Direct Oxidation of Antibiotics from Aqueous Solution by Ozonation with Microbubbles

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Abstract. In this research, antibiotics (tetracycline, ceftriaxone, and metronidazole) were removed in a batch reactor each one separately and together using ozone microbubbles (OMBs) method. The antibiotic removal efficiency was analyzed under different reaction conditions, including initial solution pH, ozonation time, ozone production rate, and initial antibiotic concentration. It was found from the experiments that the elimination of antibiotics by ozone microbubbles was higher at the basal medium between (7-8). In addition, as the ozone production rate was raised from (3.33-16.66) mg/min, the removal efficiency increased, as did the antibiotic concentration (1-100) PPM. Also, the removal tests were carried out under optimal conditions using the conventional ozonation method, and the microbubble technology and the results were compared under the same operating conditions. The microbubbles were found to save a lot of time and reduce the amount of ozone used.

Keywords: Antibiotics, Tetracycline, Ceftriaxone, Metronidazole, Ozone Microbubble.

1. Introduction

Water is a renewable, precious, and invaluable resource for human life that also helps the ecosystem to be healthy. It is spread in various parts of the planet, though, its content is not the same in all of them. More than half of the world's main waterways, for instance, are seriously degraded or poisoned due to polluting habitats that affect the health of living creatures. According to WHO and UNICEF statistics, 780 million individuals do not have access to drinking water and 185 million of them are still using surface water to serve their everyday needs [1–3]. In fact, the poor standards of water can slow down industrial development and may have detrimental health and livelihood consequences. In certain developed nations, chemical pollution of surface water is mainly caused by industrial and agricultural discharges. Pollution and toxic waste are placing water supplies at risk, affecting, and damaging the world's habitats. This risk is associated with emissions that contain persistent organic compounds (POPs), which has been one of the main environmental issues in recent decades. POPs are a category of organic compounds that withstand photochemical, chemical, and biochemical degradation into different degrees, resulting in a high average lifetime in the environment. As a result, numerous POPs in rivers, streams, and oceans around the world have been found in low concentrations (mg/l) and in drinking water as well [4]. Although the carcinogenic, mutagenic, and bactericidal properties of most POPs remain uncertain, there is a strong
interest in their exclusion from the waters to prevent their possible toxic effects and potentially harmful consequences on the wellbeing of living organisms, including humans [5]. The research community has based its attention for a long time on the study of chemical emissions that are governed by numerous laws. Some of them are polar, poisonous, chronic, and bioaccumulative toxins, such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), or dioxins. However, the emergence of modern and more precise analysis techniques has made it possible in recent years to identify the presence of other potentially harmful chemicals, known internationally as ‘emerging pollutants.’ These are classified as contaminants that are previously unknown or unrecognized, not governed by law, and whose impacts on health and the environment are not yet adequately understood. Steroids and hormones, pharmaceuticals and personal care goods, antiseptics, surfactants, disinfectants, dyes, and preservatives are common examples [1].

Pharmaceuticals are natural or man-made compounds that are used in human medications, animal medicines, and non-prescription products. These chemicals have active components which, when taken, are supposed to have pharmacological effects [6]. High levels of wastewater containing toxic organic chemicals are produced by the pharmaceutical industry. These contaminants not only contaminate drinking water, but also impair the endocrine system of fish and other marine species, causing damage to humans and animals [7,8]. Active pharmaceutical ingredients (APIs) used in medicine, as well as excipients and additives, can reach the atmosphere from several nonpoint sources, including manufacturing plants, STP effluents, household waste, and landfill effluent. Animals excrete pharmaceuticals as well which are used as growth boosters or for other uses [9]. Antibiotics, hormones, antacids, analgesics, anti-inflammatory medications, beta-blockers, antipyretics, tranquilizers, lipid-lowering narcotics, and stimulants are the most pharmaceutical pollutants most often contained in wastewater effluents [10].

Antibiotics are antimicrobial medicines that kill or prevent bacterial growth. Antibiotics are categorized according to their modes of action or chemical composition. For many decades, the use of antibiotics in vast amounts has been a subject of study in clinical settings and, in recent years, in environmental research [11]. Previous research has shown that the rate of bacterial adaptation is influenced by both the final concentration of the antibiotic (i.e. the strength of selection) and the speed at which the final antibiotic concentration is realized, even in the simplest cases (e.g. a single species evolving in response to a single antibiotic) [12–14]. The global distribution of antibiotics released into the environment is currently related to patient and animal excretion, according to available evidence [15]. Antibiotics are found in 100,000–200,000 tons each year all over the world [16]. Due to their biocidal activity, antibiotics are low biodegradable compounds that cannot be completely removed, therefore, eliminating them from wastewater is a crucial challenge [16–18]. Different techniques have been tested for the removal of antibiotics from wastewater treatment [19]. These include activated carbon [20,21], Nanofiltration [21,22] and reverse osmosis (RO) methods [21,23]. However, these treatment processes are found to be temporary solutions when only the pharmaceuticals are transported or relocated into the solid phase or be concentrated into a small quantity in the aqueous solution. Under these conditions, close to real drinking water necessitates further treatments. Chlorination processes are used for this purpose to extract multiple pharmaceuticals from synthetic wastewater in a laboratory-scale setup. However, the removal performance of certain antibiotics did not reach more than 30% in 30 minutes’ treatment, and 80% of removals took at least 24 hours [21]. Other techniques that are gained more popularity because of their higher efficiencies are advanced oxidation methods such as UV/H2O2 process, photo-catalysis [24], electrochemical method [25], ozonation processes [24], electro-Fenton process [26], and oxidation akin to Photo-Fenton [24]. These methods can inactivate multiple antibiotics simultaneously by altering their molecular structures. Ozonation has now been accepted as the best approach for eliminating antibiotics from wastewater [27]. Ozone is a potent oxidant that can be used to remove microorganisms, inorganic ions, and organic contaminants from surface and groundwater, as well as wastewater [28,29]. Antibiotic elimination by ozone has been the topic of several kinds of research works in the past years, such as amoxicillin [30], sulfadiazine, sulfamethazine, sulfamethoxazole, and sulfathiazole [31], penicillin [32],
ceftriaxone sodium [24], tetracycline [33–35] and metronidazole [36]. Almost all the research, however, was done in traditional bubble column reactors [24]. Latest studies have shown that low ozone (O3) doses (5 mg/L) can deplete certain antibacterial compounds by more than 90% in wastewaters with (DOC) concentrations as high as 23 mgC/L [37]. In aqueous solutions, ozone reacts with contaminants in two ways: direct oxidation by molecular ozone and indirect oxidation by the reaction with hydroxyl free radicals formed by ozone decomposition [38–40]. Indirect reactions by radicals are non-selective and fast, while direct reactions are always sluggish and highly selective. Additionally, OH¯ ions catalyze the decomposition of ozone. The ozone process includes all ozone reactions, which happen at the same time [24,29]. Though, poor ozone dissolution and a sluggish mass transfer rate limit the existing ozone treatment processes, resulting in low gaseous ozone utilization performance and hence high operational costs [41]. To overcome these obstacles, advanced oxidation processes (AOPs) have been developed to improve ozone solubility in water, conduct rapid oxidation of organic compounds, and reduce ozone depletion in water and wastewater. These involve the application of O3/H2O2, UV/O3, or ultrasound/O3, as well as catalytic ozonation using metal ions, metal oxides, or activated carbon. However, the advanced techniques’ implementations are constrained due to their difficulty and high cost, as well as slow ozone mass transfer. Consequently, further research into an effective methodology that increases ozone mass transfer and oxidation potential for practical use is important. In this regard, researchers have recently been drawn to a novel strategy of injection ozone in the form of micro-sized bubbles [42]. Because of the small bubble scale, large interfacial area, long stagnation time, high interior pressure, and high mass transfer rates, microbubble wastewater treatment has attracted a lot of interest [41–44]. Ozone microbubble technologies were applied in water disinfection, oxidation of organic and inorganic pollutants, and color removals [42], such as Coliform group [45], Bacillus subtilis spores [46], methylene blue [47], acrylic fiber manufacturing [41,43], and ammonia [48,49].

In this research, we have studied the removal of one of the most important and widely used emerging contaminations from wastewater (Antibiotics) such as Tetracycline (TC), Ceftriaxone sodium (CTX), and Metronidazole (MNZ). The study suggests injecting ozone into the liquid solution in the form of microbubbles to oxidize the antibiotics and thus removing them. The work also investigates the effect of different operational parameters on antibiotic degradation efficiency such as ozonation time, feed pollutant concentration, ozone concentration, and pH, as well as the comparison between using the microbubbles technique and the traditional one. Microbubbles in the current work were generated using a premium microporous ceramic diffuser that can generate exceptionally fine bubbles of approximately (100-500) micron [Pentair aquatic Eco-systems, Point four, 1 DMBDC100, USA]. Furthermore, the flat plate design of the diffuser ensures a homogenous distribution for the bubbles in the liquid solution with the lowest coalescence. The paper is organized as follows: Section 2 shows the details of the material and methods applied in this research. Section 3 presents the results and discussion of the removal experiments, considering the effect of the above-mentioned operating conditions. The conclusions from this study are drawn in section 4.

2. Material and methods

2.1. Material:

Tetracycline (C22H24N2O8) and metronidazole (C6H9N3O3) were obtained in a powder form (AR:99%) from Samara Drug Factory-Iraq, while Ceftriaxone Sodium (C18H18N8O7S3) was obtained as an injection ampoule of 1 gm (AR:98%) from a local pharmacy store. Buffer solutions were prepared for each substance at a concentration of 250 mg/l by dissolving the appropriate amount of antibiotic in deionized distilled water (DI), and the solutions were stored in 2-liter glass flasks at a temperature of 10°C. Hydrochloric acid and sodium hydroxide (AR:99%) were used for adjusting the pH of the solutions.

2.2. Experimental method:

Figure (1) shows a schematic diagram of the experimental rig used for the current work which consisted of an air compressor, a flowmeter, an ozone generator, and a microbubble tank. The experiments were performed by passing the dry air that coming out of the air compressor (50 litres, Ingico, China) at a
pressure of approximately 2.2 bar into the flowmeter to control its flowrate and then into the ozone generation device (1 g/hr., Enaly, China) to produce the ozone. After that, the gas enters the ceramic microbubble diffuser (MBD) ((Point FourTM diffuser, USA) which was placed at the bottom of the bubble tank. The microbubble diffuser (MBD) operates at a pressure between (1.9 – 3.45) bar, so we used an air compressor to reach this pressure. The bubble tank was made from acrylic and had dimensions of (7×40×20) cm in width, length, and height, respectively. All the experiments were conducted at a room temperature of 14°C, while the temperature of water was 12°C. After starting the experiment by injecting the ozone microbubbles into the liquid solution, samples of 5 ml were taken from the solution at 5 min time intervals to measure their concentrations using a UV spectrum, (UV-9200, Biotech Engineering, Management Co. LTD, UK). After knowing the wavelength and the calibration curve for each pollutant, the measurements were performed with the UV device using 1ml quartz cells. The removal efficiencies of antibiotics were calculated according to equation 1, while changing the main parameters including pH, ozone concentration, antibiotic concentration, and gas flow rate.

\[
\text{Removal efficiency (\%) } = \frac{c_0 - c_f}{c_0} \times 100\% \quad (1)
\]

Where \(c_0\) and \(c_f\) in (mg/l) are the initial and final concentrations of pollutant before and after ozonation process.

The second part of the work was focused on mixing the three antibiotics used previously in one solution and considering the ideal conditions obtained from the removal of each substance alone. The removal efficiency was measured based on the measurement of the chemical oxygen demand COD of the solution after ozonation process using a COD reactor (Lovibond Water Testing, RD 125 model, Dortmund, Germany). The measurements were accomplished by adding 2 ml of the sample in a vial containing a normal dose from a standard solution and all antibiotics in the sample were oxidized after 2 hours at 150°C. The vial was let to cool down to the room temperature and then the COD was measured using a photometer device (Lovibond Water Testing MD200, Dortmund, Germany).

3. Results and discussion:
3.1 Effect of pH.
One of the most important parameters in the ozonation process is pH because it can affect both the mechanism and the rate of the ozonation process [29]. To investigate the impact of pH on the degradation rate of tetracycline and ceftriaxone, the initial pH value of the solution was changed in the range of (3.2, 5.1, 7.2, 9.0, and 11.2) for tetracycline and (3.1, 5.0, 7.3, 9.1, and 11.2) for ceftriaxone. The antibiotic concentration, the inlet gas flow rate and the ozone concentration were fixed at 10 mg/l, 1 l/min. and 13.33 mg/min respectively for all experiments. The results of removal efficiencies at different pH values as a function of ozonation time were given in Figure 2.
As shown in Figure 2, when comparing the results of the two antibiotics, it was discovered that tetracycline was more removable and took less time than ceftriaxone. The maximum removal rates for tetracycline (95.74%) and ceftriaxone (90.29%) were achieved at pH values of (7.2) and (7.3) after 40 min and 50 min respectively. Acidic conditions (pH=3), however, showed lower removal rates of (85.89%) and (78.42%) for tetracycline and ceftriaxone, respectively. This can be ascribed to the fact that direct ozone reaction is the most frequent in acidic solutions, however, when the pH rises, the hydroxyl radicals HO• were formed by the reaction of ozone with OH‾ ions present in the solution, making the reaction of the free radicals the dominant [24]. Since both the ozone molecules and the hydroxyl radicals OH• are oxidizing agents, the contaminants involved are expected to be eliminated more quickly at pH values greater than (7) [36]. On the other hand, the lowest removal rates of tetracycline (74.98%) and ceftriaxone (76.81%) were attained when the pH value was (11.2), so, ceftriaxone has a slightly better removal than tetracycline, but it took longer. This can be understood by the fact that antibiotic elimination was mainly caused by the direct ozone oxidation reaction, however, at basic conditions, the dose of the hydroxyl radicals increased significantly, causing the indirect oxidation rate rises at the expense of the direct oxidation. Therefore, the antibiotic degradation rates were dropped [24]. The other possible explanation for this effect is that both the degradation products and the parent compound (tetracycline or ceftriaxone) are targeted simultaneously by ozone resulting in lower removal rates for the goal species [36].

Figure 2 Change of removal rates with time at different initial pH values during ozonation process for (a) tetracycline and (b) ceftriaxone. Flow 1 l/min, ozone concentration 13.33 mg/min and 10 ppm initial concentrations for (TC, CTX).

Figure 3 shows the change of pH with time at various initial pH solution. During the ozonation process, the pH value changes and as can be noticed from the results, the initial pH of 7, 9, and 11 steadily decreases with time and this most likely due to formation of carboxylic acids in the processed liquid [36]. Under acidic conditions, however, ozonation has no noticeable effect on the pH of the solution. In general, a decrease in pH during the ozonation process can have a major effect on the oxidation rate, reaction mechanism, and ozone absorption rate as observed in Figure 3.
3.2 Effect of gaseous ozone concentration:

The rate of organic oxidation is greatly influenced by the concentration of ozone in the aqueous phase [47]. For this purpose, the degradation of tetracycline and ceftriaxone by ozone microbubbles was studied at various gaseous ozone concentrations, ranging from 3.33 to 16.66 mg/min. The antibiotic concentration was maintained at 10 mg/min for both pollutants, the inlet gas flow rate at 1 l/min, and the initial pH at 7 for tetracycline and ceftriaxone and the results were given in Figure 4.

As can be seen from Figure 4, the higher the ozone concentrations, the higher the rate at which the antibiotics degraded. The results of the removal rates were (69.42%) for tetracycline and (72.46%) for ceftriaxone at 3.33 mg/min ozone concentration. These numbers increased respectively to (91.31%) and (87.46%) when the ozone concentration was 10 mg/min and to (97.96%) and (91.89%) when the ozone concentration was 16.66 mg/min.

According to Henry's law, as the gaseous ozone concentration rises, the equilibrium ozone concentration in the aqueous phase rises as well:

\[
\text{[O}_3\text{] } \bullet = \frac{\text{[O}_3\text{] } g}{\text{H}}
\]

Where [O3] g is the gaseous ozone concentration, and H is the Henry's law constant.

In this case, the mass transfer driving force will rise, resulting in an increase in the volumetric mass transfer coefficient of ozone from the gas phase to the liquid phase. Additionally, when the ozone generation rate increases, the partial pressure of ozone in the gas bubbles increases as well, leading, in turn, to an augmentation of the amount of ozone transferred to the liquid medium and thus, greater amount of the eliminated contaminants [24,50].
Figure 4 effect of ozone concentration on the removal rates for (a) tetracycline and (b) ceftriaxone. pH 7, flow rate 1 l/min and 10 PPM initial concentration of TC and CTX.

3.3 Effect of antibiotic concentration:
To investigate how antibiotic concentration, affect the rate of tetracycline and ceftriaxone degradation in simulated solutions, the concentration of the pollutant was changed between (1-100) mg/l for tetracycline and (5-100) mg/l for ceftriaxone. The pH of the solution, the ozone concentration, and the inlet gas flow rate were fixed at 7, 13.33 mg/min, 1 l/min respectively for all experiments. The results were presented in Figure 5.

According to the results in Figure 5, the higher the antibiotic concentration in the solution, the greater the efficiency of removing the antibiotic. When the concentration was 1 mg/l, the removal rate of tetracycline was (72.28%). Then, it was boosted considerably to (98.96%) when the initial concentration was 100 mg/l after 40 min of ozonation time. For ceftriaxone, the recorded removal rates were (82.94%) and (96.76%) for 5 mg/l and 100 mg/l respectively within 50 min of ozonation time. The removal of tetracycline was found to be greater than that of ceftriaxone and occurred in a shorter period in the majority of the results. The ozone-antibiotic reaction was modeled as a pseudo-first-order reaction, with the degradation period of tetracycline decreasing as the initial concentration of tetracycline increased. As the original antibiotic concentrations are raised, the first-order rate constant decreases. Ozone reacts with many molecules at lower concentrations due to its higher initial antibiotic concentration. As a result, at higher antibiotic concentrations, much more ozone is required. Finally, it can be concluded that the pseudo-first-order model corresponded to the degradation kinetics under the test conditions [16].
Figure 5 Effect of the initial antibiotic concentration on the removal rates for (a) tetracycline and (b) ceftriaxone. pH 7, flow rate 1 l/min and 13.33 mg/min ozone concentration.

3.4 Effect of gas flow rate:
Experiments were performed at different gas flow rate values ranging from 0.6 to 1 l/min, where the antibiotic concentration was 10 mg/l, the ozone concentration was 13.33 mg/min, and the pH of the medium was 7. These parameters were set constant for both experiments, and the results are plotted in Figure 6.

It can be noticed from the data that the rate of antibiotic degradation increased as the gas flow rate increased. It was also observed that tetracycline has a higher removal rates and shorter removal times than ceftriaxone. When the gas flow rate was 0.6 l/min, the removal rate for tetracycline was (84.01%) and ceftriaxone (80.79%). However, increasing the gas flowrate into 0.8 l/min leading, in turn, to enhance the removal rate for tetracycline into (90.24%) and ceftriaxone into (85.98%). These values augmented further into (95.77%) and (90.29%) respectively at a higher gas supply of 1l/min.

When the gaseous ozone injected through microbubbles, it will be absorbed into the aqueous phase and then reacted with pharmaceutical compounds. It is well known that the amount of the oxidized pollutant changes directly with the amount of ozone available in the liquid side. Also, the volumetric mass transfer coefficient of ozone improved as the gas flow rate increased and this resulted in a greater net surface area for mass transfer of ozone to the aqueous phase [24,51].
3.5 Removal of multiple antibiotics in one aqueous solution.
The second part of the current work was dedicated on exploring the possibility of treating multiple pollutants mixture. Three antibiotics were chosen for this purpose including tetracycline, ceftriaxone, and metronidazole at an initial concentration of 10 mg/l for each substance. The flow rate was kept at 1 l/min, ozone concentration at 13.33 mg/min, and pH at 7.3, during this experiment. The rate of contaminant degradation was determined by the chemical oxygen demand COD removal test and the results were shown in Figure 7.
As shown in Figure 7, the maximum COD degradation rate was (87.5%) for the mixture after 60 min of ozonation with microbubbles. The COD test was crucial in determining the rate of the antibiotics removal from the mixture because it provides an evidence for the complete mineralization of the original primary materials as well as any byproducts that might result from the reaction of the raw materials with ozone.

Figure 6 Effect of the inlet gas flowrate on the removal rates for (a) tetracycline and (b) ceftriaxone. pH 7, 13.33 mg/min ozone concentration and 10 PPM initial concentration of TC and CTX.

Figure 7 the change of (COD) removal efficiency for mixture with time. pH 7.3, flowrate 1 l/min and 10 PPM initial concentration for each material.
3.6 The effect of microbubbles

To understand the extent of benefit resulting from the addition of micro-bubbles technology in distributing ozone gas inside the liquid, several experiments were conducted to remove antibiotics (tetracycline, ceftriaxone, and metronidazole) both separately and in combination, once with using the MB diffuser and the other without the MB diffuser (i.e. with conventional bubbles). The antibiotic concentration, the inlet gas flow rate, the pH of the liquid, and the ozone concentration were fixed at 10 mg/l, 1 l/min., 7.3 and 13.33 mg/min respectively. The results were displayed in figures 8,9,10, and 11.

From the results presented here, it is evident that the removal rate of all antibiotics applied in the present study is higher when using microbubbles compared to conventional bubbles. The improvement in the degradation efficiencies were (36.46%) for tetracycline, (32.56%) for ceftriaxone, (35.12%) for metronidazole, and (32.43%) for the mixture of the three as shown in table 1.

| No. | Substance     | Removal efficiency With MB diffuser % | Removal efficiency Without MB diffuser % | Percentage of increase | Ozonation time (min) |
|-----|---------------|---------------------------------------|-----------------------------------------|------------------------|----------------------|
| 1   | Tetracycline  | 95.77                                 | 70.18                                   | 36.46                  | 40                   |
| 2   | Ceftriaxone   | 90.29                                 | 68.11                                   | 32.56                  | 50                   |
| 3   | Metronidazole | 97.10                                 | 71.86                                   | 35.12                  | 40                   |
| 4   | Mixture       | 87.50                                 | 66.07                                   | 32.43                  | 60                   |

Table 1: Comparison between the removal efficiencies with and without the use of microbubbles.

Figure 8 effect of (MBD) on removal tetracycline ceftriaxone
pH 7.3, flow 1 l/min, 13.33 mg/min OZ, 10 PPM PPM

Figure 9 effect of (MBD) on removal ceftriaxone
pH 7.3, flow 1 l/min, 13.33 mg/min, 10
Figure 10 effect of (MBD) on removal metronidazole mixture
pH 7.3, flow 1 l/min, 13.33 mg/min OZ, 10 PPM for each

Microbubbles have solved some of the conventional ozonation process's limitations, such as low ozone dissolution and slow gas–liquid mass transfer rate. When compared to coarse bubbles, microbubbles have higher surface area to volume ratio, longer residence times in the liquid and faster mass transfer rates according to Stokes’ law [52]. Therefore, the transport rate of ozone to the liquid phase is expected to increase with microbubbles. Moreover, the generation rate of hydroxyl free radicals OH• is increased by microbubbles because when microbubbles collapse in the water solution, they generate OH• radicals. Since OH• has a higher oxidation potential than molecular ozone, the oxidation effects will be boosted with the involvement of microbubbles [43].

3.7 kinetics of antibiotics degradation:
The following two equations were used to calculate the reaction rate constant and the degree of the ozonation reaction depending on the concentration of the pollutant only [53].

\[ C_t = C_o \exp(-k_1 t) \]
for first order \hspace{1cm} (2)

\[ C_t = \frac{C_o}{1 + C_o k_2 t} \]
for second order \hspace{1cm} (3)

where \( C_o \) [mg/l] is the initial concentration of the antibiotic measured at time 0, \( C_t \) [mg/l] is the concentration of the antibiotic being studied at time \( t \), and \( k_1 \) [1/min] and \( k_2 \) [l/mg.min] are the rate constants for kinetic of first and second orders respectively.

To calculate each order of the reactions and the values of the rate constant for the ozonation process, the optimal conditions for each antibiotic were used in the ozone removal experiments (pH 7, 13.33 mg/min ozone, 1 l/min flow rate, and 10 mg/min antibiotic concentration), the results of these experiments were applied to the above two equations, yielding the data shown in the figures 12,13,14, and 15 for tetracycline, ceftriaxone, metronidazole, and mixture of them respectively. The data of the rate constants and correlation coefficients are given in table 2.
Figure 12 Time dependences of experimental and calculated values of TC concentration, (a) 1st order, (b) 2nd order. pH 7, flow rate 1 l/min, 13.33 mg/min ozone, 10 PPM TC.

Figure 13 Time dependences of experimental and calculated values of CTX concentration, (a) 1st order, (b) 2nd order. pH 7, flow rate 1 l/min, 13.33 mg/min ozone, 10 PPM CTX.

Figure 14 Time dependences of experimental and calculated values of MNZ concentration, (a) 1st order, (b) 2nd order. pH 7, flow rate 1 l/min, 13.33 mg/min ozone, 10 PPM MNZ.
Figure 15: Time dependences of experimental and calculated values of the mixture concentration, (a) 1st order, (b) 2nd order. pH 7, flow rate 1 l/min, 13.33 mg/min ozone, 10 PPM for each antibiotic.

Table 2: The values of the rate constant and the correlation coefficient for the individual orders of reaction.

|  | Tetracycline | Ceftriaxone | Metronidazole | Mixture |
|---|--------------|-------------|---------------|---------|
| n | Units   | $k_n$ | $R^2$ | $k_n$ | $R^2$ | $k_n$ | $R^2$ | $k_n$ | $R^2$ |
| 1 | l/min   | 0.0914 | 0.9581 | 0.0499 | 0.9761 | 0.1025 | 0.9566 | 0.0384 | 0.9516 |
| 2 | l/mg. min | 0.0698 | 0.9001 | 0.0228 | 0.9731 | 0.1042 | 0.8786 | 0.0025 | 0.9314 |

Based on the results in table 2, it can be concluded that all reactions between ozone and the antibiotics applied in the current study were best fit for the first order kinetics. The correlation coefficient for individual orders of reaction ($R^2$) obtained from the first order equation is higher compared to the value obtained from the second order equation for all antibiotics reactions in the optimum conditions in which the experiments were conducted.

4. Conclusions:

In this study, the application of the direct oxidation process by ozone with the technique of microbubbles improves the ability of decomposing the antibiotics used (tetracycline, ceftriaxone, metronidazole) effectively and successfully. More than 90% removal rate was achieved for the contaminants utilized in the current research. It was noticed also that the removal efficiency of these contaminants differed from one material to another due to the difference in their chemical composition.

Regarding the effect of the pH, it was discovered that the best value was between 7-8, because of the formation of the effective hydroxyl radicals, however, their abundant presence when increasing the pH further will lead to an increase in the indirect reaction at the expense of the direct reaction, which leads, in turn, to a decrease in the removal rate. Also, it was found that when the ozone dose or the antibiotic concentration increased, this will lead in both cases to an increase in the removal rate.
The experiment also succeeded in treating a mixture consisting of three antibiotics (tetracycline, ceftriaxone, and metronidazole), where the overall concentration was measured by COD test, and the highest possible removal achieved was 87.5% after 60 minutes of ozonation.

When a comparison was made between the use of the microbubble technique with the traditional ozonation method, there was a pronounced improvement in the removal efficiencies of the contaminants with the use of microbubbles. The degradation rate was increased by about 35% for each contaminant alone and for the multicomponent mixture.

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