IOP Conf. Series: Materials Science and Engineering **791** (2020) 012051 doi:10.1088/1757-899X/791/1/012051

The usage of organic wastes of malt and lysine-sulphate production for reclamation of soils, contaminated with petroleum products

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Abstract. This paper presents the research findings of using two organic wastes – from feed additives production and malt production, which can optimize the process of soil remediation from petroleum products. The experiment was performed *in situ* with applying in the soil, contaminated with petroleum products (industrial oil), the excess sludge of malt production waste biological purification (1.8 or 3.6 kg/m^2 was applied). The organic matter content in the sludge was 31.79 %. With account of the high initial concentration of petroleum products (3100−8600 mg/kg) and their degradation during a short-term experiment (21 days), a conclusion was made, that the excess sludge of wastewater biological treatment had the potential for remediating soils from petroleum products. The percent reduction made up from 16.12 to 85.64% (at applying 0.44 kg/m^2 of petroleum products) and 20.37; 33.72 and 89.03 % (at applying 0.66 kg/m^2 of petroleum products). From the second waste product, which contained 83.0 % of organic matter, − corn-steep extract purification sludge, − a water extract was obtained. It has been determined that the cell colonies, which appeared from this extract (8-fold dilution) in the agarized growth medium in the presence of used petroleum products, are not inhibited, and petroleum products become a source of biogenic carbon for them.

1. Introduction

One of the most important areas of resource-saving activity is the efficient utilization of production waste, which makes possible the more rational usage of material resources, reduction of production waste amounts and the considerable decrease of raw materials consumption. Waste products can be used in building and chemical industry, and in land reclamation.

In the world practice the considerable experience of using organic wastes for soil reclamation has been accumulated. The conventional organic wastes, used for this purpose, include the decomposed agricultural waste: decomposed cattle and pig manure, chicken and duck manure. But with the development of biochemical and food industries, waste products may include valuable components, which find no practical application and are dumped at disposal sites. This is due to the fact that providing recommendations for the usage of waste requires time for its research. Organic waste can be used in polluted areas treatment technologies both *in situ* (the polluted site is treated on the spot) or *ex situ* (the polluted soil is treated in special equipment).

Traditionally soils are purified from petroleum products in suspended-media bioreactors. An example of such method is described in the publication, where the bioremediation of oil-contaminated soil (initial concentration up to 200 g/kg) was performed by means of such reactor. To the reactor with contaminated soil the bio-surfactants of actinobacteria of the *Rhodococcus* genus were added. Bio-treatment of the contaminated soil resulted in the decrease of petroleum concentration by 88% after two months. Further on, to improve the efficiency of biological purification from petroleum products, the ploughing, woodchips, wetting and biofertilizers were used. The pollution level was reduced from 1.0 to 1.5 g/kg after 5−7 weeks. In conclusion, the phytoremediation by means of three species of perennial grasses was performed [1].

To remove petroleum hydrocarbons from soil it is necessary to know the amount of bacteria in it, to regulate the moisture content and pH of the soil, to apply fertilizers and carriers [2]. The authors of the survey paper indicate the three strategies of treating contaminated soils: biological, which implies the application of bacterial strains (bioaugmentation); adding special admixtures and nutrients (biostimulation); physical-chemical methods (solvent extraction; surfactant addition; thermal pretreatment and oxidative pretreatment) [3]. At oil pollutions of soil the concentrations of polycyclic aromatic hydrocarbon (PAH) and heavy metals in it are increased. Such soil can be treated by such technology as landfarming [4].

As a rule, the combined remediation of soils, contaminated with petroleum hydrocarbons, is performed with the usage of bioaugmentation and biostimulation. As a bacterial strain the yeast *Candida-tropicalis SK21* was used, which can operate in the pH range 3−9 in the liquid medium [5]. To purify soils from petroleum products a bacterial consortium, sampled from oil-contaminated soils, (consisting of *Pseudomonas* sp.) and rice husk can be recommended. The experiment has demonstrated that the purification efficiency from petroleum hydrocarbons amounted to 95 % [6]. The similar research was carried out with using rice husk, sawdust and woodchips for treating soils, contaminated with diesel oil. The results of the research have shown that the combination of the above-mentioned additives has influence on the bioremediation of contaminated soil [7]. For the efficient removal of total petroleum hydrocarbons (TPHs) the composted chicken manure was applied to the soil. The content of lubricating oil in soils was 5; 10 and 20 % by weight. After 42 days the content of TPHs in the soil decreased by more than 60 % in case of their original content 5 % by weight [8]. In the oil-contaminated soil (Kuwaiti) two biosurfactant-producing *Pseudomonas aeruginosa* strains (KISRC1 and KISRB1) were experimentally singled out. The strains differ in carbon source, nitrogen concentration, and pH of the medium [9]. It has been determined that washing soil with surface-active agents results in the loss of fine particles in it, which influences its mechanical properties [10]. It has been proved that a large amount of microbial communities, which can reduce the petroleum products concentration in soil, is observed at petroleum oil content 3−8%. During a ∼12 months of natural accumulation, the quantity of oil-degrading microbes increased from 10^5 to 10^8 cells/g of soil [11].

There are examples of other physical-chemical methods. To remove petroleum products from soil, hydrogen peroxide can be also used (the Fenton reaction). In the process of hydrogen peroxide decomposition (H_2O_2) hydroxyl radicals are formed, which are strong oxidizers. The soils under study contained a large amount of sorbed petroleum products (10009; 4850 and 2850 mg/kg). The stepwise oxidation by the Fenton reaction increased the removal efficiency of sorbed petroleum products [12]. The similar research was carried out for the efficient purification of soils from petroleum hydrocarbons (TPHs). The optimal conditions were as follows: 4.27 wt% of catalyst content and 2.17 mol/L of hydrogen peroxide, 57% and 67% of TPH removal were achieved by magnetite (naturally mineral) and zero-valent iron (ZVI). Magnetite and ZVI can catalyze heterogeneous Fenton-like reaction [13]. To remove crude oil from the soil it was saturated with soluble free Fe (II) and humic acid (HA). This allowed catalyzing the hydrogen peroxide in the soil. It was found out that the formation of oxygen and the decomposition rate of H_2O_2 is reduced in case of presoaking with Fe (II) + HA as comparison with only pre-impregnated Fe(II) and modified Fenton (MF) [14]. The properties of subcritical water were used for purifying soil, contaminated with organic pollutants. In this state of the water there is no difference between its gaseous and liquid phase. It has been experimentally deduced that at purifying soils from crude oil by extraction method with the use of subcritical water at 300°C within 1 hour with the flow rate 1.5 ml /min, the removal efficiency is 95% [15]. The magnetic petroleum sorbent on the basis of waste kieselguhr sludge and the carbonaceous sorbents on the basis of vegetable oil extraction industry waste sludge were also suggested for purifying wastewaters from petroleum products [16, 17].

2. Materials and Methods

2.1. Physical-chemical and microbiological properties of lysine-sulphate production waste

The work studies a waste product – sludge, formed at decanting corn-steep extract (lysine-sulphate production waste). In the original extract, which is a raw stuff for microbiological lysine-sulphate production, suspended solids content is 19 %; its purification provides their removal up to the final content 1% in the decanter. The properties of corn-steep extract sludge are given in Tab. 1. The energy-dispersive research of the incinerated waste is presented in Fig. 1.The chemical analysis of ash was performed with a scanning election microscope X-MaxOxford Instrument with the integrated energy-dispersive X-ray microanalyzer.

Table 1. Physical-chemical and microbiological properties of corn-steep extract purification sludge.

Parameter	Measuring unit	Content
pH	pH unit	4.11−4.30
Moisture	%	44.8
Ash	%	17.0
Organic matter	%	83.0
Total nitrogen	%	4.71
Total phosphorus(P_2O_5)	%	4.14
Total potassium(K_2O)	%	2.59
Lead	mg/kg	0.32
Cadmium	mg/kg	0.009
Copper	mg/kg	16.5
Zinc	mg/kg	269.7
Nickel	mg/kg	3.30
Chrome	mg/kg	0.66
Mercury	mg/kg	0.008
Arsenic	mg/kg	0.25
Salmonella	Cells per gram	not found
Total coliform bacteria	Cells per gram	below 10

2.1.1. Microbiological study of the extract of corn-steep extract purification sludge in the presence of used engine oil

For cultivating microorganisms' colonies the Sabouraud's growing medium was prepared (pH = 5.6). To inhibit the bacteria growth an antibiotic (chlorfenicol) was added to the medium in amount of 0.03 g for 600 ml of distilled water (dehydrated medium weight – 43.2 g). The Petri dishes were sterilized; 1 ml was placed into each dish, in the center of some of the dishes 1 ml of a used petroleum product (used engine oil) was placed. The experiment was performed in triplicate.

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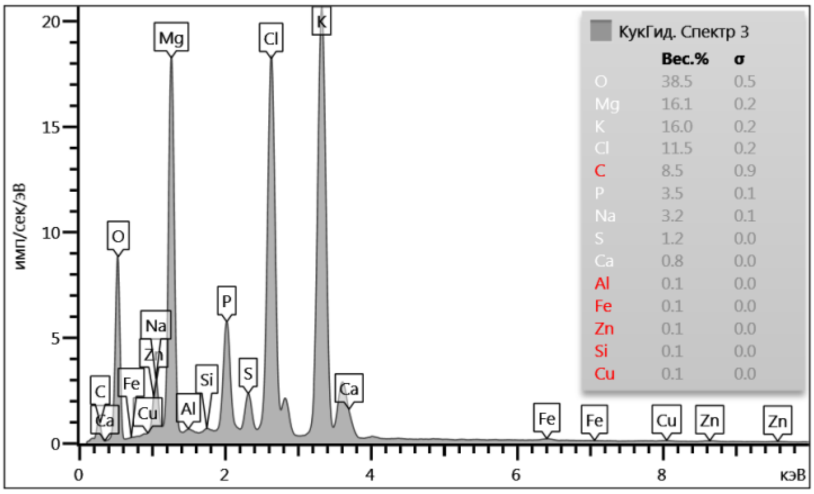

Fig. 1. Energy-dispersive spectrum of the incinerated waste.

2.1.2. Preparing of extract dilutions

To prepare dilutions the sterile distilled water was poured in amount of 9 ml into dry sterile test tubes. Then 1 ml of the extract under research was transferred with a sterile pipette into a test tube with 9 ml of sterile water – this is the first dilution (10−1). The following dilutions were prepared in the same way. At first the extract of corn-steep extract purification sludge was obtained, which had pH = 4.20, with the subsequent 8-fold dilution. Before the surface inoculation a melted agarized growth medium was poured into a number of sterile Petri dishes, from 15 to 20 ml into each. Microorganisms in the colonies, depending on their growth rate, were counted after 7 days of incubation.

2.2. Physical-chemical properties of malt production waste

In the experiment the waste product «Dewatered excess sludge of malt production wastewater biological purification» was used. Excess sludge is formed only in industrial process wastewater purification, performed at biological treatment plants. This waste finds no practical application and has the following composition (Tab. 2).

Table 2. Physical-chemical and microbiological properties of excess sludge.

Parameter	Measuring unit	Content
Moisture	%	66.24±4,64
Ash	%	< 5.00
Organic matter	%	31.79
Calcium	%	0.46±0,12
Magnesium	%	0.050±0,017
Phosphates	%	0.16±0,02
Chlorides	%	0.010±0,001
Nitrates	%	0.034±0,009
Nitrites	%	0.011±0,004
Iron	%	0.380±0,14
Aluminium	%	< 0.05
Sulphates	%	0.37±0,06
Potassium	%	0.28
Nickel	%	< 0.005
Lead	%	< 0.001

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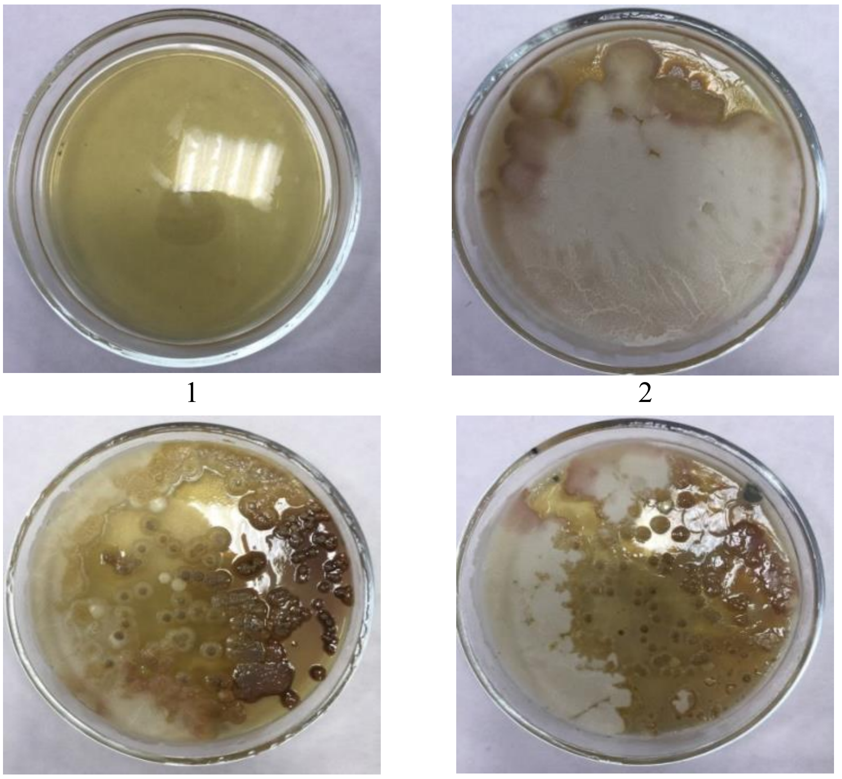
3. Results

3.1. Count of cells, cultivated on the growth medium with adding the extract of corn-steep extract purification sludge and the used engine oil

In Figure 2 the photos of cell colonies are presented. In figures 3 and 4 in Petri dishes we can see aggregations of bacterial colonies of a similar shape, formed on the surface of growth medium. The color of the colonies varies from yellow to brown. So, the extract of the waste contains a certain amount of natural oil-oxidizing bacteria, which, being placed into the Sabouraud's medium in the presence of petroleum products, begin to grow rapidly. Morphological traits of colonies in the samples are the following: round shape, convex surface, smooth edge contour, uniform consistency. The count was performed without opening the Petri dishes. The number of cells in the Petri dishes (№ 3 and 4 Fig. 2) amounts to $12.3 \cdot 10^9$ cells, in № 2 – $0.42 \cdot 10^7$ cells.



1 2

3 4

Figure 2. Photos of Petri dishes with microorganisms' colonies after 15 days of the experiment: *1* – reference sample; *2* – extract of the waste (8-fold dilution); *3, 4* – extract of the waste (8-fold dilution) + 1 ml of used petroleum products.

3.2. Experiment on purifying soil, contaminated by petroleum products, in the presence of wastewater excess sludge

In the university’s land lot 10 testing sites 1 × 1 m were made. In Tab. 3 characterization of soil is presented. The type of soil – leached chernozem – was determined by its parameters. To the depth of 15 cm petroleum products (industrial oil) were applied in amount of 0.44 or 0.66 kg/m^2. Petroleum products were distributed evenly throughout the whole area and dug over with the waste − the excess sludge. The amount of sludge was also different: 1.8 or 3.6 kg/m^2 were applied, which corresponded to 18 and 36 t/ha. The reference sites were those, where there were petroleum products.

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In Table 4 different variants of soil pollution with petroleum products are presented, with information about the amount of the applied dewatered excess sludge and the petroleum products content. After the application of petroleum products and excess sludge, the sites were watered with tap water. For the uniform distribution of the waste and the petroleum oil in the contaminated soil, and for aeration, the soil was hoed to the depth no less than 25−30 cm. The petroleum products content was studied in an accredited laboratory with fluorimetric method. This method is based on extracting from a sample with chloroform, purification of the extract by column chromatography method and the subsequent measuring of the fluorescence intensity of the extract with an analyzer.

Table 3. Characterization of soils.

Parameter	Measuring unit	Content
pH_{KCl}	pH unit	6.46
pH_{H2O}	pH unit	7.69
Dry residue	%	1.152
Mineralization	%	0.420
Moisture	%	11
Nitrates (NO_3^-)	mg/kg	40.3
Nitrites (NO_2^-)	mg/kg	1.64
Ammonium ions(NH_4^+)	mg/kg	8.4
Mobile nitrogen	mg/kg	16.49
Phosphates (P_2O_5)	mg/100 g	32.9
Humus	%	5.29
Exchangeable acidity	mg-eq/100 g	1.166
Cation exchange capacity	mg-eq/100 g	46.62

Table 4. Petroleum products concentration on the 1st and the 21st day of the experiment in the soil with the added excess sludge.

Biological treatment sludge content, kg/m^2	Petroleum products content, kg/m^2	Petroleum products concentration, mg/kg		Decrease of petroleum products concentration, %
		1st day of experiment	21st day of experiment	
0	0.44	5900	3800	35.59
0	0.66	7700	5400	29.87
1,8	0.44	5000	2905	41.90
1,8	0.44	3100	2600	16.12
1,8	0.66	8600	5700	33.72
1,8	0.66	6900	757	89.03
3,6	0.66	5400	4300	20.37
3,6	0.44	5900	847	85.64

3.3. Determination of biological activity of soils, contaminated with petroleum products, by the Mishustin method

In the experiment a natural linen canvas was used. In Fig. 3−6 the findings of the research for some soils, contaminated with petroleum products, are presented, with account of petroleum products removal efficiency (E_{re}, %). The variant, presented in Fig. 3, contained no excess sludge. During the experiment a linen canvas (10×10cm of size) was placed into the soil to the depth 15 cm, using a sterile spade. The linen is applied to a smooth section wall laterally. After the 20-day exposure the glass was excavated with the linen, and the soil particles were removed from it. Before the experiment and after finishing it the initial weight of the linen was determined and the decomposition ratio was calculated (Dr, %).

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4. Discussion

The findings of the research, concerning the influence of used petroleum products on microorganisms' cells colonies after applying the extract of corn-steep extract purification sludge (8-fold dilution) on the agarized growth medium, indicate that the used petroleum products not only don't inhibit the colonies' growth, but provide a source of biogenic carbon. The average number of colonies, grown after inoculation of this cultivation in the presence of petroleum products, amounted to 123 (№ 3 and 4 Fig. 2); without petroleum products – 28 (№ 2 Figure 2).

As we can see in Tab. 4, at the initially high concentration of petroleum products in the soil, on the 21st day of the experiment their concentration decreases more intensively at the site, where 0.44 kg/m^2 of petroleum products were applied and the created initial concentrations were within the range from 3.0 to 6.0 thousand mg/kg. The reduction of petroleum products content is directly related to the weight of the applied sludge. The percent reduction made up from 16.12 to 85.64%, respectively (at adding 0.44 kg/m^2 of petroleum products). In case of applying 0.66 kg/m^2 of petroleum products the percent reduction was 20.37; 33.72 and 89.03 %. This indicates that there are microorganisms in the biological treatment sludge, which can decompose petroleum products. The biological activity of soils increases with the increase of the applied sludge amount.

Figure 3. Dr = 38.4 %. E_{re} of petroleum products = 29.87 %

Figure 4. Dr= 34.2 %. E_{re} of petroleum products = 16.12 %

Figure 5. Dr = 20.37 %. E_{re} of petroleum products = 20.37 %

Figure 6. Dr = 92.60 %. E_{re} of petroleum products = 85.64 %

5. Conclusion
On the basis of the carried-out experiments we can make a conclusion that the application of waste products – excess biological treatment sludge and corn-steep extract purification sludge – is promising for the purpose of reducing petroleum products concentration in soil. Taking into account the high initial dose of petroleum products and their degradation during a short-term experiment, a conclusion is made, that the excess sludge of wastewater biological treatment has the potential for remediating soils, contaminated with petroleum products.

Acknowledgments
The work is realized in the framework of the Program of flagship university development on the base of the Belgorod State Technological University named after V.G. Shoukhov, using equipment of High Technology Center at BSTU named after V.G. Shoukhov.

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