Original Article

Evaluation of Baffle Fixes Film up Flow Sludge Blanket Filtration (BFUSBF) System in Treatment of Wastewaters from Phenol and 2,4-Dinitrophenol Using Daphnia Magna Bioassay

*Mohammad Javad Ghannadzadeh [1], Ahmad Jonidi Jafari[*2], Abbas Rezaee [1], Fatemeh Eftekharian [3], Ali Koolivand [4]*

Received: 01.04.2015 *Accepted: 23.05.2015*

ABSTRACT

Background: Phenol and nitrophenol are common compounds found in different types of industrial wastewater known as serious threats to human health and natural environment. In this study, *Daphnia magna* was used to evaluate the effectiveness of "baffle fixes film up flow sludge blanket filtration" (BFUSBF) system in elimination of phenolic compounds from water.

Methods: *D. magna* cultures were used as toxicity index of phenol and 2,4-DNP mixtures after treatment by a pilot BFUSBF system which consisted of baffle in anoxic section and biofilm in aerobic sections. Initial concentrations were 312 mg/L phenol and 288 mg/L 2,4-dinitrophenol (2,4-DNP).

Results: Bioassay tests showed that *D. magna* was influenced by the toxicity of phenol and 2,4 DNP mixtures. The comparison between the toxicity of initial phenol and 2,4-DNP mixtures and the output toxic unit (TU) derived from BFUSBF treatment system showed that the TU of the effluent from BFUSBF reactor was much lower than that of the solution that entered the reactor.

Conclusion: Based on the acute toxicity test, BFUSBF process could reduce phenol and 2,4-DNP in aqueous solutions. Therefore, it is possible to use BFUSBF process as an appropriate treatment option for wastewaters containing phenolic compounds.

Keywords: 2,4-Dinitrophenol, Chemical Water Pollution, Phenol, Water Purification, Daphnia.

IJT 2016 (2): 7-11

INTRODUCTION

Today, phenol and nitrophenol compounds are broadly used in different industries such as casting resins, detergents, explosives, petrochemicals, oils, pesticides, plastics, drugs and raw material manufacturing companies [1]. Consequently, these compounds can be found in wastewaters and diverse ecosystems regarding their relative constancy in the environment [2]. The concentration of these compounds in certain industries output wastewater, discharged to the environment, might exceeds 1000 mg/L [1, 3]. Phenol and its related components are relatively stable as well as water-soluble substances and can result in serious health problems [4]. Most wastewaters contain a vast range of pollutants; however, there are great gaps of information regarding the effects of these pollutants on aquatic organisms.

Even when the examined sample wastewaters, leachates and industrial sewages follow the wastewater discharge standards, bioassays show that it is better (more appropriate) not to discharge these wastewaters to the natural environment [5]. Since physical and chemical tests are not sufficient to examine the potential effects of water pollution, bioassays along with other tests are needed to examine the samples for pollution [4, 5]. A number of advantages of using bioassays to evaluate the toxicity of substances and environmental pollutants include: harmony and consistency of these tests to the environmental conditions affecting aquatics and other living creatures, and

1. Department of Environmental Health Engineering, Tarbiat Modares University, Tehran, Iran.
2. Department of Environmental Health Engineering, Iran University of Medical Sciences, Tehran, Iran.
3. Department of Environmental Health Engineering, Shiraz University of Medical Sciences, Shiraz, Iran.
4. Department of Environmental Health, Arak University of Medical Sciences, Arak, Iran.
* Corresponding Author: E-mail: jonidi.a@iums.ac.ir

identifying the type and degree of treatment needed to appropriately control water pollution as well as determining the effects of effluents and other toxic compounds on aquatics creatures [4, 6, 7]. Accordingly, in 1984 the US Environmental Protection Agency recommended a holistic approach (biological test) to identify toxic pollutants and their effects on the environment to evaluate the effectiveness of water treatment methods [8]. Due to the apparent toxicity of phenol and its derivatives, influencing aquatic organisms, a bioassay was conducted to determine the ecological effects of effluents discharging from Baffle Fixes film up flow sludge blanket filtration (BFUSBF) treatment systems [9].

Among freshwater aquatic organisms, most vulnerable species against phenol are *Daphnias*, ceriodaphniadubles and some trouts [10, 11]. *D. magna* is highly sensitive to most pollution [12] [13]; hence, it is usually used to test acute toxicity [14]. It is easy to foster and is highly adaptable to soft waters [15]. Besides, the period of reproduction in *D. magna* is shorter and it can be cultured with less duration of test compared to other bioassays [4, 6, 12].

In this study, daphnia which is a genus of small, planktonic crustaceans and a member of the order Cladocera, was used as biological indicator to evaluate phenol and 2, 4-dinitrophenol toxicity, lethal concentration 50 (LC50) and toxic unit (TU).

MATERIAL AND METHODS

D. Magna was obtained from the Faculty of Health, Tehran University, Iran. Afterwards, one *D. Magna* was used to produce other *Daphnias* with high genetic similarity. The neonates were fed until they reached sexual maturity. New cultures were set-up on weekly basis, and sick or dead animals were removed from the culture [6].

The specific system used for treatment purposes was BFUSBF, which provides an up flow filtration/flocculation mechanism with modified activity. In this study, the aerobic section was expanded by biofilm and anaerobic baffle was used as treatment system [6, 16].

To determine the toxicity of phenol and 2,4-DNP produced by Merck & Co, we primarily provided a concentrated stock solution with 312 mg/L phenol, and 288 mg/L 2,4-dinitrophenol (2,4-DNP) mixtures, then 10 samples containing 100, 75, 65, 55, 45, 35, 25, 15, 10, 5% of stock solution were produced. The Ph of all samples was near 7. As soon as the solutions were prepared, broods of daphnids (4-5 day neonates), were collected from the culture area and after three washouts, in each medium 10 samples were inoculated and one medium was singled out as control medium in which the assessed concentration was zero. To collect and transfer daphnids, we used 10 mL pipettes. Although determination of acute toxicity of *Daphnias* on 48-h exposure basis has been ideally accepted [12], a series of exposure times, including 2 ،4 8 ،24 48 ،72 and 96 h were examined. After the samples were entirely exposed, *Daphnias,* which showed no movements even after stimulation by pipette, were considered dead [4].

Data analysis and lethal concentration 50 calculation were conducted using probit regression with SPSS, version 16.0 (Chicago, IL, USA). Toxic unit (TU) also was defined as 100 divided by LC50 [8]. The toxicity reduction evaluation was based on the following expression:

$$\textbf{(1)} \qquad \mathbf{TR} = \frac{T_i - T_E}{T_i} \times 100$$

Where,
TE= External toxicity unit
TR= Toxicity reduction
Ti= Internal toxicity unit

In bioassays using *Daphnia*, every test is repeated at least three times and the mortality among control samples should not exceed 10% [6], which was the focus of the present study.

RESULTS

The results of bioassay tests of phenol and 2, 4-DNP mixtures treated with BFUSBF system are presented from probit regression in Tables 1 and 2. Given the regression analysis, this table demonstrates the death rate of *D. magna* caused by exposure to different doses of phenol and 2,4-DNP mixtures as an indicator to determine lethal concentration 50. Accordingly, toxicity indices are described in Table 3, 4.

Table 1, shows the probit regression results to determine the toxicity of phenol, and 2, 4-DNP mixtures compounds, whereas Table 2 shows the LC 50 and TU during 96-hour exposure period.

Table 1. Results from Phenol and 2, 4-dinitrophenol toxicity in *D. magna* test at the entry of expanded USBF system under optimum concentration condition.

Solution concentration (volume percent)	The number of live daphnia examined in each point	Total number of dead daphnia after conducting the test 3 times for each sample after exposure 24 hour
100	30	30
75	30	30
65	30	30
55	30	30
45	30	28
35	30	26
25	30	25
15	30	23
10	30	20
5	30	18
0	30	0

Table 2. Results from phenol and 2, 4-dinitrophenol toxicity in *D. magna* test at the exit of expanded USBF system under optimum concentration condition.

Solution concentration (volume percent)	The number of live daphnia examined in each time	Total number of dead daphnia after conducting the test 3 times for each sample after exposure			
		24	48	72	96
100	30	17	19	21	22
75	30	12	13	14	15
65	30	8	9	10	11
55	30	5	6	7	7
45	30	3	4	5	6
35	30	2	3	4	5
25	30	1	2	3	4
15	30	0	1	2	2
10	30	0	0	0	0
5	30	0	0	0	0
0	30	0	0	0	0

Table 3. Probity test conducted on daphnia magna, used to determine toxicity evaluation criteria of phenol in relation to time.

Examined solution	Exposure period (hours)	Regression coefficient	Standard deviation of regression coefficient	y-intercept	Standard deviation of y-intercept	Chi-square (X^2)	Degree of freedom	*P*.value
Phenol and 2, 4-dinitrophenol	First 24 (primary sample before treatment)	0.054	0.008	-0.454	0.360	26.53	8	0.001
	24	0.030	0.005	-2.66	0.409	2.32	8	0.969
	48	0.030	0.005	- 2.35	0.441	1.70	8	0.975
	72	0.027	0.006	-- 4.5	0.472	2.88	8	0.895
	96	0.028	0.005	- 2.12	0.388	4.49	8	0.810

Table 4. Lethal concentration 50 and toxic unit experiments for phenol and 2, 4-dinitrophenol on Daphnia magna in relation to time.

Examined solution	Exposure time (hour)	Toxicity evaluation criteria (scale) LC%50 (v/v)	Confidence interval 95% LC%50 -24(hr)	
Phenol and 2, 4-dinitrophenol	24 (first sample before treatment)	8.41	-43.3	28
	24 hour	88.3	78.7	100.2
	48	84.3	74.5	96
	72	79.6	68.5	90.5
	96	75.6	65.2	85.7

The 24-h TU for phenol and 2, 4-DNP mixtures was 11.89. However, when treated by BFUSBF in a daily basis, it declined to 1.13. These reductions for 24-hour, 48-h and 72-h 96-h exposure periods were 1.18, 1.25 and 1.32, respectively. Given the results and the initial concentration ratio of LC50 for phenol, and 2, 4-DNP mixtures was 8.41.

DISCUSSION

This study examined the effectiveness of BFUSBF system treatment on phenol and 2,4-DNP solutions using *D. magna*. Bioassay is an easy and inexpensive test that assists researcher to determine the toxicity of different substances, evaluates the efficacy of various treatments and toxics management processes, as well as monitors wastewaters.

The LC50 and TU measurements showed that after BFUSBF treatment, the levels of phenol and 2,4-DNP significantly declined. These findings were similar to outcomes of a previous study [15]. Gallego et al. investigated the decomposition and detoxification of mixtures of 2-chlorophenol, phenol and m-cresol by *Daphnias*, and suggested that the EC50 24 h (0.7% V/V) of the initial concentration of the mixture and after 99.8% elimination of all phenolic compounds (210 mg/L) reached to EC50 24 h =40.6 (%V/V). However, no more toxicity was observed after using granular activated carbon (GAC) [17]. Tisler and Zagorc-Koncan examined the toxicity of the mixture of phenol and formaldehyde. They stated that 48 h LC 50 (13.1 mg/L) phenol is more toxic in fish than in fungi, bacteria and *Daphnias* with 48 h LC50=25 mg/L [7]. In a study authors compared the efficacy of two continuous systems (active sewage treatment system and sequencing batch reactor) containing a mixture of cyanide and phenol. The toxicity levels were significantly reduced by both systems, but the maximum level of toxicity reduction at the mineral output plummets of the system was 60% and the toxicity was not fully eliminated [18]. Virginia et al. attempted to eliminate phenol and 2, 4-DNP by aerobic and anoxic processes and proposed the presence of denitrification after aerobic process. They reported different values for EC50 of 2,4-DNP in a variety of organisms [19] .

Researchers have stated that 300 mg/L of phenolic compounds consists of a mixture of phenol, (4CP) 4-chlorophenol, (4np) 4-nitrophile, 2,4-dichlorophenol(2,4 dcp), 2,4-dinitrophenol, 2-4 DNP. They showed a moderate level of toxicity of the sludge level Ec50=0.7(% V/V) in activated sludge, but the output from the system was not toxic to *Dafyta mgyna* [19].

Our bioassay tests after the treatment of phenol and 2, 4-DNP mixtures by BFUSBF system showed that the TU of phenol and 2, 4-DNP mixtures was 11.89. Based on TU, after treatment by BFUSBF system, the toxicity reduced after 96 hours to approximately 10% of the toxicity of phenol and 2, 4-DNP mixture entering BFUSBF bioreactor. However, the presence of some phenol in discharged wastewater might have been due high initial concentrations of phenol and 2, 4-DNP mixtures (600mg/L), as well as the emergence of intermediate products [3] .

The main reason for the wide range of reports on phenol and 2, 4-DNP toxicity can be attributed to the conditions ruling the system during the test. Physical, chemical and environmental factors play fundamental roles in biomarkers' behaviors and the degree of adaptation to toxic substances. However, other diverse species of fish or other organisms including bacteria showed different level of sensitivity to different compounds [20, 21]. Furthermore, lower concentrations of phenolic compounds in various treatment systems and subsequently the higher level of toxicity reduction and elimination in some studied systems may have resulted in the absence of toxins in the final solutions.

Although the BFUSBF system used in this study worked well with 600 mg/L of phenolic compounds (312 mg/L phenol and 288 mg/L 2,4-DNP), some degree of toxicity remained in the discharged wastewater, probably due to incomplete elimination of phenol as well as generation of toxic intermediate byproducts. For example, catechol is one of the common compounds usually produced during phenol degradation, which shows same degree of toxicity, or hydroquinone, derived from phenol degradation and is more toxic than phenol [3].

Given our acute toxicity test, BFUSBF system is able to eliminate phenol and 2, 4-DNP

compounds; therefore, it can be suggested as a treatment option for wastewaters containing phenolic compounds.

CONCLUSION

BFUSBF system can eliminate phenol and 2,4-DNP from wastewaters.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the Tarbiat Modares University for financial supports. The authors declare that there is no conflict of interests.

REFERENCES

1. Dehghani M, Jaafari J, Alghasi A, Porkar G. Using medium pressure ultraviolet reactor for removing azo dyes in textile wastewater treatment plant. World Appl Sci J 2011;12:797-802.
2. Paisio CE, Agostini E, González PS, Bertuzzi ML. Lethal and teratogenic effects of phenol on Bufo arenarum embryos. J Hazard Mater 2009;167:64-8.
3. Sollmann T. Correlation of the aquarium goldfish toxicities of some phenols, quinones, and other benzene derivatives with their inhibition of autooxidative reactions. J Gen Physiol 1949;32:671-9.
4. Weber CI. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms: Environmental Monitoring Systems Laboratory, Office of Research and Development, US Environmental Protection Agency; 1991.
5. Blinova I. Use of bioassays for toxicity assessment of polluted water. Institute of Environmental Engineering at Tallinn Technical University 2000.
6. Way C. Standard methods for the examination of water and wastewater. Water Environment Federation, Secaucus, NJ, USA 2012.
7. Tišler T, Zagorc- assessment of toxicity of phenol, formaldehyde, and industrial wastewater to aquatic organisms. Water, Air, and Soil Pollution 1997;97:315-22.
8. Crittenden JC, Trussell RR, Hand DW, Howe KJ, Tchobanoglous G. MWH's Water Treatment: Principles and Design: John Wiley & Sons; 2012.
9. Jin H, Yang X, Yin D, Yu H. A case study on identifying the toxicant in effluent discharged from a chemical plant. Marine Pollution Bull 1999;39:122-5.
10. Guerra R. Ecotoxicological and chemical evaluation of phenolic compounds in industrial effluents. Chemosphere 2001;44:1737-47.
11. Cairns Jr J, Buikema Jr AL, Heath AG, Parker BC. Effects of temperature on aquatic organism sensitivity to selected chemicals. Virginia Polytechnic Inst. and State Univ., Blacksburg (USA). Virginia Water Resources Research Center; 1978.
12. Mahmoodi NM, Arami M. Degradation and toxicity reduction of textile wastewater using immobilized titania nanophotocatalysis. Journal of Photochemistry and Photobiology B: Biology 2009;94:20-4.
13. Bae J-S, Freeman HS. Aquatic toxicity evaluation of new direct dyes to the Daphnia magna. Dyes and Pigments 2007;73:81-5.
14. Fernández-Alba A, Hernando D, Agüera A, Cáceres J, Malato S. Toxicity assays: a way for evaluating AOPs efficiency. Water Res 2002;36:4255-62.
15. Adams LK, Lyon DY, Alvarez PJ. Comparative eco-toxicity of nanoscale TiO 2, SiO 2, and ZnO water suspensions. Water Res 2006;40:3527-32.
16. Rajakumar R, Meenambal T, Banu JR, Yeom I. Treatment of poultry slaughterhouse wastewater in upflow anaerobic filter under low upflow velocity. Int J Environ Sci Technol 2011;8:149-58.
17. Gallego A, Fortunato M, Foglia J, Rossi S, Gemini V, Gomez L, et al. Biodegradation and detoxification of phenolic compounds by pure and mixed indigenous cultures in aerobic reactors. Int Biodeterioration Biodegradation 2003;52:261-7.
18. Papadimitriou C, Samaras P, Sakellaropoulos G. Comparative study of phenol and cyanide containing wastewater in CSTR and SBR activated sludge reactors. Bioresource Technol 2009;100:31-7.
19. Rani MR, Sreekanth D, Himabindu V. Degradation of mixture of phenolic compounds by activated sludge processes using mixed consortia. Int J Energy Environ 2011;2:151-60.
20. Mahvi AH, Maleki A. Photosonochemical degradation of phenol in water. Desalination Water Treat 2010;20:197-202.
21. Maleki A, Mahvi AH, Naddafi K. Bioassay of phenol and its intermediate products using Daphnia magna. J Water Wastewater 2007;66:19-24.