

Bioremediation of Oil Waste under Field Experiment

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Abstract: The remediation process of oily waste, selected in the tank battery, was conducted under field experiment. Compost (5 and 50%), prepared from the organic fraction of municipal solid waste, sewage sludge and sawdust, as well as two strains of soil bacteria *Bacillus thuringiensis* RG2 and *Bacillus pumilus* RG1 were used for remediation process. The lowest amount of petroleum hydrocarbons was detected when mixing waste with soil and compost at the amount of 50%. In the first case noted effect was achieved by increasing the number of hydrocarbon oxidizing bacteria, whereas in the second case it was attained by increasing the total microbial biomass and metabolic activity of the mixtures. Adding microorganisms-destroyers did not have a significant effect on the hydrocarbon decomposition process. Taking into account the relevancy for soil resources preservation, the use of compost for bioremediation seems to be the most promising technique.

Key words: Bioremediation • Oil waste • Microbial biomass • Respiration

INTRODUCTION

Oil recovery processes are inevitably accompanied by the negative impact on the environment. The most studied are the effects caused by the penetration of petroleum hydrocarbons into soil as a result of accidental spills. At the same time, waste, generated during cleaning of oilfield equipment, as well as equipment used in the oil transportation and processing, also have a negative impact on the soil [1, 2]. Amount of oil waste may reach 0.2-0.5% of produced commercial oil [1-4]. These wastes are characterized by a broad range of oil content, specifically from 17 to 862 g/kg [3-13]. Especially dangerous is the fact that the oil waste components include compounds known for their mutagenicity and toxicity [2, 10, 12, 14]. Further, during storage many compounds in the waste can be transformed into metabolites, whose toxicity and stability is unknown [14, 15]. Currently, the main practice in waste depositing is its disposal on the soil or in special storage facilities that is dangerous for the environment. To reduce the negative impact of wastes, they must be treated before the storage. According to the literature, biological methods of waste treatment, called bioremediation, are increasingly developing. These methods are more economical as compared to

physico-chemical or thermal methods and do not cause secondary contamination. According to majority of authors, various biological remediation approaches are recognized to be the most promising remediation methods [6, 7, 10, 12, 16]. When selecting the optimal method of bioremediation or monitoring the effectiveness of the process, it is important to control not only the reduction of the total amount of petroleum hydrocarbons, but also change in the biological activity and the number of specific groups of microorganisms [12]. This is due to the fact that hydrocarbons destruction is carried out exactly by the microorganisms [12, 17, 18]. Previously, under the laboratory conditions, it was shown that remediation of wastes, generated during oil production, can be performed using landfarming and bio-stimulation techniques [12, 13]. Current paper deals with modelling of remediation of waste, containing hydrocarbons, which was conducted in the field experiment with a purpose to identify the most effective remediation method.

MATERIALS AND METHODS

In this paper we used the waste produced during the purification of oilfield equipment in the Tikhonovsky tank battery (Republic of Tatarstan). During remediation we used soil (C_{org} -6.6%, N_{tot} -2860 mg/kg, soil texture:

clay-17%, sand-29%, other components-54%, oil content-362 mg/kg) and compost, prepared from the organic fraction of municipal solid waste, sewage sludge and sawdust.

Remediation was carried out in the botanical garden of Kazan Federal University in May-August, 2013 at ambient temperature from +10 to +30°C. Specially prepared trenches were filled with 300 kg of mixtures having the following composition: waste and soil in the ratio of 1:4 (sample WS); waste with compost in the rate of 5% by weight (sample WC-5); waste with compost in the rate of 5% and a mixture of two strains of microorganisms-destructors (sample WCM-5); waste with compost in the rate of 50% by weight (sample WC-05); waste with compost in the rate of 50% and a mixture of two strains of microorganisms-destructors (sample WCM-50). Waste without treatment served as a control sample (sample W). The C/N ratio of each mixture was adjusted to 15:1 by means of urea.

Two previously isolated bacteria strains of *Bacillus thuringiensis* RG2 and *Bacillus pumilus* RG1 were used in the current work. Individual strains for inoculation of mixtures were grown in a medium (g/l: KH_2PO_4 -3.0; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ -0.2; $\text{NaH}_2\text{PO}_4 \cdot 12\text{H}_2\text{O}$ -4.5; $(\text{NH}_4)_2\text{SO}_4$ -1.0) with oil (5%), then separated by centrifugation and resuspended to a final titer of 10^7 CFU/ml.

Samples for measuring test parameters were taken in three replicates on the 1st, 38th, 80th and 112th day.

Measurement of the mass fraction of oil products in the test samples was carried out by IR spectroscopy [19].

Respiratory activity and total microbial biomass (C_{mic}) was determined in accordance with [20]. Number of heterotrophic microorganisms was determined by seeding on solid medium (meat-and-peptone agar). The number of hydrocarbon-oxidizing microorganisms (HOB) was determined by limiting dilution technique in the nutrient broth with oil as the sole carbon source [21].

Measurement of all parameters was performed at least in triplicate. The figures below show mean values and standard deviation. Statistical significance of the results was evaluated based on the t-criterion.

Main Part: The first stage of the experiment was to analyze the waste, used in the work. It was found that the total petroleum hydrocarbons (TPH) content in the waste was 83.7 g/kg and pH was 6.9. Wastes with such characteristics are described in the literature [5, 7, 12, 13]. Remediation of waste was carried out in the field environment during 4 months at an average air

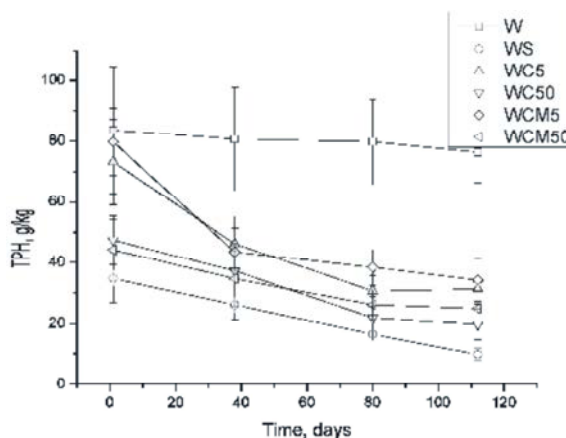


Fig. 1: Change in total petroleum hydrocarbons (TPH) during the remediation of the waste by different methods.

temperature of 22°C. Change in TPH contents is shown in Fig. 1. According to data obtained, the maximum content of TPH in the base mixtures prepared for remediation was found for samples WC-5 and WCM-5 that is due to the fact that these mixtures contain low amount of compost, which acts as a structure-forming agent. The minimum content consisting of 34.7 g/kg was found in WS sample. Differences in the TPH content are conditioned by the mechanical dilution of waste.

Throughout the entire remediation period of the sample W, in which waste underwent just mixing, reduction of TPH was negligible and amounted to 8.7% of the initial content. By the end of remediation, the minimum content of TPH, equal to 9.8 g/kg, was detected in the sample WS. Though, it should be noted that the initial content of TPH for this sample was minimal. The final content of TPH in the samples, prepared by addition of compost to soil, varied within the range from 19.7 to 34.2 g/kg, thus the reduction of TPH was 43-58% of the initial content. According to the literature, the effective degradation of oil components requires the availability of both biogenic elements and oxygen [22, 23]. The compost, used in the mixture for remediation, creates conditions fostering the flow of oxygen, as well as provides both additional nutritive substances and more pronounced mixture structuredness. In general, reduction level of oil components is comparable with the data available in the literature. Thus, the reduction of hydrocarbons when mixing waste with soil, manure, sawdust and chippings was 31-46% over 50 days at the initial concentration of 101-240 g/kg [4] and 16-31% over 120 days at the initial concentration of 44-111 g/kg [5]. When comparing the samples, differing from each other by amount of

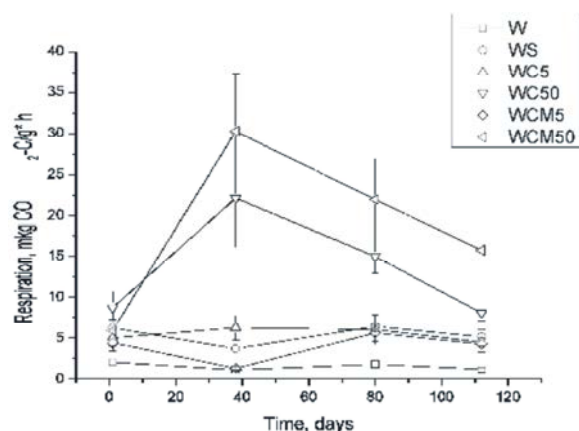


Fig. 2: Change in respiration activity during the remediation of the waste by different methods

introduced compost, it was found that, despite the higher content of TPH in the mixtures at the end of remediation, the absolute amount of hydrocarbons undergoing decomposition appeared to be 1.5 and 2.4 times higher in the test samples containing 5% of compost.

We should note the lack of reliable differences in the final content of hydrocarbons in the samples WC-5 and WCM-5, as well as in WC-50 and WCM-50. This indicates the fact that the introduction of microorganisms had no significant effect on the hydrocarbons destruction process. The literature contains data proving both increase of the hydrocarbon biodegradation efficiency by introduced microorganisms and the lack of this effect [4, 11, 24, 25].

Variation in the biological activity of the mixtures in the remediation process was evaluated by the level of respiration and general microbial biomass. These indicators adequately characterize the state of the microbial community and are mentioned in the literature most frequently [6, 26]. Respiratory activity in waste (sample W) in the remediation process varied at the level of 1.1-2.2 mkg C-CO₂/g·h (Fig. 2). In early remediation, respiratory activity for all the other samples was higher on average 2.9 times as compared with that in waste. Significant increase in respiration by factor of 19 and 27 was identified on the 30th day of remediation in the samples WC-50 and WCM-50, respectively. At a later stage these differences decreased; however, for the sample WC-50 this factor was equal to 8 and 7 on 60th and 90th day, respectively, whereas it was 12 and 13 for the sample WCM-50. Such an increase in respiratory activity in mixtures with compost in an amount of 50%, most likely was due to the presence of available organic compounds, used by the microorganisms as nutrient source. In three

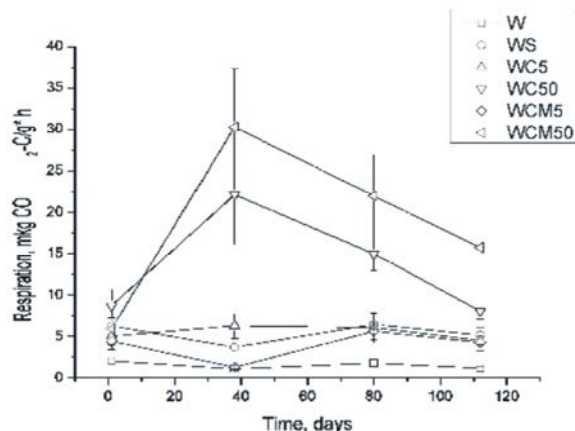


Fig. 3: Change in total microbial biomass during the remediation of the waste by different methods

samples (WS, WC-5 and WCM-5) the respiration was higher than that in waste on average by factor of 3.5 and fluctuated slightly in the process dynamics.

Determination of total microbial biomass allows one to assess integrally the totality of microorganisms in mixtures [27]. Change in the level of total microbial biomass in the remediation process is shown in Fig. 3. As is obvious from the data obtained, the minimum level of total microbial biomass (14.5-66.4 mg C/kg) was identified throughout the entire process in the waste (sample W). The maximum level of microbial biomass was determined for the samples containing compost in amount of 50% (2260 and 1920 mg C/kg for the samples WC-50 and WCM-50, respectively). This effect is associated with a high content of micro-organisms in the compost. Remediation process is accompanied by downtrend in microbial biomass; at that, minimum biomass was detected on the 38th day, however, these differences are not significant. In other samples (WS, WC-5 and WCM-5) the microbial biomass was significantly lower than that in the previous tests. This is due to the smaller amount of compost in these mixtures, as well as a low content of microorganisms in the soil as compared with the compost. At the same time, throughout the remediation process, microbial biomass in these samples was higher than that found in the waste.

We determined the change in number of oil oxidizing bacteria, since they are involved in degradation of petroleum components of waste in the remediation process. Besides, we determined a number of heterotrophic microorganisms, accounted in meat-and-peptone agar (Table 1). It is found that the number of heterotrophic microorganisms in the waste was 0.2-1.2*10⁶ CFU/g. This is significantly lower compared to

Table 1: Number of heterotrophic and hydrocarbon bacteria in the samples of remediation mixtures

Samples	Time, days			
	1	38	80	112
Heterotrophic bacteria, CFU*10 ⁶ /g				
W	0.3±0.1	1.2±0.3	0.1±0.3	0.2±0.06
WS	14.4±2.8	12.4± 3.1	14.6±3.4	18.1±4.5
WC-5	2.2±0.6	3.7±0.4	6.3±0.8	2.9±0.7
WC-50	8.0±2.1	9.9±1.6	12.7±3.1	13.4±1.5
WCM-5	2.2±0.4	10.8±2.1	11.5±2.4	8.2±1.2
WCM-50	6.0±1.2	11.2±2.9	15.7±4.2	16.7±3.9
Hydrocarbon oxidizing bacteria, CFU*10 ⁴ /g				
W	0.25±0.05	0.75±0.05	0.20±0.04	0.25±0.05
WS	7.50±1.5	2.00±0.5	9.50±2.0	7.50±2.0
WC-5	2.00±0.5	2.50±0.6	4.50±1.0	2.50±0.5
WC-50	1.50±0.3	2.00±0.5	2.50±0.5	3.00±0.5
WCM-5	2.00±0.4	1.50±0.3	4.00±0.4	5.50±0.9
WCM-50	0.95±0.2	1.10±0.3	3.50±0.7	3.50±0.6

all other options due to the toxic effects of hydrocarbons [2, 10]. This stipulates also low values of respiratory activity and total microbial biomass in concerned samples. The high number of heterotrophs was maintained throughout the entire process of remediation in the sample WS (12.5-18.5*10⁶ CFU/g). The number of heterotrophic microorganisms in other samples was lower than that in WS sample; however, due to the high variability of this parameter, the differences were not reliable.

A similar pattern was found during the analysis of hydrocarbon-oxidizing microorganisms. Maximum number of hydrocarbon oxidizing bacteria (HOB) at all stages of remediation was found in WS samples (2.0-9.5*10⁴ CFU/g). Other samples were characterized by lower values of HOB, which ranged from 0.95 to 5.5*10⁴ CFU/g. The number of HOB in the sample W varied at the level of 0.2-0.75*10⁴CFU/g. Despite the minimum number of both heterotrophic and hydrocarbon oxidizing bacteria in the sample W, the portion of hydrocarbon oxidizing bacteria in the total number of heterotrophic bacteria was maximal (up 1.9%) that is likely due to the fact that hydrocarbons are the main organic substrates in these samples.

CONCLUSION

Thus, the analysis of the waste remediation process under field experiment has shown that the smallest content of petroleum hydrocarbons was detected when mixing waste with soil as well as compost at the rates of 50%. In the first case, noted effect was achieved by increasing the number of hydrocarbon oxidizing bacteria, while in the second case it was conditioned by increasing

the total microbial biomass and metabolic activity of the mixtures. Adding microorganisms-destructors did not have a significant effect on the hydrocarbons decomposition process. However, taking into account the relevancy for soil resources preservation, the use of compost for bioremediation seems to be the most promising method.

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