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Feasibility of oil pollution elimination from water by microorganisms at the water recycling plant of refineries.

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Abstract
In this study, contamination of microbial indicators including total count of microorganisms, total count of forms, identification of Escherichia coli and Bacillus cereus counting were investigated in 80 samples with oil wastewater pollutions, oil pollutions, pollutions caused by condensates and petroleum products pollutions as well as non- oil polluted samples supplied in Khouzestan province refineries. Results showed that due to oil pollutions and diseases created by Bacillus cereus, contamination rate in 55% of studied pollutions was higher than the allowed limit. With regard to coliform contamination elimination rate, 55% of samples had a pollution rate higher than the allowed limit and also, contamination rate of samples with pollution caused by oil wastewater was significantly higher compared to samples with petroleum pollutions. With regard to the pollution dispersion among samples, conditions for coliforms and Bacillus cereus were the same. The difference was that contamination rates of products pollutions and pollutions caused by condensates were approximately equal to 41 percent. Results obtained from bacteria in pollutions caused by oil wastewater and oil pollutions indicated that contamination rates of samples with pollutions caused by oil wastewater and samples with oil pollutions were 47.5 and 15 percent, respectively. Also, with regard to contamination elimination from pollutions caused by oil wastewater, there is a significant difference among samples with pollutions caused by condensates. With regard to pollution elimination, oil pollutions were as follows: pollutions caused by oil wastewater, products pollutions and pollutions caused by condensates.

Keywords: Microbial contamination, oil pollutions, microorganism, oil wastewater

1. INTRODUCTION

Due to growing consumption of water and resource shortages, reuse of wastewater, especially in industries, to meet some of the water needs is unavoidable. The extensive activities of petrochemical complexes and refineries have caused to produce a high level of oil pollution arising from the production process, which directly enters the aqueous effluent of these complexes. Different methods have been used to remove and eliminate oil pollution. [1-3] Oil derivatives and solid and ultrafine hydrocarbon materials are among the most important oil pollutions present in wastewater solutions of industrial complexes in the field of petroleum and petrochemistry [4]. Oil pollutions with a high degree of toxicity can have a direct impact on the health of individuals and even the mortality of humans and animals [5, 6]. Therefore, the elimination of oil pollutions is essential. In recent years, the process of adsorption by feeding microorganisms has attracted a lot of attention due to its high efficiency and relatively low cost [7, 8]. Due to their high hydrophilicity, hydroxyl groups, amino groups and the flexible structure of their nutritional chains, alive microorganisms have a high potential for aqueous solution treatment. In addition, the absorption capacity of microorganisms is associated to the origin of the produced polysaccharides and the degree of N-acetylation, which through chemical modification and crosslinking, improves the performance of nutritional chains of microorganisms. The absorption ability of microorganisms is related to the contact area and concentration of dissolved matter [9]. Different methods and tools such as chemical deposition, oxidation, ion exchange, osmosis, coagulation, absorption and membrane processes are used to measure oil pollution in different environments [10]. But in most commonly used methods, elimination of oil pollution often results in large volumes of sludge production that caused to sludge treatment and disposal problems and often involve complexity and cost [11, 12]. Salination is a convenient and low-cost method, which is essential for the reuse of treated wastewater, given the country is facing a water crisis. Due to the fact that among different methods of water treatment, the method of impregnation of the oil polluted aquatic media with microorganisms has a suitable potential for removal of organic pollutants and microbial agents from water, it is highly recommended and also, it is a cost- and time – efficient method. In Chaudhary et al. [13] on removing oil pollution using two adsorption processes by feeding microorganisms and chemical deposition, it was determined that the efficiency of the adsorption process by feeding microorganisms in the removal of metals including cadmium, chromium, copper , nickel, zinc and especially lead is significant. Martinez et al. [14] showed that at initial concentration of 250 to 2000 mg / L, the adsorption process by feeding microorganisms and chemical deposition, over 99.5% lead was eliminated. The concentration of residual lead after adsorption by feeding microorganisms and chemical deposition was obtained in the range of 0 to 1.4 mg / L, which was below the recommended limit of the proposed standards in Quebec, Canada (2mg/L).
In this study, contamination of microbial indicators (total count of microorganisms, total count of forms, identification of Escherichia coli and Bacillus cereus

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counting) were investigated in 80 samples with oil wastewater pollutions, oil pollutions, pollutions caused by condensates and petroleum products pollutions as well as non- oil polluted samples supplied in Khouzestan province refineries

2. EXPERMENTAL

2-1 examined samples

In this study, contamination of microbial indicators (total count of microorganisms, total count of forms, identification of Escherichia coli and Bacillus cereus counting) were investigated in 80 samples with oil wastewater pollutions, oil pollutions, pollutions caused by condensates and petroleum products pollutions as well as non- oil polluted samples supplied in Khouzestan province refineries.

In this project, 80 samples including 40 oil polluted samples (8 samples with a pollution caused by oil wastewater, 6 samples with oil pollution, 7 samples with a pollution caused by condensates and 19 samples polluted by products) and 40 samples without oil pollution was examined. Microorganisms were incubated at 37°C.

2–2 count of Bacillus cereus, coliform and Escherichia coli

In order to cultivate the bacteria, two different concentrations of 10^{-1} and 10^{-2} were harvested. First, 100 µL of 10^{-1} and 10^{-2} concentrations was transferred to the center of Mannitol – egg yolk – polymyxin (MYP) agar plate and streaked by a L-shaped loop [15]. A separate sterile loop was used for each plate. Plate were placed at room temperature for 15 minutes to allow the cultivated liquid to be absorbed into the culture. Mentioned plates were inversely incubated at 30°C for 18 to 24 hours. In the case of not observing a clear colony, before counting, incubation was continued for another 24 hour. It should be noted that two plates were cultivated for each concentration. The number of microorganisms per gram of pollution as an average of counted colonies in petri dishes was calculated with the consideration of dilution factor. To count coliform and Escherichia coli by the same method, two concentrations of 10^{-1} and 10^{-2} were used and the average number of colonies was calculated.

2 – 3 count of pollution caused by oil wastewaters

For pollution counting of coliform and cultivation in BGBL medium, cases that was suitable in terms of gases were inoculated by a loop into a tube including peptone water at 44°C and were incubated at 44°C for 48±2 hours.

3. RESULTS

3 – 1 Bacillus cereus contamination rate for samples with condensate contaminations

Results obtained from the investigation of Bacillus cereus contamination rate by a reference method for studied samples (separately, for 40 samples without oil pollution and 40 oil polluted samples) are provided in table 1. As it can be seen from table 1, among total 80 samples with pollution caused by condensates, 44 cases (55% of samples) have colonies of Bacillus cereus more than colony limit (10^2) from which 23 cases (equivalent to 57.5% of total samples) with pollutions caused by oil wastewater and 21 cases (equivalent to 52.5% of total samples) with oil pollution were investigated. It should be mentioned that in term of P.value, there is not a significant difference among Bacillus cereus contamination rate of samples with pollution caused by condensates ($p>0.05$).

Table 1. Bacillus cereus contamination rate of samples

	Sample count	Positive	Percent of positive cases	Negative	Percent of negative cases
Pollution caused by condensates	80	44	55	36	45
Pollution caused by oil wastewater	40	23	57.5	17	42.5
Oil pollution	40	21	52.5	19	47.5

2 – 3 Bacillus cereus contamination in term of pollution type

Among all studied samples (pollution caused by condensates), cases for which contamination rate is higher than allowed limit of Bacillus cereus are tabulated and investigated. At it can be observed from table 2, among 29 samples with pollution caused by products (pollution caused by condensates), 12 cases has a pollution rate higher than allowed limit that are equal to 41% of thyme samples. Similarly, among 16 oil polluted samples, 18 samples with pollution caused by oil wastewater and 17 samples with pollution caused by condensates, 15 cases (equivalent to 94%), 12 cases and 5 cases (equivalent to 29%) have a pollution rate higher than the allowed limit. As it can be seen in statistical study, the rate of pollution elimination for oil pollution and pollution caused by oil wastewater are significantly higher compared to other pollutions ($p<0.01$). Although, the contamination rate in oil pollution is higher than that in pollution caused by oil wastewater, but it is not significant ($p>0.05$). However, the lowest rate of pollution elimination with a significant difference ($p<0.01$) occurred in pollution caused by condensates. However, despite the fact that thyme has more pollution compared to pollution caused by condensates, it is not a significant difference ($p>0.05$).

Table 2. Bacillus cereus contamination rate in term of pollution type.

Pollution type	Pollution caused by products	Oil pollutions	Pollution caused by oil wastewater	Pollution caused by condensates
Positive cases	12	15	12	5
The number of polluted samples	29	16	18	17
Percent of positive cases	41	94	66	29

3 – 3 coliform contamination rate in oil pollution

Results obtained from the investigation of pollution elimination rate of coliform for all studied samples and also, separately, for 40 samples without oil pollution and 40 oil polluted samples are presented in table 3. As it can be seen, among total 80 samples with pollution caused by condensates, 44 cases (equivalent to 55% of all samples) have coliform numbers higher than the allowed limit (10^3) from which, 29 cases (equivalent to 72.5%) of samples with pollution caused by oil wastewater and 15 cases (equivalent to 37.5%) of oil polluted samples were investigated. It should be mentioned that with regard to p. value, samples with pollution caused by oil wastewater showed a coliform pollution rate significantly higher than that of standard limit ($p<0.05$).

Table 3. Coliform pollution rate of samples.

	Sample count	Positive	Percent of positive cases	Negative	Percent of negative
Pollution caused by condensates	80	44	55	36	45
Pollution caused by oil wastewater	40	29	72.5	11	27.5
Oil pollution	40	15	37.5	25	62.5

3–4 coliform contamination rate in term of pollution type

Among all investigated samples (pollution caused by condensates), cases with a pollution rate higher than the allowed limit of coliform are presented in table 4. As it can be seen from table, among 29 thyme samples (pollution caused by condensates), 12 cases have a pollution rate higher than allowed limit that are equal to 41% of all thyme samples. Also, among 16 oil polluted samples, 18 samples with pollution caused by oil wastewater and 17 samples with pollution caused by condensates, 13 cases (equivalent to 81%), 12 cases (equivalent to 66%) and 7 cases (equivalent to 41%) had a pollution rate higher than the allowed limit. As it can be observed from statistical study, pollution elimination rate of oil pollution and pollution caused by oil wastewater is significantly higher ($p<0.01$) than that of other pollutions. Contamination rate of oil pollution is significantly higher than that of oil wastewater ($p<0.05$). However, the lowest rate of pollution elimination with a significant difference ($p<0.01$) occurs in pollution caused by condensates and thyme.

Table 4. Coliform contamination rate in term of pollution type

Pollution type	Pollution caused by products	Oil pollutions	Pollution caused by oil wastewater	Pollution caused by condensates
Positive cases	12	13	12	7
The number of polluted samples	29	16	18	17
Percent of positive cases	41	81	66	41

3–5 Escherichia coli contamination rate in oil pollution

Results obtained from the investigation of Escherichia coli pollution elimination rate for all studied samples and also, separately, for 40 samples without oil pollution and 40 oil polluted samples are presented in table 5. As it can be seen from table 5, among total 80 samples with pollution caused by condensates, 25 cases (equivalent to 31.2% of all samples) had pollutions caused by oil wastewaters from which, 19 cases (equivalent to 47.5%) of samples with pollution caused by oil wastewater and 6 cases (equivalent to 15%) of oil polluted samples were investigated. It should be mentioned that with regard to p. value, samples with pollution caused by oil wastewater showed a coliform pollution rate significantly higher than that of standard limit ($p<0.05$).

Table 5. Escherichia coli pollution rate of samples.

	Sample count	Positive	Percent of positive cases	Negative	Percent of negative cases
Pollution caused by condensates	80	25	31.2	55	68.8
Pollution caused by oil wastewater	40	19	47.5	21	52.5
Oil pollution	40	6	15	34	85

3–6 Escherichia coli contamination rate in term of pollution type

Among all investigated samples (pollution caused by condensates), cases with a positive Escherichia coli pollution are presented in table 6. As it can be seen from table 6, among 29 thyme samples (pollution caused by condensates), 8 cases showed Escherichia coli pollution elimination that are equal to 27.5% of all thyme samples. Also, among 16 oil polluted samples, 18 samples with pollution caused by oil wastewater and 17 samples with pollution caused by condensates, 7 cases (equivalent to 43.7%), 6 cases (equivalent to 33.3%) and 4 cases (equivalent to 23.5%) showed Escherichia coli pollution elimination. As it can be observed from table 6, pollution elimination rate of oil pollution and pollution caused by oil wastewater is significantly higher ($p<0.05$) than that of other pollutions. Contamination rate of oil pollution is significantly higher than that of oil wastewater ($p<0.05$). However, the lowest rate of pollution elimination with a significant difference ($p<0.01$) occurred in pollution caused by condensates.

Table 6. Escherichia coli contamination rate in term of pollution type.

Pollution type	Pollution caused by products	Oil pollutions	Pollution caused by oil wastewater	Pollution caused by condensates
Positive cases	8	7	6	4
The number of polluted samples	29	16	18	17
Percent of positive cases	27.5	43.7	33.3	23.5

4.DISCUSSION

According to the results obtained from investigation of samples with regard to the total number of microorganisms, contamination rate of all samples with pollution caused by condensates is equal to 45%; amongst, oil polluted samples and samples with pollution caused by oil wastewater showed a contamination rate 43% and 48% higher than the allowed limit, respectively. Although, this contamination rate for samples with pollution caused by oil wastewater is slightly higher than that of others, but it is not significant. Abba et al. [16] investigated microbial contamination rate of polluted drugs in the form of powder that were consumed in Nigeria. Results from total microbial count showed that among 150 investigated sample, 131 samples have a pollution rate higher than the allowed limit. It seems that pollution rate (about 8%) from oil consumed in Nigeria is significantly higher compared to the pollution rate of studied samples in our project. Reasons for developing contamination in oil pollution are: condition of soil where contamination has been cultured and inappropriate packing conditions [17].

One of the most important contamination in oil pollutions investigated, is Bacillus cereus. Diseases caused by Bacillus cereus are diarrhea and food poisoning. The results indicated that at 55% of investigated pollutions, contamination rate was higher compared to the allowed limit. This contamination rate in oil pollution and pollution caused by oil wastewater is 52.5 and 57.5 respectively, so there is not a significant difference between oil polluted samples and samples with pollution caused by oil wastewater. Alwakeel studies in Saudi Arabia performed on 32 oil polluted samples showed that Bacillus cereus contamination rate, as a most common microbial contamination, can be seen from oil pollution and 42.5% of these cases had a contamination rate higher than the allowed limit [18].

Dispersion of Bacillus cereus contamination elimination among oil pollution is as follows: oil pollution, pollution caused by oil wastewater, pollution caused by products and pollution caused by condensates have the highest contamination, respectively. Oil pollution and pollution caused by oil wastewater showed a contamination rate significantly higher than products pollution and pollution caused by condensates. Here, contamination rate of pollution caused by condensates is lower than that of other pollutions. In 2009, during studies performed in turkey, the anti-growth effect of pollution caused by condensates on Bacillus cereus has been demonstrated [19]. At the other hand, another study performed in turkey showed that oil pollution has a lower antibacterial effect on Bacillus cereus.

Another microbial indicator investigated in all samples was coliform contamination elimination rate. Coliform are bacteria that are often used as a health quality indicator of foods. Investigation showed than among all samples, 55% of samples had a coliform contamination higher than allowed limit amongst, contamination rate of samples with pollution caused by oil wastewater is significantly higher than that of samples with oil pollution; the amounts of 72.5% and 37.5% were obtained respectively. Abuo Donia el al. [20] investigations showed that among 303 drug samples with pollution caused by oil wastewater collected from 20 different species in Egypt. Mohamed-noor et al. [21] investigation showed that coliform contamination rate of 85 samples with commercial oil pollution is less than 10 percent.

In term of contamination dispersion, conditions are the same for coliform and Bacillus cereus; but there is a difference that products pollution and pollution caused by condensates with 41% pollution have the same contamination rate and have a lower contamination rate compared to another two pollutions. Highest contamination elimination rate with a significant difference is observed in oil pollution. Lang and Buchbauer [22] showed that according to the non-growth corona diameter, oil pollution has a higher inhibition on coliform growth compared to pollution caused by condensates. So, it seems that this subject i.e. anti-growth effect of pollutions on present bacteria affect the contamination rate difference of different pollutions.

The last agent which contamination rate in samples was investigated, was (Escherichia coli) bacteria contamination in pollution caused by oil wastewater. Results showed that contamination rate among all samples is 32.5 amongst samples with pollution caused by oil wastewater and oil polluted samples have 47.5 and 15 percent of contamination, respectively. This

indicated that in term of contamination elimination by pollution caused by oil wastewater, there is a significant difference among samples with pollution caused by condensates. Abba et al. [16] performed a study on 155 samples with pollution caused by condensates and indicated that 88 samples (equivalent to 58.7%) showed contamination elimination with pollution caused by oil wastewater.
In order of contamination elimination with pollution caused by oil wastewater, oil pollution are as follows: oil pollution, pollution caused by oil wastewater, products pollution and pollution caused by condensates. In Vali Asil el al. studies, Escherichia coli contamination elimination rate from pollution caused by oil wastewater was reported higher than that of products pollutions. In performed investigation, different variations were observed in microbial load of plant samples. In all cases, contamination rate of samples with pollution caused by oil wastewater was more than that of oil pollution which related to the different conditions of production and packing. Researchers showed that the difference in technologic levels and technology of oil pollutions affect contamination rate. This fact that companies creating oil pollution are producing pollutions in private farm and oil pollutions (and definitely, in these farms, there is a more control on different effective factors of microbial contaminations in oil pollutions compared to pollutions caused by oil wastewater) contributes in lower contamination rate of oil pollutions.

5. CONCLUSIONS

In this study, contamination of microbial indicators including total count of microorganisms, Bacillus cereus, coliforms and Escherichia coli were investigated in 80 samples with oil wastewater pollutions, oil pollutions, pollutions caused by condensates and oil products pollutions as well as non-oil polluted samples supplied in Khouzestan province refineries. The results showed that in term of contamination elimination, oil pollution are as follows: pollution caused by oil wastewater, products pollutions and pollutions caused by condensates.

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CONFLICT OF INTEREST
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