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Bioconversion of oil sludge into biomass of lipid metabolites for use as a source of biofuel

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Abstract. The possibilities for the generation of biofuel from the results of the accumulation of lipids in oil-contaminated environments were studied. This type of accumulation occurs in the biomass of yeast strains *Rhodotorula* sp. VKM Y-2993D; in bacteria like *Pseudomonas libanensis* B-3041D and in consortia of microalgal strains such as *Acutodesmus obliquus* Syko-A Ch-055-12, *Chlorella* sp. SYKO A Ch-011-10, *Monoraphidium* sp., and *Anabaena* sp. The most promising of these for processing petroleum hydrocarbons into biofuels was found to be the consortium of microalgal strains, the content of palmitic acid of which reached 49.0 %, thereby achieving a mid-range cetane number.

1. Introduction

The accumulation of persistent pollutants which are almost indestructible in natural environments, or are destroyed only very slowly [1], both disrupts ecosystems and the connections in the biosphere established through long-term evolution, and undermines the capability of those ecosystems to self-regulate. Such persistent pollutants include oil sludge, as well as oil products (oils, diesel fuel, petrol, resins, etc.) that often have accumulated and contaminated the environment for years.

Legislation in many countries, including Russia, encourages the inclusion of industrial wastes into economic turnover, as sources of secondary raw materials. At present, numerous non-biological technologies are evolving for the processing oil wastes. These are designed to exploit the potential of the resource to generate various commercial products, such as road-building materials, secondary hydrocarbon crude, etc. Biological methods, however, are mainly aimed at enhancing the degradation of the residual oil contaminants with a loss of energy resource.

It is well-known that, among renewable energy sources, biomass has been widely used as the basis for generating biofuels (biodiesel, bioethanol) [2, 3]. Studies are ongoing on to evaluate a range of conditions and methods for the bioconversion of various substrates into microbial biomass. The accumulation of microorganism biomass in industrial wastes is a promising energy-related and environment-friendly solution to this problem [4]. We have proposed a fundamentally new approach to obtaining secondary energy raw materials from industrial wastes by way of the bioconversion of oil sludge into lipid metabolites with petroleum-oxidizing microorganisms, and their accumulation of fatty acids that can subsequently used for generating biodiesel. Through the accumulation of useful microorganism biomass, this has the advantage of helping to solve, not only the problems of waste utilization but also of bioremediation at sites of oil spillages.

Biodiesel is a multicomponent liquid fuel consisting of methyl and ethyl esters of higher unsaturated fatty acids [5]. Fatty acids and higher fatty alcohols accumulate in the cells of the



microorganism in the form of droplets, and as the phospholipids in membranes, etc. [6]. Fatty acids obtained from microorganisms need to conform to CN standards in order to be used for biodiesel production. Diesel fuels with CN 45 to 55 ensure the best performance in up-to-date diesel engines. The higher the overall content of palmitic and stearic acids, the higher the quality of the biodiesel [7].

2. Material and methods

The purpose of this work is to identify features of the microbial bioconversion of petroleum hydrocarbons into lipid metabolites.

For our experiments, we used native microorganisms (strains of bacteria, yeast, microalgae) extracted from oil-contaminated soils of the Usinsk district. These were incubated in soil microcosms enriched with mineral oil (30%) at room temperature, under natural lighting, at 220 rpm over periods of 3, 30 and 90 days. The following microcosms were used: 1 – with an inoculum of yeasts and bacteria, 2 – with microalgae, 3 – with an alga-bacteria-yeast consortium. For the investigation we used *Rhodotorula* sp. VKM Y-2993D yeasts, *Pseudomonas libanensis* B-3041D bacteria and a consortium of microalgal strains: *Acutodesmus obliquus* Syko-A Ch-055-12, *Chlorella* sp. SYKO A Ch-011-10, *Monoraphidium* sp. and *Anabaena* sp., respectively. As a control, the concentration of fatty acids in an oil-contaminated microcosm without microorganism inoculation was determined.

Methyl esters of fatty acids were identified using the method of K. Sinyak-et al [8]. Saturated fatty acids contained in the lipid metabolites of the microbiota were determined using chromatography-mass-spectrometry [9].

3. Results and discussion

Analysis of the conditions needed for the bioconversion of oil hydrocarbons into fatty acids showed that the maximum accumulation of fatty acids occurred after 3 days of incubation. It is likely that, during their use of the easily oxidized oil products, the cells accumulated lipids as reserve materials, and that as the readily used resources disappeared and environmental conditions deteriorated the cells then accessed the reserves for continuation of their metabolic processes. As a result, after 30 and 90 days the fatty acids content had fallen again, almost to zero. The component composition of fatty acids in the system included carboxylic acids with chain lengths from C₁₂ to C₁₈ and having even numbers of carbon atoms. The strain specificity of the accumulation of stearic and palmitic acids is noteworthy. In the biomass of the bacteria, yeast and microalgal consortia, palmitic acid predominated (table 1). Table 1 shows that accumulation of palmitic acid C_{16:0} occurs in all the variants compared with the control, but that the maximum accumulation, matching the CN standards was observed in microcosm 2 with the microalgal inclusion. Furthermore, this microcosm was also high in linoleic acid – an output of 22.9%, requiring separation of these acids: palmitic acid for biodiesel generation and linoleic acid for therapeutics and cosmetics.

Table 1. Content of fatty acids in different microcosms (bacteria, yeast, microalgal, consortium), %.

Variant	C _{12:0} ^a	C _{14:0}	C _{14:1}	C _{16:0}	C _{16:1}	C _{18:0}	C _{18:1(9)}	C _{18:1(11)}	C _{18:2cis}
C	0	5.5	2.39	33.6	13.56	4.72	10.1	2.08	28.0
1	0.5	3.6	2.3	44.2	24.2	1.7	1.8	7.8	14.0
2	1.3	4.9	2.7	49.0	4.3	3.3	9.0	2.5	22.9
3	2.0	6.4	6.0	34.4	14.0	1.0	7.1	6.2	22.8

^aC_{12:00} – lauric acid; C_{14:0} – myristic acid; C_{14:1} – myristoleic acid; C_{16:0} – palmitic acid; C_{16:1} – palmitoleic acid; C_{18:0} – stearic acid; C_{18:1(9)} – oleic acid; C_{18:1(11)} – vaccenic acid; C_{18:2} – linoleic acid.

It is interesting to compare the CNs of the resulting products with the existing standards for diesel, and bio-diesel fuels from oil seed rape and from Mucorales fungal lipids *Cunninghamella japonica*

(table 2). The need to devote large areas of land for the generation of biodiesel by growing gramineous plants restricts such activity, particularly considering that, only the seed oil is used for production, and the majority of the plant biomass is not used. Furthermore, as oil-generating plants also require frequent crop rotation [10] conclusion can be drawn that, reforming oil wastes into biofuel with the help of microorganisms is a viable alternative.

Table 2. Comparative Analysis of Fuel.

Source	CN	Reference
Traditional diesel fuel	47	[11]
Biodiesel (EN14214)	>51	[12]
Plant raw material, <i>Brassica napus</i>	54.4	[11]
	51.74	[13]
Mucoral fungus biomass <i>C. japonica</i> F-1204 (-)	55.68	[14]
Biomass of a consortium of microalgal strains <i>A. obliquus</i> , <i>Chlorella</i> sp., <i>Monoraphidium</i> sp., <i>Anabaena</i> sp.	49.0	Proprietary data

However, as the overall content of palmitic and stearic acids is not very high (table 1), the resulting biodiesel cannot be considered to be of premium grade.

Thus, of the microorganisms tested the consortium of microalgal strains (*Acutodesmus obliquus*, *Chlorella* sp., *Monoraphidium* sp., *Anabaena* sp.) generating 49.0% palmitic acid and thereby providing an acceptable cetane number, was found to be most promising in respect of reprocessing waste oil products into biodiesel.

Presumably, attempts can be made to increase the yield of stearic acid, it will be necessary to include into the consortium additional microorganism capable of synthesizing this acid.

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