original article

Effects of sequential ozonation and adsorption in the removal of water-soluble fraction of crude oil, leading to total organic carbon and toxicity reduction for rainbow trout larvae

Mohsen Sadani, Hossein Movahedian[1], Maryam Faraji[1], Yaghoub Hajizadeh[1]

Department of Environmental Health Engineering, School of Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran, [1]Environment Research Center, Isfahan University of Medical Sciences, Isfahan, Iran

ABSTRACT

Aims: The purpose of this study was to evaluate the performance of the sequential application of ozonation and activated carbon processes in the elimination of water-soluble crude oil and thereby reducing total organic carbon (TOC) and toxicity for the rainbow trout larvae.

Materials and Methods: A series of water-soluble fractions of crude oil, 5-100 ml/l, were prepared. Groups of ten rainbow trout fish larvae were exposed to the solution for 24, 48, and 96 hours. Toxicity (LC_{50}: Median lethal concentration) and TOC tests were performed for the solutions before and after their treatment by sequential ozonation and activated carbon adsorption.

Results: The LC_{50} (96 hours) and TOC of the sample before the treatment process were 60 mg/l and 55 mg/l, respectively. After adsorption by 10 mg/l activated carbon, followed by ozonation with a concentration of 1 mg/l, the LC_{50}increased to 145 mg/l and TOC reduced to 36 mg/l. Those values, after treatment with 30 mg/l activated carbon, followed by 7 mg/l ozone, reached 196 mg/l and 28 mg/l, respectively. In the experiment, ozonation by 1 mg/l ozone was applied, and then adsorption was carried out by 10 mg/l activated carbon, and the $LC_{50}$was 149 and TOC was 35 mg/l. In the experiments with 7 mg/l ozone followed by 30 mg/l activated carbon, LC_{50} reached 204 mg/l and TOC reduced to 28.5 mg/l.

Conclusions: Primarily ozonation of crude oil polluted waters followed by adsorption by activated carbon can increase the removal efficiency of the process, which results in significant TOC and toxicity reduction.

Key words: Activated carbon, crude oil, ozonation, rainbow trout larvae, TOC, toxicity

Address for correspondence:
Dr. Yaghoub Hajizadeh, Environment Research Center, Isfahan University of Medical Sciences, Hezar Jarib Ave., Isfahan, Iran.
E-mail: y_hajizadeh@hlth.mui.ac.ir

Access this article online	
Quick Response Code:	Website: www.ijehe.org
	DOI: 10.4103/2277-9183.96003

INTRODUCTION

The growing demand for the use of crude oil hydrocarbons is an environmental concern throughout the world because of its toxic effects when it enters the aquatic ecosystems, either by accidental spills or via normal commercial activities.[1] Adverse health effects resulting from crude oil exposure

This article may be cited as:
Sadani M, Movahedian H, Faraji M, Hajizadeh Y. Effects of sequential ozonation and adsorption in the removal of water-soluble fraction of crude oil, leading to total organic carbon and toxicity reduction for rainbow trout larvae. Int J Env Health Eng 2012;1:16.

can vary from biochemical to organs damage effects. Acute toxicity tests or bioassays have historically played an important role in assaying the effects of pollutants on animals, and such tests have been widely applied, to evaluate the toxicities of various types and mixtures of pollutants on fish and other aquatic species.[2] The parameter of short-term mortality has been the most common measure of toxicity.[3]

Most toxicological research on crude oil has focused on the water-soluble fraction, because it is the portion that easily enters the aquatic environment and can impose immediate and acute damage on aquatic organisms.[4-7] Dissolved petroleum hydrocarbons have shown various effects ranging from reversible to long-term sub-lethal reproductive effects.[8] Crude oil spills into freshwater have been reported to reach 2.65×10^6 liters seasonally, throughout the world.[8] As the release of oil into the environment frequently involves a sudden exposure, often in a short duration, and rainbow trout larvae are very sensitive organisms, this study was set to determine the short-term lethal concentration of crude oils on rainbow trout larvae (*Oncorhynchus mykiss*). An example of a sudden accident is the one that occurred in Zayandeh-rood (a river in Isfahan, Iran), in 2008, which caused the entry of several million liters of crude oil into the river. This river is a main source of drinking water for Isfahan.

Considering the tremendous amounts of oil appearance in the environmental matrices, and thereby in the food chain and drinking water, application of an effective method for its remediation, particularly from an aqueous solution, is essential. The techniques usually adopted to minimize mineral oil pollution are largely based on the use of phase separation methods or adsorption on active suspended materials.[9] Pretreatment with an effective adsorbent and micro- or ultra-filtration are the commonly applied processes for oil cleanup from water basins.[10] Ozone is a strong oxidant that has been used successfully in water and wastewater treatment, for the oxidation of organic contaminants. It has been suggested that the application of ozone before activated carbon adsorption can significantly enhance the removal of biodegradable compounds.[11]

The purpose of this study was to compare the effectiveness of sequential ozonation and activated carbon processes in reducing TOC and toxicity of water-soluble crude oil for rainbow trout larvae. The evaluation of toxicity reduction was carried out by a bioassay test on rainbow trout larvae.

MATERIALS AND METHODS

Preparation of the water-soluble fraction of crude oil

Crude oil was obtained from the Isfahan refinery plant, and its solution (water-soluble fraction) was prepared by adding one part of the oil to nine parts water.[12] In this case, 50 ml crude oil was added to 450 ml deionized water (100 ml/l) in a 1000-ml beaker covered by aluminum foil, and then homogenized with a magnetic stirrer for 24 hours, at 200 rpm. The sample was transferred into a separation funnel and left an hour for phase separation. The water-soluble fraction was then recovered in an airtight container and refrigerated until the toxicity tests were performed. The entire procedure was carried out at 20ºC.

Biological exposure tests

For each toxicity test, groups of ten rainbow trout fish larvae (Juvenile) were exposed to a series of soluble crude oil concentrations (5 – 100 ml/l) in 30 l of dechlorinated municipal freshwater, taken from Lake Zayandeh-rood (alkalinity = 135 mg/l as $CaCO_3$, chloride 35 mg/l). The physicochemical factors such as temperature, pH, dissolved oxygen (DO), and electrical conductivity (EC) were measured daily in each experiment. The pH, DO, and EC ranges of the solution were 7 – 7.5, 7 – 9 mg/l, and 150 μz/cm, respectively. The juveniles of rainbow trout, obtained from a trout farm (Rainbow Springs, Shahr-e-kord), were acclimatized for at least one week in freshwater at 12 – 15ºC, by daily feeding them with a commercial fish food, at a rate of 3% body weight per day. Feeding was withheld for 48 hours prior to each bioassay and throughout the exposure period. All the bioassay tests were done in triplicate and the average of observations was considered. The observations were made at 24, 48, and 96 hours and the evaluated response was immobilization or death of the test specimens. All tests were carried out in a chamber at 20ºC with a photoperiod of 16 hours light and eight hours darkness. Finally, the number of larvae that survived during the desired period was recorded, and the SPSS software and Probit method were applied to determine the median lethal concentration (LC_{50}).[13] The toxicity and TOC tests were performed before and after the treatment process. A Shimadzu TOC-5050A analyzer, which used combustion-infrared methods for TOC analysis, was used for TOC measurement.

Crude oil removal by ozonation – adsorption and adsorption – ozonation

Ozone was generated from ambient air by a portable ozone generator (Model 165; Thermo). The ozonation batch experiments were performed using an experimental apparatus at 25°C with a contact time of 20 minutes, which has been reported elsewhere.[14] A 30 l capacity glass reactor, equipped with four equally spaced baffles, and a stainless steel six-blade turbine was applied. The samples were taken from the top of the reactor for analysis. The concentration of ozone was adjusted to the desired level by an ultraviolet (UV) photometric ozone analyzer (Model 49; Thermo Environmental Instruments Inc., Franklin, MA), in which, the injection of ozone was stopped when the expected concentration of each test was achieved in the solution.

The adsorption process was performed for the crude oil content samples in the jar test equipment by adding powdered activated carbon (PAC; supplied by Merck Inc.)

and stirring with a mixture at 220 rpm for 30 minutes. At the end of adsorption the samples were filtered to remove the PAC, after which the toxicity and TOC tests were accomplished. The treatment procedures were carried out in two choices of orders with the same concentrations: (i) First ozonation and then adsorption and (ii) First adsorption and then ozonation. The examined concentrations of ozone and activated carbon were 1 + 10, 3 + 10, 5 + 20, 5 + 30, and 7 + 30 mg/l (mg/l ozone + mg/l activated carbon).

RESULTS

Table 1 shows the LC_{50} for 24, 48, and 96 hours of crude oil solution, for rainbow trout larvae, and the TOC values of the solution before the treatment process and after sequential treatment by activated carbon adsorption, followed by ozonation, with different dosages of the adsorbent and ozone. The LC_{50} for 96 hours and TOC values of the samples before and after adsorption–ozonation are compared in Figure 1. In experiments using 30 mg/l activated carbon, followed by 7 mg/l ozone, the TOC content of the solution was reduced from its initial concentration of 54.98 mg/l to 28.02 mg/l, and the LC_{50} for 96 hours increased from its initial value (60 mg/l) to 196 mg/l.

The LC_{50} for 24, 48, and 96 hours and the TOC values of the solution before the treatment processes and after sequential treatment by ozonation, followed by activated carbon adsorption, with different dosages of ozone and the adsorbent, are shown in Table 2. The LC_{50} of 96 hours and TOC values of the samples before and after ozonation–adsorption are compared in Figure 2. By application of 7 mg/l ozone and then 30 mg/l activated carbon, the TOC content of the solution declined to 28.53 mg/l and the LC_{50} of 96 hours was enhanced to 204 mg/l.

Table1: LC_{50} 24, 48 and 96 hours and TOC values of crude oil solutions before the treatment process and after adsorption, followed by ozonation

LC_{50}, 96 hours (mg/l)	LC_{50}, 48 hours (mg/l)	LC_{50}, 24 hours (mg/l)	TOC (mg/l)	PAC + O_3 (mg/l)
60	148	129.5	54.98	0
145.23	152.63	210	36.11	10 + 1
148.66	179.41	206.5	33.94	10 + 3
162.33	184.23	215	30.83	20 + 5
177.22	205	216.3	28.98	30 + 5
196	211.46	238.11	28.02	30 + 7

Table 2: LC_{50} 24, 48 and 96 hours and TOC values of the crude oil solutions before the treatment process and after ozonation, followed by adsorption

LC_{50},96 hours (mg/l)	LC_{50}, 48 hours (mg/l)	LC_{50},24 hours (mg/l)	TOC (mg/l)	O_3 + PAC (mg/l)
60	153.6	129.5	54.98	0
149.43	156.4	211.8	35.22	1 + 10
151.14	183.82	209.5	33.94	3 + 10
173.75	200.56	215.5	30.83	5 + 20
193.54	214.31	218.5	28.98	5 + 30
204	218	243.7	28.53	7 + 30

DISCUSSIONS

Rainbow trout larvae is a proper case for the toxicity test, because it can be produced by many fishery farms and is very sensitive to the toxicant.[15] It is also seen to be more sensitive to low concentrations of crude oil, although in this study, the 96-hour LC_{50} value for rainbow trout larvae was 60 mg/l. However, in a similar study by Kazlauskiene *et al.*, the 96-hour LC_{50} values of 39.28 mg/l for embryos and 21.61 mg/l for its larvae have been reported.[7] The differences in these results may be attributed to the methods of LC_{50} analysis or to the methods of preparation of the water soluble fraction of crude oil. In another investigation by Vosyliene *et al.*, crude oil with a concentration of 1610 mg/l caused a 36% increase in larvae mortality rate during the 96-hour exposure, however, it did not affect the survival of adult fish.[5] It has been suggested that the oil affects the ability of the fish to regulate their body water content, as the oil increases the body water content of the fish.[4]

The commonly used techniques for the elimination of the mineral oil pollution are mostly based on the use of phase separation methods or adsorption on activated carbons.[5] These techniques, however, do not completely remove the organic pollutants from the waters, in which, significant low concentrations of contaminations can still survive.[16] The results outlined in Figure 1 show that adsorption with activated carbon and then ozonation can notably reduce the toxicity and TOC of the samples. With increasing the activated carbon dose, a significant decrease in the TOC concentration was obtained, and thereby, the toxicity of samples was reduced [Figure 1].

Laws, has reported that the LC_{50} values ranged from 2 to 28 mg/l for the aquatic invertebrates and fish, which were exposed to the simple aromatic hydrocarbons such as benzene and toluene.[17] He concluded that hydrocarbons with greater molecular weight, such as phenanthrene and fluoranthene, produced higher acute toxicity compared to those with low molecular weight. In the present study, the changes in toxicity are most likely related to the greater adsorption of high molecular weight hydrocarbons in lower activated carbon (20 mg/l), which causes a decrease in toxicity and TOC reduction, as a function of the activated carbon dose. However, an increase in the activated carbon dose (> 20 mg/l) possibly caused the adsorption of lower molecular weight hydrocarbons that might relatively have the same toxicity, with high molecular weight hydrocarbons. Therefore, causing a decrement in the amount of toxicity could be a function of the activated carbon dose, however,

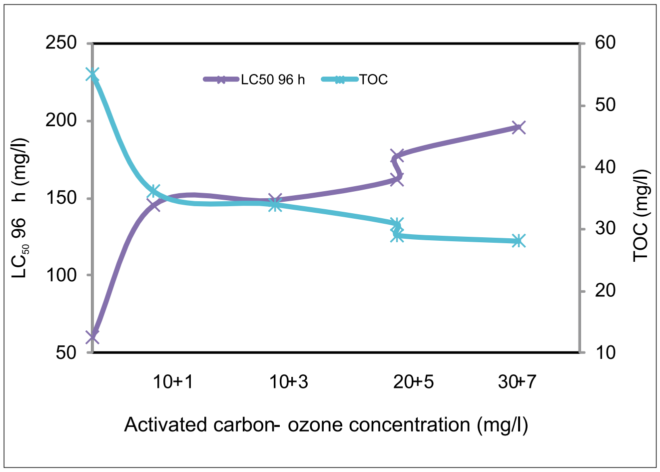

Figure1: LC_{50} (96 hours) increase in relation to TOC decrease via AC adsorption, and then ozonation

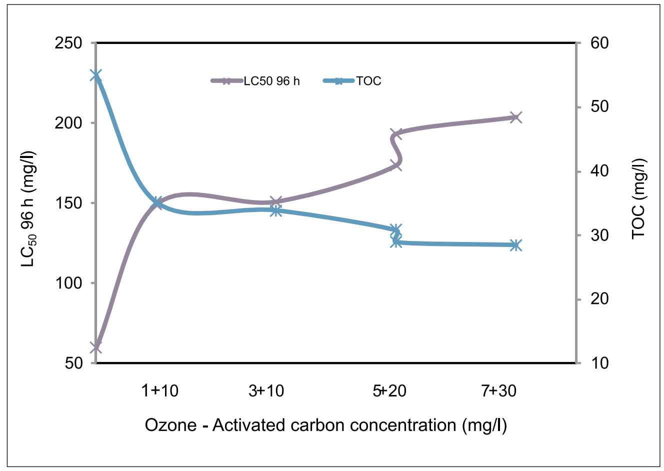

Figure 2: LC_{50} (96 hours) increase in relation to TOC, decrease via ozonation, and then AC adsorption

it could not cause a significant reduction of TOC as well as toxicity.

Previous studies indicate that advanced oxidation processes can be very profitably employed in the abatement of mineral oil pollution in wastewaters.[16] Use of a sequential treatment process, consisting of ozonation and then adsorption, for the removal of toxicity and TOC of the crude oil hydrocarbons from aqueous environment, is shown in Figure 2. This sequential treatment process causes a high level of decrement in toxicity and TOC, which is a function of the ozone and activated carbon dose. Previous studies indicate that pretreatment by ozonation is necessary for a partial oxidation of highly condensed hydrocarbons, which is followed by the biological treatment of the formed oxidation products and the accompanying oil.[18] The use of ozone most likely facilitates the oxidation of high molecular weight PAHs, and thus enhances the water solubility of the hydrocarbon.[19] Use of ozone before activated carbon can break down more and oxidize the large organic molecules, enhancing the adsorption ability of activated carbon. Therefore, the sequential treatment process by ozonation–adsorption could significantly reduce both the toxicity and TOC better than that by adsorption–ozonation.

CONCLUSIONS

The results suggest that a combined treatment protocol of chemical oxidation and adsorption may be a successful technology in the remediation of aquatic environments contaminated with crude oil, in the emergency situations, and a sequential treatment process by ozonation–adsorption shows better toxicity reduction than that by adsorption–ozonation.

ACKNOWLEDGMENT

This paper is the result of MSc. approved thesis, Research Project #388056, in Isfahan University of Medical Sciences (IUMS). The authors wish to acknowledge the financial support provided by the School of Health and the Environment Research Center, IUMS.

REFERENCES

1. Martinez-Jeronimo F, Villasenor R, Rios G, Espinosa-Chavez F. Toxicity of the crude oil water-soluble fraction and kaolin-adsorbed crude oil on Daphnia magna (Crustacea:Anomopoda). Arch Environ Contam Toxicol 2005;48:444-9.
2. Craddock DR. Use and limitations of acute toxicity tests – a review. In: Malins DC,editor. Effects of marine environments and organisms. New York: *Academic petroleum on arctic and subarctic Press*;1977. p.1-93.
3. Cowell EB, Baker JM, Crapp GB. The biological effects of oil pollution and oil cleaning materials on littoral communities including salt marshes. In: Rouvio M, editor. *Marine Pollution and Sea Life*, FAO Tech. Conf., Rome, 1972. p.359-64.
4. Lockhart WL, Duncan DA, Billeck BN, Danell RA, Ryan MJ. Chronic toxicity of the 'water-soluble fraction' of Norman Wells crude oil to juvenile fish. Spill Sci Technol Bull 1996;3:259-62.
5. Vosyliene MZ, Kazlauskiene N, Joksas K. Toxic effects of crude oil combined with oil cleaner simple green on yolk-sac larvae and adult rainbow trout Oncorhynchusmykiss. Environ Sci Pollut Res Int 2005;12:136-9.
6. Nahrgang J, Camus L, Carls MG, Gonzalez P, Jonsson M, Taban IC, *et al*. Biomarker responses in polar cod (Boreogadussaida) exposed to the water soluble fraction of crude oil. Aquat Toxicol 2010;97:234-42.
7. Kazlauskiene N, Taujanskis E. Effects of crude oil and oil cleaner mixture on rainbow trout in early ontogenesis. Polish J Environ Stud 2011;20:509-11.
8. Anderson JW, Neff JM. Characteristics of dispersions and water-soluble extracts of crude and refined oils and their toxicity to estuarine crustaceans and fish. Mar Bio 1974;l27:75-88.
9. Patterson WJ. Industrial wastewater treatment technology.2nd ed.USA: Buttereworth publishers, cha.17, p.11-4. 1985.
10. Vlaev L, Petkov P, Dimitrov A, Genieva S. Cleanup of water polluted with crude oil or diesel fuel using rice husks ash. J Taiwan Inst Chem Eng 2011;42:957-64.
11. Bourbigot MM, Hascoet MC, Levi Y, Erb F, Pommery N. Role of ozone and granular activated carbon in the removal of mutagenic compounds. Environ Health Perspect 1986;69:159-63.
12. Phatarpekar PV, Ansari ZA. Comparative toxicity of water soluble fractions of four oils on the growth of a microalga. Bot Mar 2000;43:367-75.
13. US-EPA. Methods for measuring the acute toxicity of effluents and

receiving waters to freshwater and marine organisms, 5th ed. EPA-821-R-02-012). Washington, DC: Office of Water;2002.
14. Andreozzi R, Insola A, Caprio V. Quinolineozonation in aqueous solution. Water Res 1992;26:639-43.
15. Gilbert E. Biodegradability of ozonation products as a function of COD and DOC elimination by example of substituted aromatic substances. Water Res1987;21:1273-8.
16. Andreozzi R, Caprio V, Insola A, Marotta R, Sanchirico R. Advanced oxidation processes for the treatment of mineral oil-contaminated wastewaters. Water Res 2000;34:620-8.
17. Laws EA. Aquatic pollution. An introductory text. 2nd ed. New York, NY: Wiley; 1993.
18. Kornmuller A, Wiesmann U. Ozonation of polycyclic aromatic hydrocarbons in oil/water-emulsions: mass transfer and reaction kinetics. Water Res 2003;37:1023-32.
19. Nam K, Kukor JJ. Combined ozonation and biodegradation for remediation of mixtures of polycyclic aromatic hydrocarbons in soil. Biodegradation 2000;11:1-9.

Source of Support: Isfahan University of Medical Sciences.. **Conflict of Interest:** None declared.