Effect of inorganic salts on inactivation of *Escherichia coli*form* and removal of fulvic acid by ozone in a rotating packed bed

Han Liu, Taoran Liu, Dan Wang, Wei Wang, Qi Liang, Moses Arowo and Lei Shao

**ABSTRACT**

This study investigated the effect of different inorganic salts on the treatment of simulated secondary effluent (SE) by ozone (O₃) in a rotating packed bed (RPB) – (O₃-RPB process), with *Escherichia coli* (E. coli) and UV2₅₄ of fulvic acid as the indicators. The inactivation efficiency of *E. coli* and removal percentage of UV₂₅₄ were studied under different rotation speeds of the RPB and varying concentrations of inorganic salts such as NaHCO₃, Na₂SO₄ and CaCl₂. Results indicate that both the inactivation efficiency of *E. coli* and removal percentage of UV₂₅₄ increased with an increasing rotation speed of the RPB but decreased with an increase in concentrations of the inorganic salts. Analyses on the mechanism of the treatment process reveal that the inorganic salts consume O₃ and ·OH to generate products with lower oxidation ability, and thus result in a poor treatment effect on the effluent. This work provides fundamentals for the O₃-RPB process in the treatment of SE from urban wastewater treatment plants.

**Key words** | Escherichia coli, fulvic acid, inactivation, inorganic salts, ozone, rotating packed bed

**NOMENCLATURE**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A₀</td>
<td>UV₂₅₄ value of the SE before ozonation in RPB</td>
</tr>
<tr>
<td>Aₜ</td>
<td>UV₂₅₄ value of the SE after ozonation in RPB</td>
</tr>
<tr>
<td>C_(CaCl₂)</td>
<td>CaCl₂ concentration, mg/L</td>
</tr>
<tr>
<td>C_(NaHCO₃)</td>
<td>NaHCO₃ concentration, mg/L</td>
</tr>
<tr>
<td>C_(Na₂SO₄)</td>
<td>Na₂SO₄ concentration, mg/L</td>
</tr>
<tr>
<td>C_(Ozone)</td>
<td>gaseous ozone concentration, mg/L</td>
</tr>
<tr>
<td>N₀</td>
<td>number of <em>E. coli</em> colonies in the SE before ozonation in RPB, cfu/mL</td>
</tr>
<tr>
<td>N</td>
<td>number of <em>E. coli</em> colonies in the SE after ozonation in RPB, cfu/mL</td>
</tr>
<tr>
<td>T</td>
<td>temperature, °C</td>
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<tr>
<td>β_B</td>
<td>removal percentage of UV₂₅₄, %</td>
</tr>
<tr>
<td>η_E</td>
<td>inactivation efficiency of <em>E. coli</em></td>
</tr>
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</table>

**INTRODUCTION**

Water from urban wastewater treatment plants (UWTPs) is usually used for landscaping, industrial cooling and even agricultural activities in an effort to mitigate water crisis (Ferro et al. 2015). Nevertheless, the secondary effluent (SE) from the UWTPs contains a variety of organic and inorganic compounds as well as microorganisms that limit its utilization. Soluble organic compounds in the effluent are mainly humic acids, which are refractory natural organic substances. Fulvic acid is a typical humic acid that accounts for a majority of the total soluble organic compounds in the SE. The effluent also contains a large number of microorganisms such as *Escherichia coli* (E. coli), which has a considerable survival rate and easily spreads infectious...
diseases (Rizzo et al. 2013). E. coli has thus been used as an indicator microorganism to represent the environmental bacteria (Paruch & Mæhlum 2012).

Most of the microorganisms in the SE are pathogenic and should therefore be inactivated. Existing inactivation methods include ultraviolet sterilization, chlorine disinfection, chlorine dioxide disinfection and ozone disinfection. Ultraviolet sterilization is not a thorough disinfection method since the inactivated microorganisms can easily revive their physiological activity under natural light (Wang et al. 2015). Chlorine disinfection can cause byproducts such as trihalomethanes and haloacetic acids, which are recognized as carcinogens (Watson et al. 2012). Ozone has a higher oxidation potential (2.07 V) compared with chlorine dioxide (1.15 V) and as a result exhibits a better oxidation ability. Furthermore, hydroxyl radicals (·OH) with much stronger oxidation ability (2.80 V) than ozone can be produced during ozonation. Although ozonation can produce toxic byproducts such as aldehydes and bromate ions, there are few problems with residual ozone, ozonation byproducts and its toxicity when ozonation is performed at O3 concentrations less than 20 mg/L (Hatatatsu & Suzuki 2000). Ozonation thus demonstrates outstanding advantages in inactivation of microorganisms and removal of organic substances.

High-gravity technology is an efficient process-intensification technique that is achieved through multiphase reactors such as a rotating packed bed (RPB), in which liquid is split into thin films, fine filaments and tiny droplets by a shear force created from the rotation of the rotor in the RPB, leading to a huge and violently renewed gas–liquid interface and thus a significant intensification of mass transfer and micromixing in multiphase systems. The technology has found wide applications in wastewater treatment (Arowo et al. 2015), nanomaterials preparation (Zhao et al. 2015) and gas absorption (Zhao et al. 2016).

Ozonation is generally limited by absorption efficiency of ozone into water. Inorganic compounds also have a significant effect on ozonation (Xing et al. 2016). This work therefore employed high-gravity technology via an RPB in an attempt to improve the mass transfer efficiency of ozone for inactivation of E. coli and removal of UV254 of fulvic acid in a simulated SE. The effect of inorganic salts such as NaHCO3, Na2SO4 and CaCl2 on the inactivation efficiency of E. coli and removal percentage of fulvic acid was investigated. UV254 of fulvic acid and the total number of bacteria colonies were used as the water quality indicators to analyze the effect of the O3-RPB treatment process of the SE.

MATERIALS AND METHODS

Materials

Fulvic acid (85%) was purchased from Shanghai Macklin Biochemical Co., Ltd, China. Beef extract (BR) and peptone (BR) were provided by Beijing Aobox Bio-technology Co., Ltd, China, and nutrient agar (BR) by Qingdao Hope Biotechnology Co., Ltd, China. E. coli was purchased from China Center of Industrial Culture Collection. Sodium chloride (AR), sodium bicarbonate (AR), sodium sulfate (AR) and calcium chloride (AR) were obtained from Beijing Chemical Works, China.

Water sample preparation

The simulated SE was prepared with fulvic acid, E. coli and sterile saline (0.9 wt% of NaCl) as described herein. The standard strains of E. coli were stored at −20 °C prior to activation in a broth medium at 37 °C for 5 hours to obtain the bacterial suspension, which was subsequently cultured at 37 °C for 12 hours with a shaking frequency of 120 r/min. The broth medium consisted of 0.5 wt% of beef extract, 1 wt% of peptone and 0.5 wt% of NaCl. The cultured E. coli was centrifuged at a rotation speed of 4,000 r/min for 3 min to obtain the sediment, which was then washed three times by sterile saline. The prepared E. coli was added into sterile saline to maintain the bacterial osmotic pressure prior to mixing with the fulvic acid solution. The apparatus and chemicals were disinfected at 121 °C for 20 min before use in the experiments.

Experimental setup and procedure

Figure 1 shows the experimental setup. It comprises mainly an RPB (a 3D view of the RPB is given in Figure S1 in the Supplementary Materials), an ozone generator (Tonglin
Sci. and Tech. Ltd, China), and an ozone analyzer (Double UV Light Ozone Meter, Limicen Ozone R&D Center, China). Ozone was produced from air in the ozone generator. The ozone analyzer was used to monitor and measure the concentration of ozone in the gas-stream. The ozone-containing gas was subsequently introduced into the RPB via the gas inlet and flowed from the outer edge of the packing to the inner edge, while the SE was fed into the RPB via the liquid inlet and flowed from the inner edge of the packing to the outer edge where it contacted with the ozone-containing gas in a counter-current manner to realize ozone absorption and the subsequent treatment of \textit{E. coli} and fulvic acid. Both the gas and liquid flow rates were maintained at 0.5 L/min in all the experiments.

**UV\textsubscript{254} analysis**

Fulvic acid was added into the simulated SE to adjust the UV\textsubscript{254} value to about 0.200. The removal percentage of UV\textsubscript{254} ($\beta_B$) is defined by the UV\textsubscript{254} value before and after ozonation and calculated by the following Equation (1):

$$
\beta_B = \frac{A_0 - A_i}{A_0} \times 100\%
$$

**Bacteriological analysis**

The flat counting method was employed to calculate the number of \textit{E. coli} colonies on a Petri dish with a diameter of 90 mm. The SE sample was added into the nutrient agar medium on the Petri dish and incubated at 37 °C for 24 h before the number of \textit{E. coli} colonies was counted. The number of colonies should range from 30 to 300. The inactivation efficiency based on the logarithmic value ($\eta_E$) is defined by Equation (2):

$$
\eta_E = -\log_{10}\left(\frac{N}{N_0}\right)
$$
RESULTS AND DISCUSSION

Effect of rotation speed of RPB in the presence of different inorganic salts

Figure 2 shows the effect of rotation speed of the RPB on inactivation of \textit{E. coli} and removal percentage of UV$_{254}$. It is evident that when the rotation speed increased from 200 to 1,000 rpm, $\beta_B$ increased from 9.10\% to 18.65\% (Figure 2(a)), 6.57\% to 12.63\% (Figure 2(b)), 8.54\% to 15.58\% (Figure 2(c)) and 0.95\% to 8.53\% (Figure 2(d)) in the systems with NaHCO$_3$, Na$_2$SO$_4$, CaCl$_2$ and a combination of the above three compounds, respectively. Similarly, $\eta_E$ increased from 0.13 to 4.48 (Figure 2(a)), 0.20 to 2.26 (Figure 2(b)), 0.40 to 4.41 (Figure 2(c)) and 0.06 to 3.62 (Figure 2(d)) in all of the four systems.

A higher rotation speed gives rise to a larger centrifugal force that shears the liquid into thinner films, threads and smaller droplets, leading to a larger gas–liquid interfacial area and faster surface renewal rate. Consequently, the absorption of ozone into the SE improved significantly as a result of an enhanced gas–liquid mass transfer effect, resulting in increased inactivation of \textit{E. coli} and removal percentage of UV$_{254}$.

![Figure 2](https://iwaponline.com/ws/article-pdf/doi/10.2166/ws.2019.107/597598/ws2019107.pdf)
**Effect of NaHCO₃ concentration**

Figure 3 illustrates the effect of NaHCO₃ concentration on inactivation of *E. coli* and removal percentage of UV₂₅₄. It is evident that both βₓ and ηₑ decreased from 11.11% to 4.83% and 2.13 to 0.14 respectively when the concentration of NaHCO₃ increased from 0 mg/L to 350 mg/L, indicating that NaHCO₃ has a negative effect on the inactivation of *E. coli* and removal percentage UV₂₅₄. Direct ozonation by ozone is the main pathway for inactivation of *E. coli*, while ·OH only plays a minor role (von Gunten 2003a, 2003b). Ozone attacks the cell membrane of the *E. coli* and changes its permeability. Ozone can therefore enter the cell to destroy nucleic acids, proteins and DNA, hinder the synthesis of enzymes and eventually cause the death of the bacteria (Verma et al. 2016).

Higher NaHCO₃ concentration results in a higher pH. When NaHCO₃ concentration increased from 0 mg/L to 350 mg/L, the initial pH of the SE increased from 5.41 to 8.17, leading to generation of ·OH with consumption of ozone. However, NaHCO₃ can further react with ·OH as shown in the following Equations (3)–(5) (Gottschalk et al. 2006). NaHCO₃ therefore consumed both ozone and ·OH, resulting in a lower efficiency of the inactivation of *E. coli* and removal of fulvic acid. Thus, the treatment effect of the SE declined with an increase in NaHCO₃ concentration.

\[
\cdot \text{OH} + \text{CO}_2^2 → \text{OH}^- + \text{CO}_3^- \quad k_1 = 4.2 \times 10^8 \text{M}^{-1}\text{s}^{-1} \quad (3)
\]

\[
\cdot \text{OH} + \text{HCO}_3^- → \text{OH}^- + \cdot \text{HCO}_3^- \quad k_2 = 1.5 \times 10^7 \text{M}^{-1}\text{s}^{-1} \quad (4)
\]

\[
\text{O}_3 + \text{OH}^- → \cdot \text{O}_2^- + \cdot \text{HO}_2^- \quad k_3 = 70 \text{M}^{-1}\text{s}^{-1} \quad (5)
\]

**Effect of Na₂SO₄ concentration**

The variation of βₓ and ηₑ with Na₂SO₄ concentration is presented in Figure 4. It is evident that both βₓ and ηₑ reduced from 10.73% to 7.81% and 5.11 to 3.75 respectively with an increase in Na₂SO₄ concentration from 0 mg/L to 350 mg/L.

It was noted that the pH value of the SE was maintained at 5.40 with varying Na₂SO₄ concentration. Equations (6) and (7) indicate that SO₄²⁻ can react with ·OH to produce S₂O₈²⁻ under acidic conditions (Muthukumar & Selvakumar 2004):

\[
\text{SO}_2^4^- + \cdot \text{OH} → \text{SO}_4^{2-} + \text{OH}^- \quad (6)
\]

\[
2 \cdot \text{SO}_4^{2-} → \text{S}_2\text{O}_8^{2-} + 2e^- \quad (7)
\]

·OH exhibits a stronger oxidation ability than S₂O₈²⁻ owing to its higher potential (2.80 V) compared with that of S₂O₈²⁻ (2.01 V). Thus, the consumption of ·OH by Na₂SO₄ resulted in reduction of both βₓ and ηₑ.
Effect of CaCl₂ concentration

Figure 5 shows the effect of CaCl₂ concentration on $\beta_B$ and $\eta_E$. It is evident that when CaCl₂ concentration increased from 0 mg/L to 200 mg/L, both $\beta_B$ and $\eta_E$ decreased from 8.87% to 6.90% and 3.07 to 1.27, respectively.

Intermediates formed by the reaction of ozone and organic substances can combine with Ca²⁺ to form precipitates which can increase the efficiency of degradation (Hsu et al. 2007). However, the negative impact of Cl⁻/C₀ on the removal of fulvic acid and inactivation of E. coli overrode the positive influence of Ca²⁺ in this work. The reaction mechanism between ozone and Cl⁻/C₀ at different pH has been proposed by Levanov et al. (2008) and Razumovskii et al. (2010). Under alkaline conditions, the reactions of Cl⁻ with ozone can be expressed according to the following Equations (8)–(10):

$$\text{Cl}^- + \text{O}_3 \rightarrow \text{ClO}^- + \text{O}_2 \quad (8)$$
$$\text{Cl}^- + \cdot\text{OH} \rightarrow \cdot\text{ClOH}^- \quad (9)$$
$$\cdot\text{ClOH}^- + \cdot\text{OH} \rightarrow \text{ClO}^- + \text{H}_2\text{O} \quad (10)$$

Both ozone and ·OH can react with Cl⁻ to produce ClO⁻, which as compared with ozone and ·OH, has an inferior ability for inactivation of E. coli and oxidation of fulvic acid.

When the pH is less than 3, another reaction mechanism can be established according to Equations (11)–(13).

$$\text{H}^+ + \text{O}_3 + \text{Cl}^- \leftrightarrow \text{HClO}_3 \quad (11)$$
$$\text{HClO}_3 \rightarrow \text{HClO} + \text{O}_2 \quad (12)$$
$$2\text{H}^+ + \text{Cl}^{-1} + \cdot\text{ClO}^{-1} \rightarrow \text{Cl}_2 + \text{H}_2\text{O} \quad (13)$$

Since the pH of the SE with CaCl₂ is 5.34, it is assumed that both of the above mechanisms played a role in reduction of the inactivation of E. coli and removal of fulvic acid in the presence of CaCl₂.

CONCLUSION

This work investigated the inactivation of E. coli and the removal percentage of UV₂₅₄ by ozonation in an RPB in the presence of different kinds of inorganic salts. Results indicate that NaHCO₃, Na₂SO₄ and CaCl₂ have negative influence on inactivation of E. coli and removal percentage of UV₂₅₄. The reaction mechanisms of the three inorganic compounds were analyzed, and it was noted that the compounds can consume ozone and ·OH to generate products with lower oxidation ability and consequently lead to a poor treatment effect on the simulated SE. It was observed that a higher rotation speed of the RPB enhances mass transfer efficiency, leading to improved absorption of ozone and hence increased inactivation efficiency of E. coli and removal percentage of UV₂₅₄. This work lays the foundation for actual application of the O₃-RPB process in the treatment of SE in UWTPs. It also demonstrates that the O₃-RPB process may be an efficient route to achieve the reuse of SE.

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