

## Removal of Endotoxin in Water Using Ozonation Process

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**Abstract:** The object of this research was to study the inactivation of endotoxin in water by ozonation process. The effects of initial endotoxin concentrations in pH values using the ozone were evaluated. The efficiency of ozone for endotoxin inactivation increased with increasing exposure time and ozone concentration, but it was independent of preliminary endotoxin concentrations and pH variation. Under the conditions of this experiment, a ninety minute exposure to 1 l/min ozone was adequate to inactivate 200 EU/ml of endotoxin. During the experiment, it was observed that the conditions existing during exposure of the endotoxin to the ozone affected the rate of inactivation. These conditions included excess foaming and bubble size, which related to the contact time of the ozone to endotoxin. By controlling these variables, however, reproducible end points were achieved. These results suggest that ozonation process will be able to inactive endotoxin from water.

**Key words:** Water, Endotoxin, Ozonation process, Inactivation, LPS

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### INTRODUCTION

Rising concerns about the quality of drinking water have led both public and private parties to invest considerable human and economic resources in development of invest water treatment processes to more effectively remove organic micropollutants (Rivera-Utrilla, *et al.*, 2006). Recently some of pollutants are considered as emerging contaminants, which means that they are still unregulated or in process of regulation (Esplugas, S., *et al.*, 2007). Endotoxin is one of organic micropollutants that has several adverse health effects for human (Rapala, J., *et al.*, 2002). Endotoxins, also called pyrogen are component of the lipopolysaccharide (LPS) complexes, which constitute a part of the outer layer of the cell walls of most Gram-negative bacteria and some Cyanobacteria (Hanora, A., *et al.*, 2006; Oehmen, A., *et al.*, 2007). The structure of this macromolecule composed of three main regions: lipid A, core polysaccharide and “O” antigens (Anderson, W.B., *et al.*, 2003). Endotoxin are typically released either during cell lyses or multiplication and have a high stability even at high temperature (stable at 121°C for 1 h) and pH values due to its amphoteric structure (Anderson, W.B., *et al.*, 2003; Hyrayama, C., *et al.*, 1999). The lipid A component is critical for all biological responses to endotoxin. Inhalation of moisture-saturated air in showers, swimming pools, hot tubs, saunas and exposure to endotoxin in drinking water used for preparation or dilution of solutions for intravenous injection or dialysis may be more important (Castor, M.L., *et al.*, 2005). General symptoms of endotoxin exposure in humans include fever, diarrhea, vomiting, hypotensions, shock, intravascular coagulation and death (Tessarolo, F., *et al.*, 2006). To date, outbreaks of endotoxin-related illness associated with drinking water have been documented infrequently. This may be due to the facts that outbreaks of fever-related illness in water are never identified by routine medical and bacteriological analyses and since endotoxin related fevers symptoms are typically short-lived (Anderson, W.B., *et al.*, 2003). Fever is an extremely common symptom, as endotoxins stimulate host cell to release proteins known as endogenous pyrogens, which affects the temperature regulating portion of the brain (hypothalamus). Endotoxin is not only injurious by itself but it also augments the toxic agents, so that together they cause greater and more sustained damage. For example endotoxin, may be boost the toxicity of ethanol and variety of drugs of abuse (Anderson, W.B., *et al.*, 2002). Unfortunately although adverse effects of this pollutant had been distinguished, there is only limited information available on untreated surface water, ground water and drinking water endotoxin concentration. Data from four separate studies indicate that endotoxin level in surface water range from <1 to 1049 ng/ml. These levels would

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certainly be significant if they were found in treated drinking water. There are several ways in which endotoxin can be detoxified but, from the point of view of drinking water treatment, oxidation with hydrogen peroxide or permanganate is of most interest. Although application of these compounds is the most important method but control of required dosage of oxidants, safety of operators, storage problems and control of their by products are difficult (Anderson, W.B., *et al.*, 2002). Recently water purification with ozone as an option to disinfection and degrade organic micropollutants has been widely developed (Sano, N., *et al.*, 2007; Smeets, P.W.M.H., *et al.*, 2006). Ozonation is the dark oxidation method can be used in the removal of new emergent pollutants. Approximately 90% of dark oxidation treatments found in the scientific literature corresponds to ozonation (Esplugas, S., *et al.*, 2007). Because, this process has several advantages as follows: reactivity of ozone is quite strong so that organic compounds can be degraded effectively, ozone can be generated easily by use of ultraviolet light or electric discharge and can be converted to O<sub>2</sub> easily (Sano, N., *et al.*, 2007). In this research we studied inactivation of different concentrations of endotoxin in pH values by the ozonation process.

## MATERIALS AND METHODS

### **Reagents and Equipments:**

All chemicals used for solutions (buffer, eluents, etc.) were at least reagent grade and were used without further purification (Merck and Sigma). Water used for solutions was purified with a MQ-UV water device (Millipore). Electrical conductivity of the distilled water was been used for preparation of culture medium and other reagents was lower than 3 µmohs/cm. The solution was acidified to pH 4 with HCl and alkalified to pH 11 by NaOH. Measurement of pH, EC and DO was done using Hach pH meter (Hach Co., USA).

### **Glassware Preparation:**

All glassware was rendered pyrogen free by heating at 350 to 400°C for at least 30 min, none of glassware was autoclaved. Some products such as distilled water were purchased pyrogen free. The plastic apparatuses were treated with 33 % H<sub>2</sub>O<sub>2</sub>, then rinsed with pyrogen-free water and dried at 80°C for 8 h (Zhang, Y., *et al.*, 2007). A fresh bottle of pyrogen-free water was used for each experimental run to avoid potential contamination problems.

### **LPS Extraction:**

*E.coli* ATCC 25922 was obtained from reference laboratory of health, treatment and medicine education ministry (Tehran, Iran). The strain was maintained on standard methods agar slants at 5-6°C. The culture were grown on the nutrient agar medium and kept at 37°C. For the LPS preparation, pure culture of above mentioned *E.coli* strain was propagated at brain heart infusion broth (BHI) medium with concentration of 37 g l<sup>-1</sup> in 200 ml erlenmeyer flasks in shaking incubator at 35°C and 208 rpm for 18 h (Vigorously shaking is necessary for inducing of bacteria cell growth). After the required growth phase has been reached, the inact cells and cell debris were concentrated by centrifugation (12000 g for 15 min) and the supernatant were discarded. Subsequent extraction of endotoxin from pellet was conducted with boiling of the cell pellet for 15 min in depyrogenated distilled water and the resulting debris concentrated through centrifugation (12000 g for 15 min) (Venter, P., *et al.*, 2006). The extracted endotoxin (Supernatant) was preserved in 4°C and spiked as a target pollutant to water for producing of identified concentrations (100, 200 and Eu/ml) .

### **Endotoxin Detection:**

Detection of endotoxin was been carried out by Nowotny and Keler method with using of spectrophotometrically absorbance (Unico model, USA) in 535 nm. For detection of endotoxin 50 µl of sample or standards was added to 1 ml of color reagent of following composition: 5 mg of 1,9 dimethylmethylene blue, 0.43 g glycine, 0.33g NaCl, 4.7 ml NaOH 1N., 0.5 ml 80% ethanol, which dissolved in pyrogen-free water and diluted up to 100 ml (Keler and Nowotny, 1986). Calibration curve was plotted with using of standard endotoxin of *E.Coli* O55:B5 (Choa chrom Co, USA).

### **Ozone Production:**

Ozone was generated by OZOKAV ozone generator and pure oxygen with maximum flow rate of 1 L/ min (Fig.1). The mass of produced ozone was determined with Semi-batch method and using of 2% KI solution (APHA, 2005) that was 0.45 g/h.

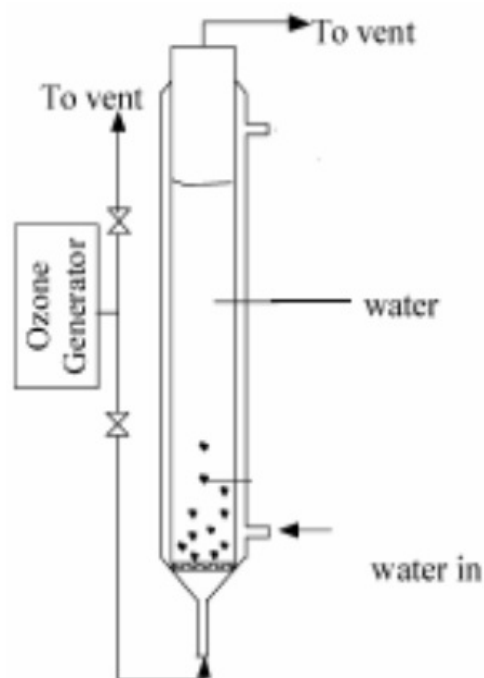


Fig. 1: Schematic of experimental setup.

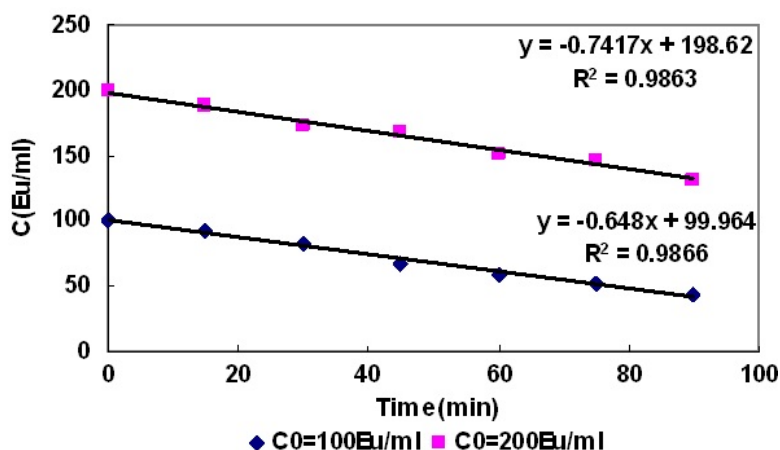


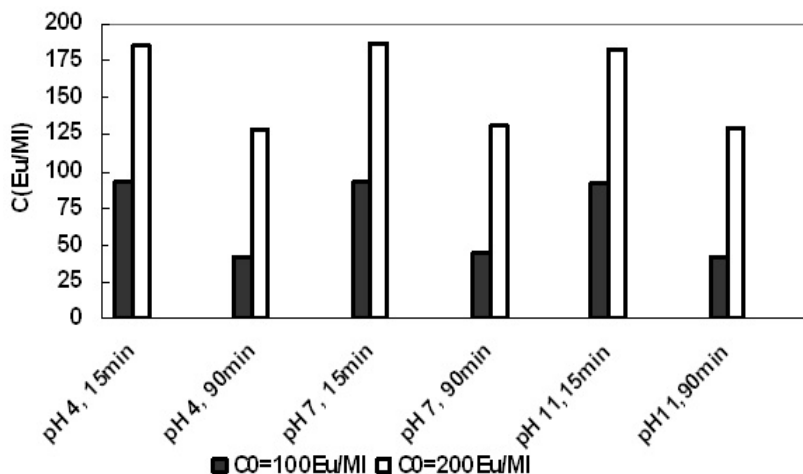
Fig. 2: Endotoxin inactivation versus reaction time (pH 7,  $T=20\pm 0.5$ , ozone dose=0.45g/h)

## RESULTS AND DISCUSSION

### Results:

Batch experiments were performed in this study to investigate the effects of pH values and preliminary concentrations for inactivation of endotoxin. The results of the triplicate endotoxin determination for tests are shown. The results of this study showed that endotoxin can be inactive by using of ozonation process and the inactivation rate that showed in figures is independent of preliminary concentrations. Also, our result showed that variation of pH is not effective for the inactivation of endotoxin with ozonation process. Fig. 2 shows the effect of ozonation in pH 7 for endotoxin inactivation in 100 and 200Eu/Ml. Endotoxin levels in the control samples (endotoxin present but no ozone added) remained constant throughout the experiment. As can be seen from the equations in Fig. 2 the inactivation rate of endotoxin was approximately 0.7 Eu/ml min and was almost identical at various pH and concentrations (a similar procedure can be seen in Figs. 3). Also our results showed that ozonation is appropriate in concentrations of lower than 200 Eu/ml. In higher concentrations

(data not shown), handling of white resistant foam is very hard task and is required for recycling installations. We concluded that the stability of molecular structure of endotoxin may be considered as a factor that variation of pH to be ineffective on ozonation process efficacy.



**Fig. 3:** Endotoxin inactivation versus reaction time and various pH ( $T=20\pm 0.5$ , ozone dose=0.45g/h)

**Discussion:**

It is important to note that high concentrations of endotoxin could occur as a result of contamination of open finished water storage reservoirs by Gram-negative bacteria or Cyanobacteria and biofilm growth in distribution systems which may confound observations in full-scale systems<sup>[1]</sup>. Anderson *et al* reported that inactivation rate of endotoxin with using of medium pressure UV lamp is 0.55(Eu/ml)/(mj/cm<sup>2</sup>) but ,no information available in about operational conditions. Although , several studies reported that chlorination is effective for inactivation of water endotoxin (Rapala, J., *et al.*, 2002). but, it was showed that in presence of 2 and 100 mg/l free chlorine residual the inactivation rate is 1.3-1.4 Eu/ml and this rate is independent of starting concentration<sup>[1]</sup> , so free chlorine would be relatively ineffective at distribution systems residence times which are typically available. According to Figs. we found that inactivation rate of endotoxin in presence of ozone is very higher than rates that was reported (Anderson, W.B., *et al.*, 2003) and varied from 0.65 Eu/ml min ( $C_0=100\text{Eu/ml}$ ) to 0.74Eu/ml min( $C_0=200\text{Eu/ml}$ ) , this higher inactivation rate can be related to oxidation power of ozone that higher than chlorine. This result in agree with the results of Anderson, *et al.* that was reported zero order reaction for inactivation rate of endotoxin by chlorine (Anderson, W.B., *et al.*, 2003). Because variation of pH have not effects for inactivation rate of endotoxin we suggest that ozonation process can be used in neutral point of pH that this phenomena is very appropriate from the point of view of drinking water treatment . Since it has been reported several adverse health effects for endotoxin and this organic micropollutant can be augments the toxic agents further studies to assess endotoxin concentrations associated with Gram-negative bacteria and biofilm in distribution systems and efficiency of operating water treatment plants to remove them are important. Also studies should be directed to determine safe endotoxin levels in drinking water since no guideline values for endotoxin concentration exist.

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