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Influencing Factors on Degradation of Penicillin G by Fenton-like Reaction

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Abstract

Penicillin G (PEN G) was discovered and has been used for treating bacterial diseases in humans, animals, and plants. The fact that it contains **β**-lactam rings is a cause for concern due to its large production and application. Highly common usage in clinical treatment leads to antibiotic resistant bacteria which render medical treatment of the diseases ineffective. PEN G is not degraded during conventional activated sludge processes or adsorption. To eliminate PEN G in the wastewater from a PEN G production facility and the receiving river, influencing factors on PEN G degradation in synthesized wastewater by Fenton-like reaction using calcium peroxide as an oxidant were studied in this work. A High-Performance Liquid Chromatography (HPLC) was used for analyzing PEN G concentration. Experiments were designed by Box-Behnken design (BBD) and conducted in a batch reactor with 150 ppm of initial concentration of PEN G. For the degradation of Penicillin G by Fenton-like reaction, the influencing factors were found to include pH, $FeSO_4$ and CaO_2.

Keywords : Antibiotic; Beta lactam; Penicillin G degradation; Fenton-like reaction; Box-Benhken Design

Introduction

Since Antibiotics are used to inhibit pathogenic microorganisms. They are widely used and their annual consumption is estimated to be between 100,000 and 200,000 tons per year [1]. Large amounts of antibiotics are produced, consumed and applied to treat bacterial diseases in humans, animals, and plants. Bacterial resistance to antibiotics has become a serious problem encountered frequently in clinical treatment of diseases. Overuse of antibiotics and the existence of residual antibiotics in the environment have been linked with the formation of antibiotic resistance. Penicillins are antibacterial agents belonging to the group known as the β-lactam antibiotics. As penicillin antibiotics have strong antimicrobial activities, they have been used extensively both in clinical and animal breeding practices. However, penicillins may cause allergic reactions, even death for some individuals. The use of penicillins in food-producing animals may lead to the emergence of penicillin-resistant bacterial strains, and the residues in milk and tissues are potential risks for individuals who are hypersensitive to penicillins [2]. Among various penicillins, penicillin G is widely used in humans and animals [3-6]. Penicillin G has the most activity among natural antibiotics and more than 11,000 tons are produced annually in 20 countries [7, 8]. However, with increased use, penicillin G contamination has been found in wastewater, groundwater, and sometimes in drinking water [9, 10]. Penicillin G and its intermediates could cause water pollution [11, 12]. Antibiotics in the environment may also increase the problem of development and spread of antibiotic resistance, posing a potential threat to public health, since they can be released into the environment after their use [13-15].

Penicillin G was the first antibiotic found by Sir Alexander Fleming and applied for human bacterial disease [16]. It belongs to the β-lactam class of antibiotics, which all contain β -lactam rings, and is a cause for concern due to its large production, mostly common usage in clinical treatment and widely appearing PEN G-resistant bacteria in medical treatment and the environment [17]. From the view of discharging sources, antibiotic contaminated wastewater cannot be neglected because it contains high concentrations of antibiotics, as exemplified by the penicillin contamination in groundwater. Nowadays, antibiotics have been found in the effluents of many pharmaceutical industries and hospitals, sanitary sewage, surface water and groundwater [18]. Therefore, development of an effective method is greatly desired for the treatment of Penicillin G effluents in order to reduce antibiotic resistance problem and protect the public health in communities.

Many studies have been conducted to degrade PEN G by Advance oxidation processes (AOPs) which have been increasingly applied in the treatment of industrial wastewater. Regarding AOPs methods, the formation of a hydroxyl radical is formed. The processes use H_2O_2, CaO_3, potassium manganese for hydroxyl radical formation as in Fenton process [19], photo Fenton process, photo catalysis [19, 20]. Several researchers have offered a positive assessment of Fenton process degradation technology. Such high concentrations of antibiotics in production wastewater have been reported to significantly influence the treatment of wastewater [21, 22]. The fates of antibiotics during different treatment processes and their environmental behaviors should be illuminated first. Fenton-like reaction can be considered as an effective method for various contaminants of wastewater. The previous studies have suggested that CaO_2 is a more efficient source of hydroxyl radicals ($OH^{\bullet}$). Among other reagents able to release OH, CaO_2 has been selected for these studies due to its relatively low cost [23]. The process of dissolving CaO_2 to obtain H_2O_2 and $Ca(OH)_2$ takes place according to Eq. (1) [23].

$$CaO_2 + 2H_2O \longrightarrow H_2O_2 + Ca(OH)_2 \qquad (1)$$

This work aimed to investigate an appropriate method and influencing factors for degrading PEN G in synthesized wastewater using Fenton-like reaction. Calcium peroxide was chosen as the oxidant in the reaction.

Materials and Methods

In order to determine the factors which influence the degradation of Penicillin G, experiments were designed and conducted. Experimental conditions for PEN G degradation by the Fenton-like reaction were designed using the Box–Behnken design (BBD) [24-25] technique. Based on the review of literature, independent variables were considered to include pH (2-7), CaO_2 content (1-5 g/L), and ferrous ion (0.04-0.12 g/L). One hundred and fifty mg/L of PEN G was placed in a 500 mL batch reactor and the pH was adjusted by HCl and NaOH. Then Ferrous sulfate Heptahydrate ($FeSO_4 \cdot 7H_2O$), a source of ferrous ion, and CaO_2 were individually added into three batch reactor. The reaction temperature was at room temperature. During the first 20 minutes of reaction, 5 ml of samples were drawn from the reactor at an interval of 5 minutes and filtrated by 0.45-micron filters. After that, the samples were drawn at an interval of 10 minutes and filtrated by the filters, and the experiment was terminated after 150 minutes to ensure that no further degradation of PEN G was observed. A High-Performance Liquid Chromatographer, HPLC (Hitachi Chromaster-5000, Japan) equipped with a 5C18-Ar-II (250 mm × 4.6 mm ID, COSMOSIL Packed Column) was used for analyzing PEN G concentration. The mobile phase was solvent A (30% water) and B (70% methanol) with a constant flow rate of 1.0 ml/min during the 15 min analysis. The peaks of PEN G were detected using a UV detector (Hitachi Chromaster 5420 UV/VIS, Japan) at a wavelength of 230 nm.

Results and Discussion

1. Influence of pH on Penicillin G degradation

The effect of pH on Penicillin G degradation was observed at pH 3 5 and 7 for Fenton-like reaction which was shown in Figure 1a The figure indicated that 39.54% of PEN G was degraded at pH 3 while degradation at pH 5, and pH 7 was much lower. Reaction time was not found to be significant since most of the degradation took place during a short period of time from the beginning. The figure indicating the high level of degradation was in the acidic range. Then, the second experimental set was performed at pH in the range of 2-4 for degradation of PEN G as shown in Figure 1b at pH 3, 38.67% of PEN G was degraded, and higher than at pH 2 and pH 4. It should be noted that the percentage degradation at pH 3 in a Figure 1b was different from that of Figure 1a for the same pH because they were the results of different experiments. It is also seen that degradation of PEN G was higher at lower pH. Higher values of pH resulted in more precipitation of $Fe(OH)_3$ and more decomposition of H_2O_2 to water and oxygen. In addition, lower pH values lead to the formation of oxonium ion by proton solvating of H_2O_2 according to Eq.2 and Eq.3 [26], causing the Fenton-like reaction to continue, thus more degradation of Penicillin G.

$$2H_2O_{2\,(aq)} \longrightarrow 2H_2O_{(l)} + O_{2\,(g)} \qquad (2)$$

$$H_2O_{2\,(aq)} + H+_{(aq)} \longrightarrow H_3O^{2+}_{(aq)} \qquad (3)$$

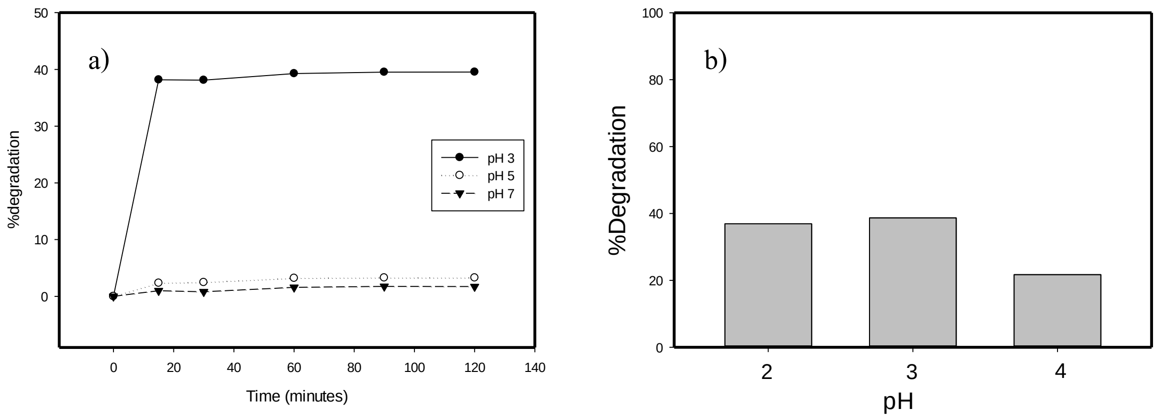

Figure 1 a). pH effect at 3, 5 and 7 on condition of $[CaO_2]$ = 1.15 g/L, Iron dosage = 0.08 g/L.
b). pH effect at 2, 3 and 4 on condition of $[CaO_2]$ = 1 g/L, Iron dosage = 0.08 g/L.

2. Influence of Calcium peroxide content on Penicillin G degradation

Figure 2 shows that Penicillin G degradation for 1, 3 and 5 g/L of CaO_2 was 38.67%, 36.96% and 28.21%, respectively. An increase of CaO_2 tends to decrease Penicillin degradation because of the reaction of hydroxyl radical with H_2O_2 and recombination of hydroxyl radicals [26] according to Eq.4 and Eq.5.

$$HO^{\bullet}_{(aq)} + H_2O_{2(aq)} \longrightarrow H_2O_{(l)} + HO_2^{\bullet}{}_{(aq)} \quad (4)$$

$$HO^{\bullet}_{(aq)} + HO^{\bullet}_{(aq)} \longrightarrow H_2O_2 \quad (5)$$

3. Influence of iron dosage on Penicillin G degradation

Figure 3 shows 7.65%, 36.96% and 21.82% of Penicillin G degradation at 0.04, 0.08 and 0.12 g/L of ferrous dosage, respectively. It can be seen that Penicillin G degradation increased with the increasing ferrous dosage from 0.04 to 0.08 g/L. However, the increasing ferrous dosage from 0.08 to 0.12 g/L decreased Penicillin G degradation. This can be explained by the fact that a higher ferrous dosage results in more $Fe(OH)_2$ precipitation according to (Eq.6) making the solution more basic according to Eq. 7, and leading to the discontinuation of the reaction [26].

$$Fe^{2+}_{(aq)} + 2OH^{-}_{(aq)} \longrightarrow Fe(OH)_{2(s)} \quad (6)$$

$$Fe^{2+}_{(aq)} + HO^{\bullet}_{(aq)} \longrightarrow Fe^{3+}_{(aq)} + HO^{-}_{(aq)} \quad (7)$$

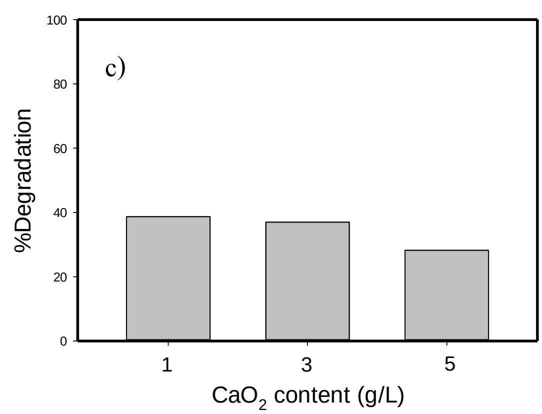

Figure 2 Effect of CaO_2 content on condition of pH 3, Iron dosage = 0.08 g/L

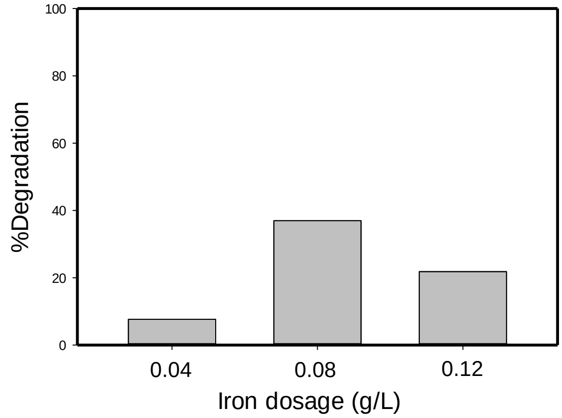

Figure 3 Effect of iron dosage on condition of pH 3, [CaO_2] = 1 g/L

Conclusion

In this study, Fenton-like reaction was demonstrated to be a cost-effective and expedient method for PEN G degradation. The experimental work of this research also revealed that the influencing factors of the reaction were pH, Fe and CaO_2 as stipulated prior to the actual experiment. The results indicate that Penicillin G degradation was better at lower pH values, and lower dosage of CaO_2. For ferrous dosage, it was found that it must not exceed a certain value which was 0.08 g/L in this experiment. The next phase of this research is to investigate the interactions of these parameters and determine the optimal condition for the Fenton-like reaction to degrade Penicillin G. Such information will be useful for future actual application of the reaction for the treatment of Penicillin-contaminated wastewater.

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