Bacterial Exotoxin (Streptolysin O) Removal from Water Using Ozone Gas

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Abstract: High concentrations of bacterial toxins could occur as a result of contamination of open water. The object of this paper was focused on the inactivation of bacterial toxin (streptolysin O) in water by ozonation process. The interest in ozone as a water disinfectant is based on its high biocidal efficacy, wide antimicrobial spectrum, absence of by-products that are detrimental to health and the ability to generate it on demand, "*in situ*", without needing to store it for later use. The efficacy of ozone for bacterial toxin inactivation increased with increasing exposure time (5 minutes) and ozone concentration (7 g/m^3). A bacterial strain (*streptococcus pyogenes* ATCC 19615) was allowed to grow on toxin production medium. Subsequent extraction and precipitation of toxin was conducted for detection of toxin. Finally the crude toxin was treated with ozone gas. The ozone was generated using coaxial dielectric-barrier-discharge (DBD) technique. In the present work, the cell of the discharge consists of coaxial electrodes. Ozone was applied directly into the tubes containing 1 ml of the crude SLO diluted with PBS at pH 7.4.
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1. Introduction

Water resources in Egypt are limited to the Nile River, groundwater in the Delta, Western deserts and Sinai, rainfall and flash floods as well as drainage and wastewater. The Nile is the predominant source of fresh water in Egypt (Phillip *et al*., 2003). Degradation of water quality is a major issue in Egypt. The severity of water quality problems in Egypt varies among different water bodies depending on: flow, use pattern, population density, extent of industrialization, availability of sanitation systems and social and economic conditions. Discharge of untreated or partially treated industrial and domestic wastewater, leaching of pesticides and residues of fertilizers; disposal of solid wastes; and navigation are often factors that affect the quality of water. Illegal polluting practices are numerous and widespread where there is little or no possibility of direct control. Sources range from intentional dumping of night soil, garbage, washing of animals and domestic utensils, to seepage from landfills, run-off from animal farms and accidental releases of chemicals (Phillip *et al*., 2003).

Waterworks encounter difficulties connected with exploitation of water intakes and water mains. This results from an increasing level of pollution of waters used as the source of drinking water. One of many reasons triggering off the deterioration of water quality and causing difficulties in making water drinkable and in forwarding it are living organisms, namely bacteria, fungi, plants and animals. They influence many features of water quality, including its smell, color, turbidity, pH value, the content of organic substances, the content of nitrogen, and among other things they also influence the concentration of toxic organic compounds (Makowski and Wardas, 2001). These toxins may be hepatotoxins, which affect the liver and kidney, neurotoxins, which affect the central nervous system, or dermatotoxins, which are skin irritants, capable of causing both acute and chronic illnesses (Haney *et al*., 2002).

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 micro-pollutants (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). Slaughterhouse wastewater is very harmful to the environment (Masse and Masse, 2000). Effluent discharge from slaughterhouses has caused the deoxygenation of rivers (Quinn and Farlane 1989) and the contamination of groundwater (Sangodoyin and Agbawhe 1992). Blood is one of the major dissolved pollutants in slaughterhouse wastewater (Tritt and Schuchardt 1992).

Streptococcus pyogenes is one of the most frequent pathogens of humans that can grow in blood contaminated water (specially in effluents discharged from slaughterhouses) and produce many toxins. In the last century, infections by *S. pyogenes* claimed many lives especially since the organism was the most important cause of pharyngitis (strep throat), scarlet fever (rash), impetigo (infection of the superficial layers of the skin) or cellulitis (infection

of the deep layers of the skin). Invasive, toxigenic infections can result in necrotizing facilities, myositis and streptococcal toxic shock syndrome. Patients may also develop immune-mediated post-streptococcal infection, such as acute rheumatic fever and acute glomerulonephritis, following acute infections caused by *Streptococcus pyogenes* (Kenneth Todar,2008).

Endotoxin and exotoxins are examples of organic micro-pollutants that has several adverse health effects for human (Rapala *et al.,* 2002). Endotoxin are typically released either during cell lyses or multiplication and have a high stability even at high temperature (stable at 121ºC for 1 h) and pH values due to its amphoteric structure (Hyrayama *et al.*, 1999; Anderson *et al.*, 2003). General symptoms of endotoxin exposure in humans include fever, diarrhea, vomiting, hypotensions, shock, intravascular coagulation and death (Tessarolo *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 *et al.*, 2003). Rezaee *et al.*, (2008) studied the inactivation of endotoxin in water by ozonation process.

Klaus (2009) indicated that exotoxins are toxic proteins that are secreted by many microorganisms. The have a high toxicity and they are heat labile. Many exotoxins consist of an A part and a B part. The A part has the enzymatic activity whereas the B part binds the toxin to the tissue cell and translocated the A part across the tissue cell membrane into the cytoplasm. One of the most important exotoxins is streptolysin (haemolysin) which acts by destroying membrane phospholipids. Streptolysin 0 (SLO) belongs to a family of cytolysins produced by four genera of gram-positive bacteria, Streptococcus, Bacillus, Clostridium, and Listeria, most of which are pathogenic for humans (Dieter *et al.*, 1993). Streptolysin O (SLO) is a potent membrane-disrupting protein produced by *Streptococcus pyogenes*, and it has been reported to bind to cholesterol in the red blood cell membrane, form polymers, and generate very large trans-membrane channels, through which haemoglobin (Hb) can escape (Jun Suzuki, 2009).

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 *et al.,* 2002). Recently water purification with ozone as an option to disinfection and degrade organic micropollutants has been widely developed (Smeets *et al.,* 2006; Sano *et al.,* 2007). 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 *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_2 easily (Sano *et al.,* 2007).

2. Material and Methods

2.1. Strain

The strain *S. pyogenes* ATCC 19615 (group A) was obtained kindly from the department of microbiology , Memphis Pharmaceutical Company (Cairo, Egypt). The strain was maintained on nutrient agar slants at 4 ºC.

2.2. Culture medium

The reference strain (*S. pyogenes* ATCC 19615) was cultivated on Mueller–Hinton broth supplemented with 3–5% rabbit blood (Steininger *et al*., 2002), and incubated at 37°C for 7 to 8 hours to be used in the preparation of crude streptolysin O toxin.

2.3. Preparation of crude streptolysin O (SLO) toxin

One liter of cultivation medium was inoculated with 5 ml of bacterial suspension cultured for 7 to 8 h, and incubated at 37°C with shaking, the pH was held at 7.5 for 16 to 18 h of incubation. After incubation, culture supernatants were harvested by centrifugation at 12000 rpm for 15 minutes (Jun Suzuki, 2009).

2.4. Precipitation of crude streptolysin (O) toxin

The supernatants fluid containing SLO toxin were brought to 0, 20, 40, 60, 80 and 100 % saturation by the addition of solid ammonium sulfate. The precipitates were recovered by centrifugation, collected in approximately 100 ml and stored at 4 ºC and spiked as a target pollutant to water for producing of identified concentration of 200 IU/ml (Grushoff *et al*, 1975).

2.5. Assay of SLO hemolytic activity

Hemolytic activity of SLO was spectrophotometrically determined using 50%-end-

point titration (Jun Suzuki, 2009). SLO preparations were diluted with Phosphate buffered saline (PBS) supplemented with 2-mercaptoethanol (2-ME) and bovine serum albumin (BSA), and 0.5 ml of a 2.5% suspension of rabbit erythrocytes (RE) in PBS (pH 7.4) was added to 1.5 ml of the SLO solutions. After incubation at 37°C for 60 min, tubes were centrifuged and supernatant (containing haemoglobin) in the tubes was measured spectrophotometrically at 510 nm against a blank tube containing the diluent in stead of SLO. One hemolytic unit is defined as the smallest amount of streptolysin required to produce 50% lysis of 1 ml of a 2.5% of rabbit erythrocytes suspension upon incubation at 37 ºC for 60 min (Grushoff *et al*., 1975). Calibration curve (Fig. 2) was plotted with using of standard exotoxin SLO (Sigma No., S5265, USA) with concentration of 25.000 – 50.000 U/vial. Hemolysis percentage as well as optical densities of 200 U/ml of SLO standard at different doses i.e 3, 5, 7, 9, 11, 13, 15, 17 and 19 µl/ml were used (Table 1) and a standard curve was constructed between log of SLO doses and hemolysis percentage and a linear relationship was obtained.

2.6. Protein estimation

Protein in the supernatants was estimated by the method of Lowry *et al*, (1951) , and determined at absorbance of 280 nm. Comparison was made with a standard curve of bovine albumin.

2.7. Ozone generation and treatment

In the present work, the cell of the discharge consists of coaxial electrodes (Fig. 1), the inner electrode is made of brass with radius (0.5 cm) and the outer electrode is made of graphite coating on the outer wall of a glass tube. The dielectric material between the two electrodes is Pyrex glass of 0.1 cm thickness. The gap space between the inner electrode and the inner wall of glass tube is 0.2 cm. The length of the reactor region is 50 cm and the gas pressure used is 1.005 bar. The working gas is pure oxygen (99.9 %) and the gas flow rate was adjusted to 5 L/min (Garamoon *et al.*, 2009). Ozone was applied directly into the tubes containing 1 ml of the crude SLO diluted with PBS at pH 7.4.

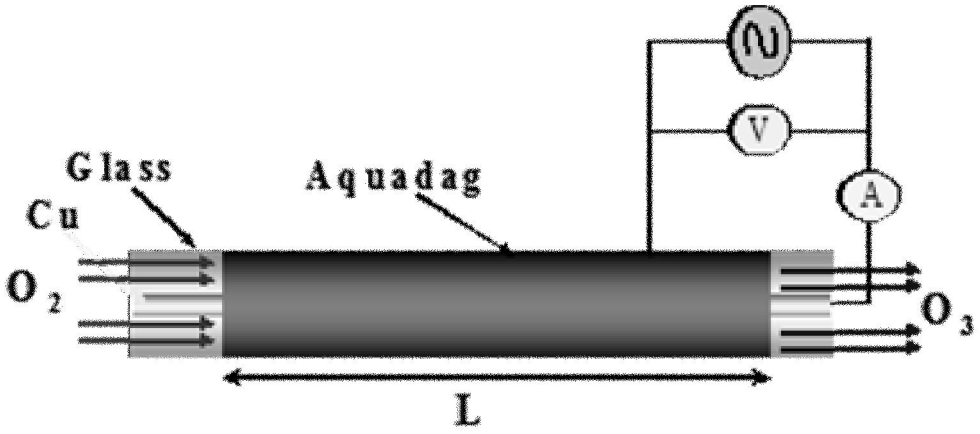


Figure 1. Schematic of the dielctric coaxial electrodes

3. Results

3.1. Construction of a standard curve for assaying a standard SLO toxin

As shown in Table (1), different doses of a standard SLO (Sigma, USA) showed varying hemolysis percentages. One hundred % of hemolysis was achieved at 1.7 log doses of SLO (45 µl/ml) of 200 U/ml of SLO standard with an optical density (O.D.) of 0.59 g/l (hemolysed rabbit erythrocytes) at 510 nm. Results also revealed that a linear relationship was obtained (Fig.2).

3.2. Precipitation of crude SLO produced by *S. pyogenes* ATCC 19615

As can be seen from Table (2), the crude SLO produced by the cultivation of the reference strain *S. pyogenes* ATCC 19615 on the production medium showed a maximum hemolytic activity with an optical density of 0.59 g/l at 80 % ammonium sulphate fractionation (Fig. 3). The protein content was estimated for each fraction.

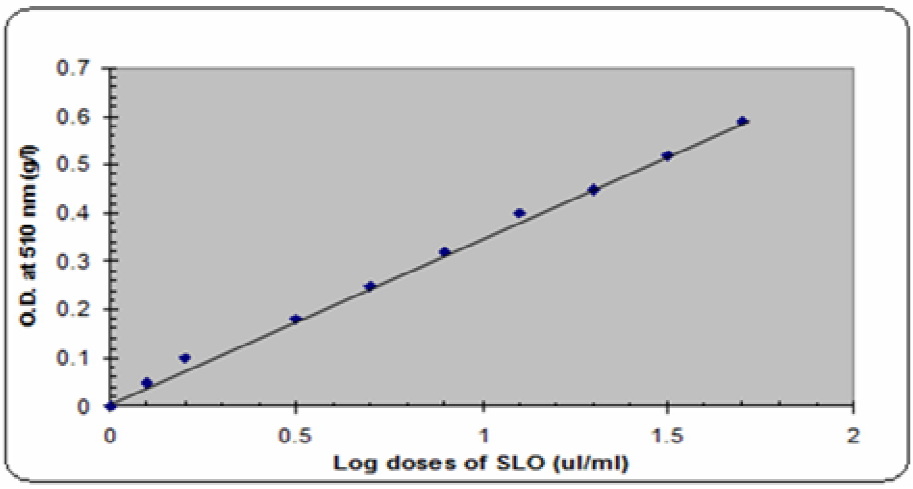


Figure 2. A linear relationship between log doses of SLO and optical densities of supernatants at 510 nm

Table 1. Relation between different SLO doses, hemolysis (%) and their optical densities

SLO doses (µl/ml)	Log dose of SLO	Hemolysis %	O.D. at 510 nm (g/l)
1.50	0.1	8	0.05
2.00	0.2	17	0.10
3.00	0.5	31	0.18
6.00	0.7	42	0.25
9.00	0.9	54	0.32
13.0	1.1	68	0.40
18.0	1.3	76	0.45
30.0	1.5	88	0.52
45.0	1.7	100	0.59

Table 2. Ammonium sulphate fractionation of crude SLO produced by *S. pyogenes* ATCC 19615

Ammonium sulphate conc. (%)	Protein content (mg/ml)	O.D. of supernatant at 510 (nm)
0	4.5	0.11
20	7.3	0.29
40	9.5	0.34
60	10.6	0.46
80	12.7	0.59
100	12.6	0.59

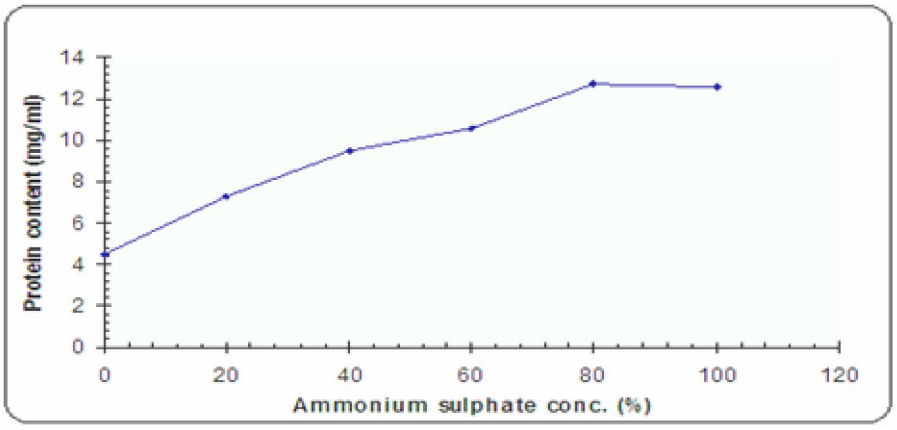


Figure 3. Precipitation of crude SLO produced by *S. pyogenes* ATCC 19615 using different ammonium sulphate concentrations

3.3. Effect of ozone on different concentrations of crude SLO

Different doses of crude SLO (i.e. 2.5, 5, 15, 30 and 45) with concentration of 200 IU/ml were exposed to 1 g/m^3 ozone at 37 ºC for 1 min Table (3). An obvious decrease in hemolytic activity of crude SLO was detected when compared with the results of the control (untreated). Data were illustrated and represented in Fig (3).

Table 3. Effect of 1 g/m^3 ozone on different doses of crude SLO at 37 ºC for 1 min.

Doses of crude SLO (µl/ml)	Log doses of SLO (µl/ml)	Treated SLO		Untreated SLO	
		O.D. (g/l)	Hemolysis (%)	O.D. (g/l)	Hemolysis (%)
45	1.7	0.37	64	0.58	100
30	1.5	0.35	60	0.50	86
15	1.2	0.27	47	0.43	74
5	0.7	UD*	UD	0.23	40
2.5	0.4	UD	UD	0.15	26

* UD = Undetected

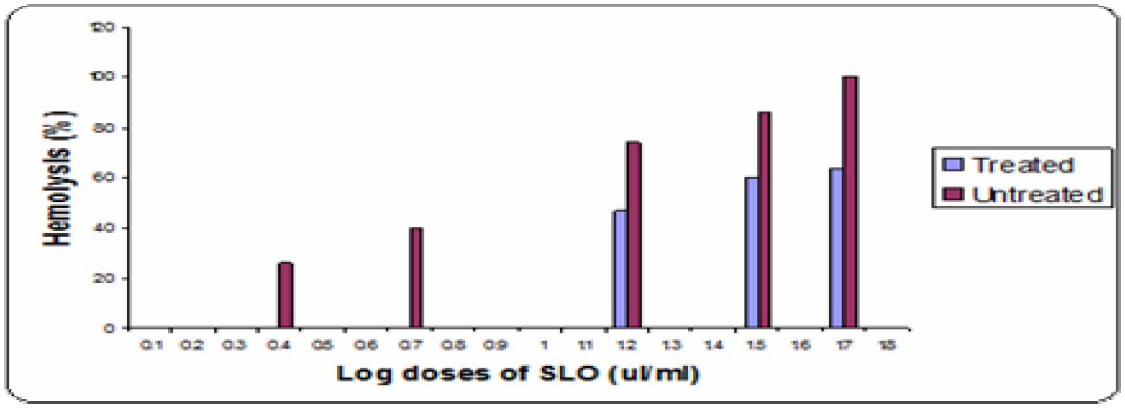


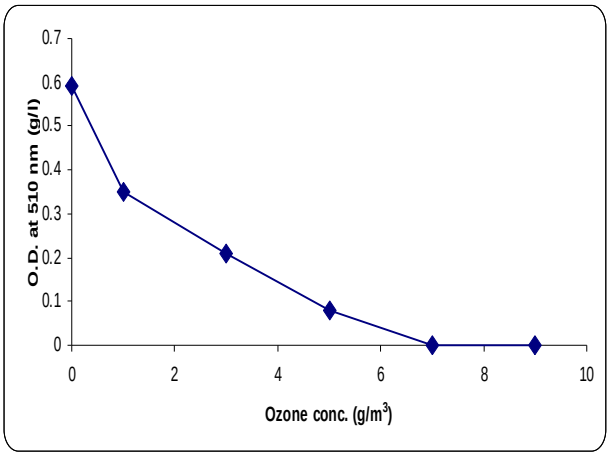
Figure 4. Exposure of different doses of crude SLO to 1 g/m^3 ozone for 1 min at 37 ºC

3.4. Effect of different ozone concentrations on crude SLO

An appropriate dose (45 µl/ml) of crude SLO (200 U/ml) was exposed to different ozone concentrations (i.e. 1, 3, 5, 7 and 9 g/m^3 for a defined time (1 min) at 37 ºC. results recorded in table (5) and represented graphically in figure (4) revealed that there was a continuous decrease in percentage of hemolysis due to increasing of ozone concentrations. Both of hemolysis as well as optical densities of the crude SLO supernatant were undetected at ozone concentrations of 7 and 9 g/m^3 respectively.

Table 4. Inactivation of crude SLO by different Ozone Concentrations for 1 min at 37 ºC

Ozone Conc. (g/m^3)	Hemolysis (%)	O.D. at 510 nm (g/l)
0	100	0.59
1	59	0.35
3	36	0.21
5	14	0.08
7	UD*	UD
9	UD	UD



*UD= Undetected

Figure 5. Crude SLO inactivation by different conc. of ozone for 1 min at 37ºC.

3.5. Inactivation of crude SLO in relation to time of ozone exposure

The crude SLO (200 U/ml) was exposed to 1 g/m^3 ozone at specific dose 45 (µl/ml) for various exposure times (0, 1, 2, 3, 4, 5 and 6 min) at 37 ºC. the represented data (Fig. 5) showed that by increasing the time of ozone exposure, the optical density of the studied samples decreases. Hemolysis was found to be disappeared at 5 min of ozone exposure (Table 6).

Table 5. Effect of different exposure times on the activity of crude SLO.

Exposure time (min)	Hemolysis (%)	O.D. at 510 nm (g/l)
0	100	0.59
1	59	0.35
2	49	0.29
3	29	0.17
4	12	0.07
5	UD*	UD
6	UD	UD

* UD = Undetected

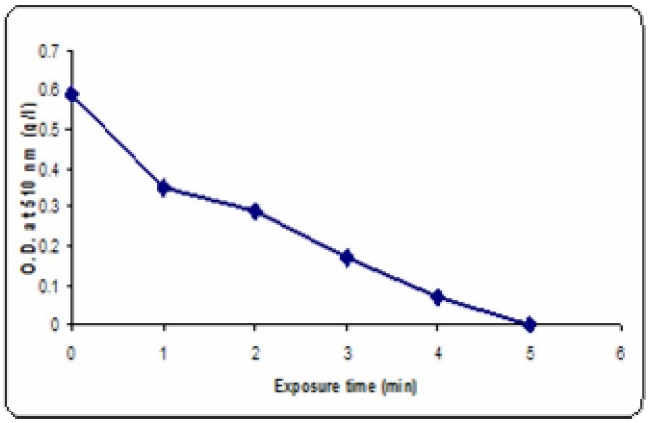


Figure 6. Inactivation of crude SLO in relation to increasing of exposure time.

4. Discussions

This study was focused on bacterial toxin removal during drinking water treatment with the primary focus on the removal of extracellular toxins (Streptolysin O). The recent work suggests that the degradation of streptolysin O by ozone. In contrast with SLO, higher ozone concentrations increased the inactivation rate of SLO. This result is in agreement with the results obtained by Himberg *et al*, (1989) who mentioned that the best results for removal of bacterial extracellular toxins were obtained with the application of ozonation process. Other studies on the uses of ozonation process in the removal of bacterial exotoxins were carried out by Keijola *et al.,* 1988, Himberg *et al*., 1989, Bruchet *et al.,* 1998, Hart *et al.,* 1998, Rositano *et al.,* 1998 and 2001, Newcombe 2002b. They stated that microcystin and anatoxin (a) are inactivated at different ozone doses. Oxidation with ozone is largely effective at destroying most cyanotoxins (WD, 2009). Makowski *et al*, (2001) studied the effect of ozonation and chlorination on the removal of hepatotoxins from water. The arising of highly reactive hydroxyl-radicals with unspecific activity can lead to removal of the toxins from water (Makowski *et al.,* 2001). Rezaee *et al*, (2008) studied the inactivation of bacterial toxin in water by ozonation process. They found that at 90 min exposure to 1 l/min, ozone was adequate to inactivate 200 EU/ml of bacterial toxin. In contrast, ozone exposure values of greater than 6.9 mg min L^{-1} resulted in 40 % destruction of the saxitoxins. (Archuleta and Manwaning 2002) revealed that microcystins were destroyed to below detection by HPLC and the toxicity was also removed with the action of different ozone concentrations.

Results obtained at this study proved that by increasing the ozone concentrations, the hemolysis percentage decreases gradually to be undetected at ozone concentration of 7 g/m^3. This is in accordance to the results obtained by Rezaee *et al*, (2008), since they stated that the efficiency of ozone for bacterial toxin inactivation increased with increasing ozone concentration. Brooke (2009) found that saxitoxins (Cyanobacterial toxins) were removed with the using of different ozone concentrations and he indicated that conventional ozone treatment was effective in removing hepatotoxicity from drinking water.

Inactivation of SLO was also studied in relation to time of ozone exposure. The data obtained t this study also proved that by increasing the time of ozone exposure, the optical density of the studied samples decreased and the hemolysis was found to be disappeared at 5 min of ozone exposure. This is in agreement with Rezaee *et al*, (2008) results, where they found that the inactivation of bacterial toxin was increased by increasing exposure time. A residual of at least 0.3 mg L^{-1} of ozone for 5 minutes will be sufficient for removal of the most common microcystins (Nicholson *et al*., 1994; Newcombe 2002; Rositano *et al*., 1998; Rositano *et al*., 2001; Ho *et al*., 2006a and Acero *et al*., 2005).

The crude SLO toxin produced in this paper was precipitated by 80 % ammonium sulphate precipitation. Grushoff *et al,* (1975) also precipitated at 80% of ammonium sulphate precipitate.

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References

1. Acero J., Rodriguez E. and Meriluoto J (2005) Kinetics of reactions between chlorine and the cyanobacterial toxins microcystins. Water Research, 39, 1628-1638.
2. Anderson, W. B., Slawson R. M. and Mayfield C.I. (2002) A review of drinking-water associated endotoxin,including potential routes of human exposure. Can.J. Microbiol., 48: 567-587.
3. Anderson, W.B., Mayfield C. and Dixon D. G. (2003) Endotoxin inactivation by selected drinking water treatment oxidants. Water Res., 37: 4553-4560.
4. Archuleta G. Edmund and Manwaning F. James (2002) Removal of algal toxins from drinking water using ozone and GAC. Awwa research foundation and American Water Works Association, USA. ISBN 1-58321-225-6.
5. Brooke J. L. Samuel (2009) The destruction of cyanobacterial toxins with oxidants used in drinking water treatment. PH.D. Thesis, School of Pharmacy and Medical Sciences of the University of South Australia.
6. Bruchet A, Bernazeau F, Baudin I, Peironne P (1998) Algal toxins in surface water analysis and treatment. Water Supply 16 (1–2):619–623.
7. Dieter G., Werner K., Elisabeth G., and Karlheinz M. (1993) Purification and Characterization of Streptolysin 0 Secreted by Streptococcus equisimilis (Group C) Institute of Experimental Microbiology, DO-6900 Jena, 1 and Max-Planck-Institut fur Biochemie, DW-8033 Martinsried,2 Germany, Infection and immunity, Vol. 61, No. 6 p. 2727-2731.
8. Esplugas, S., Bila D. M., Krause L. G. T. and Dezoti M. (2007) Ozonation and advanced oxidation technologies to remove endocrine

disrupting chemicals(EDCs) in water effluents. J.Haz.Mater., 149: 631-642.
9. Garamoon A. A., Elakshar F. F. and Sawah M. (2009) Optimization of ozone generation at low resonance frequency. Eur.Phys. J. Applied Phys., 48, 21002.
10. Grushoff P. S., Shany S. and Bernheimer A. W. (1975) Purification and properties of streptococcal nicotinamide adenine dinucleotide glycohydrolase. Journal of bacteriology, Vol. 122, No. 2, P 599:605.
11. Haney, James F., John J. Sasner, Myoshi Ikawa and Jeffrey Schloss (2002). Brief summary of findings of the survey of biotoxins in New Hampshire lakes. Prepared for: N.H. Department of Environmental Services, Concord.
12. Hart J, Fawell J, Croll B (1998) The fate of bath intra and extracellular toxins during drinking water treatment. Water Supply 16(1–2):611–616.
13. Himberg K, Keijola A, Hiisvirta L, Pyysalo H, Sivonen (1989) The effect of Water Treatment Processes on the removal of hepatotoxins from Microcystis and Oscillatoria Cyanobacteria: A Laboratory Study. Water Research 23 (8): 979–984
14. Ho L, Onstad G, von Gunten U, Rinck-Pfeiffer S, Craig K and Newcombe G (2006a) Differences in the chlorine reactivity of four microcystin analogues. Water Research 40 (6): 1200-1209.
15. Hyrayama, C., M. Sakata and M. Nakamara, 1999. Preparation of poly adsorbents and application to selective removal of lipopolysaccharide. J. Chromatogr. B., 721: 187-195.
16. Jun Suzuki (2009) Characterization of acidic and neutral streptolysin O. Department of food biochemistry, School of life and environmental Science, Azabu University. Journal of 35 : 45.
17. Keijola A, Himberg K, Esala A, Sivonen K, Kiisvirata L (1988) Removal of cyaonbacterial toxins in water treatment processes: laboratory and pilot plant experiments. Toxicological Assessment 3:643–656.
18. Kenneth Todar (2008) Streptococcus pyogenes and Streptococcal Disease. Todar's online textbook of Bacteriology. Vol 304: p. 1421).
19. Klaus D. Elgert (2009) Immunology: Understanding the immune system, Second edition Book, Published by Wiley-Blackwell.
20. Klein G.C. (1976) Manual of clinical immunology. ASM 264-273.
21. Lowry, 0. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall (1951) Protein measurement with Folin phenol reagent. J. Biol. Chem. 193:265-275.
22. Makowski A. and Wardas W. (2001) Photocatalytical degradation of toxins secreted to water by cyanobacteria and unicellular algae and photocatalytical degradation of the cells of selected microorganisms. Department of General and Analytical Chemistry, University Medical School of Silesia, Jagiellonska 4, 41-200 Sosnowiec, Poland, Current Topics in Biophysics 2001, 25(1), 19-25.
23. Masse D.I. and Masse L. (2000) Characterization of wastewater from hog slaughterhouses in Eastern Canada and evaluation of their in-plant wastewater treatment systems. Canadian Agricultural Engineering Vol. 42, No. 3.
24. Newcombe, G. (2002) Removal of algal toxins from drinking water using ozone and GAC. AWWA Research Foundation Report, American Water Works Association, Denver, CO.
25. Newcombe G. (2002b) Removal of Algal Toxins from Drinking Water Using Ozone and GAC. Denver, Colorado: American Water Works Research Foundation.
26. Nicholson, B. C., Rositano, J. and Burch, M. D. (1994) Destruction of cyanobacterial peptide hepatotoxins by chlorine and chloramine. Water Research 28(6), 1297-1303.
27. Phillip B., Fatma El Gohary, Mohamed A. T., Emad I. H. and Shaden Abdel-Gawad (2003) Nile River Water Quality Management Study. Egypt Water Policy Reform Contract No. LAG-I-00-99-00017-00 Task Order 815, Report No. 67.
28. Quinn J.M. and P.N. McFarlane. 1989. Effects of laughterhouse and dairy factory wastewaters on epilithon: comparison in laboratory streams. Water Research 3:1267-1273.
29. Rapala, J., Lahti K., Rasanen L. A., Esala A. L., Niemela S. I. and Sivonen K. (2002) Endotoxin associated with Cyanobacteria and their removal during drinking water treatment. Water Res., 36: 2627-2635.
30. Rezaee A., Ghanizadeh Gh., azdanbakhsh A. R. Y., Behzadiannejad Gh., Ghaneian M. T., Siyadat S. D., Hajizadeh E. (2008) Removal of Endotoxin in Water Using Ozonation Process School of Medical Sciences, Tarbiat Modares University. Tehran, I.R.Iran. Australian Journal of Basic and Applied Sciences, 2(3): 495-499.
31. Rivera-Utrilla, J., Mendez_Diaz J., Sanchez-Polo M. and Ferro-Garcia M.A. (2006) Removal of the surfactant sodium dodecylbenzenesulphomate from water by simultaneous use of ozone and powder activated

carbon: Comparision with systems based on O3 and O_3/H_2O_2. Water Res., 40: 1717-1725.
32. Rositano J, Nicholson B, and Pieronne P (1998) Destruction of cyanobacterial toxins by ozone. Ozone Science Engineering 20:223–238.
33. Rositano J, Newcombe G, Nicholson B, Sztajnbok P (2001) Ozonation of NOM and alglal toxins in four treated waters. Water Research 36(1):23–32.
34. Sangodoyin, A.Y. and Agbawhe O. M. (1992) Environmental tudy on surface and groundwater pollutants from abattoir ffluents. Bioresource Technology 41:193-200.
35. Sano, N., T. Yamamota, D. Yamamota, S.I. Kim, A. Eida-Ua, H. Shinimiya and M. Nakaiwa, 2007. Degradation of aqueous phenol by simultaneous use of ozone with silica-gel and zeolite. Chem. Eng.Proc., 46: 513-519.
36. Smeets, P.W.M.H., A.W.C. Van der Helm, Y.J. Dullemont, L.C. Rietreld, J.C. Vandijk and C.J. Medema, 2006. Inactivation of Escherichia Coli by ozone under bench-schale plug flow and full-schale hydrolic conditions. Water Res., 40: 3239-3248.
37. Steininger C., Allerberger F. and Gnaiger E. (2002) Clinical significance of inhibition kinetics for Streptococcus pyogenes in response to penicillin. Journal of Antimicrobial Chemotherapy 50, 517–523.
38. Tessarolo, F., I. Caola and G. Nollo, 2006. Efficiency in endotoxin removal by a reprocessing protocol for electrophysiology catheters based on hydrogen peroxide plasma sterilization. Int. J. Hyg. Environ-Health., 209: 557-565.
39. Tritt, W.P. and F. Schuchardt. 1992. Materials flow and ossibilities of treating liquid and solid wastes from slaughterhouses in Germany. Bioresource Technology 1:235-245.
40. WD, D.W., G.B. (2009) Cyanobacteria and Drinking Water: Guidance for Public Water Systems. Environmental fact sheet. New Hampshire Dept. of Environmental services. (603) 271-3503.

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