

Removal of Penicillin G by combination of sonolysis and Photocatalytic (sonophotocatalytic) process from aqueous solution: process optimization using RSM (Response Surface Methodology)Ali Almasi¹, Abdollah Dargahi², Mitra Mohamadi³, Hamed Biglari⁴, Farhad Amirian⁵, Mehdi Raei⁶

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Abstract

Introduction: Penicillin G (PG) is used in a variety of infectious diseases, extensively. Generally, when antibiotics are introduced into the food chain, they pose a threat to the environment and can risk health outcomes. The aim of the present study was the removal of Penicillin G from an aqueous solution through an integrated system of UV/ZnO and UV/WO₃ with Ultrasound pretreatment.

Methods: In this descriptive-analytical work dealing with the removal of Penicillin G from an aqueous solution, four significant variables, contact time (60-120 min), Penicillin G concentration (50-150 mg/L), ZnO dose (200-400 mg/L), and WO₃ dose (100-200 mg/L) were investigated. Experiments were performed in a Pyrex reactor (batch, 1 Lit) with an artificial UV 100-Watt medium pressure mercury lamp, coupled with ultrasound (100 W, 40 KHz) for PG pre-treatment. Chemical Oxygen Demand (COD) was selected to follow the performance of the photo-catalytic process and sonolysis. The experiments were based on a Central Composite Design (CCD) and analyzed by Response Surface Methodology (RSM). A mathematical model of the process was designed according to the proposed degradation scheme.

Results: The results showed that the maximum removal of PG occurred in ultrasonic/UV/WO₃ in the presence of 50 mg/L WO₃ and contact time of 120 minutes. In addition, an increase in the PG concentration caused a decrease in COD removal. As the initial concentration of the catalyst increased, the COD removal also increased. The maximum COD removal (91.3%) achieved by 200 mg/L WO₃ and 400 mg/l ZnO, a contact time of 120 minutes, and an antibiotic concentration of 50 mg/L. All of the variables in the process efficiency were found to be significant ($p < 0.05$). Catalyst dose and contact time were shown to have a positive effect on the response ($p < 0.05$).

Conclusion: The research data supported the conclusion that the combination of advanced oxidation process of sonolysis and photocatalytic (sonophotocatalytic) were applicable and environmentally friendly processes, which preferably can be applied extensively.

Keywords: Integrated process, Penicillin G, Advanced oxidation, COD removal, Response Surface Methodology

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1. Introduction

Recently, the pharmaceutical drug presence in the environment is considered as a noteworthy environmental concern worldwide (1). Antibiotics are one of the most important groups of pharmaceuticals that are commonly used in human infections, veterinary medicine, and agriculture. Meanwhile the harmful effect of antibiotics in the environment, including release into soil, surface water, and creating biological resistance are undeniable (2). The annual antibiotic consumption in the world has been estimated at around between 100000-200000 tons (3). Approximately, 90% antibiotics after their use are active in the human body. Therefore, it can be deduced optimistically that nearly 30,000 tons and at worst, approximately 180,000 tons of active antibiotics are being introduced into the environment, annually (4, 5). The presence of antibiotics has been detected in surface waters, ground water aquifers, and even in drinking water in a range of nanogram/L to microgram/L, which have been released into environment by various pathways, such as the pharmaceutical industry's wastewater, hospitals, as well as human and animal elimination (6, 7). The main purpose of evaluation and control of pharmaceuticals in the environment is the release of antibiotics into food chain and the building of pharmaceutical drug resistance, which threatens the environment and human health. Penicillin G ($C_{16}H_{17}N_2NaO_4S$) is used for diverse category of infectious diseases, generally, it has a biological half-life of 30-60 minutes and is completely water soluble. Penicillin G (PG) prevents the production of peptidoglycan through the destruction of the bacterial cell-wall (8). Up to now, various physical, chemical, and biological methods have been utilized for the removal of pharmaceutical drugs from an aqueous solution (9, 10). In recent decades, an advanced oxidation process (AOPs) has been experienced widespread use, in order to reduce antibiotic residue in aqueous solutions. Advanced oxidation processes have become more advanced including, combination of processes such as ozonation, peroxide process, wet oxidation, H_2O_2 /ozonation/ultraviolet, photocatalytic, and sonolytic processes. (11-13). Generally, AOPs processes are more acceptable for wastewater containing pharmaceutical drugs in comparison to other technologies because it is able to completely remove all of the pharmaceutical drug compounds (14). Among the advanced photo oxidation processes, non-homogenous photocatalyst has been employed as an applicable technique for the degradation of contaminants, such as ultrasound/photocatalytic that has come to be known as the sonophotocatalytic degradation process (15, 16). The sonochemical being able to achieve the oxidation of the antibiotics and then, be converted into byproducts that have a lower toxicity and that are harmless for microorganisms. (17). Ultrasound radiation enhances the chemical and physical processing in an aqueous solution and the collapse of bubble cavitation, subsequently (18). The main objective of this study was to investigate the removal of PG by a combination of the advanced oxidation process of ultrasound (sonolysis) and the photocatalytic process (UV/ZnO and UV/ WO_3). The present study modeled and analyzed the removal of PG from an aqueous solution by Response Surface Methodology (RSM).

2. Material and Methods

2.1. Study design

This descriptive-analytical study was conducted beginning October 2015 until December 2015 at Kermanshah University of Medical Science, Kermanshah, Iran.

2.2. Chemical

All chemicals used were of analytical reagent grade and were used without any further purification. Penicillin G, Zinc Oxide (ZnO), and Tungsten Trioxide (WO_3) were purchased from Merck Company, Germany. Synthetic solutions were prepared with deionized water.

2.3. Collection of samples and the operation of the ultrasound and photocatalytic reactor

The variables in this study were contact time (60-120 min), initial PG concentration (50-150 mg/L), ZnO dose (200-400 mg/L), and WO_3 dose (100-200 mg/L). All of experiments were carried out in batch mode with 3 repetitions. To reduce the data scattering, the mean value of experiments were reported. The Pyrex reactor was designed and fabricated with a working volume of 1 liter. In order to investigate the effects of the ultrasound, the whole reactor assembly was immersed into an ultrasonic bath (DSA 100-SK 2-4, 0) having the capacity of 4 liters and with a submerged contact time of 60 minutes (100W, 40 KHz, 30°C). The efficiency of ultrasonic was evaluated by Chemical Oxygen Demand (COD) measurement. For the following step, the PG solution was blended by a magnetic stirrer during the reaction period (at a velocity of 500 rpm). The synthetic wastewater was introduced batch by batch into the reactor. The irradiation source was a low pressure mercury lamp with a wavelength of 254 nm (Philips Co, Germany) that had been adjusted at 5 cm above of reactor and was surrounded with aluminium foil. The measurement of the concentrations of chemical oxygen demand (COD) were carried according to the standard methods of water and wastewater analysis (19). For the COD, a colorimetric technique with a closed reflux method

was developed. Spectrophotometer (DR 5000, Hach, Jenway, USA) at 600 nm wavelength was used to measure the absorbance of the COD samples. The experimental runs were designed using Design Expert Software (DOE) (Stat-Ease Inc., version 6.0) as described in Section 2.4.

2.4. Experimental design

The DOE software provides a platform for the design of the experimental runs. In accordance with the DOE software, the optimum operating conditions must be established with the aim of eliminating the systematic error and by estimate the error and decreasing the number of experiments and the analysis of the significance relative to several affecting factors in terms of their multifaceted interaction (20). In the present study, the Central Composite Design (CCD) was employed since it is the most commonly used with RSM. Among several classes of response surface design such as the Central Composite Design, the Box-Behnken design, Hybrid design, and a three-level factorial, in the present study like most of the other similar studies, the central composite design (CCD) was employed since it is most commonly used with RSM (21). CCD was used to study the three different factors such as antibiotic concentration (A), dose of catalyst (B), and contact time (C), which were enclosed by antibiotic concentration (50-150 mg/L), contact time (60-120 min), ZnO dose (200-400 mg/L) and WO₃ dose (100-200 mg/L) to evaluate the response. The design consists of 2k factorial points augmented by a 2k axial points and a center point, where k is the number of variables and the range of the mentioned parameter are displayed in Table 1. The variables were considered at three levels, 1 (minimum), 0 (central) and 1 (maximum), which were assessed based on the full face-centered CCD experimental plan. In total, 20 experimental runs were performed and 5 runs were repeated, in order to validate the accuracy of experimental runs.

Table 1. Experimental range and levels of the independent variables

Variables	Range and Level		
	-1	0	+1
Antibiotic concentration (mg/L)	50	100	150
Catalyst concentration of ZnO (mg/L)	200	300	400
Catalyst concentration of WO ₃ (mg/L)	100	150	200
Reaction time(min)	60	90	120

2.5. Research Ethics

This study was approved by the Ethics Committee at Kermanshah University of Medical Sciences (approval number 94127).

2.6. Mathematical Modeling

RSM, which has been acknowledged as an effective statistically sound method for optimizing the parameter of the processes and assessment for fitting the model. In present study, RSM was applied with respect to identifying the independent variable and to determine if its interaction affect elicits the desired response. A model in the form of Eq. 1 describes how the RSM fits the experimental data and elaborates about the optimization, and the coefficient for the model was calculated. In order to identify the input, response, as well as the quadratic and linear equation models for predicting the optimal variable, the following was used: $Y = \beta_0 + \beta_i X_i + \beta_j X_j + \beta_i X_i^2 + \beta_{ij} X_j^2 + \beta_{ij} X_i X_j + \dots$

Where: Y, i, j, β , X shall be considered as the process response, linear coefficient, quadratic coefficient, regression coefficient, and coded independent variables, respectively (20). A multiple regression analysis was used in order to analyze the coefficient variables. Model terms are chosen or neglected in accordance with the probability of error (P) value with a 0.95 of confidence level. The statistical results that were obtained by means of CCD were surveyed by an analysis of variance (ANOVA). The analyses of the data, the experimental design, and the operative optimization of the interaction effect of independent variables on the response were done by design expert software. The results of the experimental data were illustrated using a 3 dimensional plots diagram with respect to the simultaneous effect of variables on the response.

3. Results

3.1. Statistical analysis

In the present study, CCD was selected for determining the relationship between variables and their response. The variables (A, B and C) are listed in terms of actual and coded units and are illustrated in Table 2. The number of 20 experimental runs that were performed according to Table 2. The reduction model illustrates in terms of the coded factors with significant model terms and the ANOVA results are presented in Table 3. The results achieved from the ANOVA analysis were used to determine the applicable level of significance. The significance of the model term

has been determined via the p-value and the F-Value that were used for response. Meanwhile, the value of probability $> F$, indicates that the corresponding models and the individual coefficients are more significant. In this study, the higher significance of corresponding model were implied with respect to the larger amount of F-value and smaller amount of probability. The probability value was found to be quite low (0.000), which implies that the term was significant for the model. With respect to equation addressed here, by increasing antibiotic concentration, the COD removal decreased incrementally with increased units of contact time as well as the dose of the catalyst led to an increasing of COD removal. The fitting model has been verified according to the predicted model via the correlation coefficient, R^2 , adjusted R^2 , and predicted R^2 between the experimental and the predicted model values (Table 3). The correlation coefficients, R^2 , adjusted R^2 , and the predicted R^2 are near to one another and close to 1.0. In the UV/ WO_3 process, the predicted R^2 and adjusted R^2 were found to be 0.927 and 0.946, respectively. In the UV/ZnO process, the predicted R^2 and adjusted R^2 were found to be 0.945 and 0.955, respectively. The validity and reliability of this analysis was determined with adequate precision and it should be valued at 4 or more. There was adequate precision of present study scoring between 35.55 and 45.57 for UV/ZnO and UV/ WO_3 , respectively, and it was remarkably found to be greater than 4 in value, which meant the proper validity of the analysis was supported.

Table 2. The Experimental condition and design expert software result of this study

Run	Antibiotic concentration (mg/L)	Contact time (min)	ZnO (mg/L)	WO_3 (mg/L)	COD removal (%)	
1	75	60	300	150	UV/ZnO	UV/ WO_3
2	50	120	400	200	54.1	72.4
3	75	90	300	150	79.6	91.3
4	75	90	200	100	58.3	76.2
5	100	120	400	200	46.6	67.4
6	100	120	200	100	62.3	70.8
7	50	60	200	100	45.4	56.3
8	50	120	200	100	56.5	70.2
9	100	60	200	100	60.4	74.4
10	75	120	300	150	40.6	53.6
11	75	90	400	200	64.4	77.6
12	50	90	300	150	69.5	84.3
13	50	60	400	200	67.2	83.5
14	100	60	400	200	75.2	88.1
15	100	90	300	150	57.6	70.4

Table 3. The ANOVA result for the equation of the design expert software

Processes	Modified equations with significant	Type of Model	Adeq. precision	R^2	Adjusted R^2	Predicted R^2	p-value
UV/ WO_3	$-75.34-9-9.39 A +7.65 B +1.94C -3.4B^2$	Quadratic	35.55	0.958	0.946	0.927	0.000
UV/ZnO	$2.81C + 9.47B+ 8.34A-58.86$	Linear	45.47	0.962	0.955	0.945	0.000

3.2. Ultrasound process (Effect of antibiotic concentration on COD removal)

It was observed that the efficiency of the ultrasound process increased with a decrease in the concentration of the PG ($p < 0.05$). The maximum degradation was achieved with 50 mg/l of PG. Figure 1 depicted the Penicillin G concentration according to influent and effluent of COD. The maximum and minimum COD removal (24.82% and 12.45%) were observed at the antibiotic concentration was at 50 mg/l and 100 mg/l, respectively. Also, with respect to the ANOVA analysis and the Duncan test in SPSS version 18 software, the COD removal in different antibiotic concentration was found to be significant ($p < 0.05$).

3.3. Photocatalytic process

3.3.1. Effect of contact time on the COD removal:

The effect of the variables on COD removal in terms of 3D plot is presented in Figure 2 and 3. According to these obtained results, a high rate of decomposition is observed at the initial time of photodegradation. For example, in initial antibiotic concentration of 50 mg/L and WO_3 dose of 100 mg/L, by increasing the contact time from 60 to 120 minutes, the COD removal increased by nearly 4.2%.

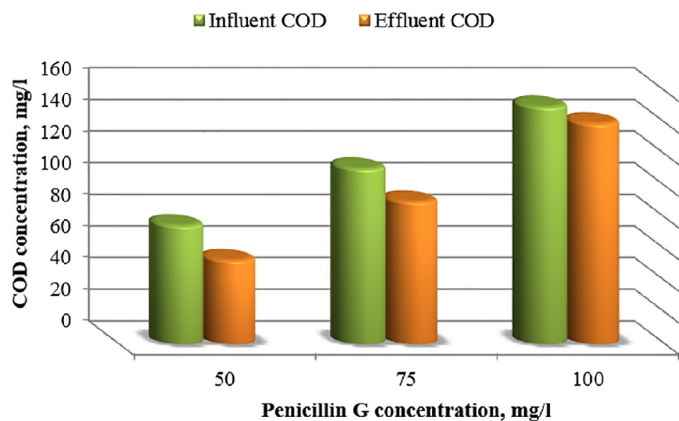


Figure 1. The influent and effluent COD Concentration in Ultrasound process

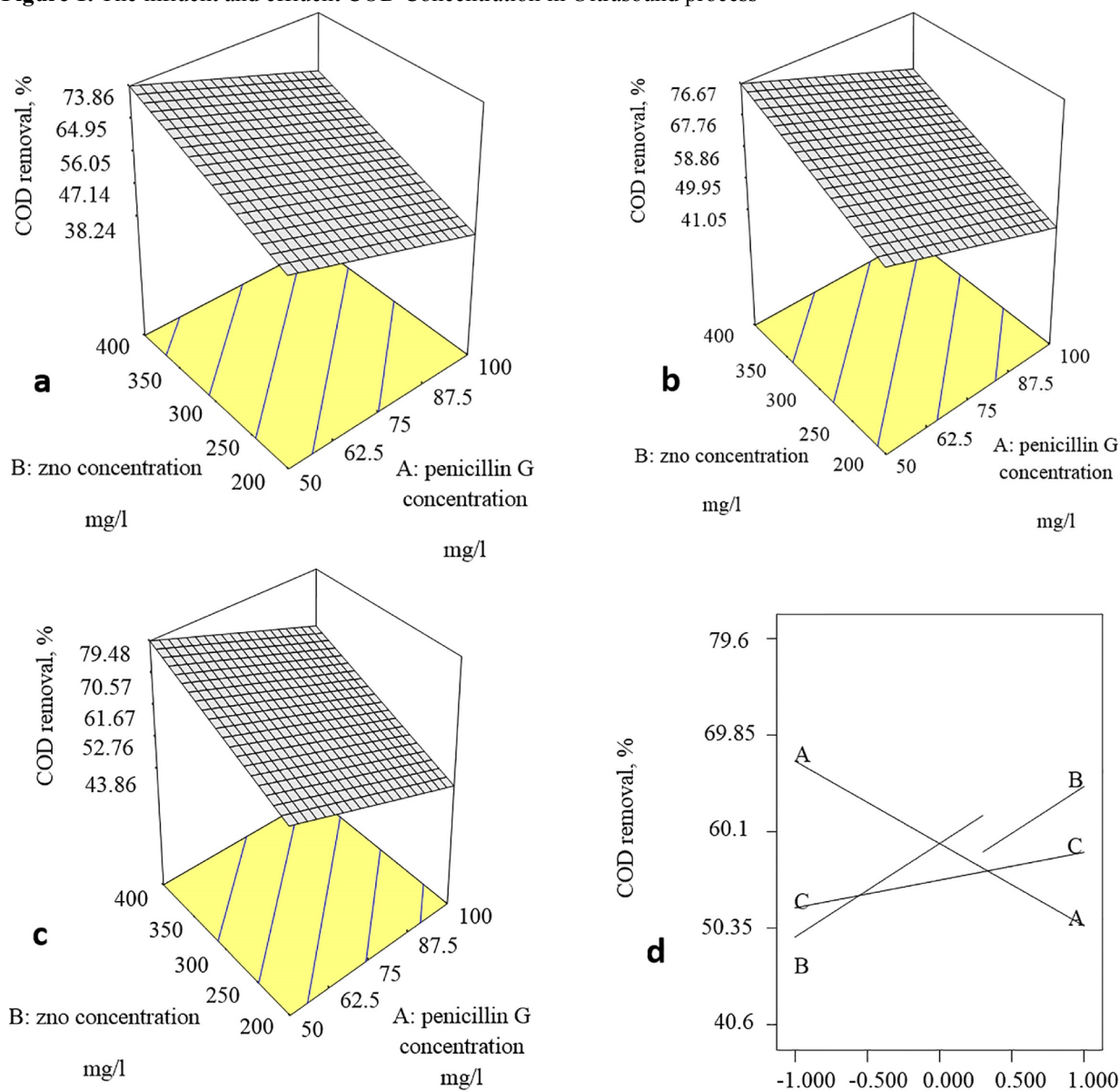


Figure 2. Response surface plot for COD Removal of Penicillin G with UV/ZnO in contact time of (a) 60 min (b) 90 min (c) 120 min (d) Perturbation plot for COD removal rate

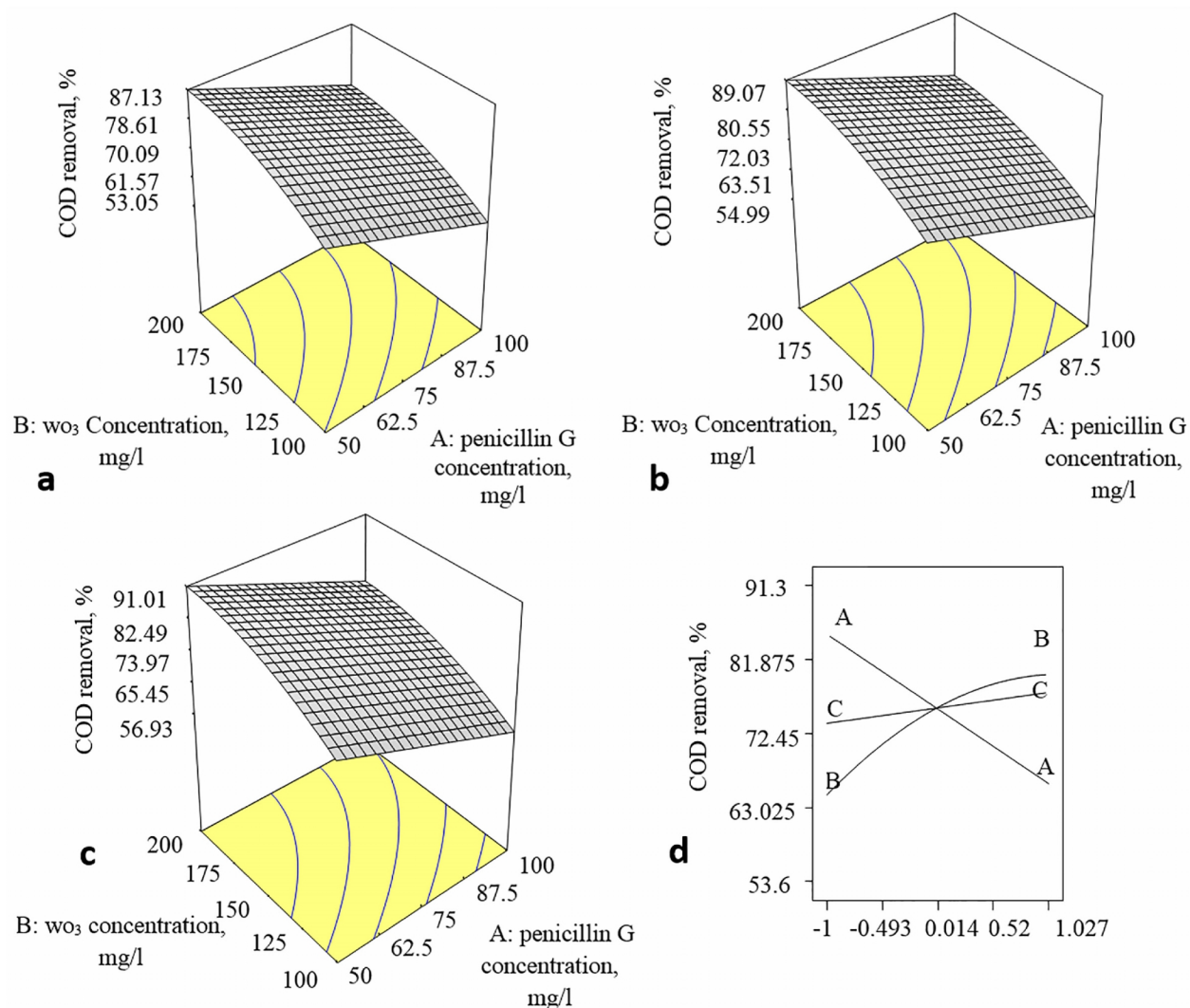


Figure 3. Response surface plot for COD Removal of Penicillin G with UV/WO₃ in Reaction time of (a) 60 min (b) 90 min (c) 120 min (d) Perturbation plot for COD removal 3-4- Process optimization and verification

3.3.2. Effect of the initial antibiotic concentration and of ZnO and WO₃ dose on the COD removal:

A three dimensional plot of the model with respect to the simultaneous effect of the antibiotic concentration, WO₃ dose, and ZnO dose over different contact times are shown in Figures 2 and 3. The initial concentration of PG was an effective parameter on the photodegradation efficiency. By increasing the initial concentration of PG up to 100 mg/L, the removal efficiency decreased. For instance, for a contact time of 120 minutes in UV/ZnO, the COD removal for the initial antibiotic concentration of 50 mg/L (79.6%) was more than 100 mg/L (62.3%). By exploring the effect of the catalyst dose on process efficiency; it was also revealed by increasing of catalyst dosage that the performance of photocatalytic has advanced. The maximum COD (79.6% and 91.3%) removal efficiency was observed at the ZnO dosage of 600 mg/l and WO₃ dosage of 200 mg/l, respectively. Meanwhile, the lowest predicted removal efficiency of COD (40.6%) for UV/ZnO and (53.6%) for UV/WO₃ was obtained at the contact time of 120 minutes and the PG concentration of 50 mg/l. The influence of the antibiotic concentration and the catalyst dose on COD removal efficiency was found to be more significant than the variable of contact time ($p < 0.05$).

3.4. Process optimization and verification

The graphical optimization of the overlay plot demonstrates that the regions meet the proposed criteria. The optimum region corresponding to the COD removal was higher than 70%. The yellow area was the region that satisfies the constraints. The optimal region enclosed by the the Penicillin G concentration (75 mg/l) and WO₃ dose

(150 mg/l) in UV/ WO₃; and Penicillin G concentration (50 mg/l) and ZnO dose (400) in UV/ZnO. In order to validate the accuracy of the models, a point within the optimum region was chosen (conditions shown by flags in Figure 4). The actual value along with the predicted value response was compared. The accuracy of optimum condition through DOE software was investigated by examining the standard deviation for each response, and very close to the actual values to predict model values were obtained.

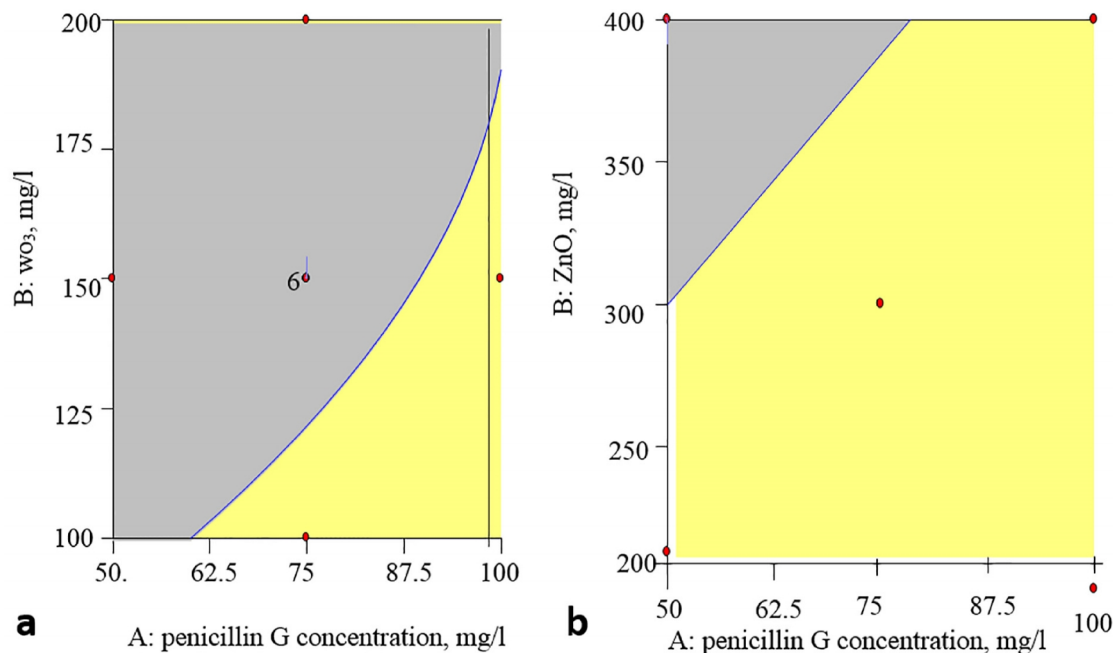


Figure 4. Overlay plot for optimal region (a) WO₃ (b) ZnO

4. Discussion

Generally, it can be concluded that the sonophotocatalytic degradation could achieve high COD removal efficiency in a low amount of contact time and with a low catalyst dosage. Based on results obtained, the COD removal efficiency significantly increased along with an increase in the catalyst dosage ($p < 0.05$). As a result, when the PG was decreased, a lesser catalyst dose was required. An inverse relationship between the PG concentration and the COD removal was observed in this study. This supports the assertion that all three variables have significant effects on COD removal. Ultrasonic pre-treatment could not achieve a high level of COD removal. It may be attributed to low amount of hydroxyl radical (OH•) generation. The effectiveness of this ultrasonic process is associated with the water matrix. According to various studies, the increasing of ionic strength can accelerate the free radical activity in an aqueous solution and cause increased efficiency of COD removal. Hence, the low COD removal may be attributed to the use of deionized water (15, 22).

4.1. Effect of initial antibiotic concentration

Based on the results that were obtained, the initial concentration of PG was an effective parameter for predicting the photodegradation efficiency. By increasing the initial concentration of PG up to 100 mg/L, the removal efficiency decreased. The catalyst dose and contact time had a similar effect on the COD removal in the photocatalytic reactor. The reaction of the antibiotic with the hydroxyl radicals in the low concentration lead to degradation improvement due to free radicals (23). In the high concentration of PG, the active site has been covered through antibiotic ions that cause a reduction in the generation of hydroxyl radicals on the surface of the catalyst (24). Therefore, the high concentration of PG, absorbs photons from UV light readily, and as a result, it tends to reduce the UV photons for catalyst activation (25). Another reason may be attributed to the intervention of intermediate by-products, which are generated during the degradation of antibiotic molecules.

4.2. Effect of contact time

As shown in Table 3, the increasing contact time enhanced the COD removal. This may be due to the exiting ZnO and WO₃ particles, and increasing of hole creation in the catalyst surface and as a result, leading to the incremental absorption surface area and as well as COD removal enhancement (26). A high rate of degradation was observed in

the first 60 min of photodegradation and after that the rate of PG degradation became slower. This is probably due to a large amount of available active sites in the beginning. Additionally, the generated intermediate organic compounds in the photochemical degradation leads to decreasing the COD removal.

4.3. Effect of catalyst dose

According to the results obtained, by increasing the catalyst dosage the rate of PG decomposition by absorbing more photons on its surface was also increased. So, the available active sites increased and as well as causing an increasing of the absorbed antibiotic molecules (16, 24, 27). Contrastingly, the content of ZnO and WO₃ has significantly influenced the COD removal ($p < 0.05$). Generally, the slight increase in catalyst dose leads to a reduction of reaction time in the photocatalytic process. The catalyst has two opposite effects on the process efficiency including the COD removal increment due to increasing of available site and decreasing of COD removal due to the solution turbidity increasing and the decrease in UV light penetration. Hence, the optimal set up and operation of the photocatalytic reactor should be considered in order to ensure the photon absorption and preventing extra catalyst use (4).

4.4. The effect of UV light

The organic matter photolysis by the photocatalytic processes through optimal simulation semiconductor and subsequently by the electron-hole pair's generation in the surface of catalyst. High oxidative potential holes (h valence band) in the catalyst provide direct oxidation of the organic matter with the intermediate product. Likewise, the reactive hydroxyl radicals are produced by the water catalysis or the reaction between holes with hydroxyl radical (OH). The hydroxyl radical is a strong non-selective oxidant for organic compound degradation. Also, electrons in the conduction band generated the hydroxyl radicals, which are the principal reason for organic matter mineralization (16).

5. Conclusions

In the present study, PG has been successfully degraded by the sonophotocatalyst. The advantages of the processes are a high rate, a lesser cost for wastewater treatment in a UV/ZnO process in comparison to other advanced oxidation processes, a lack of sludge generation, simple operation, and an industrial scale applicability. The maximum COD removal in UV/ WO₃ was found to be 91.3% in catalyst dose of 200 mg/L, contact time of 120 minutes and antibiotic concentration of 50 mg/L. Finally, it can be deduced that the effect of the combined photochemical processes in water, direct photolysis by UV radiation, acoustic cavitation, and hydroxyl radicals have been identified as main reason for high COD removal in this study. The photocatalytic process has been confirmed as an applicable technology and is an environmentally friendly process that can be used extensively, in comparison to the high financial component and possibility of creating poisonous intermediate byproduct generation in the chemical process, which would be caused by employing of photocatalytic processes to solve the problem.

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Conflict of Interest:

There is no conflict of interest to be declared.

Authors' contributions:

All authors contributed to this project and article equally. All authors read and approved the final manuscript.

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