RESEARCH OF THE BIOREMEDIATION OF HYDROCARBONS IN SOIL BY THE USE OF SILICA NANOCOMPOSITE

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Abstract. Decades ago, oil spill has become a global issue. It effects not only environment but also economic life. Oil spills occur due to tanker disasters, wars, operation failures, during transportation, storage, use of oil and other accidents. Soil contaminated with petroleum effects human health, causes organic pollution of groundwater, which limits its use and decreases the agricultural productivity of the soil. Therefore, it is important to clean up oil spills as quickly as possible. Nowadays researchers are looking for new technologies that tackle three most important factors related with the oil spill clean-up: money, efficiency and time. The aim of this study was to evaluate the potential of bioremediation of petroleum hydrocarbons in oil-contaminated soil using silica nanocomposite. According to the findings, silica nanocomposite might increase microbial activity during biodegradation of petroleum hydrocarbons in soil because of the ability of nanoparticles to absorb water and keep moisture in soil thus creating a favourable environment for microorganisms. The study of biodegradation with the use of silica nanocomposite was carried out for a period of ten weeks in cooperation with the company Grunto Valymo Technologijos.

Keywords: oil spill, soil bioremediation, petroleum contamination, silica nanocomposite, petroleum degradation, petroleum hydrocarbons.

Introduction

Oil spills have become a global issue decades ago. It has polluted the environment and marine life and had global effects on the economy (Ibrahim *et al.* 2013). Soil contaminated with petroleum poses a serious hazard to human health, causes organic pollution of ground water, which limits its use and decreases the agricultural productivity of the soil (Thapa *et al.* 2012). Oil spills occur in the seas, water bodies and land surfaces due to tanker disasters, wars, operation failures, equipment breaking down, accidents, and natural disasters, as well as during the production, transportation, storage and use of oil (Karan *et al.* 2010). Spilled oil, which is denser than water, reduces and restricts permeability: organic hydrocarbons, which fill the soil pores, expel water and air, thus depriving the plant roots from the much–needed water and air (Essien, John 2010). The toxic components of spilled oil eventually enter the human food chains and affect human health. Thus, spilled oil causes enormous environmental problems unless it is removed as quickly as possible (Behnood *et al.* 2013).

The growing quantity of soil contamination with oil products urges to optimize cleaning operations. Only novel research and development strategies for soil purification from oil pollution can solve the emerging problems.

Cleaning the soil from oil and its products using microorganisms is one of the biological treatment methods. This method has long been used to remove oil contamination from soil. Recently, new materials have been looked at aiming to increase the efficiency of microorganisms used for eliminating oil pollution from soil. Nowadays nanoparticles and its composites are widely used. Nanocomposites (where one of the phases has nanoscale additives) exhibit unique physical and chemical properties: they can be easy developed, processed and modified (Paul, Robenson 2008). The current research is based on the hypothesis that, depending on the properties of nanocomposites, nanoparticles may intensify microorganism activity.

Materials and methods

The research was done in cooperation with the company Grunto Valymo Technologijos.

The investigation was carried out in order to test the hypothesis that nanoparticles may increase microorganism activity. All processes were investigated under optimal conditions for diesel biodegradation in laboratory which belongs to the company Grunto Valymo Technologijos.

Experimental design

The study was carried out for a period of ten (10) weeks. Samples of soil polluted with diesel were placed into six (6) different containers of similar size and geometry. 1 kg of soil was placed in each of the containers, all of them were polluted with 10 g of diesel and all were exposed to the same atmosphere and environmental conditions. Two containers with pollutants treated with microorganisms (without nanocomposites) were used as controls (CT), and in four other containers pollutants were treated with microorganisms and different proportions of nanosilica.

The research was carried out in the company Grunto Valymo Technologijos located in the Birbinciu str. 59, Kiskenu village, Dovilu district, Klaipeda region, Lithuania.

Preparation of contaminated soil

Bulk soil from agricultural fields was taken from 15 cm to 20 cm depth with a shovel, air was dried and passed through a 2 mm sieve. For optimal diesel biodegradation in bioremediation operations, soil was fertilized with NH_4NO_3 (ammonium nitrate), NPK (nitrogen phosphorus potassium) and KCl (Potassium chloride). The amount of fertilizer per kilogram was as follows: NH_4NO_3 – 0.416 g, NPK – 0.576 g, KCl – 0.084 g.

Soil was divided into 1 kg samples and stored in special containers of similar size and geometry (Fig. 1).

Fig. 1. Soil samples

All containers with bulk soil were artificially contaminated with diesel. To do so, hydrocarbon standard solution was prepared according to LST EN ISO 16703: 2004.

Hydrocarbon standard solution was prepared by mixing roughly the same masses of 2 different types of mineral oil (petroleum hydrocarbon and lubricating oil without any additives). Concentration of hydrocarbon mass was 8 g/l; to obtain this concentration, the mixture of mineral oils was weighted accurately and dissolved in the RTW (retention-time window) standard solution (International organization for standardization 2004).

Concentration of diesel in all these samples was 1% of the mass of soil (10 g/kg). Diesel was sprayed so that the whole soil sample would be polluted homogenously. The containers were left in the open air for 2 weeks. To avoid petroleum hydrocarbon layering in soil it was stirred twice a week.

Microorganisms used

Biopreparation GVT-1 that was used in the experimental research was provided and prepared by the company Grunto Valymo Technologijos.

Experimental design and soil treatment

Samples of soil polluted with diesel were divided into 6 treatment containers which were similar in size and geometry. Containers were chosen such that the depth and exposed surface-area of the soil, and in turn its temperature, nutrient concentration, moisture content and oxygen availability, could be controlled. Two containers were used as control samples; they were polluted with 10 g of petroleum and treated with 6.7 g of biopreparation GVT-1. The remaining 4 containers with diesel polluted soil received the same amount of biopreparation GVT-1 as controls plus 10, 20, 30 and 40 g of nanosilica respectively. For this experiment nanosilica was used in the form of white powder.

Treated soils samples were kept under optimal conditions for diesel biodegradation: humidity was 20% of field capacity (FC), room temperature was kept at 20 ± 2 °C, pH was kept at 5.5–6.5 to maintain appropriate environment for the activity of diesel decomposing microorganisms.

In order to remove the effect of the lack of oxygen and preparing aerobic soils conditions, soil in containers was stirred twice per week for 10 weeks.

Experimental soil sampling

The experimental soil was analyzed in the laboratory at set intervals. The soil was tested at the start of the experiment and then throughout the treatment every 14

2

days. The samples were thoroughly mixed and homogenized before collection. The soil samples were immediately taken to laboratory for analysis.

Physicochemical characteristics of soil samples were also determined. Every week humidity of soil was measured to make sure 20% of field capacity is maintained, pH is kept at 5.5–6.5 level, soil temperature is constant (temperature determines the activity of microorganisms). TPH (total petroleum hydrocarbon) amount was determined by gas chromatography using Shimazu 2010+ gas chromatograph (Fig. 2). In parallel, CO_2 emissions from soil were measured with Q-Box SR1LP (Fig. 3).

Fig. 2. Shimazu 2010+gas chromatograph

Total petroleum hydrocarbons (TPH) are a large family of several hundred chemical compounds that originally come from crude oil. Because there are so many different chemicals in crude oil and in other petroleum products, it is not practical to measure each one separately. However, it is useful to measure the total amount of TPH at a site.

Since side products of bioremediation consuming hydrocarbons are water and carbon dioxide it is necessary to measure CO_2 emissions for comparison. CO_2 was measured with Q-Box SR1LP (see Fig. 3).

Fig. 3. Q-Box SR1LP

To ensure the accuracy of results, each analysis was carried out twice. After recording the measurements, the average values were calculated.

Results

Hydrophobic silica nanocomposite A-300 was used in this research. A microphotographic image of the silica nanocomposite was made with TEM (transmission electron microscope) (see Fig. 4). Silica aggregates consist of primary particles with a 10–15 nm diameter.
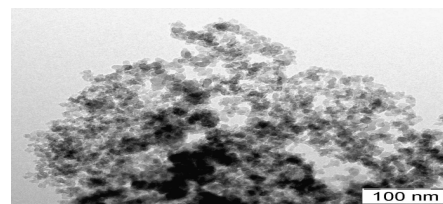

Fig. 4. TEM microphotographic image of hydrophilic silica A-300

Researchers have reported that changes in particle properties might be seen when the particular level is higher than particle size (Table 1). When particle dimensions reach the nanometre level, reaction at phase interfaces improves, which is very important in trying to enhance the materials' properties (Camargo *et al.* 2009).

Table 1. Feature size and changes in properties
(Camargo *et al.* 2009)

Properties	Feature size (nm) at which changes might be expected
Catalytic activity	<5
Making hard magnetic materials soft	<20
Producing refractive index changes	<50
Producing super paramagnetism and other electromagnetic phenomena	<100
Producing strengthening and toughening	<100
Modifying hardness and plasticity	<100

Silica nanocomposite belongs to polymer matrix nanocomposite group, which is known for their ability to increase modulus, yield stress, tensile strength and high creep resistance.

The main assumption of this work is that nanosilica might increase microbial activity of biodegradation of hydrocarbons in soil because of nanoparticle ability to absorb water and keep moisture in soil. Ability to absorb water creates a favourable environment for microbial activity. For this reason, investigation of nanosilica's (A-300) ability to absorb water was done (Table 2).

Thus, it can be seen that silica nanoparticles can absorb 40% of water when the moisture is 100% and 2.7% when moisture is 20%. However, most of water evapora-

tes, while in the composite, prepared on the basis of the hydrophobic nanosilica, the absorbed water is practically undetectable by gravimetric method, but it is believed that depending of the ambient humidity, water adsorption might be promoted or impeded by the biological component of the composite system.

Table 2. Characteristics of water absorbtion of nanosilica

% of solid phase, g	Mass of the sample, g	Mass of the sample after one day kept in desiccators, g	% of water absorbed 100% moisture/ 12 °C	% of water absorbed 20 % moisture/ 12 °C
89.63	0.108	0.180	40	2.7

Physicochemical properties

Samples were analysed for some physicochemical parameters every week during the research period.

Throughout the whole study period pH was kept constant at 6.5. Microbial activity depends on many factors, such as soil moisture (dry biomass stops microbial activity), temperature (microbial activity increases as temperature increases and vice versa), organic carbon (microbes use it as food) and pH (it is best to keep pH around neutral). Table 3 shows that temperature fell down during the study period. However, the decrease of temperature was not drastic – it decreased about 2–1 °C per sample. Since temperature is one of the indicators showing microbial activity, it can be concluded that the lowest activity was during the 5th week of the research, when temperature in all samples was about 17–18 °C. During other weeks temperature was more or less stable, except at the beginning of the study where it was a little bit higher than average because microorganisms had not prepared suitable media for themselves yet. Soil moisture during the research varied a lot. After one week of research moisture in all samples was rather low – about 7–10% per sample (while recommended moisture for the best microbial activity is 20%), but after two weeks a drastic increase in moisture was recorded rising up to about 40% of moisture in each sample. Supposedly that might have happened because of the high activity of microbes during that period (which was noticed during determination of petroleum degradation) since water is the side product of bioremediation. During the rest of the research moisture was more or less stable (around 20%) and variations during that period might be attributed to the changing environmental conditions or evaporation.

Formation of carbon dioxide

Microorganisms utilize hydrocarbons and mineralize total petroleum hydrocarbons into harmless carbon dioxide. CO_2 emissions describe microbial activity during bioremediation. Since this research involves relatively low petroleum pollution, CO_2 emissions remained low and constant (around 3000 mg/m^3).

Biodegradation of petroleum hydrocarbons

Results of biodegradation of hydrocarbons in soil contaminated with petroleum products using silica nanocomposites are showed in Table 3.

Table 3. Petroleum degradation in different samples

Data/ Parameters	16.12.12	16.12.19	17.01.04	17.01.19	17.02.02	17.02.15
Control sample No. 1, g/kg	9.9	8.1	7.67	6.55	6.15	5.2
Control sample No. 2, g/kg	10.7	8.3	7.24	6.91	6.55	5.9
Sample No. 1 + 10g nanocomp., g/kg	10.5	7.92	6.97	6.9	6.54	5.6
Sample No. 2 + 20g nanocomp., g/kg	10.1	7.63	7.02	6.98	6.83	5.3
Sample No. 3 + 30g nanocomp., g/kg	10.7	8.12	7.24	6.94	6.87	5.2
Sample No. 4 + 40g nanocomp., g/kg	10.3	8.7	8.24	6.47	6.74	5.6

Samples of hydrocarbon biodegradation were analyzed every 2 weeks. From the results given in Table 3 it can be inferred that biodegradation occurred in all samples of hydrocarbons. The highest change occurred after two weeks of research when the amount of petroleum hydrocarbons all samples decreased ~2 g. High decrease of petroleum hydrocarbons during the first two weeks of research might mean that light fractions of hydrocarbons vaporized and heavy fractions remained and were later cleaned by microorganisms. During the rest 8 weeks of the research constant variation was observed in the degradation of petroleum hydrocarbons. The percentage change of petroleum hydrocarbon is illustrated in Figure 5.

As it was mentioned before, the highest change of petroleum degradation occurred during the first two weeks of research. Figure 5 shows that during this period
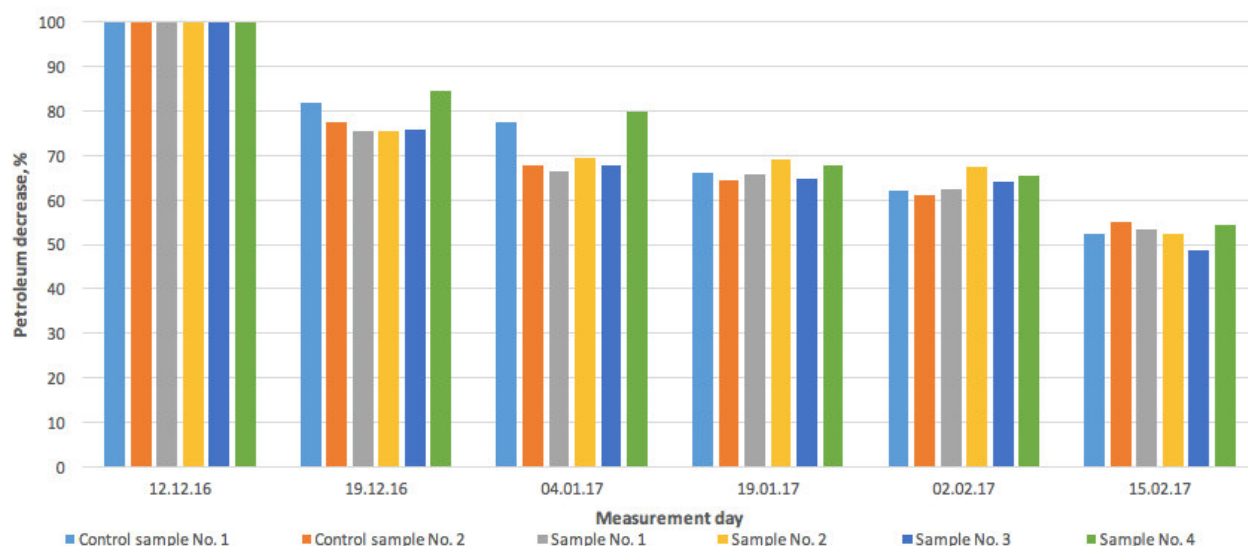
Fig. 5. Decrease in petroleum hydrocarbons in samples investigated, %

three samples showed almost similar results. The amount of petroleum in sample No. 1 was 24.6% lower than at the beginning of the research, sample No. 2 – 24.5%, and sample No. 3 – 24.1%. The remaining samples (except sample No. 4) also showed similar results – in control sample No. 1 petroleum decreased by 18.2% and in control sample No. 2 – 22.4%, while sample No. 4 showed slightly worse results as the amount of petroleum decreased only by 15.5%.

Biodegradation during the remaining six weeks in control sample No. 2, sample No. 1, sample No. 2, sample No. 3 was without drastic changes and stable, while control sample No. 1 and sample No. 4 showed a sharp decrease of petroleum products after 6 weeks of research. The percentage change of petroleum between the results obtained after 4 weeks of research and after 6 weeks of research in control sample No. 1 was 11.3% and in sample No. 4 – 12.2%.

Over the last week of research it was noticed that microbial activity in samples that contained nanosilica was higher than in samples without nanocomposites. Sample No. 2 (that contained 20 g of silica nanocomposite) and sample No. 3 (that contained 30 g of silica nanocomposite) showed a decrease of petroleum by 15.1% and 15.6% respectively when it was compared with the results obtained after 8 weeks of research, while the change in control sample No. 1 and control sample No. 2 was 9.6% and 6.1 % respectively. This might mean that nanocomposites start to effect the activity of microorganisms after some time period as heavier fractions of pet-

roleum hydrocarbons remain in the soil that requires more time to be cleaned up by microorganisms.

After 10 weeks of research the total percentage of petroleum decrease in samples was about 50%. The highest decrease was observed in sample No. 3 where the amount of hydrocarbons dropped to 48.6%, while other samples showed quite similar results: control sample No. 1 – 52.5%, control sample No. 2 – 55.1%, sample No. 1 – 53.3%, sample No. 2 – 52.5%, and sample No. 4 – 54.4%.

Although sample No. 3 (which contained 30 g of silica nanocomposite) in the end showed the best results, they were not significant enough compared to the results of the samples that contained only microorganisms to confirm that the use of silica nanocomposite increased microbial activity, especially when it can be seen that results from other samples that contained silica nanocomposite did not differ from samples that did not contain silica nanocomposite. However, increased petroleum degradation in samples that contain nanocomposites, during the last two weeks shows that nanocomposites influence the heaviest fractions of petroleum hydrocarbons.

Acknowledgements

Conclusions

1. In this research, silica nanocomposite belonging to polymer matrix nanocomposite group and known for its ability to increase modulus, yield stress, tensile strength and high creep resistance was used.

2. The research aimed at finding out if nanosilica might increase microbial activity of biodegradation of hydrocarbons in soil because of nanoparticles' ability to absorb water and keep moisture in soil.

3. The research results show that silica nanoparticles can absorb 40% of water when the moisture is 100% and 2.7% when moisture is 20%. At the end of the research the total percentage of petroleum decrease in samples was about 50%.

4. The best results were observed in sample No. 3 where the amount of petroleum hydrocarbons decreased to 48.6%, while other samples showed quite similar results: control sample No. 1 – 52.5%, control sample No. 2 – 55.1%, sample No. 1 – 53.3%, sample No. 2 – 52.5%, and sample No. 4 – 54.4%.

5. Based on the results obtained, it can be concluded that nanocomposites did not influence bioremediation during this research. However, increased petroleum degradation in samples containing nanocomposites during the last two weeks of the experiment shows that nanocomposites' influence on biodegradation may occur during the latest stages when there are the heaviest fractions of petroleum hydrocarbons remaining in the soil.

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NAFTOS ANGLIAVANDENILIŲ BIOREMEDIACIJOS DIRVOŽEMYJE PANAUDOJANT SILICIO NANOKOMPOZITUS TYRIMAI

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Anotacija

Ne vieną dešimtmetį naftos išsiliejimai yra laikomi pasaulinio lygio problema. Naftos produktai ne tik veikia gamtą, bet taip pat sukelia ir milžiniškus ekonominius nuostolius. Naftos produktai į dirvožemį patenka įvairiais būdais: dėl technikos gedimų, karų, transportavimo, laikymo, naftos naudojimo ar tiesiog nelaimingų atsitikimų. Naftos produktais užterštas dirvožemis neigiamai veikia žmogaus sveikatą, užteršia požeminį vandeninį organiniais junginiais, kurie sumažina dirvožemio derlingumą. Pagrindinis tyrimų tikslas buvo įvertinti silicio nanokompozito potencialą naudojant jį kartu su mikroorganizmais šalinant naftos angliavandenilius iš dirvožemio. Remiantis ankstesniais tyrimais, silicio nanokompozitas gali padidinti mikroorganizmų aktyvumą bioremediacijos metu – dėl nanodalelių galimybės sugerti vandenį ir išlaikyti dirvožemio drėgmę taip sukuriant geresnę aplinką mikroorganizmų veiklai. Tyrimai buvo atliekami 10 savaičių, bendradarbiaujant su „Grunto valymo technologijos" institutu.

Reikšminiai žodžiai: naftos išsiliejimai, dirvožemio atkūrimas, naftos tarša, silicio nanokompozitas, naftos degradacija, naftos angliavandeniliai.