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Search for biological means of oil adhesion decrease

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Decrease of oil adhesion is important for accelerating the degradation of oil contaminants in soil. Microorganisms are able to act as desorbents of insoluble substrata from surfaces. The effectiveness of microorganism filtrates and CO_2 – a product of polysaccharide fermentation – to decrease oil adhesion to soil was estimated. It was noticed that the process of oil removing from oil containing sandstone and polluted soil was more intensive when yeast *Candida lipolytica* and fungus *Trichoderma harzianum* strains were grown together. The oil film was more remarkable at 50 °C than at 20 °C. Various substrata were used for fermentation by yeast *Kluyveromyces marxianus,* but the highest content of CO_2 and the best extraction of oil from the soil were found in the case of glucose and whey fermentation. Sodium alkylbenzenesulfonate may be used as a washing agent together with microorganisms for decreasing oil adhesion in polluted soil cleaning.

Key words: microorganisms, biodegradation, oil-polluted soil, adhesion

INTRODUCTION

Decrease of oil adhesion to soil is important for degradation of contaminants in the environment and enables to extract oil from poor occurrences. The activation of microorganisms able to extract insoluble substrata from the surface may be used for this purpose.

Dichloromethane, chloroform, acetone and other solvents are used for removing oil products from soils and sediments (Genouw et al., 1994). The adhesion of oil to soil particles depends on the quantity of humus. Shaking contaminated soil with hot hexane steam effectively extracted oil products from rust soil, but it was less effective in black earth (100% and 75% during 30 min, respectively) (Груздкова, Лановенко, 1993). The technological cycle (submerging by nutrient solvents – drainage – aeration) was effective for elimination of oil products from soil under laboratory conditions (Widrig, Manning, 1995).

Microorganisms produce a wide spectrum of bioemulsifiers, which increase the surface area of hydrofobic insoluble substrata desorbing them from surfaces and regulate the sorption or desorption of microorganisms to/from surfaces. Consequently, biological emulsifiers may be used for cleaning oil-polluted soil or water, or for decreasing oil extraction (Rosenberg, Ron, 1999; Kuyukina et al., 2004; Jacobucci et al., 2005).

The aim of the current investigation was to estimate the effectiveness of microbiological means to decrease oil adhesion and remove oil from polluted soil.

MATERIALS AND METHODS

Quartz sandstone from an oil well (containing 3–6 mg/kg of oil) and loamy soil contaminated with oil products (5–6 mg/kg of fuel oil) were used for testing oil removal by microbiological means. Microorganisms (oil oxidizing yeasts and fungi) were selected using standard methods (Kurtzman, Fell, 1988; Звягинцев, 1991).

Microalgae were grown in tap water inoculated with water from a lake (100 ml/l) and enriched with 0.7 g/l of the Kemira Kombi complex mineral fertilizer (NPK 14-11-25). After three weeks the medium with microalgae was supplemented with the following substances (g/l): glucose – 50.0; KH_2PO_4 – 1.6; K_2HPO_4 – 0.3; $MgSO_4 \times 7H_2O$ – 1.0; $(NH_4)_2SO_4$ – 2.0; $CaCl_2 \times 6H_2O$ – 0.5; NaCl – 0.5; $Na_2SO_4 \times 10H_2O$ – 0.5; $FeCl_3$, H_3BO_3, $ZnSO_4$, $MnSO_4$ – traces; oil – 0.2; barley-water – 20 ml/l. The medium was inoculated with selected oil-oxidizing microorganisms: *Candida lipolytica* (F. C. Harrison) Diddens et Lodder C.6.1-5 (current name *Yarrowia lipolytica* (Wick., Kurtzman et E. A. Herrm.) van der Walt et Arx)*, Trichoderma harzianum* Rifai VNB-16 and the *Candida lipolytica+Trichoderma harzianum* complex. The inoculation dose was 10^6 colony forming units/ml (Repečkienė et al., 1999). The biomass of microorganisms was filtered off after 7 days of growth and the filtrates were used for oil-polluted soil treatment. 25 g of sandstone or oily soil was submerged in filtrates and exposed at 20 °C and 50 °C for 5 days. Oil extraction was estimated visually.

The removal of oil products from soil and sandstone under the action of CO_2 produced by yeasts during the alcoholic fermentation process of different carbon sources was studied in a specially constructed system. The produced CO_2 accumulated in a graduated test-tube and was measured in cm^3. The filtrate of microalgae was enriched with nutrient substances and one of carbon sources (glucose, molasses, flour waste, whey, 50 g/l). The medium was inoculated with *Kluyveromyces marxianus* (E. C. Hansen) van der Walt Kl.m1, and alcoholic fermentation lasted 24 h at 33–35 °C. The amount of CO_2, change of medium pH and removal of oil from the substrata were monitored.

The sensibility of microorganisms to the washing substance alkylbenzenesulphonate (ABSNa) was studied adding this surfactant to a malt agar medium at a concentration of 0.25% (recommended by producers), 0.5% and 1% (control – malt agar). The medium was inoculated with the test microorganisms and the diameter of colonies was measured after five days. The percentage of growth inhibition was calculated (Билай, 1982).

RESULTS

Selected active oil-oxidizing microorganisms able to pursue alcoholic fermentation of various substrata as well as products of their metabolism were examined for reduction of oil adhesion. The strains *Candida lipolytica* C.6.1-5 and *Trichoderma harzianum* VNB-16 were grown in media where green algae had been grown previously. Green algae release large amounts of organic compounds favourable for the development of microorganisms. Green algae developed intensively and spread gradually over all medium within three weeks. The growth of oil-oxidizing microorganisms in the media with algae, enriched with nutrient substances, was different. Fungus *Trichoderma harzianum* grew as a thick film on the surface, while yeast *Candida lipolytica* spread over all media. The yeasts stimulated the growth of the fungus: the dry biomass of *Trichoderma harzianum* was 8.4 g/100 ml, while grown in a complex with yeasts it reached 8.9 g/100 ml after 7 days. This may be important in preparing oil-oxidizing biopreparations for practical usage.

We noticed that oil was more easily extracted from polluted soil than from sandstone. The process of oil removal was more intensive at 50 °C. The most remarkable slick of oil was noted on the surface of the filtrate where a complex of the fungus and yeasts was grown, and contaminated soil was kept in the filtrate at 50 °C (results evaluated on a 5-point scale). The effectiveness of all variants of treatment is shown in Table 1. The removal of oil from sandstone visually was more difficult to estimate because of the low concentration of oil in them.

The yeast *Kluyveromyces marxianus* Kl.m1 strain exhibits a broad spectrum of alcoholic fermentation of carbon sources (Table 2). During the fermentation of

Table 1. **Intensity of oil extraction from oily soil and sandstone at 20 °C and 50 °C in filtrates of microalgae and oil-oxidizing microorganisms (5 point scale)**

Substrata	Temperature °C	Microorganisms		
		Trichoderma harzianum VNB-16	*Candida lipolytica* C.6.1-5	*Trichoderma harzianum* + *Candida lipolytica*
Sandstone (containing 3–6 mg/kg of oil)	20	1	1	1
	50	1	0	1
Soil (contamination level 5–6 mg/kg)	20	2	1	3
	50	5	3	5

Table 2. **Estimation of action on oily substrata of CO_2 produced by *Kluyveromyces marxianus* Kl. m1 during alcoholic fermentation of different carbon sources**

Oily substrata	Carbon source	Amount of CO_2, cm^3	pH		Estimation of removal of oil products
			At the beginning	At the end	
Soil (contamination level 5–6 mg/kg)	Glucose	24	6.4	5.7	Very thin slick of oil
	Molasses	21	6.3	5.9	Very thin slick of oil
	Flour waste	11	6.1	5.8	Traces of oil
	Whey	20	4.18	3.34	Thin slick of oil
	Whey with washing water	0	4.21	4.09	No fermentation. Extraction of oil invisible
Oily sandstone (containing 3–6 mg/kg of oil)	Glucose	23	6.4	5.6	Traces of oil
	Molasses	20	6.3	5.8	Traces of oil
	Flour waste	12	6.1	5.7	Extraction of oil invisible
	Whey	19	4.19	3.39	Traces of oil
	Whey with washing water	0	4.21	4.12	No fermentation. Extraction of oil invisible

different substrata 11–24 cm^3 of CO_2 was produced. The results show that the best removal of oil from contaminated soil was obtained under the action of CO_2 produced by yeasts during the fermentation process of glucose, molasses and whey. In these cases a thin slick of oil products was visible on the surface of the media. The fermentation of flour waste was weaker, and removal of oil was not remarkable. No alcoholic fermentation was noted in media with whey and washing water, maybe due to inhibition of yeast growth. The extraction of oil from sandstone was not as intensive as from oil-contaminated soil.

Surface-active agents may be used together with microorganisms to decrease oil adhesion. The alcoholic fermentation of whey mixed with washing water was very weak. Therefore the sensibility of microorganisms to the washing substance alkylbenzenesulphonate (ABSNa) was examined. The obtained results showed that *Kluyveromyces marxianus* Kl.m1 was rather tolerant to the test material (Figure). The diameter of *Kluyveromyces marxianus* colonies decreased from 6.7% (ABSNa concentration 0.25%) to 40% (ABSNa concentration 1%) as compared with control. The yeast *Candida utilis* C.6.1-5 was more sensitive: the diameter of colonies decreased by 31.8–81.8%. The growth of the test strain *Trichoderma harzianum* VNB-16 was inhibited almost completely. The test surfactant is suitable to use together with yeasts for oil removal from polluted substrata.

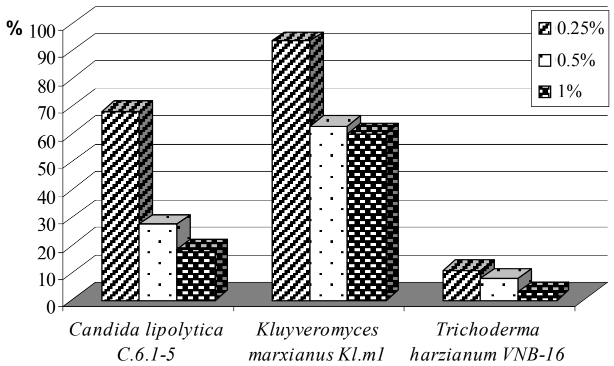

Figure. Inhibition (%) of microorganism growth in media with surfactant sodium alkylbenzenesulphonate

DISCUSSION

At present, microorganisms are widely used for bioremediation of oil-polluted soil (Onwurah-Ine, 1999; Isikhuemhen et al., 2003; Jankevičius, Liužinas, 2003; Vinas et al., 2005). Metabolites of microorganisms may be efficient as biosurfactants for cleaning soils contaminated with oil (Grigiškis, Žunda, 2001). The strains *Candida lipolytica* C.6.1-5 and *Trichoderma harzianum* VNB-16 screened as active oil-oxidizing agents (Repečkienė et al., 1999) were used in oil-adhesion decreasing studies. The results showed that adhesion of oil products to soil or sandstone was decreased under the action of filtrates of microalgae and active oil oxidizing strains *Trichoderma harzianum*, *Candida lipolytica,* and especially under the action of a mixture of these two cultures. The oil removal process was more intensive at 50 °C. Extraction of oil from sandstone was difficult to estimate visually because of the low concentration of oil (3–6 mg/kg) or possibly due to a stronger adhesion.

Hydrocarbon-degrading microorganisms produce surface-active materials which increase the surface area of hydrophobic water-insoluble substrata, thereby enhancing the growth of microorganisms and the rate of bioremediation (Ron, Rosenberg, 2002). Filtrates of media in which microalgae and oil-oxidizing microorganisms were grown, and CO_2, a product of microbiological polysaccharides fermentation, were used to decrease oil adhesion to soil.

The yeast strain *Kluyveromyces marxianus* Kl. m1 during alcoholic fermentation of various carbon sources produced remarkable quantities of CO_2 which decreased adhesion and enabled removing oil products from the substrata. Fermentation of molasses and whey may be recommended as a comparatively economic and effective source for alcoholic fermentation and CO_2 production.

Soil wash technologies are used for cleaning the soil from contaminants. Oil removal is caused by reduction of surface and interfacial tensions. Some oil-oxidizing microorganisms produce natural emulsifiers of hydrocarbons (Bicca et al., 1999). Biolological and synthetic surfactants are able to remove significant amounts of crude oil from contaminated soil (Urum, Pekdemir, 2004). In our research, the negative action of the washing material alkylbenzenesulphonate at a concentration of 0.25% on the growth of yeasts was rather insignificant. Non-toxic washing materials together with microorganisms characterized as pursuing alcoholic fermentation may be used for decreasing the adhesion of oil products to various substrata.

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BIOLOGINIŲ PRIEMONIŲ PAIEŠKA NAFTOS PRIEKIBAI SUMAŽINTI

S a n t r a u k a

Naftos ir jos produktų priekibos prie dirvožemio dalelių sumažinimas yra svarbus siekiant pagreitinti teršalų degradaciją ir apvalyti aplinką. Mikroorganizmai gamina medžiagas, kurios veikia kaip emulsifikatoriai ar desorbentai. Tirta mikroorganizmų augimo terpės filtratų ir polisacharidų alkoholinės fermentacijos produkto CO_2 įtaka naftos priekibos prie smiltainio ir priemolingo grunto sumažėjimui. Naftą oksiduojantys mikroorganizmai *Candida lipolytica* ir *Trichoderma harzianum* bei jų kompleksas gerai augo terpėje, kurioje pradžioje buvo auginti žalieji dumbliai. Augimo terpių filtratai pagreitino naftos išsiskyrimą iš naftingo smiltainio ir naftos produktais užteršto grunto. Procesas geriau vyko 50°C temperatūroje. Mielės *Kluyveromyces marxianus* vykdė gliukozės, melasos, miltų atliekų ir išrūgų alkoholinę fermentaciją, kurios metu išsiskyrusios anglies dvideginio dujos sumažino naftos priekibą prie grunto ir smiltainio. Geriausias naftos atsiskyrimas stebėtas, kai mielės fermentavo gliukozę ir išrūgas. Išrūgos gali būti naudojamos kaip pigus substratas fermentacijai vykdyti ir CO_2 gauti. Natrio alkilbenzensulfonatas neslopino mielių augimo, todėl ši sintetinė paviršiaus aktyvioji medžiaga gali būti naudojama kartu su mikroorganizmais naftos priekibai sumažinti.

Raktažodžiai: mikroorganizmai, biodegradacija, nafta užterštas dirvožemis, priekiba