Original Article

Photodegradation of the Antibiotic Penicillin G in the Aqueous Solution Using UV-A Radiation

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Abstract

Background and purpose: Highly consumption of antibiotics and their entrance into the environment has increased concerns all over the world. These compounds enter to the environment through an incomplete metabolism and a considerable amount of them cannot be removed using usual waste filtration systems. Therefore, the present study aimed to investigate the feasibility of using ultraviolet radiation (UV-A) to remove penicillin G (PENG) from aqueous phase and determining its removal efficiency.

Materials and Methods: The experiments were carried out in the batch mode. The samples were assessed in a 2-liter reactor. In order to investigate the effect of UV-A radiation on the removal rate of antibiotic penicillin G (PENG), the following parameters were studied. Three concentration levels of PENG antibiotic (10,25, and 45 mg/l) were exposed to UV-A at three pH levels (3,7,11) and were evaluated at four reaction times (30,60,90, and 120 min). Antibiotic penicillin G (PENG) was determined using HPLC instrument (Waters YL9100,USA) and results analyzed using factorial design software.

Results: The finding demonstrated that antibiotic removal rate increased by decreasing pH and decreasing the initial concentration of antibiotic and increasing contact time. The maximum rate of penicillin G removal occurred in acidic pH (pH=3) is as much as 38%. All of the variables in the process have been statistically significant effect ($p<0.001$).

Conclusion: Results showed that by reducing the pH, increasing contact time and reducing the antibiotic concentration, the removal rate increases. In conclusion, photodegradation process using UV-A may enhance the rate of penicillin G degradation in polluted water and could be used as a complementary step for other chemical and biological processes to remove penicillin G from the aqueous solution. Therefore, UV-A process in conjugate with the other processes is an appropriate method for reducing antibiotic penicillin G in polluted water resources.

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***Key words*:** Antibiotic, Penicillin G, UV-A Radiation, Photodegradation, Removal, Aqueous Solution

1. Introduction

Antibiotic refers to a material that can be used for the elimination of microorganisms, such as bacteria, fungi and parasites. Up to now, 250 antibiotics have been recorded for human, livestock, and plant consumption. The annual consumption rate of antibiotics has been estimated to be around 100000-200000 tons in the world (1). Antibiotics are among the most beneficent drugs, however, they have harmful effects on the environment, including entrance into the soil and water resources and causing the development of antibiotic resistance in microorganisms (2). Different drug compounds have been found in water sources such as surface water, seawater and wastewater sewage treatment (30). The other concerns of inappropriate use of antibiotics are their remaining in animals tissues that humans consume their meat (4). Penicillin G ($C_{16}H_{17}KN_2O_4S$ with the molecular weight 372.48 and pKa =2.75) is a conventional antibiotic which is used for treatment of different kinds of infectious diseases, such as soft tissues, bacterial, and respiratory infections (5). It is soluble in water and the mechanism of destruction of bacteria's cell wall is by preventing production of peptidoglycan layer (6,7). The biological half-life is 30-60 min and excreted mainly by kidney (6). Benzyl penicillin (penicillin G) is composed of a core of 6-amino penicillanic acid plus a side chain of benzyl.

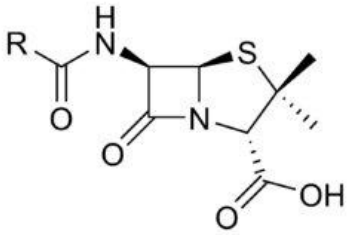

Figure 1. Structure of penicillin core (8)

Penicillin G contain b-lactam ring which is very sensitive to pH, heat and b-lactamase enzymes (8). Although many studies have not able to detect penicillin G in environmental samples (9), the degradation products are detected in water resources. During the past few years, the presence of different kind of antibiotics in the environmental samples has been reported. Antibiotics are detected in the higher concentration (mg/l range) in hospital effluents, lower concentration (mg/l range) in municipal wastewater and very low concentration (ng/l) in surface water and groundwater (10-12). Many different antibiotics and one degradation product were detected in water samples collected downstream from the effluent discharge at Mud Creek (13). Many studies have reported the presence of several kinds of antibiotics in many environmental samples (14,15). Kim and Carlson demonstrated that the amount of antibiotics in the water column is less than sediments. Therefore, sediments have the highest potential for antibiotics accumulation after receiving antibiotics into the aquatic environment (11). The considerable amount of antibiotics residue was detected in the soil analysis (16). In addition, the run-off from the agricultural fields has very high antibiotic concentrations (1). Many antibiotics such as sulfamethoxazole,trimethoprim, ciprofloxacin, ofloxacin, lincomycin and penicillin G were detected in hospital wastewater (300-35000 ng/l). Sulfonamides and fluoroquinolones were found at very high concentrations in hospital wastewater and could not be removed by wastewater treatment. Only antibiotic ofloxacin (1300 ng/l) was detected in the residential wastewater (10). Thomas et al.

showed that the mass loadings for three analgesics (paracetamol, ibuprofen, and diclofenac) and four antibiotics (tetracycline, trimethoprim,ciprofloxacin,sulfamethoxazole) and three estrogens (17 bestradiol, estriol, estrone) were in the range of 90 and 64 g/day for two hospitals in Norway (17). Azithromycin was detected at a very high concentration at Mud Creek (2.35 µg/l). Although the secondary and advanced wastewater treatment processes reduce the antibiotic concentrations, this effluent discharge concentration could be a source of antibiotic in the water resources (18). The concentrations of penicillin G detected in raw sewage and in treated were 153 mg/l and 1.68 mg/l, respectively. Therefore, the partial transformation of penicillin G was occurred during the anaerobic, aerobic processes at the wastewater treatment plant (19). Antibiotics cannot be effectively removed by the usual processes of filtration systems, such as biologic filtration, surface absorption with activated carbon, and reverse osmosis (20-22). Kim et al studied the removal of pharmaceutical compounds from aqueous environment using ultraviolet radiation. The medicine iopromide and diclofenac removed to the extent of 15% and 27%, respectively (23). Laoufi et al removed the antibiotic tylosin in the aqueous media (30%) by ultraviolet radiation (24). Sona et al studied the removal of recalcitrant contamination in water using ultraviolet radiation. They showed that sulfamethoxazole could be removed almost 100% and fuel-derived contaminants such as MTBE 26% could be removed as well (25). In the recent years, incorrect and arbitrary consumption of drugs, especially antibiotics, have become one of the basic challenges in the field of health in Iran. In fact, Iran is one of the first 20 countries in antibiotic consumption, and penicillin G is one of the most widely used antibiotics in this country (10). Therefore, the present study aims to assess the possibility of using UV-A radiation for removing antibiotic penicillin G in aqueous environment.

2. Materials and Methods

The experiments were carried out in duplicates in the batch mode. The study parameters were pH, reaction time and initial antibiotic concentration. Factorial design was used for the analysis of the parameters and their interaction effects were studied as well. To reduce the scatter in the data, log of transformation and geometric mean were used.

2.1. Chemicals and analytical method

Penicillin G with 99% purity was purchased from Sigma-Aldrich Company (USA). The rest of chemicals were purchased from Merck (Germany). UV lamp (F8T5) with the length of 25 Cm, with the intensity of 8 Watt and 356 nm wave length, (Hitachi, Japan) was used as the radiation source. For penicillin G detection in the aqueous phase a Waters Model high performance liquid chromatography (HPLC) (Waters YL9100HPLC SYSTEM, USA) system with C18 column columns (CP-SIL 5 CB column model, 250*4.6 mm, 5 µm) was calibrated and tested prior to injection of the samples. The mobile phase was included methanol and water (20/80 V/V) with the flow rate of 0.5 ml/min. A UV absorbance detector at 210 nanometer wavelength was used to detect Penicillin G in the samples (5). The retention time for the antibiotic is 7 minutes. The detection limit for the sample was 1nanogram/ l. Penicillin G chromatogram is presented in Figures 2.

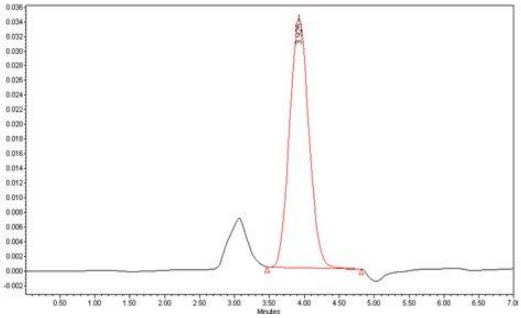

Figure 2. Penicillin G chromatogram

2.2. Reactor specifications

The specification of photochemical reactor is shown in Figure 3. The experiment was performed in a 2-liter volume reactor. Test was performed in a closed glass reactor with adjustable mixer. The source of radiation was a UV lamp (8 Watt, Hitachi Japan) which was protected by a Quartz tube with the height of 30 Cm and inside diameter of 5cm. The UV radiation source was immersed in the solution for better radiation. The whole system was wrapped in an aluminum foil in order to prevent reflection.

Figure 3. Photochemical reactor

2.3. Effects of pH and contact time on the removal rate of penicillin G by UV-A process

To measure the influence of different parameters on the removal rate of penicillin G by UV-A process in the aqueous phase, different pH from 3-11 (interval of 4) with two replications was used at the antibiotic concentration (10, 25, and 45 mg/l) and the contact time of 30, 60, 90, and 120 minute intervals. Ammonium hydroxide and hydrochloric acid were used to adjust pH in the samples. RH Basic 2 magnetic mixer (IKA Company, Germany) was used for mixing the samples. A blank without UV-A was also used for all the experiments. Then, the samples were passed through a Whatman filter cellulose acetate membrane with 0.45 micron pore size (Germany). After that, the residual of penicillin G was measured using HPLC. All the experiments were done in two replications in the presence of the control samples.

3. Results

3.1. The effect of pH on penicillin G removal

The variations of pH on the removal rate of penicillin G by UV-A process at initial concentration of the antibiotic penicillin G (C0=10 mg/l) was shown on Figure 4. Data regarding the effect of pH shows that as pH increased from 3.0 to 11, the rate of penicillin G reduction decreased (Figure 4). Based on the data obtained in the present study, pH of 3 is optimal for penicillin G degradation. The reduction rate was 38% in this case. According to regression analysis, it can be concluded that there was a significant difference between pH and penicillin G removal rate ($p<0.001$).

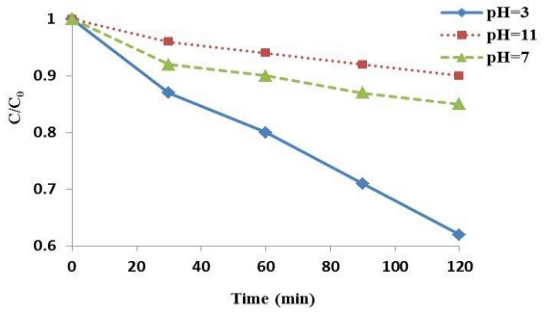

Figure 4. Effect of pH at different time interval on the removal of penicillin G by UV-A process (penicillin G =10 mg/l)

3.2. Effects of initial antibiotic concentration of penicillin G (PENG) and contact time on the removal rate of penicillin G by UV-A process

The effect of initial antibiotic concentration of penicillin G on the removal rate at the optimal condition (pH=3) was shown in Figure 5. Antibiotic removal rate decreased as the initial penicillin G concentration increased from 10 to 45 mg/l. The maximum removal rate of penicillin G (38%) occurred at the lowest initial antibiotic concentration (PENG=10 mg/l) with the maximum UV-A radiation time (Time=120 min). Regression analysis showed that there was a significant difference between initial antibiotic concentration on penicillin G and the antibiotic removal rate ($p<0.001$).

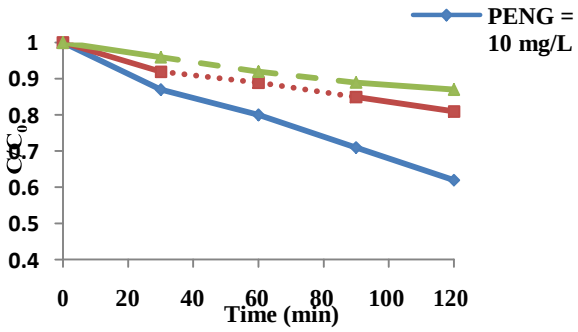

Figure 5. Effect of initial antibiotic concentration (PENG) at different time interval on the removal of penicillin G by UV-A process (pH=3)

4. Discussion

Nowadays the use of ultraviolet radiation to degrade the refractory contaminants such as a pharmaceutical compound is an improving technology. In this study, different parameters were evaluated for the removal of penicillin G from aqueous phase using UV-A radiation with emphasis on reaction time, the antibiotic initial concentration and pH. Data demonstrated that the removal of penicillin G is higher at acidic pH due to the presence of H+ ions which consequently produce H° radicals. pH is one of the most important factors affecting on the efficiency of chemical and biological processes (26). The reduction rate of penicillin G reduced at higher pH, because of the formation of insoluble compounds which in turn reduced the intensity of UV radiation and the potential of hydroxyl radical production as well (22). Other studies also demonstrated that better removal of antibiotic occurred at lower pH. Studies conducted by Guo et al (27) and Sauna et al (25) also showed that H+ ions have an important role in the formation of radicals H°. The antibiotic initial concentration plays a major role in many photo-reaction processes. Data obtained in the current study demonstrated that as the initial penicillin G concentration is increased, the removal rate of antibiotic is reduced(Figure5). Since UV-A radiation is the same in all the samples, the feasibility of removing antibiotic is lower at higher initial concentration of antibiotic. Therefore, the samples of lower initial penicillin G concentration with the same amount of UV radiation have a higher chance of removal. Our results agree with Borji et al et al (28). Determining the

equilibration time is another important factor to achieve the maximum rate of antibiotic reduction in the aqueous phase. According to the results illustrated in the current study, at first the rate of penicillin G reduction increases very fast as the contact time increases. After that, its rate becomes slower until reaching a plateau (Figures 4-5). At equilibrium, the degradation reached a plateau. If the reaction time exceeds equilibrium, the process will be no longer cost-effective. Basically, an optimal contact time is a very important parameter for any chemical reactions. Based on the data obtained in the present study, 120 min reaction time is optimal for penicillin G degradation (Figure 5). In general by increasing the reaction time, the potential formation of radicals is higher and therefore the higher removal rate of penicillin G will be achieved. The studies of Kim et al (11) and Prados et al (29) achieved the same results. In water and soil, parent penicillin G are subjected to various biotic and abiotic degradation processes such as photolysis, oxidation, hydrolysis and biodegradation, leading to production of different degradation products. Penicillin G rapidly transforms to penicilloic acid at the alkaline conditions. Then, it converts very fast to penilloic acid or penillic acid under acidic conditions. The end hydrolytic degradation products of penicillin G are penicilloaldehyde and penicillamine (30). In addition, ß-lactamase enzymes released from resistant bacteria can also transform penicillin G to penicilloic acid by opening the ß -lactam ring (31). These degradation by-products have not been detected in the environment either. The main penicillin G degradation by-products in surface water are penilloic acid, penicilloic acid and isopenillic acid (32). Therefore, determining the main degradation products of penicillin G is very important. In conclusion, the results of this research showed that UV-A process had effectively reduced penicillin G in liquid phase. The removal of the antibiotic was increased with decreasing initial concentration of penicillin G. Moreover, penicillin G removal in the aqueous solution was relatively high at pH=3 and contact time=120 min. According to the current study, the reduction rate of the penicillin G from aqueous solutions was 38% at optimal conditions. Therefore, UV-A process in conjugate with the other processes is an appropriate method for reducing antibiotic penicillin G in polluted water resources and makes it feasible to reduce antibiotic concentration in drinking water to the desirable level.

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