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INFLUENCE OF HYDROCARBONS ON THE COUNT OF BACTERIA IN WATER OF THE AERATOR OF A LABORATORY SETUP FOR BIOREMEDIATION OF CONTAMINATED SOIL

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ABSTRACT. Oil and oil derivatives may cause disastrous ecological damages in the case of their accidental spilling, such was the one during the destruction of the Novi Sad Oil Refinery.in 1999. As the Refinery is located in the hinterland of the water source "Ratno Ostrvo", this was a potential threat to the overall water source, endangering thus the drinking water supply to the city of Novi Sad. One of measures that have been undertaken was the attempt in bioremediation of contaminated soil. Kinetics of this process was examined on a laboratory setup in which functioned a vessel designated as aerator. Microbiological degradation of oil may be effective to a smaller or greater extent, and monitoring of the dynamics of population of particular bacteria groups in the aerator water and concentration of hydrocarbons should provide information about the bioremediation process efficiency. Studies showed that the changes of certain technological parameters (e.g. increase of the water flow rate in the setup) in time, yielded a negative effect of hydrocarbon concentration on water microflora in the aerator. An abrupt drop was observed of both bacteria counts and enzyme activity of the present microflora.

KEY WORDS. bacteria, hydrocarbons, water, aerator

INTRODUCTION

Oil and oil derivatives can have disastrous ecological consequences when, in various accidents, they reach natural environment. Very diverse techniques have been used to remove oil and remediate the endangered site. Among them, there are also techniques

based on biodegradation and processes that can make it possible and/or facilitate it. Processes of biological degradation - known as bioremediation, are very complex, and they are proceed thanks to activity of microorganisms. Oil as a contaminant undergoes biodegradation under aerobic or anaerobic conditions, and the degree of biological degradation is dictated by numerous factors related to oil composition, presence of microorganisms, necessary nutrients, temperature, and oxygen concentration. Microorganisms capable of degrading hydrocarbons from oil are widespread in natural acosystems.

Although they use a specific substrate, hydrocarbon-oxidizing bacteria do not represent a narrowly-specialized group, as they, most probably, possess enzymes of both constitutive and adaptive nature. In view of the complex nature of oil and its derivatives as environmental contaminants, the assessment of counts of this group of organisms is not a simple task. Their activity is limited by the availability of oxygen and nutrient elements (nirogen and phosphorus). Concentration of contaminants can have a two-fold effect on the present microorganisms: if it is extremely low, the microorganisms simply cannot "feel it", and if it is extremely high, there may appear toxic effects. Under natural conditions, especially when a complex organic substrate such as oil, is involved, an important role play also metabolic interactions (of commensalistic relationship type) between different populations.

During the destruction of the Novi Sad Oil Refinery in 1999, large amounts of oil and oil derivatives were spilt over the ground. In view of the fact that the Refinery is located in the hinterland of the water supply source "Ratno Ostrvo", the entire water source was endangered, and thus the supply of drinking water to the city of Novi Sad. After the accident, complex investigations have been carried out related to the filed monitoring of groundwater quality, along with laboratory examinations of water and soil contamination, as well as the possibility of its remediation. One of measures undetaken has been the attmpt to bioremdiate the contaminated soil. Kinetics of this process has been studied on a laboratory setup for soil bioremediation, encompassing also a part designated as "aerator" or "quasi-bioreactor". The role of this unity of the laboratory setup is of great importance as the water from the bioreactor is introduced to it, where it is aerated and then returned to the top of the soil layer in the bioreactor. Such a procedure is to ensure the oxygen front to move down through the soil layer and thus contribute to the acceleration of biodegradation processes. The purpose of water aeration in the aerator is also to increase population of micoorganisms, to support their growth and activity, so that the introduction of such water onto the soil surface should contribute to an enhanced efficiency of the biological degradation of contaminants.

The aim of this work was to study the dynamics of counts and activities of bacteria in the unit called that can be called "aerator" or "quasi-bioractor" of the laboratory setup for bioremediation of oil-contaminated soil. The objective was also to examine the influence of the concentration of hydrocarbons in water, on the present microflora.

MATERIAL AND METHODS

Laboratory setup

Bioremediation of oil-contaminated soil was investigated on a laboratory setup (Figure 1), a cylindrical reactor of dimensions 3200 x 800 mm. The reactor was filled first with sand to a height of about 135 mm and then a layer of contained soil (about 475 mm) was placed above it. Finally, the groundwater originated from the contaminated soil taken from the same site was poured over to a level of 510 mm. This water was recirculated with the aid of a membrane pump at a rate that was of the same order of magnitude as that of groundwater on the same location (about $1x10^{-7}$ m/s). The recirculation rate has been increased in time, in order to examine transport of contaminants and nutrients. The room temperature was held at about 20^0C. The water from the reactor was conducted to the aerator (volume, 2 l), and from that poured over the soil layer at the entrance to the reactor. The water in this part of the setup was intensively aerated, so that oil contaminants were flotated on the surface, whereas at the bottom accumulated the precipitated metal hydroxides.

Analysis of the aerator water quality

Standard methods were used to monitor some relevant chemical and microbiological parameters of the aerator water quality (oxygen concentration, concentration of total hydrocarbons and mineral oils, count of physiological bacteria groups, phosphatase activity).

Chemical investigations

Concentration of total hydrocarbons and mineral oils in water was determined by FTIR spectrometry, preceded by extraction with carbon tetrachloride, and, in the case of mineral oils, by adsorption of polar compounds on aluminum oxide (Eaton et al., 1995; Škunca-Milovanović et al., 1990). Dissolved oxygen concentration was measured by membrane electrode (Eaton et al., 1995).

Microbiological investigations

Counts of bacteria in the aerator water were followed by conventional microbiological techniques and methods (Rodina, 1972., Petrović et al., 1998) and were expressed as the number of cells per ml of liquid. Use was made of cultivation methods on agarosed media, specific for each group of physicological bacteria (organotrophs, facultative oligotrophs, lipolytic and hydrocarbon-oxidizing bacteria). Lipolytic bacteria with their hydrolytic enzymes actively participate in the transformation of oil degradation intermediates, so that they can be regarded as good bioindicators of hydrocarbon contamination. Their counts were followed in parallel in two media (Tween and Tributyrine). Physiological group of hydrocarbon-oxidizing bacteria was also determined in two different supporting media, with addition of paraffine base. These were MSWYE (MS) medium – with addition of easily taken up organic matter and Tauson medium – a purely mineral medium, in which only the oil present served as a source of carbon for microorganisms.

In the frame of microbiological investigations, in addition to bacteria count, enzymatic activity was also determined. Such biochemical approach is very important having in mind that microorganisms significantly faster react at the level of metabolic activity than it could be evidenced as a change in their count. Phosphatase activity was determined as an indicator of the load of the environment by biodegradable contaminants. Its activity was expressed as the *index of phosphatase activity* (IPA, $\mu mol/s/dm^3$ pNP, 30°C), and it reflects the state of total organic load of an aqueous ecosystem (Matavulj, 1986).

RESULTS AND DISCUSSION

In Figure 2 is presented the change in counts of investigated bacteria groups and concentration of total hydrocarbons and mineral oils in the course of 435 days of experiment.

In the beginning, the aerator was filled with pure water and a flow rate of $1.9 * 10^{-7}$ m/s was adjusted, whereby a jump in the concentration of total hydrocarbons and mineral oils was observed. In parallel with that, the bacterial microflora, introduced with the water, showed a tremendous jump in its count (hundreds of millions). After that, due to the favorable conditions in the aerator, such as the presence of oxygen and lower concentration of hydrocarbon contaminanats than in the reactor (toxic action decreased), the count of hydrocarbon-oxidizing increased by 6 to 10 times. At the same time, the count of lipolytic bacteria decreased by 6 to 53 times, whereas the counts of organophils and facultative oligotrophs remained essentially unchanged.

After increasing the flow rate to $5.7 * 10^{-7}$ m/s, starting from the 54th day, an increase in concentration of hydrocarbons could be expected as a consequence of intensified washout from the reactor. However, a drop in concentration was registered. This can be explained in terms of the action of the billions of bacteria present in one ml of the aerator water, especially of hydrocarbon-oxidizing bacteria, which were predominanat.

Starting from the 107th day, the flow rate was increased again, now to $7.6 * 10^{-7}$ m/s, and on the 132th day it was expected to detect an increased hydrocarbon concentration, as the bacteria involved could not propagate at a rate that would match the increase in substrate. However, on the 138th day of experiment, the aerator had to be cleaned because of the system clogging caused by the precipitation of iron and manganese hydroxides. After the cleanup, 300 ml of the "old water" was poured in to the pure water in the aerator, to preserve high population of the adapted microflora. The dilution of the aerator water resulted in a decrease in the concentration of hydrocrabons and bacteria count, but in the subsequent month, new amounts of hydrocarbons reached the aerator from the reactor, and bacteria had time again to multiply. Thus, on the 167th day, the same hydrocarbon concentration was registered as before the flow rate increase, which resulted from the water dilution in the course of the aerator cleanup, new washout from the reactor, and action of the present

microflora. Although billions of bacteria were still present in one ml of water, their count was still lower than 100 days before that, and the structure of groups changed: the counts of organotrophs, facultative oligotrophs and lipolytic bacteria increased (in the Tributyrine medium, even 20 times), whereas the count of lipolytic (Tween medium) and hydrocarbon-oxidizing bacteria (in Tauson medium, even 28 times). The decrease in the count of microflora and change in the fraction of particular physiological groups were the result of removal of part of microflora in the course of aerator cleanup, and an increase in the biomass at a lowered substrate concentration and a constant supply of new amounts of substrate.

On the 169th day, a new cleanup of the aerator had to be undertaken, but this time, all the previous water was retained. Also, on the 173th day, the flow rate was increased to $19 * 10^{-7}$ m/s. After a one-month time, an increased concentration of hydrocarbons was registered as a consequence of washout from the reactor, as well as a drastic decrease in the counts of all physiological groups of bacteria, from several billions to several millions per ml of water. Obviously, the hydrocarbons involved exhibited a toxic effect on the aerator microflora, so that a washout of microflora from the aerator to the reactor also took place.

After another month (on the 232nd day), bacteria counts showed a further decrease (below one million per ml), excpet for lipolytic bacteria (Tweenn medium). At the same time, a drop in hydrocarbon concentration was observed, which suggests that no millions and billions of bacteria per ml are needed in order to have an efficient biodegration process.

On the 272nd day, an increase in hydrocarbon concentration and in bacteria count, especially of hydrocarbon-oxidizing bacteria (MSWYE medium), lipolytic (Tributyrine medium) bacteria and facultative oligotrophs, were also oberved.

On the 299th day, the concentration of hydrocarbons was lower, while the counts of bacteria were higher, but their structure was also changed - fractions of lipolytic and hydrocarbon-oxidizing bacteria decreased and that of facultative oligotrophs increased.

After that (on the 306th day), the aerator was disconnected from the bioreactor in order to monitor the changes in bacteria counts without substrate supply and without washout of the microflora from the aerator. On the 341st day, a twice lower hydrocarbon concentration and a three times higher bacteria counts were found. Further, on the 406th day, the hydrocarbon concentration was not essentially changed, but the bacteria count was significantly lower.

It is important to notice that the fraction of mineral oils in total hydrocarbons has been low all the time, which can be a consequence of microbiological transformation of non-polar to polar compounds on the one hand, and an intensified washout of polar

compounds from the reactor soil and their higher mobility, on the other. Under such dynamic conditions, involving a great number of factors that influence the bioremediation process, it is very difficult to exactly define contribution of hydrocarbons to the count of microbial population in the bioreactor.

In Figure 3 is shown the change of the oxygen concentration in the aerator with time, along with the change of the index of phosphatase activity.

In the beginning, there was no problem in establishing an effective aeration. However, the subsequent accumulation of iron and manganese hydroxides caused the clogging of the aeration system, so that the precipitate from the bottom of the aerator had to be removed and, from time to time, the whole aerator had be cleaned up. After two months of experiment, the most serious problem was how to establish aeration, which is visible from Figure 3. However, the problem with aeration was later overcome. After 306 days of experiment, the aerator was disconnected from the reactor, and this yielded an increased oxygen concentration in the water of the aerator, whereas all the oxygen from the precipitate was consumed.

The high index of phosphatase activity coincided with a high count of the present bacteria groups, and later, it decreased with a decrease in counts and had not essentially changed to the day of diconnecting the aerator, when it continued to decrease, which is in congruence with the decrease in microbiological activity due to a decreased concentration of the substrate.

CONCLUSION

Monitoring of bacteria counts in the aerator of the laboratory setup for remediation of oil-contaminated soil showed, first of all, that bacteria of all the investigated groups were present in the water. However, populations of particular groups were not the same, and significant fluctuations of counts within the same physiological groups have also been observed in the course of aerator operation. Generally, a constant decrease in the count, and thus in their biochemical activity, expressed via the index of phosphatase aqctivity in the aerator water, have been observed. The reason for such, occasionally drastic, drops of bacterial population in general, and especially of some physiological groups, should be sought at the level of their microenvironment and of the pertaining technological parameters of the setup operation.

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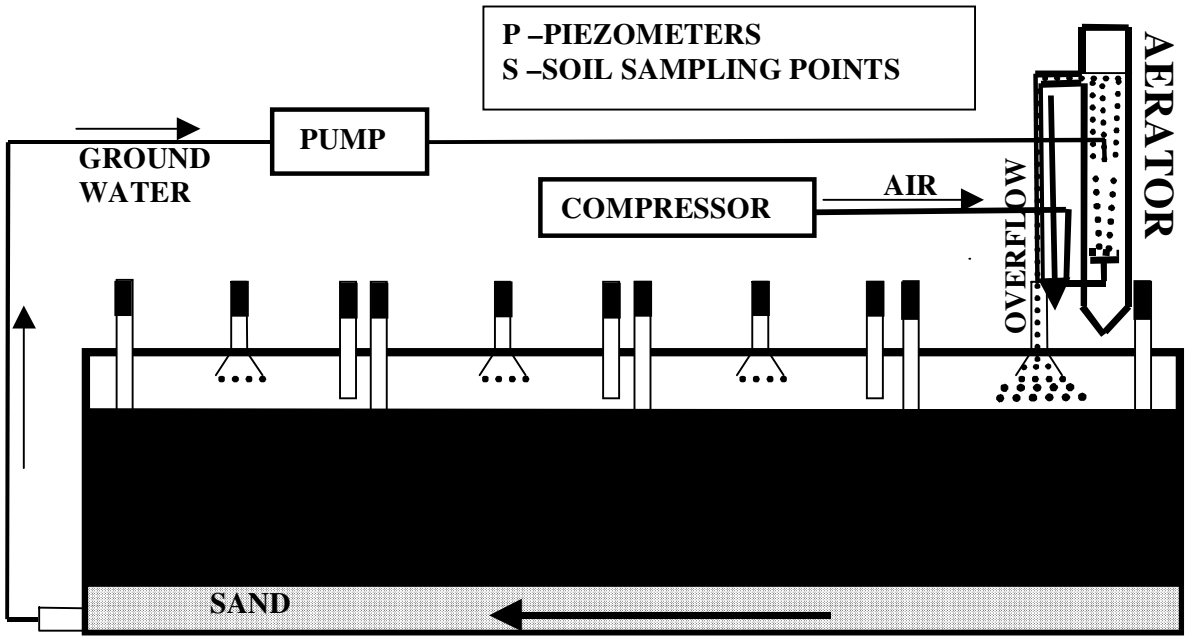

Figure 1. Laboratory setup for investigation of bioremediation of soil and groundwater contaminated with oil and oil derivates

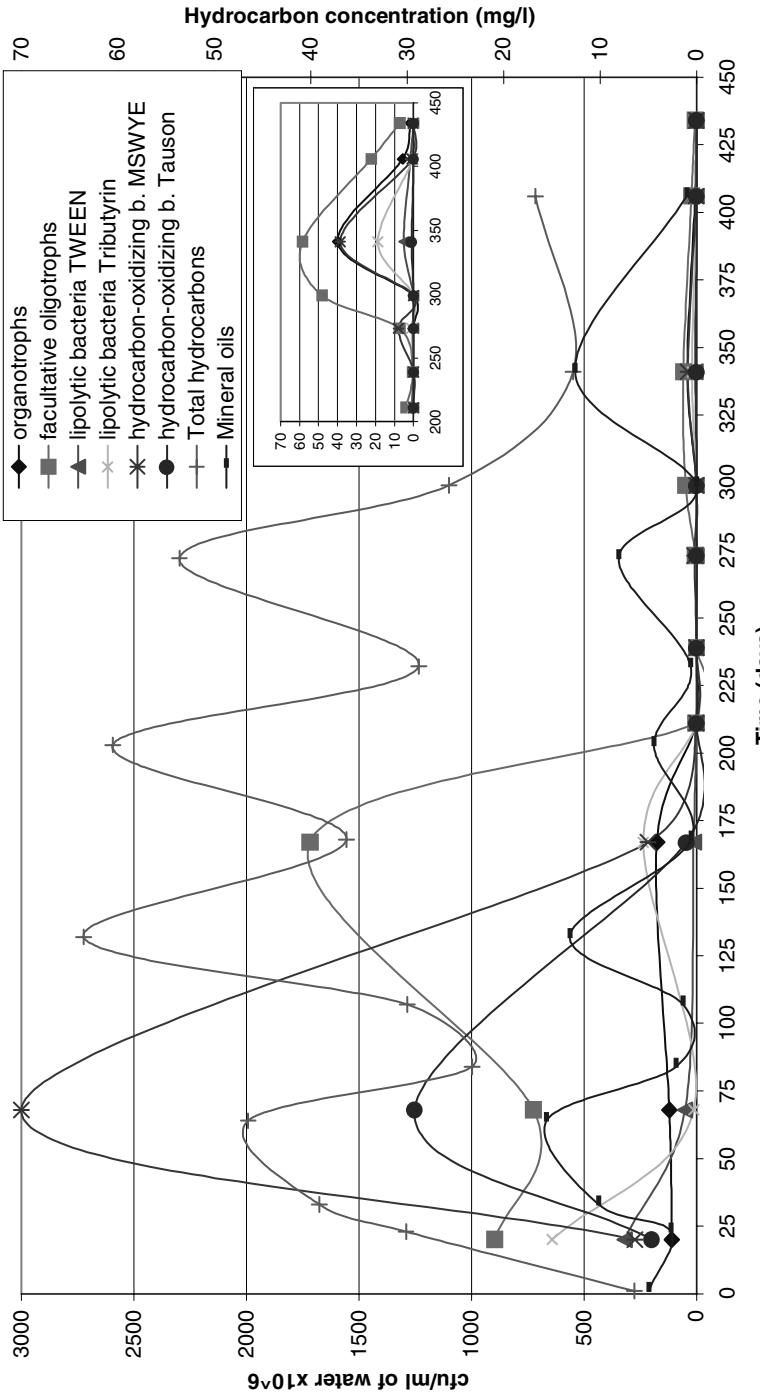

Figure 2. Changes in counts of investigated bacteria groups and concentration of total hydrocarbons and mineral oils in the aerator water during 435 days of experiment.

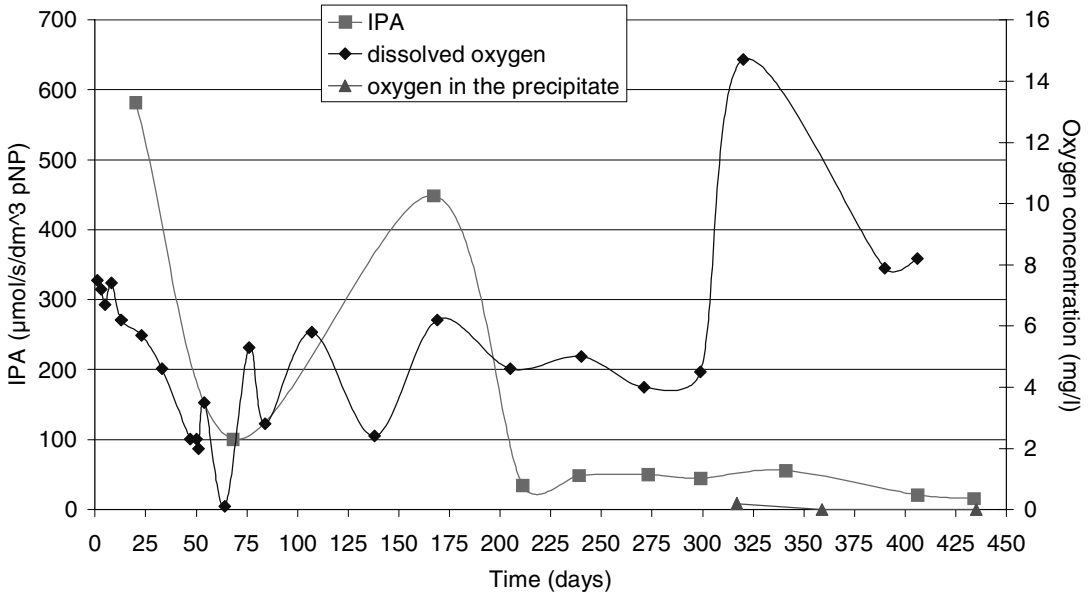

Figure 3. Changes of oxygen concentration (mg/l) and index of phosphatase activity (IPA, µmol/s/dm^3 pNP) in the aerator water during 435 days of experiment.