Feasibility Study of Tetracycline Removal by Ozonation Equipped with an Ultrafine-Bubble Compressor

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Abstract: In this study, a combination of an ozone gas producer and an ultrafine-bubble compressor was used to degrade tetracycline, which is a well-known antibiotic and medicine commonly used in human and animal care, and effects of varying the reaction parameters were studied. Experiments indicate that each gram of introducing ozone can degrade 2.72 g of tetracycline at pH 3 and 1.48 g at pH 11. However, basic conditions contribute to increased mineralization of tetracycline because of the ·OH radical oxidation mechanism. Higher reaction temperatures and higher ozone dosages enhance the reactivity between the ozone molecules, ·OH radicals, and tetracycline, resulting in a decline in the toxicity of the tetracycline solution as measured by cell viability. The mineralization of organic compounds is the key to decreasing the toxicity of the solution. Ultrafine-bubble ozonation can provide homogeneity of gas bubbles in solution hence it not only reduces the requirement of ozone and thus the operational cost of the reaction, but also extends the efficacy of the method to the treatment of solutions with high tetracycline concentrations.

Keywords: cell viability; mineralization; tetracycline; toxicity; ultrafine-bubble ozonation

1. Introduction

More than 4000 pharmaceutical medicines are currently in use around the world, and the annual total global antibiotic consumption is estimated to be 100,000–200,000 tons. Because of excessive consumption and the disposal of unused or expired antibiotics, these compounds are released into the environment in large quantities. In Europe, about 15,000 tons of antibiotics are discharged into the environment every year [1,2]. In Taiwan, more than 1600 tons of antibiotics are used each year, of which 70% are used in animal husbandry feed additives. In China, annual antibiotic consumption per person was about 138 g, which is 10 times that of the United States [3]. The abuse of antibiotics all over the world has significantly increased the resistance of bacteria to current antibiotics and led to more than 25,000 deaths due to drug-resistant bacteria in Europe each year [4,5].

Tetracycline was discovered in 1940 and is one of a family of antibiotics that inhibits protein synthesis by preventing aminoacyl-tRNA from connecting to the ribosomal receptor (A) seat [6]; its molecular structure is shown as Figure 1. Its broad effectiveness and strong therapeutic performance have led to the widespread use of tetracycline as a broad-spectrum antibiotic. Tetracycline has been widely used in animal husbandry, agriculture, aquaculture, fisheries, veterinary medicine, and human treatment globally [7]. It is often used in the treatment or control of human diseases and as an additive to animal feed to prevent diseases and promote growth [8]. Because tetracycline is not completely metabolized by humans or animals, approximately 60% of the tetracycline ingested is excreted in urine and discharged to the environment [9]. Even though the concentrations of tetracycline discharged are usually low, it can accumulate in the environment and result in severe contamination over time because of its high consumption by humans and animals [10].
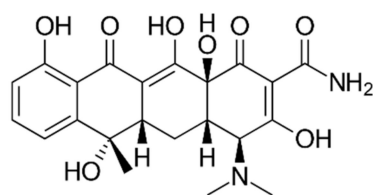
Figure 1. Molecular structure of tetracycline.

The formation of hydroxyl radicals (·OH) in solution is the key to converting organic compounds or pollutants into harmless products, especially CO_2 and H_2O, because of the high reactivity and short lifetimes of ·OH radicals [11]. Once ·OH radicals are generated, they attack organic pollutants in three ways: electron transfer (redox reaction), Equation (1), hydrogen atom extraction abstraction (dehydrogenation), Equation (2), and electrophilic π-addition (hydration), Equation (3). In these equations, RX and PhX represent aliphatic and aromatic halides, respectively. These reaction mechanisms not only open the phenyl rings in organic compounds but also lead to reactions with oxygen to produce hydroperoxyl radicals. This results in the formation of intermediates or by-products such as $HO_2·$ and H_2O_2 that undergo further oxidation reactions until the organic compounds are fully mineralized [12]. Therefore, ultra-violet light, Fenton reactions, ultrasound, and ozonation have been used to destroy, degrade, or oxidize organic compounds, either by themselves or with the addition of H_2O_2, TiO_2, and many types of iron [13–16] or non-iron based heterogeneous catalysts such as zeolites [17].

$$·OH + RX \rightarrow RX^+· + ·OH^- \tag{1}$$

$$·OH + RH \rightarrow R· + H_2O \tag{2}$$

$$·OH + PhX \rightarrow PhX(·OH)· \tag{3}$$
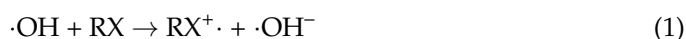
Ozone is a triatomic oxygen molecule composed of an oxygen atom and a diatomic oxygen molecule. It is an unstable gas that rapidly decomposes into oxygen with a half-life of about 40 min at 20 °C [18,19]. This unstable O_3 has a strong oxidizing ability to destroy pollutants. Ozone can easily degrade aromatic organic pollutants by destroying the aromatic rings to form intermediate products such as short-chain carboxylic acids, aldehydes, and ketones. However, direct ozone oxidation may not be sufficient to achieve the required degradation efficiency. Indirect oxidation by ·OH radicals augments the degradation efficiency because the ·OH radicals resulting from the reaction between ozone and water have a high oxidation potential [20].

An ultrafine-bubble compressor equipped with an ozone gas production unit was used for the degradation of tetracycline because ultrafine gas bubbles have a large specific surface area, high stability in solution, long life, self-pressurization, and long residence time [21,22]. If the diameter of the ozone bubbles is smaller than 50 μm, the mass transfer between the gas and solution phases is better than with a millimeter scale bubble, due to the higher specific interfacial area, slower rising velocity, and the higher inner pressure of the ozone gas bubbles, resulting in improvements to the solubility and treatment efficiency of target compounds in water [23]. The objective of this study was to investigate the feasibility of an ultrafine-bubble ozonation in the degradation, mineralization and detoxification of tetracycline at different reaction conditions. Degradation kinetics, operational costs, and degradation capacity of each gram of ozone on tetracycline were also investigated.

2. Materials and Methods

2.1. Materials

Commercial analytical-grade tetracycline (Merck, Germany, purity of 98%) was used as the target species for ozonation. Other chemical reagents including sulfuric acid (H_2SO_4), phosphoric acid (H_3PO_4), sodium hydroxide (Na·OH), potassium hydrogen phthalate

($C_8H_5KO_4$), and *tert*-butanol ((CH_3)$_3$C·OH) were of the purest commercially available grade (from Merck or FERAK, Germany) and were used without further purification.

2.2. Experimental Apparatus

A schematic diagram of the experimental apparatus designed for tetracycline degradation has been shown in our previous study [24]. The reactions were performed in a Pyrex-glass cylindrical reactor (1 L working volume) with a cooling jacket and a circulating temperature controller to keep the reaction temperature constant. The reactor was equipped with an ozone generator (Triogen LAB2B, Glasgow, UK), an external feed flow rate control device (Bronkhorst® EL-FLOW select F-201 CV/F-211 CV, Dutch, The Netherlands), and an ultrafine-bubble compressor (Fluid Metering, FMI Lab Model MA5S, Hack UFB Co., Ltd., Yamanashi, Japan) to provide the required ultrafine-bubble ozone gas, transferring into the solution as dissolved ozone for the degradation of tetracycline; the diameters of the ultrafine bubbles were 0.5–3 μm. The maximum O_3 dosage produced was 10 g/h; as the ozone flow rates were maintained at 30 and 40 mL/min, the ozone production rates were 0.06 and 0.08 g O_3/h, respectively. Several instruments (Eutech/CyberScan pH 510, Taiwan) were placed in the reactor to monitor parameters such as pH, oxidation reduction potential (ORP) and dissolved oxygen (DO) concentration. The concentration of dissolved ozone (DO_3) was measured by the ozone monitor (Kasahara OZ-301, Gifu Prefecture, Japan) conducted with a thin film electrode method.

2.3. Experimental Conditions

Figure 2 shows profiles of dissolved ozone concentrations during ultrafine-bubble and milli-bubble ozonation with deionized water (DI water, produced by ultrapure water producer, ELGA option-Q, Taiwan), where the ozone flow rate was 40 mL/min, pH 6.5 and 25 °C. After 60 min of milli-bubble ozonation, the dissolved ozone concentration reached 4.2 mg/L. When ultrafine-bubble ozonation was used instead, the dissolved ozone concentration exceeded this level after just 2 min of ozonation. After 20 min of ozonation, the maximum dissolved ozone concentration reached 9.2 mg/L, which demonstrates that a smaller size of the ozone gas bubbles is preferable to enhance the dissolved ozone concentration, which was integrated with respect to time to calculate the quantity of ozone produced, as shown in Figure 2. The grey area, which was calculated to be 484.2 mg·O_3 min/L, represents the real quantity of ozone present during the ozonation; in this study, the area was equivalent to 0.08 g O_3/h. The initial concentration of tetracycline varied from 50 to 500 mg/L. Other parameters such as pH levels (3–11), ozone dosages (0.06–0.08 g/h), the addition of *tert*-butanol (50–400 mg/L), and temperatures (15–55 °C) were also systematically varied.
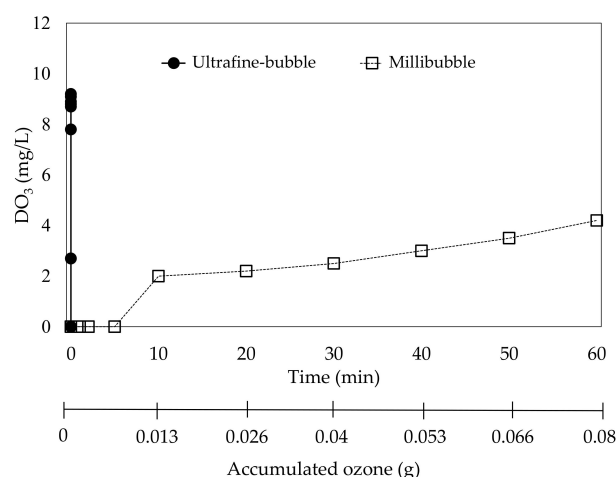

Figure 2. Concentration of dissolved ozone during ultrafine-bubble ozonation with deionized (DI) water (flow rate 40 mL/min; temperature 25 °C; pH 6.5).

2.4. Sample Analysis

A UV-visible spectrophotometer (Thermo Scientific GENESYS 10S, Madison, WI, USA) and a total organic carbon (TOC) analyzer (TOC-500, Shimadzu, Japan) were used to measure the concentration of tetracycline and TOC. Prior to measuring tetracycline concentration during the experiments, a calibration curve with satisfactory linear regression value was developed based on UV-Vis spectrophotometry. A full scan of UV-visible spectrophotometer indicated that two major absorption peaks were observed at wavelengths of 276 and 357 nm [25]. However, based on the literature, 357 nm was identified as the maximum absorption wavelength for the determination of tetracycline concentration [26,27]; the limit of quantification of tetracycline in this study was 0.024 mg/L. Toxicity of the solution before and after ultrafine-bubble ozonation was determined as described in an earlier study [27]. In all figures, each experiment was carried out three times and the data shown in each curve were the averages.

3. Results and Discussion

3.1. Effect of pH

It is well-known that direct attack of organic molecules by mechanisms at acidic condition is the major reaction, and $\cdot$OH radicals oxidation mechanisms dominate the degradation of refractory organic pollutants at basic conditions [28]. In addition, tetracycline contains two acidic groups and one alkaline group so that there are four different species in the aqueous phase at different pH values [26], and these different pH values would induce the tetracycline oxidation to take place in different functional groups. In this study, the initial pH levels were varied from 3 to 11 to investigate the effect of pH on the degradation and mineralization of tetracycline. The profiles of the tetracycline residues during ultrafine-bubble ozonation are shown in Figure 3. The residual concentrations of tetracycline after 60 min' reaction (accumulated ozone = 0.08 g O_3) at all pH levels were comparable, with values between 0.24 and 0.44 mg/L, and the degradation percentages were all higher than 99%. However, at low pH, the degradation of tetracycline occurred within a reaction time of 20 min (0.026 g O_3), which indicates that lower pH assists tetracycline degradation, due to a cationic tetracycline species observed in aqueous solution, and the oxidization potentials of $\cdot$OH radicals are higher in acidic medium than in basic medium [26–29]. Besides the type of tetracycline presented in solution, the concentration of dissolved ozone may be the key parameter affecting the degradation of tetracycline. Figure 4a shows that at pH 3, no dissolved ozone was detected for the first 10 min. The dissolved ozone concentration then increased to 7.8 mg O_3/L by the end of the reaction. At pH 11, the concentration of dissolved ozone was still zero after 20 min' reaction, after which it steadily increased to 6.3 mg/L (Figure 4b). Based on the integration, the grey areas in Figure 4a,b were 289.2 and 364.2, respectively, which estimates that the consumption of dissolved ozone for tetracycline degradation is equivalent to 0.048 g O_3 and 0.060 g O_3 at pH 3 and pH 11; this indicates that each gram of O_3 can degrade 1.041 g and 0.833 tetracycline, respectively. However, as the results show in Figure 3, 20 min of ultrafine-bubble ozonation was adequate to degrade most tetracycline at pH 3 to pH 9, which indicated that the required ozone dosage was 0.018 g O_3 (grey area was 108) to degrade 49 mg/L tetracycline. Hence, at those conditions, each gram of ozone could degrade 2.72 g tetracycline. At pH 11, ultrafine-bubble ozonation should be carried out for at least 30 min to degrade 49 mg/L tetracycline, which indicated that the required ozone dosage was 0.033 g O_3 (grey area was 199) and each gram ozone could degrade 1.48 g of tetracycline.

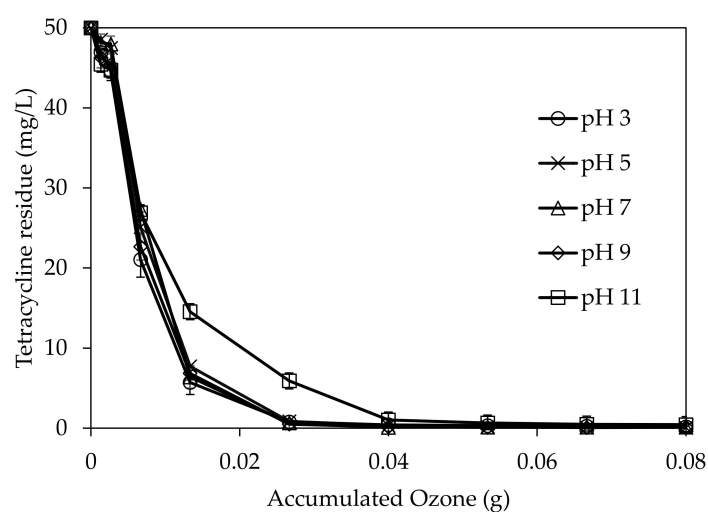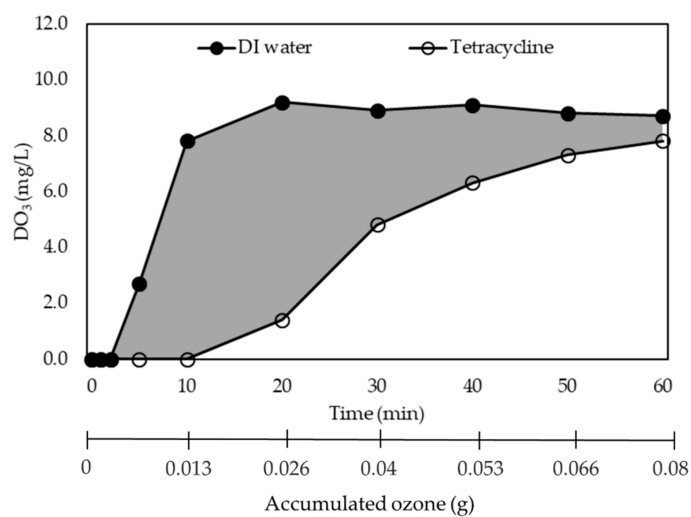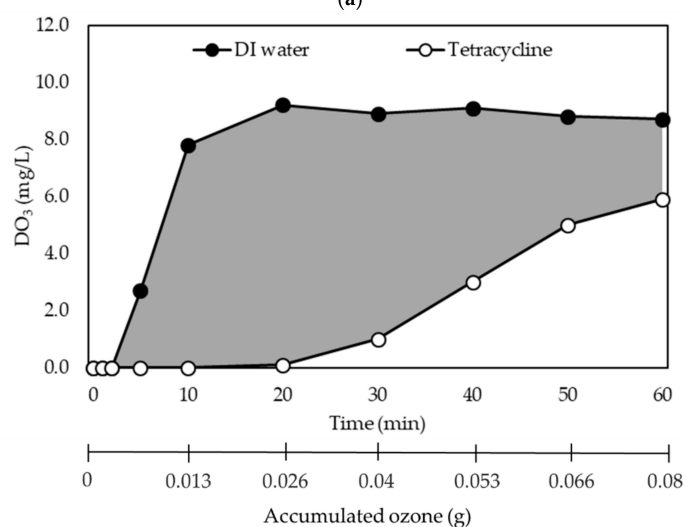**Figure 3.** Residual tetracycline in solution during ultrafine-bubble ozonation at different pH levels.

(**a**)

(**b**)

Figure 4. Ozone usage during tetracycline degradation calculated by integrating method at (**a**) pH 3 and (**b**) pH 11.

As the tetracycline was degraded by the introduction of ozone gas bubbles, it reacted with ozone molecules or ·OH to form intermediates, then become further oxidized and mineralized to CO_2 and H_2O, as shown as Equations (4) and (5). Therefore, it is estimated that the reaction rate can be obtained from the calculation of tetracycline concentration and ozone (or ·OH) concentration, based on Equations (6) and (7), where the reaction kinetics should be defined as a second-order type. However, the ozone gas is continuously introduced into the reactor and then the concentration of ozone reaches a stable level, so that the ozone or ·OH concentration in the solution phase may be considered at a consistent stage. Then the calculation of rate constant can be achieved by pseudo first-order kinetics, based on Equation (8). Table 1 shows the tetracycline degradation percentages, the pseudo first-order rate constants for tetracycline degradation (reaction time was 60 min), mineralization of tetracycline (presented as TOC removal), and toxicity (presented as cell viability) at different reaction conditions. The pseudo first-order rate constant at pH 3 was greater than other pH levels, which indicates that the degradation of tetracycline at this pH level was fastest, though the final degradation efficiencies at all five pH levels were comparable. However, the levels of mineralization of tetracycline at basic pH levels were higher than those at acidic levels; this corresponds with Figure 4b in that the higher dose of ozone was used in the further degradation of organic compounds into CO_2 [30]. The cell viability at pH 11 was higher than at other pH levels, which indicates that the toxicity of the solution at pH 11 had been reduced sufficiently by the higher mineralization of the organic compounds [28–31].

$$\text{Tetracycline} + O_3 \rightarrow \text{Intermediates} \rightarrow CO_2 + H_2O \qquad (4)$$

$$\text{Tetracycline} + \cdot OH \rightarrow \text{Intermediates} \rightarrow CO_2 + H_2O \qquad (5)$$

$$\text{Rate} = k \times [\text{Tetracycline}] \times [O_3] \qquad (6)$$

$$\text{Rate} = k \times [\text{Tetracycline}] \times [\cdot OH] \qquad (7)$$

$$\text{Rate} = k' \times [\text{Tetracycline}] \qquad (8)$$

Table 1. Degradation and mineralization of tetracycline by ultrafine-bubble ozonation at different reaction pH and temperatures.

Concentration of Tetracycline (mg/L)	pH	Temperature (°C)	Degradation Efficiency of Tetracycline (%)	Mineralization Efficiency of Tetracycline (%)	Pseudo First-order Degradation Kinetic Rate (min^{-1})	Cell Viability (%) *
50	3	25	99.6	19.1	0.121	66.3 ± 14.5
50	5	25	99.8	20.7	0.119	67.6 ± 18.2
50	7	25	99.8	22.2	0.117	71.1 ± 18.1
50	9	25	99.7	24.6	0.112	74.7 ± 15.8
50	11	25	99.1	31.9	0.088	84.3 ± 13.5
50	3	15	99.7	11.3	0.122	62.2 ± 10.8
50	3	25	99.6	19.1	0.121	66.3 ± 14.5
50	3	35	99.6	25.2	0.119	76.5 ± 23.5
50	3	45	99.7	29.5	0.116	82.9 ± 21.2
50	3	55	99.5	36.2	0.115	87.5 ± 15.0

* Cell viability of a blank sample (DI water) was 100%.

3.2. Effect of Temperature

Several parameters of reaction mechanisms involving ozone, such as Brown hydrogenation of organic compounds by ozone, mass transfer coefficients, and ozone gas solubility, are temperature-dependent [32,33]. As the mass transfer to the solution or the ozone gas solubility increases, the degradation of organic compounds is enhanced. Five reaction temperatures were used to investigate the effect of temperature on ultrafine-bubble

ozonation, and the results are also shown in Table 1. The degradation of tetracycline after 60 min was approximately 99.5% at all five temperatures.

Though temperature had little effect on the degradation of tetracycline, the rate of TOC removal and change in cell viability were both affected. Increasing the reaction temperature increased the TOC removal and cell viability, which demonstrates that higher reaction temperatures resulted in the conversion of organic molecules to CO_2, thus reducing the toxicity of the solution. This is explained by the increased reactivity between organic compounds and ozone at higher temperatures [5,34]. However, lower reaction temperatures increase the solubility of ozone and the mass transfer of ozone from the gas to the solution phase [32–35]. This explains why, in Figure 5, the residual tetracycline concentration after ten minutes' reaction at 15 °C was lower than at 55 °C. The degradation rate was slightly faster at lower solution temperatures because of the higher dissolved ozone concentration. In fact, only 20 min' reaction was needed to achieve this high treatment performance. The ultrafine-bubble ozonation method effectively degrades tetracycline regardless of the reaction temperature, so controlling the reaction temperature need not be prioritized.
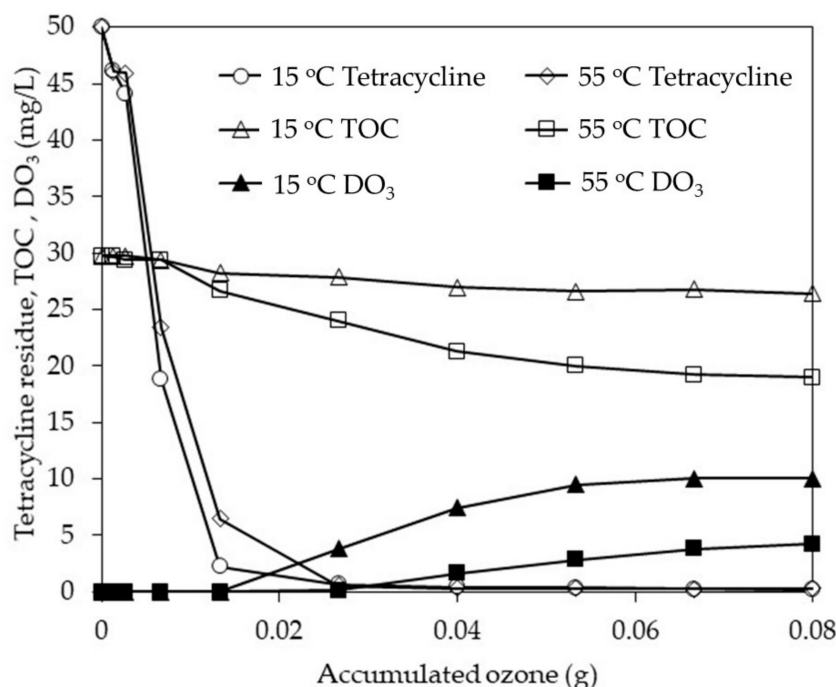

Figure 5. Residual tetracycline and total organic carbon (TOC) in solution during ultrafine-bubble ozonation at different reaction temperatures.

3.3. Effect of Tetracycline Concentration

Almost 100% of the tetracycline was degraded within 20 min' reaction when the initial tetracycline concentration was 50 mg/L (Figure 5). This suggests that ultrafine-bubble ozonation with 0.08 g of accumulated ozone can degrade more tetracycline than 50 mg/L. Hence, four different initial tetracycline concentrations, 50, 100, 200, and 500 mg/L, were carried out for ultrafine-bubble ozonation, and the results are shown in Table 2 and Figure 6. More than 90% of the tetracycline was degraded even when the initial tetracycline concentration was as high as 500 mg/L. The exact amount of tetracycline removed by 60 min' reaction was 454 mg. In addition, dissolved ozone was not detected in solution during the 60 min reaction, which indicated that the introduced ozone was fully used to degrade tetracycline. This can be explained by the fact that increasing tetracycline initial concentration causes an increase in the concentration of degradation intermediates and, as a result, extra ozone consumption for increased removal and oxidation of by-products [36]. Therefore, even though the pseudo first-order rate constant at 500 mg/L tetracycline solution was much lower than at the other concentrations, the tetracycline removal rate was still

high enough to suggest that the technology is effective even at high initial tetracycline concentrations. As the initial concentration of tetracycline increased, the amount of carbon present in the solution also increased. More dissolved ozone was therefore consumed in the degradation and mineralization of the organic compounds. Hence, in Figure 6, residual dissolved ozone concentration and the TOC removal ratio both decreased as the initial concentration of tetracycline increased. The cell viability also decreased because fewer tetracycline molecules were fully decomposed [35–37]. This suggests that the ozone dosage (or gas flow rate) should be augmented for the treatment of solutions with higher initial tetracycline concentrations. However, based on the results of ozone usage and tetracycline degradation, it is found in Table 2 that each gram of O_3 could degrade 2.54 to 5.68 g of tetracycline. This indicates that ultrafine-bubble ozonation with an accumulated ozone of 0.08 g can not only be used to degrade lower concentrations of tetracycline solution but also to degrade very high concentration solutions.

Table 2. Degradation and mineralization of tetracycline by ultrafine-bubble ozonation at different initial tetracycline concentrations (pH 3, temp. 25 °C).

Concentration of Tetracycline (mg/L)	Degradation Efficiency of Tetracycline (%)	Mineralization Efficiency of Tetracycline (%)	Degradation of Tetracycline by Each Gram of O_3 (g Tetracycline/g O_3)	Pseudo First-Order Degradation Kinetic Rate (min^{-1})	Cell Viability (%) *
50	99.7	19.1	2.72	0.121	66.3 ± 14.5
100	98.6	16.1	2.90	0.098	65.1 ± 7.9
200	99.1	14.8	2.54	0.074	62.9 ± 5.8
500	90.8	2.9	5.68	0.043	57.6 ± 11.7

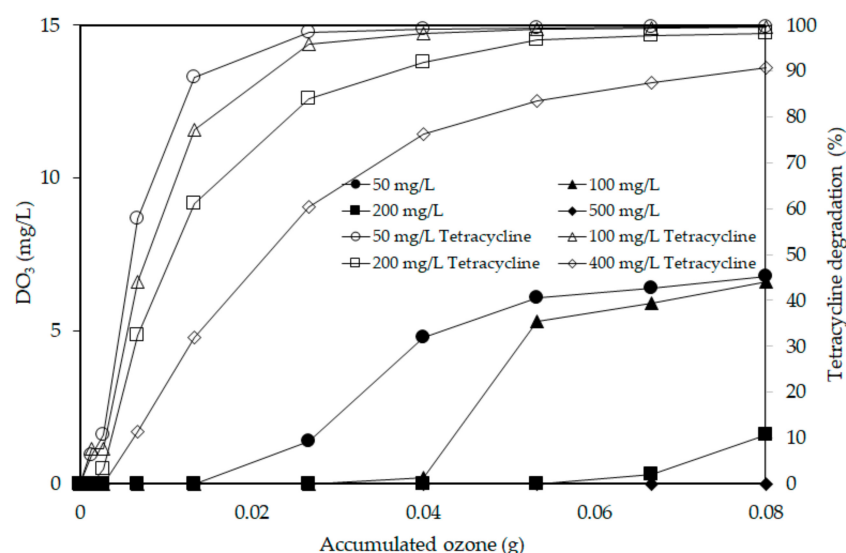* Cell viability of a blank sample (DI water) was 100%.

Figure 6. Tetracycline degradation percentage and dissolved ozone concentration during ultrafine-bubble ozonation at different initial tetracycline concentrations.

3.4. Toxicity Profiles

Although ultrafine-bubble ozonation effectively degraded tetracycline with a maximum TOC removal rate of 36.2%, more than 60% of the degraded tetracycline was not mineralized to CO_2 by the oxidation process. The toxicity of tetracycline and its degradation by-products of environmentally relevant bacteria was investigated and found that the minimum inhibitory concentration of tetracycline was 2.0 mg/L, much lower than most tetracycline degradation by-products [38]. However, even tetracycline might be degraded as a by-product by oxidation methods, the possible by-products still contained four aro-

matic rings in the molecular structures. Hence, full mineralization should be considered as the priority. Changes in the toxicity of the solution based on cell viability measurements must also be evaluated along with the degradation efficiencies. Twelve experimental runs were carried out (Tables 1 and 2), and the rate of TOC removal is plotted against cell viability in Figure 7. Prior to degradation, the cell viability of untreated 50 mg/L tetracycline was 55.3% $\pm$ 11.6%, while the cell viability of a blank sample (DI water) was 100%. The TOC removal rate was strongly correlated with the cell viability (R^2 = 0.94), which indicates that the higher mineralization of tetracycline decreased the toxicity of the solution. This demonstrates that TOC removal during the degradation of organic compounds is the critical factor in reducing toxicity [30]. After the toxicity of tetracycline is reduced by the oxidation process, the wastewater should be subjected to further biological treatments to reduce the concentrations of other chemicals.
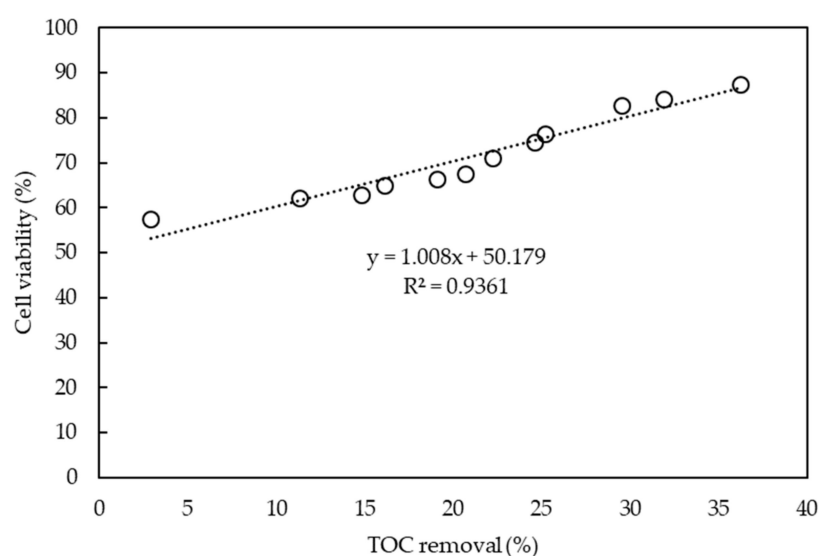

Figure 7. Correlation between cell viability and TOC removal in the tetracycline degradation tests.

In Table 3, the effect of ozone dosage and *tert*-butanol addition, which is a well-known $\cdot$OH radical scavenger, on the degradation of tetracycline was investigated. It is shown in Table 3 that increasing the dosage of ozone profits the degradation and mineralization of tetracycline. Even though the results of tetracycline degradation after 60 min reaction are comparable, as the ozone dosages are 0.06 and 0.08 g O_3/L, it is still different in the first 10 min, where the degradation results are 80% and 90%, respectively. This result proves that tetracycline can be degraded quicker with higher ozone dosages. Not only the results of tetracycline degradation but also tetracycline mineralization, increase of cell viability, and reaction rate constant show that higher ozone dosages are useful to increase the reactivity of tetracycline treatment. In previous observations, it is known that the reaction between organic compounds and $\cdot$OH radicals predominate in the mineralization mechanism of tetracycline. Therefore, in this study, four different concentrations of *tert*-butanol, which has been generally used as an $\cdot$OH radicals scavenger, were added in the solution to investigate the effect. It is found that the degradation efficiencies at different *tert*-butanol additions are similar, but mineralization is found to slightly decrease with increasing *tert*-butanol additions. It is a fact that degradation of tetracycline is mainly contributed to by ozone molecule direct oxidation, so that even the addition of *tert*-butanol will scavenge the formation of $\cdot$OH radicals, with degradation of tetracycline comparable to the data without *tert*-butanol addition. In addition, mineralization of tetracycline is influenced by *tert*-butanol addition, where the higher addition of *tert*-butanol led to lower mineralization results.

Table 3. Degradation and mineralization of tetracycline by ultrafine-bubble ozonation at different ozone dosages and *tert*-butanol additions.

Dosage of Ozone (g)	Addition of *tert*-Butanol (mg/L)	Degradation Efficiency of Tetracycline (%)	Mineralization Efficiency of Tetracycline (%)	Pseudo First-Order Degradation Kinetic Rate (min^{-1})	Cell Viability (%) *
0.06	-	99.3	14.5	0.113	60.9 ± 11.2
0.08	-	99.6	19.1	0.121	66.3 ± 14.5
0.08	50	99.7	7.9	0.121	68.5 ± 5.8
0.08	100	99.8	7.6	0.122	68.6 ± 7.6
0.08	200	99.7	7.1	0.119	73.3 ± 11.7
0.08	400	99.6	6.5	0.119	74.5 ± 22.8

* Cell viability of a blank sample (DI water) was 100%.

3.5. Operational Cost

The operational costs of several oxidation processes for degrading tetracycline, such as ultrasound, Fenton reactions, milli-bubble ozonation, and their combinations, have been proposed [27], and the results are shown in Table 4 (items g to j). It is found in Table 4 that the operational costs of ultrasound and the Fenton process applied individually were 2466.53 USD and 263.76 USD, respectively, to remove 1 kg of tetracycline from solution (i.e., if the tetracycline concentration is 50 mg/L, removing 1 kg of tetracycline equates to treating 20 m^3 of wastewater). When ultrasound was combined with the Fenton process (a sono-Fenton system), the costs of the chemical reagents (Fenton) and electricity charges (sonicator) led to a higher operational cost of 393.20 USD per kg of tetracycline removed. When the milli-bubble ozonation was used either as the sole process in the treatment or in combination with other processes, the operational costs to remove 1 kg of tetracycline were 193.63–405.59 USD. The operational costs for O_3 + Fenton and O_3 + sono-Fenton processes were lower than the Fenton process alone because of the shorter reaction times. In this study, we used an ultrafine-bubble compressor to reduce the bubble size and increase the solubility of various gases for the treatment of tetracycline. When the air was used, the operational costs were much higher than for oxygen or ozone, even though the only cost was that of electricity for the pump and compressor. This was because of the extremely low degradation efficiency of tetracycline after 60 min' treatment with air.

Table 4. Degradation and mineralization of tetracycline by ultrafine-bubble ozonation at different reaction conditions.

Methods	Tetracycline Removal	Tetracycline Concentration	Operational Cost	Operational Cost
	Percentage (%)	(mg/L)	(USD/run)	(USD/kg Tetracycline Removal)
Ultra-fine bubbles ozonation				
50 mg/L [a]	98.5	50	0.185	125.27
100 mg/L [b]	98.3	100	0.278	94.13
200 mg/L [c]	98.3	200	0.555	94.11
500 mg/L [d]	90.8	500	0.555	40.77
Oxygen [e]	3.0	50	0.250	551.88
Air [f]	0.6	50	0.014	1555.57
Milli-bubble O_3 [g]	98.0	50	0.539	330.06
Milli-bubble O_3+Ultrasound [h]	98.0	50	0.655	405.59
Milli-bubble O_3+Fenton [i]	98.0	50	0.313	193.63
Milli-bubble O_3+ultrasound +Fenton [j]	99.8	50	0.371	229.71

[a] O_3 = 0.04 L/min, pH 3, 20 min; [b] O_3 = 0.04 L/min, pH 3, 30 min; [c] O_3 = 0.04 L/min, pH 3, 60 min; [d] O_3 = 0.04 L/min, pH 3, 60 min; [e] O_2 = 0.04 L/min, 60 min; [f] Air = 0.04 L/min, 60 min; [g] O_3 = 0.2 L/min, 55 °C, pH 3, 20 min; [h] O_3 = 0.2 L/min, ultrasonic watts 100 W, 55 °C, pH 3, 20 min; [i] O_3 = 0.2 L/min, Fe^{2+} = 0.2 mM, H_2O_2 = 2 mM, 55 °C, pH 3, 10 min; [j] O_3 = 0.2 L/min, Fe^{2+} = 0.2 mM, H_2O_2 = 2 mM, ultrasonic watts 100 W, 55 °C, pH 3, 10 min.

Table 4 also shows that the treatment performances of milli-bubble ozonation (200 mL O_3/min) and ultrafine-bubble ozonation (40 mL O_3/min) were comparable. With 20 min'

treatment, the degradation efficiencies of both processes were greater than 98%. Decreasing the bubble size increased the reactivity of ozone with organic compounds, allowing the ultrafine-bubble ozonation to achieve satisfactory treatment performance with lower gas flows or ozone dosages. In addition, the operational cost for ultrafine-bubble ozonation shown in Table 4 is only 38% of the milli-bubble ozonation operational cost. The operational costs for treating higher initial tetracycline concentrations were also lower: 100 mg/L tetracycline solution was treated for 30 min with ultrafine-bubble ozonation, resulting in 98.3% tetracycline degradation at a cost of 94.13 USD per kg of tetracycline; for 500 mg/L tetracycline solution, 90.8% degradation was achieved in 60 min reaction at a cost per kilogram of tetracycline (20 m3 tetracycline wastewater) as low as 40.77 USD. This demonstrates that 40 mL/min ultrafine-bubble ozonation has sufficient oxidation performance to degrade solutions with high tetracycline concentrations. In this study, we used a commercial pure oxygen steel cylinder to supply oxygen to the ozone gas producer. The unit price of pure oxygen was about 3.6 times the unit price of air (Table 4), so the operational costs will be lower still if air can be used instead of oxygen to generate ozone.

4. Conclusions

The performance of tetracycline degradation and detoxification by an ultrafine-bubble compressor equipped with an ozone gas producer was found to be effective, where each gram of ozone could degrade as high as 5.68 g tetracycline if the initial tetracycline concentration was 500 mg/L. The decrease in solution toxicity was due to the removal of TOC or the mineralization of organic compounds. The results of rate constant indicated that the degradation of tetracycline was faster at acidic solution than that at basic ones. In addition, within the short treatment period, the tetracycline degradation capacity at pH 3 was 1.84 times that at pH 11. The effect of ·OH radicals scavenger by adding *tert*-butanol and higher reaction temperatures on tetracycline degradation can be neglected at acidic conditions, which indicates that the major reaction oxidation mechanism is the ozone molecule attack. Alkaline condition leads to the increase of tetracycline mineralization because of the higher concentrations of ·OH radicals contributing to the removal of TOC. The results of operational cost in this study indicate that the ultrafine-bubble ozonation process is cheaper than other processes and can be used to effectively degrade tetracycline and reduce its toxicity, even if the initial tetracycline concentration is as high as 500 mg/L.

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