



Degradation of amoxicillin, ampicillin and cloxacillin antibiotics in aqueous solution by the UV/ZnO photocatalytic process

Emad S. Elmolla*, Malay Chaudhuri

Dept. of Civil Engineering, Universiti Teknologi PETRONAS, Bandar Seri Iskandar, 31750 Tronoh, Perak, Malaysia

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ABSTRACT

The study examined the effect of operating conditions (zinc oxide concentration, pH and irradiation time) of the UV/ZnO photocatalytic process on degradation of amoxicillin, ampicillin and cloxacillin in aqueous solution. pH has a great effect on amoxicillin, ampicillin and cloxacillin degradation. The optimum operating conditions for complete degradation of antibiotics in an aqueous solution containing 104, 105 and 103 mg/L amoxicillin, ampicillin and cloxacillin, respectively were: zinc oxide 0.5 g/L, irradiation time 180 min and pH 11. Under optimum operating conditions, complete degradation of amoxicillin, ampicillin and cloxacillin occurred and COD and DOC removal were 23.9 and 9.7%, respectively. The photocatalytic reactions under optimum conditions approximately followed a pseudo-first order kinetics with rate constant (k) 0.018, 0.015 and 0.029 min^{-1} for amoxicillin, ampicillin and cloxacillin, respectively. UV/ZnO photocatalysis can be used for amoxicillin, ampicillin and cloxacillin degradation in aqueous solution.

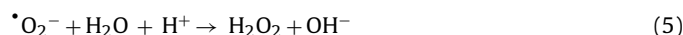
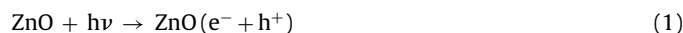
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1. Introduction

From an environmental engineering point of view, pharmaceuticals including antibiotics are a new group of man-made chemicals of concern entering the environment at concentrations such that the health effects are unknown. Problem that may be created by the presence of antibiotics at low concentration in the environment is the development of antibiotic resistant bacteria [1]. Amoxicillin, ampicillin and cloxacillin are semi-synthetic penicillin obtaining their antimicrobial properties from the presence of a beta-lactam ring. They are widely used in human and veterinary medicine. Some authors have found amoxicillin and cloxacillin in wastewater [2,3].

Certain semiconductors, notably zinc oxide (ZnO) and titanium dioxide (TiO_2) when illuminated by photons having an energy level that exceeds their band gap energy excites electrons (e^-) from the valence band to the conduction band and holes (h^+) are produced in the valence band. The photogenerated valence band holes react with either water (H_2O) or hydroxyl ions (OH^-) adsorbed on the catalyst surface to generate hydroxyl radicals ($\bullet\text{OH}$) which are strong oxidant. The photogenerated electrons in the conduction band may react with oxygen to form superoxide ions ($\bullet\text{O}_2^-$). The superoxide ions can then react with water to produce hydro-

gen peroxide and hydroxyl ions. Cleavage of hydrogen peroxide by the conduction band electrons yields further hydroxyl radicals and hydroxyl ions. The hydroxyl ions can then react with the valence band holes to form additional hydroxyl radicals. Degradation of organic substances can be achieved by their reaction with hydroxyl radicals ($\bullet\text{OH}$) or direct attack from the valence band holes. Recombination of the photogenerated electrons and holes may occur and indeed it has been suggested that preadsorption of substrate (organic substance) onto the photocatalyst is a prerequisite for highly efficient degradation. Reactions ((1)–(6)) show the formation of $\bullet\text{OH}$ by photocatalytic process [4].



Daneshvar et al. [4] reported ZnO to be a suitable alternative to TiO_2 since its photodegradation mechanism is similar to that of TiO_2 . ZnO can absorb a larger fraction of the solar spectrum than TiO_2 , and hence ZnO photocatalyst is considered more suitable for photocatalytic degradation in the presence of sunlight [5]. There are many reported studies using ZnO as catalyst in photocatalytic degradation of organic pollutants such as kraft black liquor [6], 2-phenylphenol fungicide [7], phenol and chlorophenols [8] and

* Corresponding author. Tel.: +60 14 904 7313.

E-mail addresses: em.civil@yahoo.com, emadsoliman3@gmail.com (E.S. Elmolla).

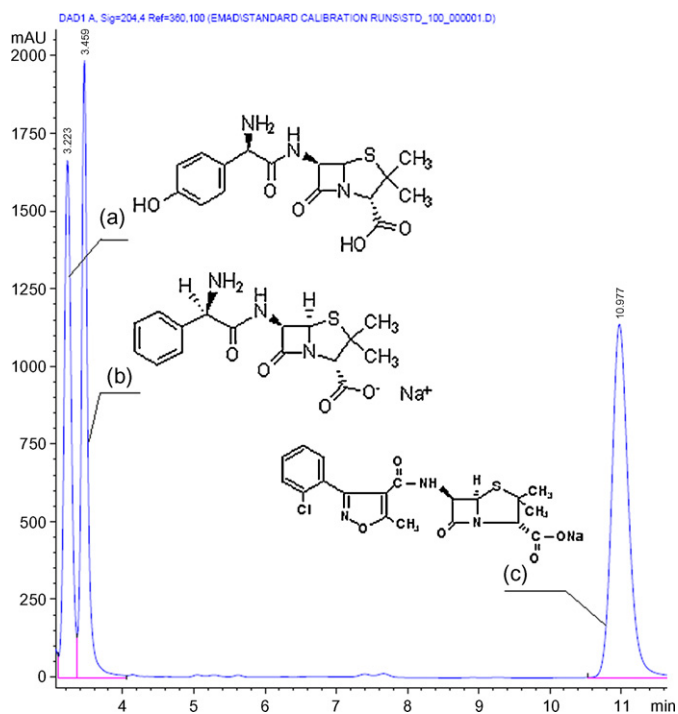


Fig. 1. Chemical structure and HPLC chromatograph of (a) amoxicillin, (b) ampicillin sodium and (c) cloxacillin sodium.

lignin [9]. Photocatalytic degradation of some dyes such as azo dye acid red 14, acid yellow 23, rhodamine 6G (R6G) and methyl orange (MO) have been reported [4,10,11]. Paola et al. [12] have reported that the use of UV/TiO₂ was able to achieve complete mineralization of 50 mg/L lincomycin antibiotic in 8 h. Abellán et al. [13] have reported 82% of sulfamethoxazole degradation and 23% TOC reduction by UV/TiO₂ in 6 hr. No study on degradation of amoxicillin, ampicillin and cloxacillin antibiotics in aqueous solution by UV/ZnO has been reported. Thus, it is important to examine the feasibility of using UV/ZnO photocatalysis for degradation of amoxicillin, ampicillin and cloxacillin antibiotics in aqueous solution.

The present study was undertaken to evaluate the optimum operating conditions for degradation of amoxicillin (AMX), ampicillin (AMP) and cloxacillin (CLX) in aqueous solution and the kinetics of degradation by UV/ZnO photocatalysis.

2. Materials and methods

2.1. Chemicals and antibiotics

Zinc oxide (ZnO) was purchased from R & M Marketing, Essex, U.K. Analytical grade of the antibiotics amoxicillin (AMX) and ampicillin (AMP) were purchased from Sigma, Malaysia and cloxacillin (CLX) from Fluka, Malaysia to construct high performance liquid chromatograph (HPLC) analytical curves for the determination and quantification of the antibiotics. AMX, AMP and CLX used to prepare antibiotics aqueous solution were obtained from a commercial source (Farmaniage Company, Malaysia). The commercial products were used as received without any further purification. Sodium hydroxide (NaOH) and sulfuric acid (H₂SO₄) were purchased from HACH Company, USA. Potassium dihydrogen phosphate (KH₂PO₄) was purchased from Fluka, Malaysia and acetonitrile HPLC grade from Sigma, Malaysia. Fig. 1 shows the chemical structure and HPLC chromatograph of amoxicillin, ampicillin sodium and cloxacillin sodium.

2.2. Analytical methods

Concentration of AMX, AMP and CLX were determined by HPLC (Agilent 1100 Series) equipped with micro-vacuum degasser (Agilent 1100 Series), quaternary pump, diode array and multiple wavelength detector (DAD) (Agilent 1100 Series) at wavelength 204 nm. The data were recorded by a chemstation software. The detection column was ZORBAX SB-C18 (4.6 mm × 150 mm, 5 μm). The column temperature was 60 °C. Mobile phase was 55% 0.025 M KH₂PO₄ buffer solution in ultra pure water and 45% acetonitrile. Chemical oxygen demand (COD) was determined according to the Standard Methods [14]. Biodegradability was measured by 5-day biochemical oxygen demand (BOD₅) according to the Standard Methods [14]. Dissolved oxygen (DO) was measured by a YSI 5000 dissolved oxygen meter. Bacterial seed was obtained from a municipal wastewater treatment plant. A pH meter (HACH Sension 4) with a pH electrode (HACH platinum series pH electrode model 51910, HACH company, USA) was used for pH measurement. A TOC analyzer (Model 1010; O & I Analytical) was used for determining dissolved organic carbon (DOC).

2.3. Antibiotics aqueous solution

Antibiotics aqueous solution was prepared by dissolving specific amounts of AMX, AMP and CLX in distilled water. The aqueous solution characteristics were: AMX, AMP and CLX concentration 104, 105 and 103 mg/L, respectively, COD 520 mg/L, BOD₅/COD ratio ~0, DOC 145 mg/L and pH 5.5. The antibiotics aqueous solution was prepared weekly and stored at 4 °C.

2.4. Experimental procedure

A 500 mL aliquot of the antibiotics aqueous solution was placed in a 600 mL reactor with required amount of ZnO and was mixed by a magnetic stirrer. pH was adjusted to the required value by 1N H₂SO₄ or 1N NaOH and the mixture was kept in dark for 30 min for dark adsorption. Thereafter, the mixture was subjected to UV irradiation and the source of UV light was a UV lamp (Spectroline model; EA-160/FE, 230 V, 0.17 A, Spectronics Corporation, New York, USA) with a nominal power of 6 W, emitting radiation at 365 nm. Samples were taken at pre-selected time intervals using a syringe and filtered through 0.45 μm membrane filter for COD, BOD₅ and DOC determination, and through 0.20 μm membrane filter for determination of antibiotics concentration by HPLC.

3. Results and discussion

3.1. Effect of UV irradiation

Photolysis occurs when chemical substances absorb light. It is important to know separately the effect of photolysis on the antibiotics. The degradation of the antibiotics due to UV irradiation *per se* was studied. The experimental conditions were initial AMX, AMP and CLX concentration 104, 105, 103 mg/L, respectively and pH 5. By 5-h UV irradiation, degradation was 2.9, 3.8 and 4.9% for AMX, AMP and CLX, respectively. Fig. 2 shows that amoxicillin and cloxacillin absorb light below 300 nm. Hence, no significant degradation was expected due to 365 nm UV irradiation and the degradation was due to antibiotics hydrolysis. The hydrolysis reaction would proceed through the attack of the nucleophile H₂O to the β-lactam ring followed by ring opening [15].

3.2. Effect of ZnO concentration

To observe the effect of ZnO concentration, initial ZnO concentration was varied in the range 0.2–2.0 g/L. The experimental

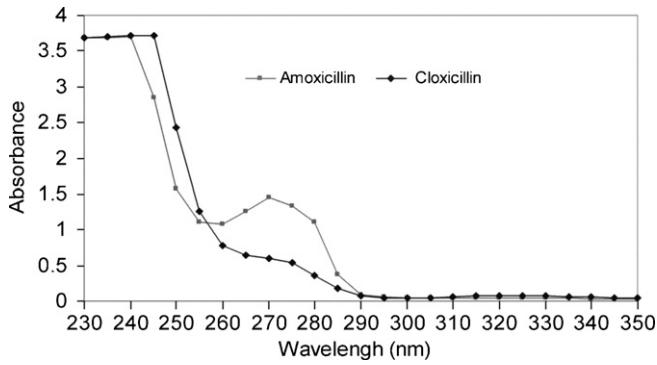


Fig. 2. Absorption spectra of AMX and CLX.

conditions were AMX, AMP and CLX concentration 104, 105 and 103 mg/L, respectively, pH 8, irradiation time 300 min and initial COD 520 mg/L. Fig. 3 shows the degradation of AMX, AMP and CLX, respectively. Degradation after 300 min irradiation were 44.2, 51.2, 72.1, 71.2, 70.2 and 68.6% for AMX (Fig. 3(A)), 60.0, 68.6, 73.3, 70.5, 68.6 and 67.6% for AMP (Fig. 3(B)), 96.1, 100, 100, 100, 100 and 100% for CLX (Fig. 3(C)) at ZnO concentration 0.20, 0.35, 0.50, 1.0, 1.5 and 2.0 g/L, respectively. It is seen that degradation of antibiotics increased with ZnO concentration presumably due to increase

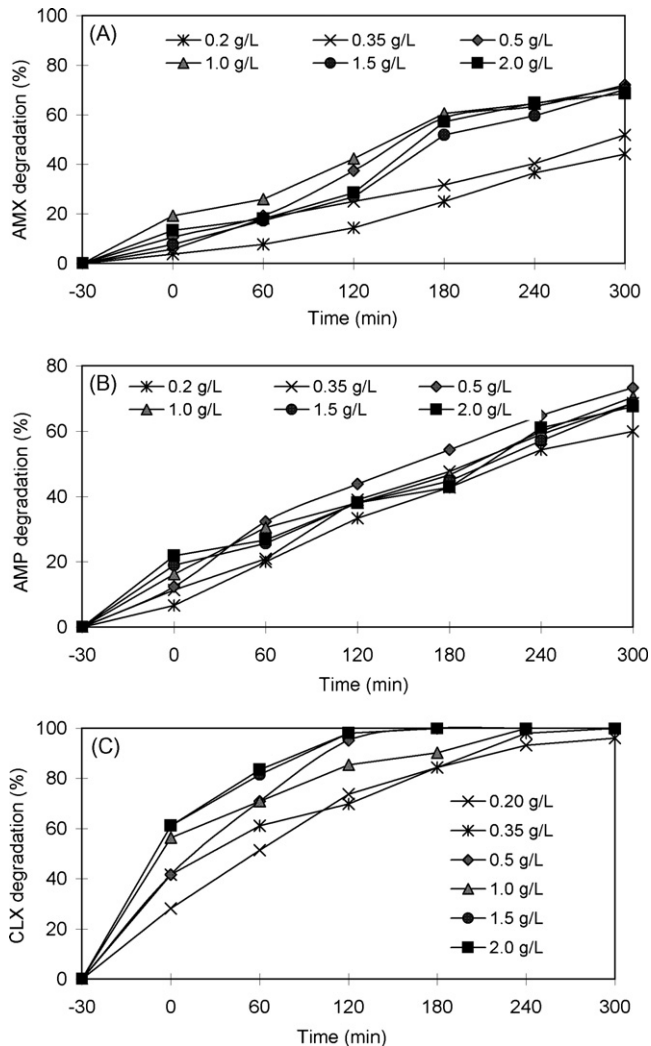


Fig. 3. Effect of ZnO concentration on (A) AMX, (B) AMP and (C) CLX degradation.

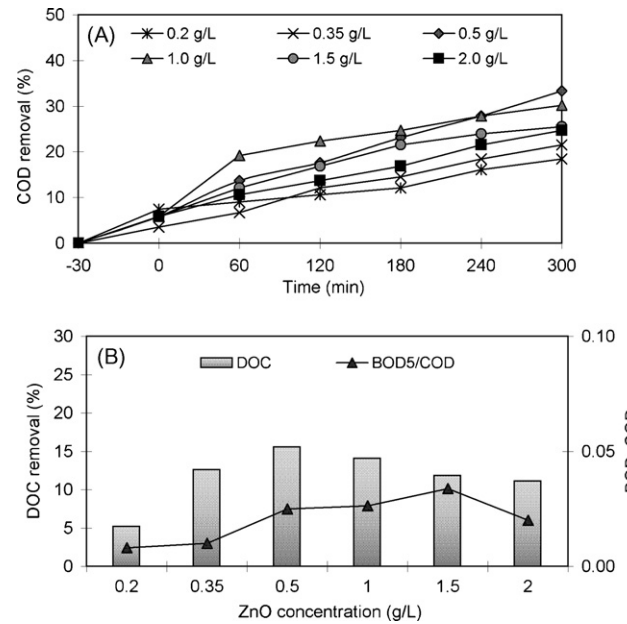


Fig. 4. Effect of ZnO concentration on (A) COD removal and (B) DOC removal and biodegradability improvement.

of $\cdot\text{OH}$ production. However, increasing ZnO concentration above 0.5 g/L did not produce any significant improvement in antibiotics degradation. This may be due to decreasing UV light penetration as a result of increasing turbidity and thus decreasing the photoactivated volume of the suspension [4].

Fig. 4(A) shows the effect of ZnO concentration on antibiotics degradation in terms of COD removal. The results show that COD removal after 300 min irradiation time were 18.4, 21.5, 33.3, 30.2, 25.5 and 24.7% at ZnO concentration 0.20, 0.35, 0.50, 1.0, 1.5 and 2.0 g/L, respectively. Fig. 4(B) shows the effect of ZnO concentration on antibiotics degradation in terms of DOC removal and biodegradability (BOD_5/COD ratio) improvement. The results show that DOC removal after 300 min irradiation time were 5.2, 12.6, 15.6, 14.1, 11.9 and 11.1% at ZnO concentration 0.20, 0.35, 0.50, 1.0, 1.5 and 2.0 g/L, respectively. No significant improvement in biodegradability was observed and the maximum BOD_5/COD ratio was 0.034. Low biodegradability may be due to the toxicity of antibiotics degradation products and dissolved zinc. Based on the results, the optimum ZnO concentration for degradation of amoxicillin, ampicillin and cloxacillin in aqueous solution is 0.5 g/L.

A statistical analysis (one-way ANOVA) performed on the results at a 5% level of significance indicated that AMX, AMP and CLX degradation were significantly affected by ZnO concentration (Table 1).

3.3. Effect of pH and irradiation time

pH is considered an important factor since it influences the surface charge properties of the semiconductor. To study the effect of

Table 1

One-way ANOVA for AMX, AMP and CLX degradation at different ZnO concentration and pH.

Parameter	Antibiotics	No. of groups	F	P-Value	F crit
ZnO	AMX	6	211.9	5.3014E-26	2.4
	AMP	6	211.9	5.3014E-26	2.4
	CLX	6	99.8	1.6E-20	2.4
pH	AMX	3	4.9	0.007	2.8
	AMP	3	3.6	0.023	2.8
	CLX	3	158.6	4.7E-12	2.8

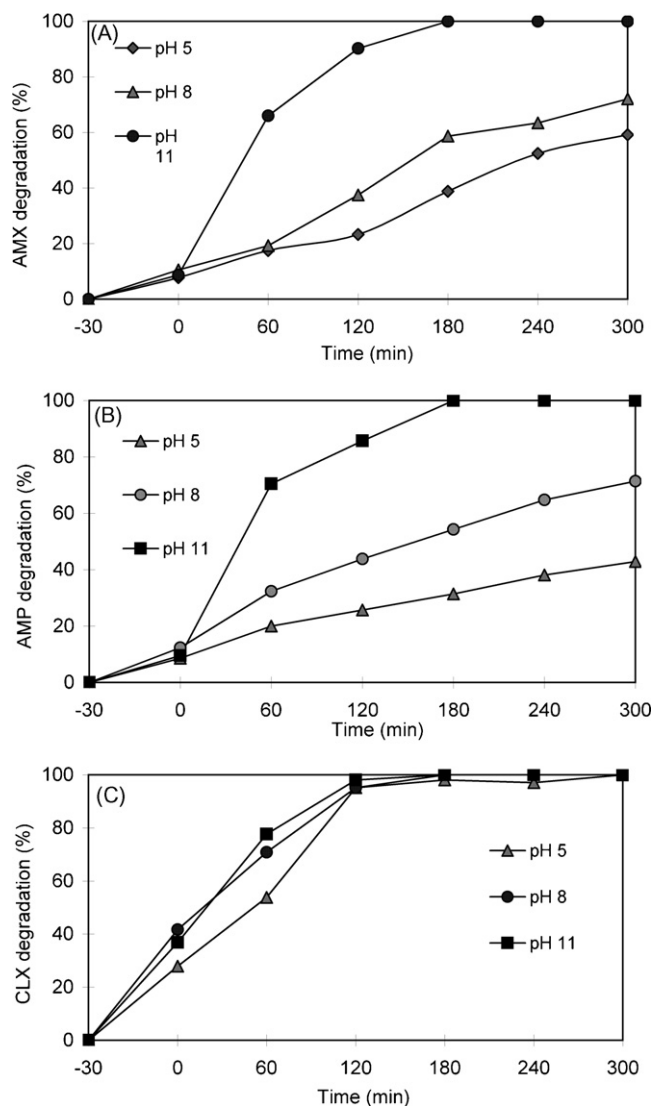


Fig. 5. Effect of pH on (A) AMX, (B) AMP and (C) CLX degradation.

initial pH on the degradation of AMX, AMP and CLX, experiments were conducted by varying the pH in the range 5–11. The experimental conditions were AMX, AMP and CLX concentration 104, 105 and 103 mg/L, respectively, ZnO concentration 0.5 g/L, irradiation time 300 min and initial COD 520 mg/L. Fig. 5 shows the effect of pH on the degradation of AMX, AMP and CLX. The degradation after 300 min irradiation were 59.2, 72.1 and 100% for AMX (Fig. 5(A)), 42.9, 71.4 and 100% for AMP (Fig. 5(B)) and complete degradation for CLX (Fig. 5(C)) at pH 5, 8 and 11, respectively. Based on the results, the optimum pH and irradiation time for degradation of amoxicillin, ampicillin and cloxacillin in aqueous solution are 11 and 180 min, respectively.

A statistical analysis (one-way ANOVA) performed on the results at a 5% level of significance indicated that AMX, AMP and CLX degradation were significantly affected by pH (Table 1).

Fig. 6(A) shows the effect of pH on antibiotics degradation in terms of COD removal. COD removal after 300 min irradiation time was 24.7, 26.3 and 28.2% at pH 5, 8 and 11, respectively. Fig. 6(B) shows the effect of pH on antibiotics degradation in terms of DOC removal and biodegradability (BOD₅/COD ratio) improvement. DOC removal after 300 min irradiation was 11.1, 15.6 and 16.3% at pH 5, 8 and 11, respectively. No significant improvement in biodegradability was observed and the maximum BOD₅/COD ratio at pH 11 was 0.036.

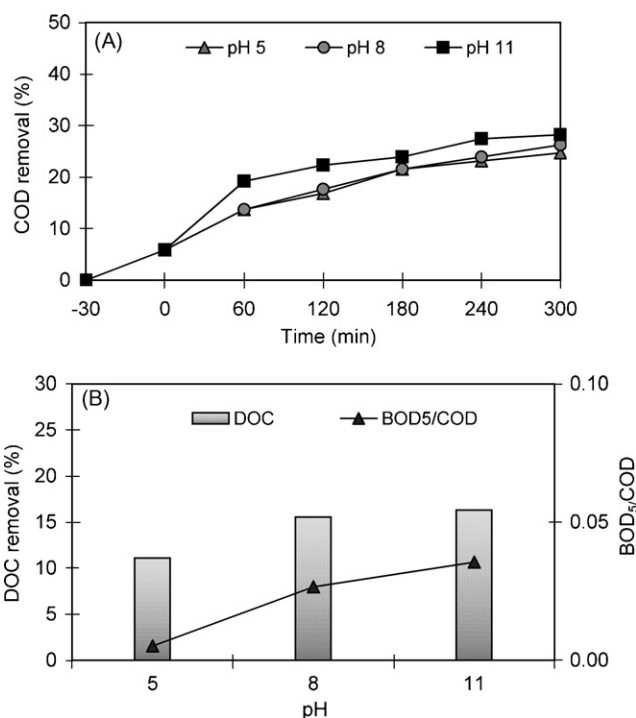
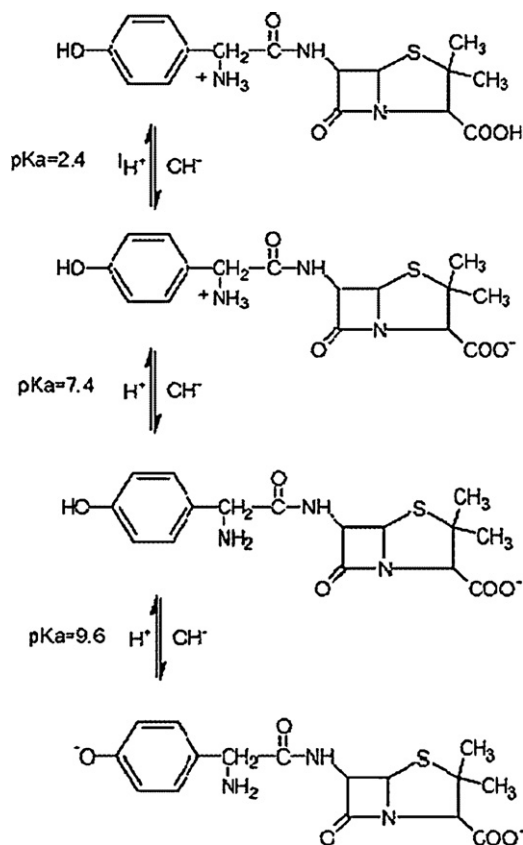


Fig. 6. Effect of pH on (A) COD removal and (B) DOC removal and biodegradability improvement.

The effect of pH on antibiotics degradation can be explained by taking into consideration the properties of both the catalyst and antibiotics at different pH. For ZnO, the zero point charge is 9.0 ± 0.3 [16] and hence the ZnO surface is positively charged at $\text{pH} < 9$ and is negatively charged at $\text{pH} > 9$. It is reported that ionic amoxicillin species changes from positive charge at acidic pH to negative charge at alkaline pH as shown in Scheme 1 [17]. At acidic pH, both ZnO and amoxicillin are positively charged and hence, the adsorption on the surface of ZnO is limited. At near neutral pH, amoxicillin has negative charge, whereas ZnO still has positive charge favouring adsorption. At $\text{pH} > 9$, both amoxicillin and the ZnO are negatively charged and so repulsive forces between the catalyst and the antibiotics are developed. The results show that the adsorption of the antibiotics after 30 min dark adsorption was high at pH 8 compared to pH 11 and 5, and the adsorption percent varied for each antibiotic depending on their property (Fig. 5). High degradation of antibiotics in alkaline condition may be due to two facts. First is the presence of large quantities of OH^- ions on ZnO surface favoring formation of $\cdot\text{OH}$ radicals [11]. Second is the hydrolysis of the antibiotics due to instability of the β -lactam ring at high pH as reported by Hou and Pool [18].

3.4. Kinetics of photocatalytic degradation of amoxicillin, ampicillin and cloxacillin

To study the kinetics of photocatalytic degradation of amoxicillin, ampicillin and cloxacillin, experiments were conducted under optimum operating conditions (ZnO concentration 0.5 g/L, irradiation time 180 min, pH 11). Initial AMX, AMP and CLX concentration were 104, 105 and 103 mg/L, respectively. Fig. 7 shows the plots of $\ln([\text{antibiotic}]/[\text{antibiotic}]_0)$ vs. irradiation time for amoxicillin, ampicillin and cloxacillin. The linearity of the plots suggests that the photocatalytic reaction approximately followed the pseudo-first order kinetics. Degradation of cloxacillin exhibited the highest rate constant (0.029 min^{-1}) followed by amoxicillin (0.018 min^{-1}) and ampicillin (0.015 min^{-1}).



Scheme 1. Anionic species of amoxicillin at different pH [17].

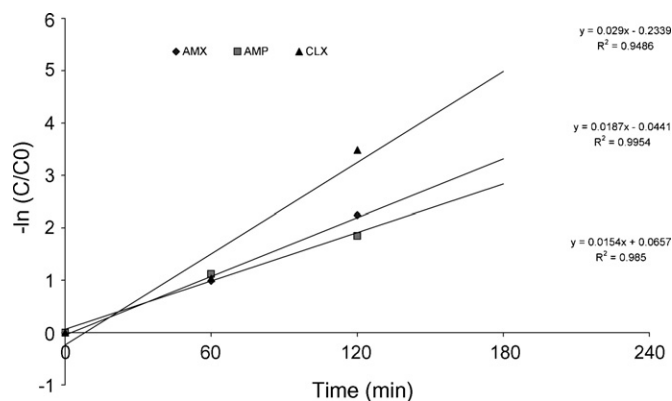


Fig. 7. Kinetic analysis of AMX, AMP and CLX degradation under optimum conditions.

4. Conclusions

In UV/ZnO photocatalysis, pH has a great effect on amoxicillin, ampicillin and cloxacillin degradation. The optimum operating conditions for complete degradation of the antibiotics in aqueous solution containing 104, 105 and 103 mg/L amoxicillin, ampicillin, and cloxacillin, respectively were: ZnO concentration 0.5 g/L, irra-

diation time 180 min and pH 11. Increase of organic carbon release (mineralization) with increasing illumination time, ZnO concentration and pH was observed and 9.7% of DOC was removed under optimum operating conditions. Degradation of cloxacillin exhibited the highest rate constant (0.029 min^{-1}) followed by amoxicillin (0.018 min^{-1}) and ampicillin (0.015 min^{-1}). UV/ZnO photocatalysis can be used for amoxicillin, ampicillin and cloxacillin degradation in aqueous solution.

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