Original Article

Effect of metal plating industry effluents on biological wastewater treatment

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ABSTRACT

Aims: In this study, the toxicity of three metal plating wastewaters were assessed using sequencing batch reactor (SBR) bacteria.

Materials and Methods: For determining the growth inhibition in each metal plating wastewater concentration, the cultured SBR bacteria on nutrient broth media were used and after exposure they cultured on nutrient agar media. Each test was performed in three replicates. Mean of three replicate were analyzed, and no observed effect concentration (NOEC), 50% effective concentration (EC_{50}) was calculated using the probit analysis in SPSS version 16.0 software (SPSS Inc., 233 South Wacker Driv).

Results: According to the probit analysis, there is no effect on tested bacteria at the concentrations of 1 mL/L, >50 mL/L and >100 mL/L of metal plating wastewaters in sample 1, 2 and 3, respectively. While almost all bacteria will be dead in the concentration of 1000 mL/L of each metal plating wastewater. Maximum and minimum EC_{50} were recorded for the metal plating wastewater number 3 (with value of 960 mL/L) and number 1 (with value of 6.5 mL/L). On the other hand, the calculation of NOEC using probit analysis showed that sample 1 is toxic even in minimum concentrations because its NOEC was 1.1 mL/L.

Conclusion: Results of this study showed that some metal plating wastewaters in the very low concentrations could induce an adverse effect on the bacterial community structures of WWTPs biological units. Hence, it could be recommended that effluent standards and *in-situ* treatment rules should be applied according to the kind of process in such industries.

Key words: Bacteria, the effective concentration, metal plating wastewater, toxicity

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INTRODUCTION

Wastewater treatment systems are a main part of quality management of water resources.[1,2] The main subject of wastewater treatment plants (WWTPs) is the removal of pathogens and chemical pollutants so that they have the

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minimum health risk.[3,4] On the other hand, biological processes of WWTPs are handled with the different microorganisms such as bacteria, fungus, rotifers, viruses, and nematodes.[5] Therefore, the maintenance of such microorganisms is necessary for appropriate operation of biological units. Among the chemical contaminants existed in the wastewaters, the heavy metals are the resistant compounds that their effects are well-studied.[6,7] on the other hand, the bacteria are the most important microorganisms in the WWTPs that heavy metals are considered to be toxic on their activity.[8] However, some studies proved that some microorganisms can be adapted in the environments with low concentration of heavy metals.[9,10] Such adaptation will resulted in appropriate treatment of wastewaters and even heavy metal removal.[9,11] In fact, some heavy metals are necessary for macro and microorganisms but in high concentrations of the same metals they can act as toxic agents.[12,13] These compounds in high concentrations can induce the disturbance in the metabolic activity that resulted in low effectiveness of biological processes in WWTPs.[2,14] Toxicity of heavy metals in wastewater treatment systems is depended on the kind of heavy metal and its concentration. Other factors such as pH, quantity and species of microorganism, nature of wastewater and nutrients can also affect the toxicity of heavy metals.[15] Nowadays, it is obvious that such physical and chemical characteristics have a wide variations.[16] Hence, for detecting the industries that cause the toxicity in WWTPs, it is necessary to study each unit separately and for control of such problems, each industry unit should be subject of toxicity studies. In this regards in some studies, the toxicity of some compounds was investigated separately, but for detecting the synergistic effects of effluent compounds, it is necessary to conduct the toxicity experiments that assess the whole effluents toxicity.[15]

The aim of this study was an investigation the probable toxicity of three kind of metal plating industry wastewater against current bacteria of sequencing batch reactor (SBR) unit. This SBR unit is a main biological part of WWTPs for treatment the industrial wastewaters. The results of the present study will improve the effluent standards of metal plating and similar industries.

MATERIALS AND METHODS

The three metal plating industry samples were obtained from Isfahan, Iran. To separate the suspended solids, samples were allowed to settle at room temperature for 2 h and then clear supernatants were analyzed for pH, alkalinity, electrical conductivity (EC), color, total suspended solids (TSS), volatile suspended solids (VSS), chemical oxygen demand (COD), and total organic carbon (TOC) contents according to the standard methods.[16]

Bioassays using the SBR bacteria were performed according to standard methods.[16] The bacterial species were obtained from SBR unit of WWTP (Isfahan, Iran.). For this aim, the wastewater samples cultured on the nutrient broth medium. This media incubated for 48 h at 35°C and then bacteria species were isolated using the centrifuge apparatus. After culturing the isolated bacterial species, toxicity tests of metal plating industry wastewater were performed by inoculating bacterial cells on nutrient broth media containing different concentrations of metal plating industry wastewater (1-1000 mL/L) and without metal plating industry wastewater (as a control). All tests were measured in triplicate and mean the value of mortality in comparison with the controls were determined. The inoculated cells were estimated to be 200 colony forming unit (CFU) per plate. Optical density of the inoculated bacteria was set using a Hach's DR 5000 spectrophotometer. The optical density was 0.9. The bacterial growth inhibition was calculated according to the Equation 1.

$$\text{Percent of bacterial growth inhibition} = A - B/A \times 100 \quad \text{Eq. 1.}$$

In this equation, A and B are the CFU value before and after the exposure to metal plating industry wastewater, respectively. The results of Equation 1 were used for calculation of no observed effect concentration (NOEC), 50% EC_{50} and 100% mortality concentration.

The results were recorded as the quantity of effluents in mL/L required for reducing the growth of bacteria to two standard deviations (NOEC), to 50% (EC_{50}), and to 100% (100% mortality concentration) of the mean growth level of control cultures. These values were calculated by probit analysis using the SPSS version 16.0 software (SPSS Inc., 233 South Wacker Driv).

RESULTS

In the present study, sampling periods was 3 time, and the samples were obtained from 3 different metal plating industry. The effluent characteristics of these industries are shown in Table 1. According to this table, the range of dissolved oxygen was 1.5-2.3 mg/L in 3 effluents. The range of COD was 603-1836 mg/L and for TOC this value was 2.4-27.3 mg/L. Other characteristics of effluents are shown in Table 1. Values of EC and TSS parameters were significantly high ($P < 0.01$) in industrial wastewater number 1. While COD, TOC, and VSS were significantly high ($P < 0.01$) in industrial effluent number 2.

The toxicity assessment of these effluents were performed after cultivation and isolation the SBR bacteria. In the present study, the growth inhibition of metal plating wastewaters on the bacteria was determined in the different concentrations of metal plating industry effluents. However, the results are reported in 5 concentrations of each effluent. The mortality within these concentrations were between 0% and 100% and the difference between them were statistically significant ($P < 0.05$). Table 2 shows the results of the

Table 1: Raw wastewater characteristics of the three metal plating industries

Parameters	Metal plating industry #1	Metal plating industry #2	Metal plating industry #3
Chemical oxygen demands (mg/L)	806	1863.8	603.2
Dissolved oxygen (mg/L)	2	1.5	2.3
Total organic carbon (mg/L)	2.4	27.3	8.1
Electrical conductivity (mS/cm)	15.3	13.6	8.0
Total suspended solids (mg/L)	93.1	66.0	8.4
Volatile suspended solids (mg/L)	12.1	5.7	1.2
Alkalinity	2	0.0	0.0
Color (Pt-Co)	187	756	165
pH	3	1.41	1.65

Table 2: SBR bacteria death ratio (%) of the three performed experiments due to exposure to different concentration of metal plating industrial wastewater

Kind of wastewaters	Wastewater concentration (mL/L)	Growth inhibition (experiment 1)	Growth inhibition (experiment 2)	Growth inhibition (experiment 3)	Mean of growth inhibition (3 experiments)	SD
Metal plating industrial wastewater #1	1.0	0.0	0.0	0.0	0.0	0.0
	2.5	16.8	0.1	7.7	8.0	8.3
	5.0	41.7	18.7	44.4	34.2	14.1
	10.0	72.7	65.2	86.1	74.0	10.6
	20.0	92.4	71.9	99.3	87.1	14.3
Metal plating industrial wastewater #2	50	0.5	0.2	0.7	0.5	0.3
	100	14.5	18.6	8.6	13.6	5.1
	200	23.8	29.1	23.1	25.2	3.3
	400	55.5	75.8	81.2	71.2	13.5
	600	99.7	100.0	99.3	99.6	0.3
Metal plating industrial wastewater #3	100	0.2	0.0	0.8	0.4	0.4
	250	1.2	0.7	2.2	1.4	0.8
	500	19.4	13.1	9.3	13.5	5.1
	750	20.2	20.1	17.4	19.1	1.6
	1000	70.0	41.5	62.4	58.1	14.7

SD: Standard deviation, SBR: Sequencing batch reactor

bacterial inhibition percent at the different concentrations of metal plating industry effluent. According to this table, industrial effluent of #1, #2 and #3 there are no adverse effect on the tested bacteria at the concentration of <1, <50, and <100 mL/L, respectively. Furthermore, results proved that the concentration of 1000 mL/L (a pure solution) of all three effluents can induce at least 50% growth inhibition in tested bacteria. Obtained data proved that the increment of effluent concentration will resulted in increment of bacterial growth inhibition. In present study probit analysis was uses for determination of EC_{50}, NOEC and also their confidence interval limits. Probit analysis is generally used in toxicology to determine the relative toxicity of compounds to living organisms. Such application can be achieved by testing the response of an organism under various concentrations of each of the compounds in question and then comparing the concentrations at which one encounters a response. The response is always binomial, and the relationship between the response and the various concentrations is always sigmoid. Probit analysis acts as a transformation from sigmoid to linear and then runs a regression on the relationship. Once a regression is used; we can use the output of the probit analysis to compare the amount of the compound required to create the same response in each of the various toxicants. There are many endpoints used to compare the differing toxicities of toxicants, but the EC_{50} or lethal concentration 50 are the most commonly used outcomes of the modern dose-response experiments.

Results of EC_{50}, NOEC and 100% mortality obtained by probit analysis are shown in the Table 3. In fact, these parameters are calculated using the data in Table 2. According to the Table 3, maximum and minimum of EC_{50} was for effluent #3 (EC_{50} = 960 mL/L) and #1 (EC_{50} = 6.5 mL/L). On the other hand, calculation of NOEC shows that the lowest concentrations of the effluent #3 is toxic for most of the bacteria (NOEC = 1.1 mL/L). While this value for effluent 2 and 3 was 66 and 166 mL/L.

DISCUSSION

Metals in real industrial effluent samples do not find as pure solutions. In real situations, effluents are complex mixtures and may have unrecognized effects on biological processes.[15] To minimize potential shock loading of wastewater plants, it would be necessary to have an appropriate activity assay to detect the potential effect of imported industrial waste on the plant processes as a whole. In the previous studies, toxicity assessment of industrial wastewater has been performed using bioassay of an aquatic plant *Lemna minor*.[17] Growth

Table 3: Results of EC_{50}, NOEC, and 100% growth inhibition tests (with 95% CI) for SBR bacteria exposed to metal plating samples

Kind of wastewaters	Parameters	Value (mL/L)	Upper bounds	Lower bounds
Metal plating industrial wastewater #1	NOEC	1.155	1.477	0.836
	EC_{50}	6.531	7.286	5.857
	100% growth inhibition	36.931	51.180	28.842
Metal plating industrial wastewater #2	NOEC	66.406	94.190	34.274
	EC_{50}	215.449	263.682	174.522
	100% growth inhibition	699.014	1311.714	500.087
Metal plating industrial wastewater #3	NOEC	166.736	380.312	0.815
	EC_{50}	960.236	1223.023	830.191
	100% growth inhibition	1753.737	2713.937	1408.994

The results were recorded as the concentration of wastewaters in mL/L required for reducing the growth of bacteria to 2 SD NOEC to EC_{50} and to 100% mortality concentration of the mean growth level of control cultures. The EC_{50}s were calculated by probit analysis using the SPSS version 16.0 software (SPSS Inc., 233 South Wacker Driv). Values are mean of triplicate measurements. CI: Confidence interval, SD: Standard deviation, SBR: Sequencing batch reactor, NOEC: No observed effect concentration, EC_{50}: 50% effective concentration

inhibition was studied as reduction in dry weight and fresh in industrial wastewater and sewage water, exposed *L. minor* plants. Results indicate a decrease in chlorophyll content was significant in comparison to control. Decrease in total protein content was 30.6%, 14.7%, and 32.5% at 96 h of exposure in industrial wastewater in 3 different seasons. Exposure of industrial wastewater to *L. minor* indicates that it is a highly sensitive plant to the pollutants present in industrial wastewater. In the present study, the effect of time was not assessed, but results showed the application of whole bacterial community can show toxicity of the effluent better than *L. minor* plants. In fact, the present method was more sensitive. In this study, all of the bacteria were inhibited in their growth at the concentration of 50 mL/L of effluent number 1. However, there is no adverse effect in the same concentration of effluent number 2 and 3. However, in these effluents the run time of the present method was very lower than previous ones.[17] This result also proved that the variation of the toxicity of metal plating industry wastewater is very high. Hence, it can be suggested that all similar industrial effluent should be assessed periodically for detection of their toxicity. If the toxicity was proved for an industry, the discharge regulation and standards can be legislated and executed for the protection of downstream units. In this regards in previous studies, it was suggested an on-line respirometer, in combination with chemical analysis, for the protection of the operation of a municipal WWTP from toxic shocks.[18]

The obtained data related to the characteristic of industrial effluents [Table 1] and their toxicity [Table 3] showed that there is no relationship between toxicity and COD or TOC parameters. In fact, the change of toxicity was not proportional to the drop of COD or TOC value. Such conclusion was proved by previous studies.[19] In previous study, the analysis of time characteristics obviously showed the time delay between the COD and toxicity changes. When COD values stabilized, toxicity showed further tendency to decrease. Similar observations were concluded for maleic acid anhydride and urea-formaldehyde resin adhesive effluents. It confirms the conclusion that the efficient optimization of treatment methods requires both removal of organic constituents and reduction in wastewater toxicity.[19]

According to the results of this study, SBR bacteria are susceptible to toxic effects of metal plating wastewater. Therefore, where the wastewater contains heavy metals, wastewater treatment facilities, which are dependent to bacterial activity, should employ such bioassays for control of the system. Moreover in the wastewater treatment systems that rely on the bacteria, such toxicity tests should be performed for each industrial effluent to control the toxic shocks of all industrial wastewaters.

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