Environmental Engineering and Management Journal July 2018, Vol.17, No. 7, 1679-1683
http://www.eemj.icpm.tuiasi.ro/; http://www.eemj.eu

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SURVEY OF INDUSTRIAL WASTEWATER TREATMENT PERFORMANCE WITH BIOLOGICAL MONITORING OF PROTOZOA IN THE INDUSTRIAL PARK OF ABADAN

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Abstract

Industrial wastewater is of particular importance. In most wastewater treatments, physicochemical parameters are used to determine the performances which are costly while protozoa can be used as less expensive biological indicators to determine the efficiency of the treatment plant. The purpose of this study was to investigate the relationship between protozoa and physic-chemical characteristics of the Abadan wastewater treatment industry.
Samples were collected from wastewater stabilization ponds in the Abadan industrial park. A total of 66 samples for biochemical and chemical tests and protozoa counts were taken. The sites of sampling were influent of sewage and effluent from maturation pond. For the parameters from influent, first 300 mL of samples was collected in depths of 0.1, 0.35 and 1 m and then mixed to provide a composite sample. 300 mL sample was directly taken from the effluent.
The results of this study indicate that there is a positive relationship between the number and population percentage of amoeba and mastigophora (flagellate) with BOD and COD, whereas is the negative relationship between sticking ciliates with BOD and COD. Furthermore there is a positive relation between DO, the percentage, number of ciliates and amoeba population and there is a correlation, with flagellate population. Increase of flagellate numbers is indicative of high effluent Kjeldahl nitrogen. There was also an increase of input TSS and amoeba population percentage and a reduction of free ciliates numbers.
The type and abundance of protozoa in industrial wastewater treatment following biological methods is an indicator of the system performance and effluent quality. This study shows the use of biological indicators can be used for monitoring as a much cheaper alternative resulting in a better economy. It is also a good indicator for the detection of toxic materials entering the plant or refinery and also its organic load shocks.

Key words: Abadan, amoeba, ciliate, industrial wastewater, mastighophora, monitoring, protozoa,

Received: August, 2013; Revised final: September, 2014; Accepted: September, 2014; Published in final edited form: July, 2018

1. Introduction

The nature of industrial waste disposal for elimination in the environment is of special interest and nowadays, due to rapid industry and population growth, the importance of wastewater treatment is even more significant than before. In most refineries the correct treatment filtration is determined by the physicochemical parameters. It has also been well established that industrial wastewater contains high concentration of dissolved organic and various toxic compounds (Luna-Pabello et al., 1990; Popescu et al., 2009). For the aerobic treatment of wastewater, protozoa play a significant role in removal of colloidal and suspended organic matter (Madoni, 1994; Luna-Pabello et al., 1990). It is demonstrated that the

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presence of organisms such as protozoa, their abundance and species diversity are associated with quality, type of waste and emissions which can be used to evaluate the performance of refineries or plants (Puigagut et al., 2005). In recent decades, regulatory authorities in industrialized regions have also endeavored to improve water quality by advanced wastewater treatment (Eslami et al., 2016; Foley et al., 2010). Therefore, evaluation and monitoring of industrial wastewater treatment is necessary. The reason that the use of stabilization ponds in wastewater treatment are growing rapidly around the globe and especially in tropical areas is due to their methods of function.

Since the type and frequency of biological agents represent the performance of wastewater treatment plants, therefore the possibility of monitoring refineries performance by measuring protozoan is possible. So due to its low cost and high speed, this technique for monitoring of wastewater treatment plants have been recommended (Papadimitriou et al., 2010; Zhou et al., 2006). Variety of studies such as the following research has reported the existence of protozoa in sewage in many countries.

Papadimitriou et al. (2010) studied protozoa as an indicator of wastewater treatment efficiency in wetlands. They also showed there is a significant relationship between the total coliform and phosphorus removal rate and by increasing the removal of loaded organics, the number and variety of protozoa also increase. Dubber et al. (2011) in Ireland during the study of SBR reactors evaluated the effect of anoxic and anaerobic process on protozoa population. They measured chemical and biochemical parameters such as BOD, COD, TKN in four different times of the reactor use and also determined the abundance and diversity of protozoan. Their studies showed that prolonged anoxic and anaerobic processes were reducing the entire population of protozoa in the reactor. Chen et al. (2005) conducted a survey on wastewater sturgeon fish in south of China. They used protozoa as bio-indicators to deposit effluent quality assessment. They found that the protozoa populations are dependent on distribution and pollution rotation period that this property can be used as a treatment bio-indicator. They also reported that more than 80 percent of the 216 ciliates species are omnivorous (Chen et al., 2005). Perez-Uzet al. (2010) studied the likely assessment of bio-indicators to determine the performance of treatment in advanced wastewater treatment systems.

Their results showed that the reduction of nitrogen removal efficiency increased ciliates population or the high existence of Ciliates resulted in low efficiency in system nitrogen removal. Poole compared the species of parasites where they operated activated sludge systems in which nitrification and operating systems were not performed and indicator organisms were introduced to the system (Poole, 1984). The organic load entering relation to plant and protozoa population were studied by Salvador and Gracia (1993) in Spain.

Since the use of stabilization pond treatment system is rapidly increasing specially in the tropical areas and due to the difficulties and the high cost of wastewater quality control testing, in this study, we have tried to propose appropriate solutions for monitoring and evaluation of system's performance by investigating the relationship between protozoan and physicochemical indicators.

2. Material and methods

In this study, samples were collected from wastewater stabilization ponds in the Abadan industrial park. Abadan is a region with a warm climate in the Iran. The pool was made of concrete and the average of raw sewage was 800 cubic meters per day.

The major wastewater productions were from soft drink industry, tuna fish and dairy and food industry. The pond design specifications are given in Table 1.

2.1. Sampling

In this study the samples were collected within four months (during spring and summer). Eight samples were collected per month through duplicate sampling. A total of 66 samples for BOD, COD, TSS, TKN tests and protozoa counts were taken. DO and pH parameters were measured daily in place by a Hach portable. The sites of sampling were influent of sewage and effluent from maturation pound. For the parameters from influent, 300 mL of samples were collected in a depth of 0.1, 0.35 and 1 m and mixed that to provide a composite sample and 300 mL sample was taken from the effluent directly.

2.2. Determination of physicochemical parameters

For measuring BOD, COD and TKN, the samples were filtered by fiber glass filters. In order to determine the amount of TSS the effluent was passed through Whatman filter and then dried at 103 to 105$^{\circ C}$ and the weight difference filter was calculated. All physic-chemical parameters were measured according to standard methods (APHA, 2005).

2.3. Protozoan population monitoring

For protozoa counting after stirring the sample with distilled water, the sample was prepared diluted in a ratio of 1 to 3. Then 1 mL of the diluted sample was placed on counting slide of Sedgwick-Rafter and observed with optical microscope with 100X enlarging and protozoa were counted.

Field method was used to count protozoa and 10 randomly selected fields and protozoa counts were performed in four major categories of moving ciliates (free), sticking ciliates, amoeba and mastigophore (flagellate). This count for each sample was repeated twice and the results were calculated based on the number of mL from Eq. (1) (APHA, 2005).

Table 1. Details of wastewater stabilization ponds design of Abadan industrial park

Pond	*Depth (m)*	*Water depth(m)*	*Width (m)*	*Length (m)*	*Area (m^2)*	*Width/Length*	*Influent flow rate (m^3/day)*
Anaerobic	4	3.4	32	48	1536	1:1.5	400
Facultative	2.3	1.9	100	153	15300	1:1.53	800
Maturation	1.8	1.4	100	252	25200	1:2.52	800

$$NO = \frac{C \times 1000}{A \times D \times F \times f} \quad (1)$$

C: Number of organisms counted
A: The area of each field view
D: Depth of field view
F: The number of field counted
f: Dilution of the sample

2.4. Data analysis

Data were analyzed by using SPSS v.18 software. For data interpretation, the mean and standard deviation (mean ± SD) were used. Data normality was evaluated by Shapiro-Wilk test and for normal data the average. Comparing of input and output variables paired T-test was used. The Pearson correlation was used to determine the correlation severity and linear regression models to determine the relationship between variables studied in input and output places. P-value of 0.05 was considered as statistical significance for all tests.

3. Results and discussion

Table 2 provides an important list of genus or species of protozoan were identified during the study. Moreover, some of the achievements and results of this study are summarized in Tables 3 to 6. These Tables (3 to 6) shows the general characteristics of quality and statistical indicators during the review.

3.1. Relationship between BOD and COD with protozoa

The results of this study indicate that there is a positive relationship between the number and population percentage of amoeba and mastigophore (flagellate) with BOD and COD whereas is negative the relationship between sticking ciliates with BOD and COD. Furthermore the results demonstrated that there is a positive relation between DO, the percentage, number of ciliates and amoeba population and there is a correlation with flagellate population.

Table 2. The important genus or species of protozoa that were found in wastewater treatment plants

Category	*Genus or species*
Ciliates	Paramecium sp (180-300um) Trachelophyllum pusillum (40-50um) Tetrahymena pyriformis (40-80um) Colpidium sp. (90-150um) Euplotes sp. Aspidisca sp. Vorticella sp. Opercularia Carchesium polypinum Epistylis sp. Didinium (80-200um)
Mastigophore (flagellate)	Bodo sp. Euglena sp. Peranema sp.
Amoeba	Acanthamoeba sp. Amoeba proteus Amoeba sp.

Table 3. The quality and statistical indicators of the raw wastewater entering the anaerobic treatment pond in industrial park

Parameter	*Unit*	*Mean*	*SD*	*Maximum*	*Minimum*
BOD	mg/L	905	204.7	1266	541
COD	mg/L	1711	300.5	2406	1090
DO	mg/L	0.4	0.41	1.2	0
TKN	mg/L	151.4	25.7	198.1	98.1
TSS	mg/L	893	173.7	1299	628
pH	-	8.3	0.32	8.8	7.7
Temperature	ºC	26.2	3.8	32.5	19.4

Table 4. The quality and statistical indicators of the final effluent in established pond of the industrial park of Abadan

Parameter	*Unit*	*Mean*	*SD*	*Maximum*	*Minimum*	*Mean Efficiency* (%)*
BOD	mg/L	107	36.3	211	68	88
COD	mg/L	232	58.3	370	128	86.4
DO	mg/L	3.3	0.49	4.4	2.5	-
TKN	mg/L	22.9	7.4	44	13.8	84.8
TSS	mg/L	219	50.7	298	102	75.4
pH	-	8.9	0.26	9.2	8.2	-
Temperature	ºC	27.8	4.4	35.4	21.6	-

**Mean Efficiency=Mean of Influent (Table 3) – Mean of Final effluent (Table 4) / Mean of Influent ×100*

Table 5. The quality and biological indicators of the effluent entering the maturation pond of industrial park of Abadan

Parameter	*Unit*	*Mean*	*SD*	*Maximum*	*Minimum*
Mastigophora(flagellate)	No/mL	18692	6250	32146	10846
Free Ciliates	No/mL	5911	5904	18972	864
Attached Ciliates	No/mL	1879	595	2916	808
Amoeba	No/mL	728	647	2536	93

Table 6. The quality and biological indicators in final effluent of industrial park of Abadan

Parameter	*Unit*	*Mean*	*SD*	*Maximum*	*Minimum*
Mastigophora(flagellate)	No/mL	12082	4082	24752	6471
Free Ciliates	No/mL	12597	7727	29714	1379
Attached Ciliates	No/mL	3127	828	4136	1211
Amoeba	No/mL	561	524.9	1946	64

In other words, with the increase in flagellate population it can be expected that the output effluent quality will not be satisfactory and with the increase in number of attached ciliates it can be expected to achieve satisfactory effluent quality. These findings are consistent with Dubber reports on the terms of ciliates populations.

According to Dubber's study there is a direct relationship between the reduced amount of BOD and organic matter with the total population of protozoa (Dubber et al.2011).

3.2. Relationship between DO concentration and Protozoa

The results of this study show that there is a direct relation between DO and the number and percentage of Ciliates and Amoeba population and also a correlation with flagellate population ($P<0.05$). But the highest correlation is related to the association of DO and attached (sticking) ciliates. The existence of high dissolved oxygen can cause spreading in attached ciliates population.

3.3. Relationship between Kjeldahl nitrogen and protozoa

Since Kjeldahl nitrogen amounts in sequential wastewater ponds is decreasing, comparing Kjeldahl nitrogen amounts with the number and percentage of ciliates and also the number of amoeba results in a direct relationship. Whilst, total Kjldal nitrogen in pond and the final effluent has a significant direct correlation with the number of flagellate ($P<0.05$). In other words, increasing of flagellate numbers is indicative of high effluent Kjeldahl nitrogen. The research findings were consistent with Puigagut and colleagues on nitrogen amount and total population of ciliates (Puigagut et al. 2005).

3.4. Relationship between TSS and Protozoa

The Relationship between incoming suspended solids and protozoa population showed that there is a significant relation between this parameter and the percentage of amoebas population and the numbers of ciliates ($P<0.05$) which both have direct and inverse relationship. This means that by increasing input TSS, the amoeba population percentage and free ciliates numbers were reduced. Moreover the results of input TSS measurements have a direct correlation with flagellate population**.**

4. Conclusions

Changes in the abundance of biologic indicator species of the microbial community can give advance warning of impending problems in wastewater treatment plant. Changes in operating conditions of the wastewater treatment plant may cause rapid changes in the structure of the communities of microscopic organisms, and it is hoped to work towards an understanding of how to regulate the operating conditions to maintain an ideal balanced community of protozoa.

The type and abundance of protozoa in industrial wastewater treatment working on biological methods are the indicator of system performance and effluent quality. Among protozoa, the presence of certain variety of ciliates is considered highly important compared to other species. This study shows increase in the ciliates population reduced concentrations of BOD, COD, TSS and nitrogen in output wastewater effluent and leads to satisfactory quality of wastewater. Whereas the increasing in the number of amoeba and flagellates indicates the sewage treatment is incomplete. It is also demonstrated that there is susceptibility between DO concentration and flagellate population in which by increasing dissolved oxygen, the number of flagellate decreases.

Thus, it can be deduced from the findings of this study that low cost biological indicators can be used for monitoring and hence result in a better economy especially in tropical area. Furthermore it is also a good indicator for the detection of toxic materials entering the plant or refinery and also its organic load shocks. Especially in industrial wastewater because of the cost of testing is expensive, also in developing countries and technical problems of the analysis, the use of bio-indicators for operating plant can be very helpful**.**

Acknowledgments

Special thanks to the head of environmental health research center and company officials of industrial park in Khuzestan province as well as Dr. Bijan Bina and Dr. Jafarzadeh for their efforts in providing research facilities and their guidance.

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