-1	0	+1
MLSS concentration (mg/l)	A	1000	2000	3000
HRT (hr)	B	4	8	12
Packing rate (%)	C	20	40	60

Statistical analysis

The model was selected after the experiments and entering the response values into the test design table. ANOVA was applied to analyze the data, where the lack of fit, P-value, f-value, and R² were determined. The R² correlation-coefficient indicated that the model data were close to the laboratory data; as the data became closer to the unity of the R² value, the selective model was considered to be more valid.²⁷

Based on the CCD, Eq. 5 for azithromycin showing the experimental relationship of the coded variables in the MBBR system was presented in the form of the 2FI model, which was obtained after eliminating the ineffective variables and their interactions.

$$Y = +47.68 +9.38 A +6.19 B +21.09 C +2.18 AB +6.02 A^2 \quad (5)$$

In the Eq. 5, *Y* is the antibiotic removal efficiency (%), *A* represents the MLSS concentration (mg/l), *B* shows the HRT (hour), and *C* is the packing rate (%).

Results and Discussion

Pollution Assessment

Based on Eq. 5 for azithromycin, factor *C* had the most significant impact on the removal efficiency, while factor *AB* exerted the least significant impact. Furthermore, the least significant effect among the three main study variables was observed in the HRT. The positive and negative signs also indicated the direct and inverse correlation between the studied parameters and response, respectively.²⁸

In the present study, the three terms of single effects (*A*, *B*, and *C*) and two terms of double effects (*AB* and *A*²) were considered significant (*P*<0.0500). Table 2 shows the antibiotic removal efficiency values based on the RSM. Table 3 shows the results of linear regression analysis for the 2FI model in the response surface methodology for the removal of azithromycin using the MBBR system. Table 4 shows the results of ANOVA for the 2FI model regarding the removal of azithromycin using the MBBR system.

Table 2. Antibiotic removal efficiency values based on RSM

Run	MLSS (mg/l)	HRT (hr)	Packing rate (%)	Removal efficiency azithromycin (%)
1	3000.00	12.00	20.00	50.2
2	3000.00	12.00	60.00	91.2
3	3000.00	8.00	40.00	64.3
4	2000.00	4.00	40.00	38.8
5	2000.00	8.00	40.00	46.3
6	2000.00	12.00	40.00	54.7
7	3000.00	4.00	60.00	79.5
8	2000.00	8.00	40.00	48.5
9	1000.00	8.00	40.00	40.1
10	2000.00	8.00	40.00	46.5
11	1000.00	12.00	20.00	28.6
12	3000.00	4.00	20.00	30.2
13	1000.00	12.00	60.00	69.3
14	2000.00	8.00	40.00	48.7
15	2000.00	8.00	60.00	71.3
16	2000.00	8.00	20.00	28.2
17	1000.00	4.00	60.00	60.2
18	1000.00	4.00	20.00	23.4
19	2000.00	8.00	40.00	46.1

Table 3. Results of linear regression analysis for 2FI model in RSM for azithromycin removal using MBBR system

Model	Prob > F	R ²	Adj.R ²	Predict.R ²	Adeq. precesion	Std. Dev.	C.V. %
2FI	0.0001	0.9847	0.9789	0.9535	49.125	2.66	5.22

Table 4. ANOVA results on 2FI model for removal of azithromycin by MBBR system

Response	ANOVA					
	Source	Sum of Squares	DF	Mean square	F value	P value
Azithromycin Removal (%)	Model	5920.52	5	1184.10	167.86	< 0.0001
	A-MLSS	879.84	1	879.84	124.73	< 0.0001
	B-HRT	383.16	1	383.16	54.32	< 0.0001
	C-Packing Rate	4447.88	1	4447.88	630.53	< 0.0001
	AB	37.85	1	37.85	5.36	0.0375
	A ²	171.79	1	171.79	24.35	0.0003
	Residual	91.70	13	7.05	-	-
	Lack of Fit	85.26	9	9.47	5.88	0.0518
	Pure Error	6.45	4	1.61	-	-
	Cor Total	6012.23	18	-	-	-

Effect of the UV/H₂O₂ process

To investigate the removal efficiency of azithromycin using the combined UV/H₂O₂ process and MBBR based on the RSM and CCD, the effects of the initial antibiotic concentration, pH, contact time, and H₂O₂ concentration on the UV/H₂O₂ process were assessed. According to the findings, the highest removal efficiency was obtained at the azithromycin concentration of 2 mg/l in the acidic media; therefore, this concentration was selected as the optimum concentration.

Accordingly, among the four study variables, the initial antibiotic concentration has the highest effect on the process efficiency, so that increasing the initial concentration of antibiotics would result in an inverse effect on the removal efficiency for azithromycin through the UV/H₂O₂ process. In addition, increasing the initial concentration was associated with reduced removal efficiency.

According to the results of the present study, higher antibiotic concentration was associated with the increased substance under radiation, which required more time to perform the process, and the second material acted as a filter. As a result, the penetration of UV radiation decreased, and the increasing trend of the process velocity increased with the trend of the increased substance concentration (antibiotic) as two opposite points.^{29, 30} In a

similar research, Belghadr *et al.* investigated the removal of cefixime from aqueous solutions using the advanced oxidation process (UV/H₂O₂) and obtained consistent results, so that the removal efficiency reduced at higher antibiotic concentrations.³¹

Effects of MLSS concentration

The results of this phase of the experiments are presented in Table 2 and Fig. 2. At this stage, the effects of MLSS concentration (1,000, 2,000, and 3,000 mg/l) on the removal efficiency of azithromycin were investigated. According to the findings, higher concentration of MLSS was associated with the increased removal efficiency of azithromycin. As is depicted in Fig 2, at the MLSS concentration of 1,000 mg/l, HRT of four, eight, and 12 hours, and packing rate of 20%, 40%, and 60%, the removal efficiency of azithromycin was 27.58%, 44.32%, and 61.05%, respectively. At the MLSS concentration of 2,000 mg/l, the removal rate was estimated at 29.49%, 47.67%, and 65.86%, while at the MLSS concentration of 3,000 mg/l, the removal efficiency of azithromycin was 43.44%, 63.08%, and 82.71%, respectively. According to the results of the present study, the highest removal efficiency of azithromycin was 82.71% at the MLSS concentration of 3,000 mg/l. Therefore, this MLSS concentration was selected as the

optimum concentration.

The high system efficiency could be attributed to biofilm growth and increased number of microorganisms.²¹ In a research in this regard, Shokoohi *et al.* investigated the removal of LAS anion detergent from hospital

wastewater using the MBBR (determination of the removal model based on RSM), proposing similar results; accordingly, the removal efficiency increased at higher MLSS concentrations.³²

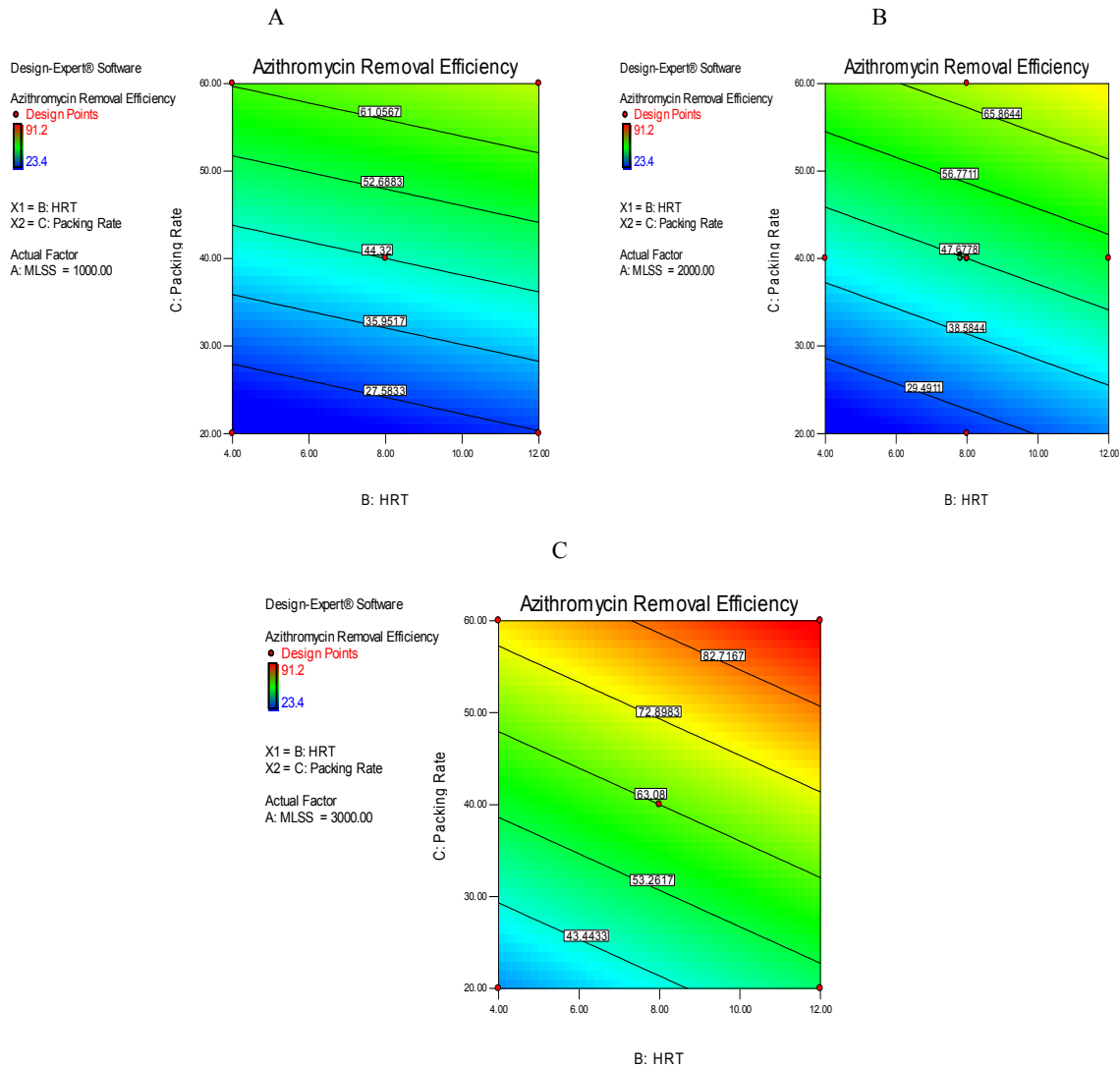


Fig. 2. Removal efficiency of azithromycin at MLSS concentration of A) 1,000 mg/l, B) 2,000 mg/l, and C) 3,000 mg/l using combined system of UV/H₂O₂ process and MBBR reactor

Effect of HRT

The results of this phase of the experiments are presented in Table 2 and Fig. 3. At this stage, the effect of HRT (4, 8, and 12 hours) on the removal efficacy of azithromycin was investigated. According to the findings, increased HRT was associated with the higher removal efficiency of azithromycin, which could be attributed to the increased contact time

between the enzymes that were secreted from the biofilms and azithromycin, which ultimately results in the increased biodegradability of azithromycin.³³ As is shown in Fig. 3, at the HRT of four hours, MLSS concentrations of 1,000, 2,000, and 3,000 mg/l, and packing rate of 20%, 40%, and 60%, the removal efficiency of azithromycin was 27.83%, 47.02%, and 66.21%. At the HRT of eight hours, the removal

efficiency of azithromycin was 33.14%, 53.55%, and 73.96%, and at the HRT of 12 hours, the removal efficiency of azithromycin was 38.11%, 59.88%, and 81.65%, respectively. Therefore, the highest removal efficiency of azithromycin was 81.65% at the HRT of 12 hours. Therefore, the HRT of 12 hours was selected as the optimum HRT.

In another study, Golshahi *et al.* investigated the efficiency of the MBBR system in reducing the municipal wastewater COD

compared to the conventional activated sludge systems, proposing similar results. Accordingly, increased HRT was associated with the higher removal efficiency of municipal wastewater COD. Furthermore, the efficiency of the system in the removal of COD was close at both the eight-hour and 12-hour HRT. Therefore, due to the higher energy consumption saving of aerators or mixers, the eight-hour HRT was selected as the optimum retention time.³⁴

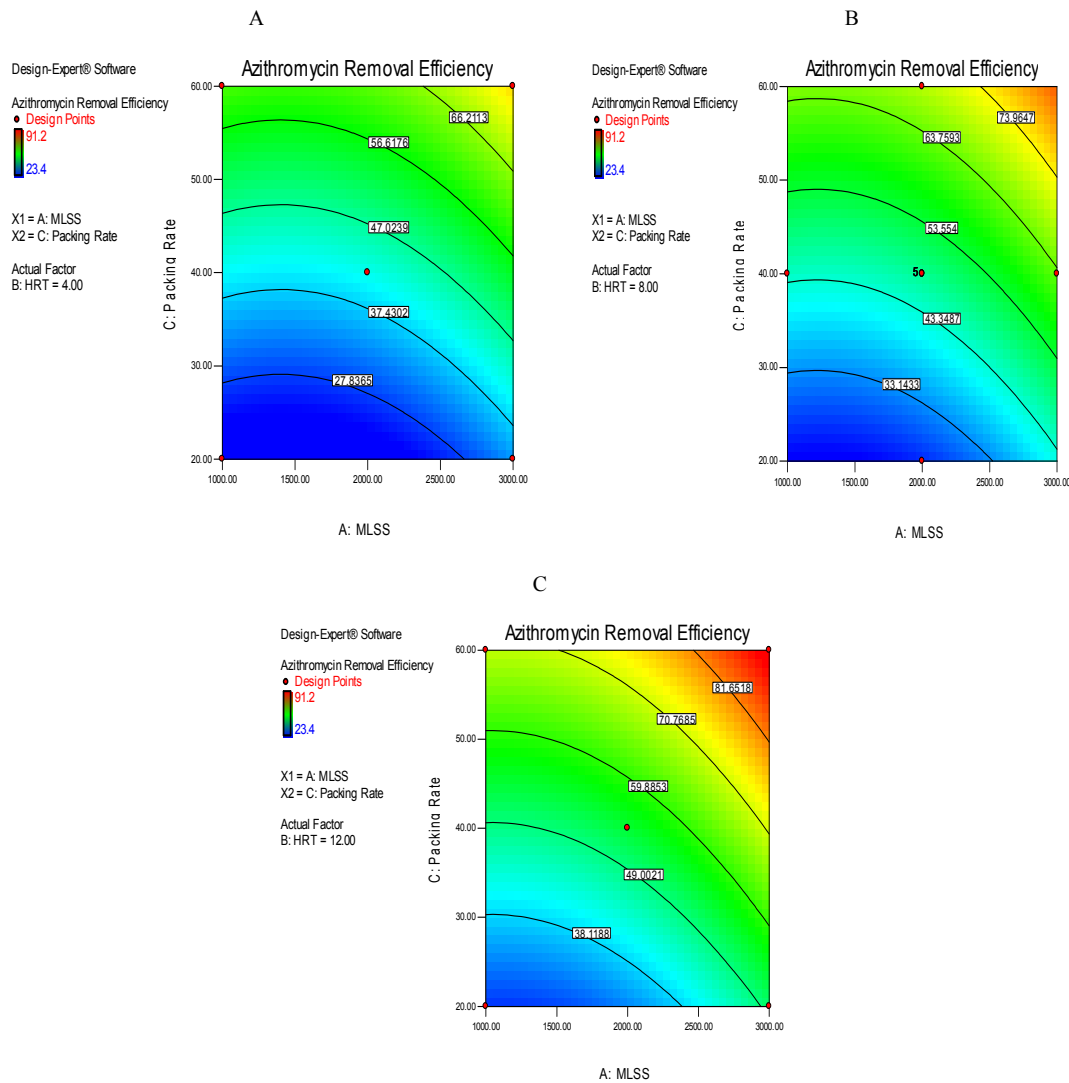


Fig. 3. Removal efficiency of azithromycin with HRT at A) 4 hours, B) 8 hours, and C) 12 hours using combined system of UV/H₂O₂ process and MBBR reactor

Effect of the packing rate

The results of this phase of the experiments are presented in Table 2 and Fig. 4. At this stage, the effect of filling percentage (20%, 40%, and

60%) on the removal efficiency of azithromycin was investigated. According to the obtained results, increased packing rate was associated with the higher removal efficiency of

azithromycin. Therefore, it could be concluded that the packing rate directly affects the activity of microorganisms.³³ As is shown in Fig. 4, with 20% packing rate and at the MLSS concentrations of 1,000, 2,000, and 3,000 mg/l and HRT of four, eight, and 12 hours, the removal efficiency of azithromycin was 23.59%, 34.29%, and 45%, respectively. At 40% packing rate, azithromycin removal efficiency was 44.68%, 55.38%, and 66.09%, and at 60% packing rate, the removal efficiency of azithromycin was 65.77%, 76.47%, and 87.18%, respectively. Therefore, the highest azithromycin removal efficiency was obtained

at 60% packing rate and estimated at 87.18%, and the mentioned packing rate was selected as the optimum value.

According to the current research, system design in terms of aeration, HRT, bed, and reactor packing rate are effective in attaining the desirable results.³² Shokoohi *et al.* assessed the application of the MBBR in the removal of ciprofloxacin from hospital wastewater in operation conditions, proposing similar results. Accordingly, increased packing rate was associated with the higher ciprofloxacin removal efficiency.³⁵

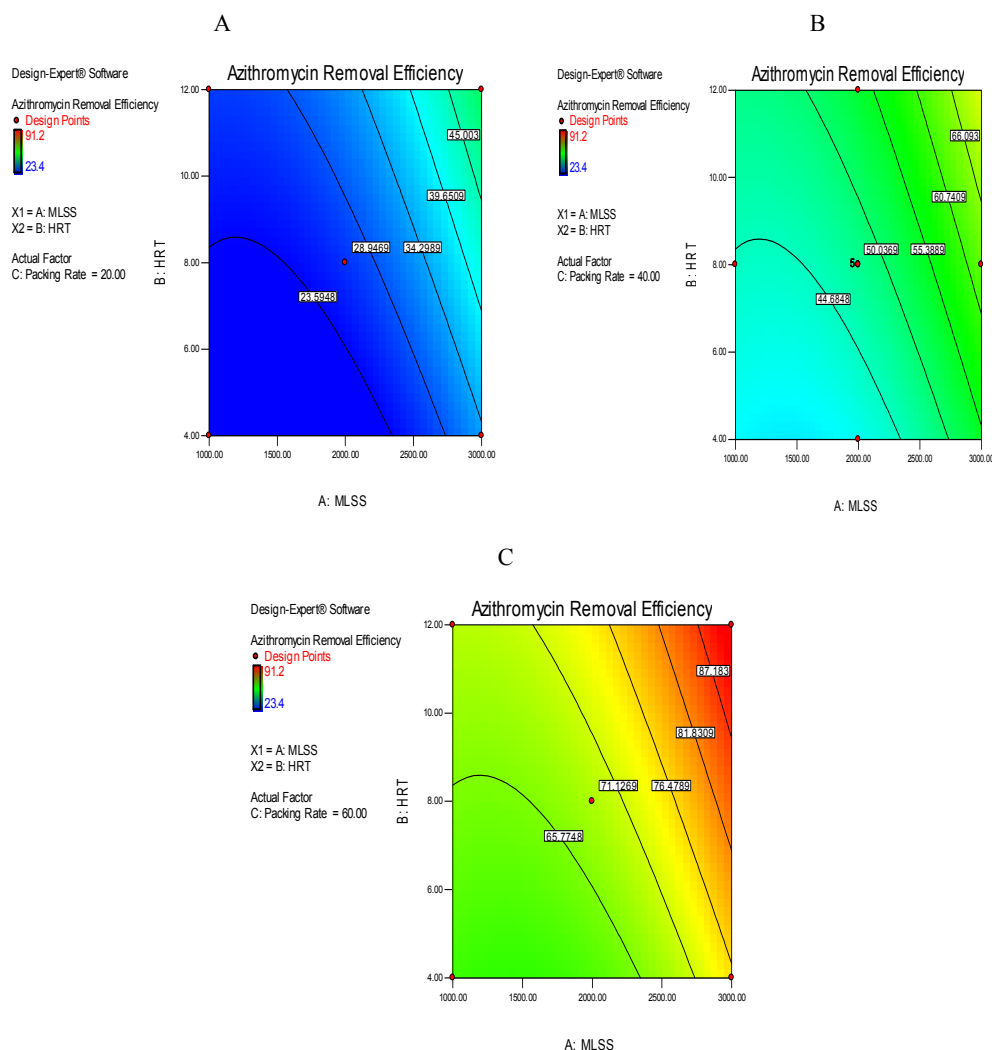


Fig. 4. Removal efficiency of azithromycin with packing rates of A) 20%, B) 40%, and C) 60% using combined system of UV/H₂O₂ process and MBBR reactor

Conclusion

This study aimed to describe a simple, cost-efficient, sensitive, accurate, and economical

visible spectrophotometric method for the estimation of azithromycin. According to the findings, the combined system of UV/H₂O₂

process and MBBR could effectively remove azithromycin from aqueous solutions. In addition, the results of the acclimation period indicated that the acclimation period in the MBBR reactor was short, and the system was put into operation very quickly. The maximum removal efficiency of azithromycin (91.2%) was obtained at the MLSS concentration of 3,000 mg/l, HRT of 12 hours, and 60% packing rate. The increased MLSS concentration, HRT, and MBBR packing rate were associated with the higher efficacy of the treatment process. On the other hand, the combined system of the UV/H₂O₂ process and MBBR reactor had high efficiency in the removal of azithromycin from aqueous solutions. Therefore, it is suggested that this system be used as an environmentally friendly method to remove azithromycin from hospital wastewater, and further investigations in this regard must be focused on the effects of the heat induced from UV lamps on aqueous solutions.

Acknowledgments

This article was extracted from a doctoral dissertation conducted by Mr. Rouhollah Shokri under the supervision of Dr. Reza Jalilzadeh Yengejeh, Dr. Ali Akbar Babaei, other advisors, Dr. Ehsan Derikvand, and Prof. Ali Almasi. We would like to thank Ahvaz Islamic Azad University for supporting this research project.

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