The algae-macrophytes of the Barents Sea in bioremediation of marine environment of oil products

G.M.Voskoboynikov¹, M.V.Makarov¹, L.O.Metelkova², I.V.Ryzhik¹ and D.V.Pugovkin¹

¹Federal State Budgetary Institution of Science Murmansk Marine Biological Institute of Kola Scientific Centre of Russian Academy of Sciences
Murmansk, Russia
²Federal State Budgetary Institution of Science Saint-Petersburg Science and Researches Center of Ecological Safety of Russian Academy of Sciences
Saint-Petersburg, Russia

E-mail: grvosk@mail.ru

Abstract. There are shown the results of the researche of the probable role of marine algae-macrophytes in cleaning the coastal waters from oil products. The results of researches demonstrate a high ability of sorption of algae Fucus vesiculosus and Ulvaria obscura to oil products: decreasing the containment of oil products in water, in parallel its accumulation in algae. A destruction of oil products of ulvaria goes as of fucus, the whole thalli: starts at the surface of algae with epiphytic hydrocarbonoxidizing bacteria which provides absorbing and neutralization of oil products by the cells of plants. The received information makes a contribution to an evidence base of participance of algae-macrophytes in bioremediation of a marine environment, allow to estimate its role in clearing the coastal waters from oil products much more higher than it was considered before. The information about the possible role of symbiotic association of marine algae and hydrocarbonoxidizing bacteria in bioremediation have become a base for creation the technology of sanitary algae plantation (SAP) – plantation-biofilter, directed to cleaning the coastal waters of the Arctic Seas. The approbation of plantation at the bays of the Barents sea and the White Sea has already shown it's high efficiency.

1. Introduction

Previously there was identified an ability of some species of algae-macrophytes to survive for a long time at the litoral of the Barents Sea in conditions of a constant oil pollution(1). There was shown an ability of algae Fucus vesiculosus to form a symbiotic association with epiphytic hydrocarbonoxidizing bacteria, and the number of hydrocarbonoxidizing bacteria on the syrface of fucus in oil-polluted waters is much more higher compared to the algae from clear places of habitats. It has been suggested that hydrocarbonoxidizing bacteria transforms oil products, accumulated on the surface of fucus, making them available to be absorbed by the algae (2-4).

Laboratory experiments on the effect of oil products on algae have demonstrated a decreasing of content of added diesel fuel in water in the presence of fucus in several times (5). Parralely during 15 days of experiment a content of oil product in a thalli of algae have increased. The gross content of oil products in algae have increased from 3,9 mkg/g (0 days) to 179 mkg/g (15 days).
But it was still unclear if only *F. vesiculosus* has such stability to oil products and an ability to its absorbing or the other algae also actively participate in a process of bioremediation?

To answer this question we have conducted extra experiments with algae *F. serratus*, *Ulvaria obscura*, inhabiting in a litoral zone of the Zelenetskaya bay of the Barents Sea, and *Saccharina latissima* (*Laminaria saccharina*) of sublitoral. Such experiments showed that all these dominant species demonstrate different level of stability to toxicant. All the three species of algae remained viable up to 15 days in concentration of oil products of 20 % of maximum permissible concentration which we can observe in a number of bays of the Barents Sea. But in higher concentrations *S. latissima* was dying, but at the same time ulvaria and focus still have maintained their vitality.

To find out an ability of algae of absorbing and further transforming oil products there were conducted extra experiments on *U. obscura*, one of the inhabitants of constantly polluted waters of the Barents Sea. The new information of biology of algae-macrophytes of the Barents Sea, mechanisms of its probable participating in cleaning the coastal waters from oil pollution (6) is a base for creation the technology of sanitary algae plantations, which acquires special significance in the Arctic latitudes, where the process of natural degradation of oil and oil products is significantly slowed down (low temperature, polar night).

2. Materials and methods

The experiments have been conducted in 3-litres containers with marine water salinited 33%. In water, previously filtered through the cotton gauze filter, there was added summer diesel fuel in amount of 2.87 mg/l. The concentration of diesel fuel is nearly 60 times exceeded the maximum permissible concentration for marine water. The vegetative thalli of *U. obscura* have been selected on the litoral of the Zelenetskaya bay of the Barents Sea (69°07'09" n.latitude, 36°05'35" e.longitude), cleaned if the probable foulings, weighted and immerse in the containers with water. The experiment has been conducted in thermostatic counters at the temperature 7-8ºC, the lightness 16-18 vt/m² and with constant aeration of water. The whole duration of the experiment was 15 days. Every 5 days the samples of water and algae have been selected and researched with a method of GC/MS (gas chromatography / mass spectrometry) to show the content of oil products in them. The algae haven't effected by the oil products, were researched as a control sample (the content of individual n-alkanes in a control sample hasn't exceeded 0.1 mg/kg/l, which is incomparably small to concentrations measured in experiment).

The process of sample preparation and instrumental analysis were conducted on the base of the method EPA 8270 (Semivolatile Organic by GC/MS). For preparing samples of water there were used liquid-liquid extraction in neutral value of indicator of acidity of environment. The samples of algae were extracted and ultrasound installation. The solute of inner standart (2-fluoronaphthalene) was added before extraction in probes of water and right before GC/MS analysis in extracts of algae. The analysis of extracts was conducted using chromato-mass spectrometer of a low resolution DSC II of a company Thermo Finnigan. The calculation of the mass fraction of content of components of diesel fuel was made using methods of inner standart. Calculating the meanings of concentration it's taken in account a coefficient of sensitivity of mass-spectromatic detector in pairs 2-fluoronaphthalene/deuterated pentadecane (D32) and 2-fluoronaphthalene/deuterated eicosane (D42).

3. Results and discussion

The visual observations of thalli and also with a light microscope of the construction of cells of ulvaria haven't revealed any signs of degradation of algae during the whole experiment. The research showed a decreasing of a gross content of oil products and a whole content of individual components of diesel fuel (alkanes C11-C25) in water during the experiment. The gross content – from 6.6 mg/l (5 days) to 2.2 mg/l (15 days), an amount of alkanes – from 3.2 mg/l to 0.95 mg/l. The changes of correlation C17/priestane can testify of actively running process of degradation of oil products in water.

At the same time with the increasing of content of oil products in samples of water there was observed an increasing of its content in water. The changes of content of individual components of diesel fuel of
ulvaria in the process of experiment is shown at pict.1 The achieved information demonstrates the increasing of concentration of each component. The gross content of oil products in algae is also increasing over time from 6.1 mkg/g (0 days) to 1927 mkg/g (15 days). The changes of correlation of C17/priestane and water can point on degradation of oil products.

The analysis of a place of degradation of oil products (at the surface or inside the algae) has shown that in both cases the samples are saturated with hydrocarbons. The significant difference of the qualitative or quantitative compositions of alkanes isn’t observed. Logical to imagine that either in case of ulvaria or in case of fucus (5, 7) the destruction of oil products starts at the surface of algae which provides absorbing and further destruction of oil products by the cells of a plant. This suggestion is supported by information on the presence of epiphytic bacteria on the ulvarium surface and also preliminary information about the ability of a representative of the ulv family, Ulva lactuca, to metabolize the gasoline hydrocarbons (8).

The research showed the decreasing of gross content of oil products and the whole content of individual component of diesel fuel (alkanes C11-C25) in water during the experiment. The gross content – from 6.6 mg/l (5 days) to 2.2 mg/l (15 days), an amount of alkanes – from 3.2 mg/l to 0.95 mg/l. The marked changes of correlation C17/priestane can testify of actively running process of degradation of oil products in water.

The results of researches demonstrate a high ability of sorption of algae Fucus vesiculosus and Ulvaria obscura to oil products: decreasing the containment of oil products in water, in parallel its accumulation in algae. A destruction of oil products of ulvaria goes as of fucus, the whole thalli: starts at the surface of algae with epiphytic hydrocarbonoxidizing bacteria which provides absorbing and neutralization of oil products by the cells of plants (6). The received information makes a contribution
to an evidence base of participance of algae-macrophytes in bioremediation of a marine environment, allow to estimate its role in clearing the coastal waters from oil products much more higher than it was considered before, and also become a base for creation satitary algae plantation (SAP) – plantation-biofilter for cleaning the coastal waters from oil pollution.

4. From theory to practise
The sanitary algae plantation (the SAP) is an engineered construction with a bio component represented by the symbiotic association of laminaria and (or) fucus algae and hydrocarbonoxidizing bacteria. Especially the properties of bio component as a technology of staging the plantation provides a successful annual functioning of SAP, directed to bioremediation of coastal waters (5).

5. The biotic component of sanitary algae plantation
Before the selection of biotic component of SAP there were researches of biological features of algae and microorganisms, mostly their range and mechanisms of tolerance to factors of outer environment, ability of bioremediation, and the taxonomic affiliation. In the base of technology of mutually beneficial symbiosis of algae and hydrocarbonoxidizing bacteria. The algae as a substrate enriches the environment of microorganisms with oxygen. The bacteria partly neutralize the oil products and make them available to be absorbed by algae (7).

6. The algae component of symbiotic association
*Fucus vesiculosus* L. is spread in the Atlantic and the Arctic Ocean. In the Barents Sea it grows in the upper and in the middle horizontal layers of litoral. It has a high environmental plasticity, inhabits in open and protected areas, in conditions of oceanic salinity and in highly desalination areas (to 10%), stable to ultraviolet, draining, influence of negative and positive temperatures, oil pollution. Practically all the fucus, selected in polluted areas at the litoral, demonstrated an ability of growth, photosynthesis, pigment synthesis, hydrocarbons, lipids. Because of this in the suggested technology of SAP the main charge of preventing the spread of oil film and neutralization of oil products in the surface layer of water goes on *F.vesiculosus*.

*Laminaria saccharina (L.) Lamour = Saccharina latissima* in the Barents Sea forms wide thicket in sublitoral zone (12-15 meters deep), in areas with intensive movement of water. Instead of fucus, Saccharina is less stability to the influence of factors of outer environment: ultraviolet, desalination, and as the experiments have shown, the oil pollution (9, 10). *Fucus serratus* demonstrates less stability than *F.vesiculosus* to factors of outer environment but it is more stable to these factors than *S. latissima*. *F.serratus* grows in the lower horizont of litoral, reaches sublitoral. It can be set on the plantation at a greater depth than *F.vesiculosus*. Phytophagous. On the horizontal ropes of plantation set on the surface of water, there are a lot of phytophagous, including Ulvaria, considering that they can participate in absorption of oil products from marine water.

7. The microbiological component of symbiotic association
The questions connected with a state of epiphytic bacterial community of coastal waters of the Arctic seas haven't been discussed until recently. During several years we have conducted researches of epiphytic bacteria of fucus algae (*F.vesiculosus*), their number and taxonomic structure. And special attention was paid to the influence of anthropogenic factors (the oil pollution of environment) on the bacteria and on the algae which is a substrate for them.

The number of saprtrophic bacteria (including hydrocarbonoxidizing bacteria) on the surface of macrophytes in polluted areas is higher than on the algae selected in clear areas (3, 11). Previously using the standard methods of microbiolog we have picked out and identified the dominant species of cultivated hydrocarbonoxidizing bacteria (11). In polluted and in clear areas on the surface of algae there was noted the representatives of different species: *Pseudomonas fluorescens* and *Ochrobactru anthropi*. In clear areas there were also noted epiphytic *Pseudomonas guinea*, in polluted
areas there were Rhodoccocus fascians. Pseudomonas and Rhodoccocus are cultivated bacteria, easily adapt to the conditions of cultivation, which gives a possibility for the future researches in laboratory conditions.

The methods of molecular biology allow to get a more complete picture of bacterial association in water areas with different levels of pollution of oil products. All the representatives of epiphytic bacterial association are in Actinobacteria, Bacteroidetes, Planctomycetes, Proteobacteria, Verrucomicrobia, Acidobacteria, Cyanobacteria, Firmicutes, Fusobacteria, TM7 (12). Their correlation in associations varied depending of the level of pollution of the environment.

The least variety of epiphytic bacterial association have been noted on the algae from relatively clear water area of the Zelenetskay bay (12). The dominated groups were the representatives of Proteobacteria (Alphaproteobacteria and Gammaproteobacteria) and Bacteroidetes (Sphingobacteria and Flavobacteria). On the fucus from the polluted water area of the Marine port (the Kola bay) there were a prevalence of bacteria of Proteobacteria (~63%) wit a domination of representatives of Acinetobacter (32% of content of bacteriocoenosis). On the fucus from moderately polluted by oil products water area near the village of Abram-mis there were detected a maximum number of bacterial genera (66), and there were also a domination of representatives of Proteobacteria, among them there were a majority (35%) of alphaproteobacteria (11,12).

At all both in clear and in polluted water areas there were detected 82 bacterial genera.

8. The bases of technology of staging SAP in coastal waters of the Barents Sea.
In the bases of SAP there is a technology of a plantation growing of laminaria algae (Figure 1) developed in the Murmansk Marine Biology Institute of KSC RAS and approbated at the Zelenetskaya bay (the Eastern Murman) of the Barents sea (13, 14).

![Figure 1](image1.png)

Figure 2. The scheme of sanitary algae plantation (1 – gravitation anchors of cement or natural stone weighted 60-100 kg ; 2 – vertical synthetic supporting ropes (10-20 m length) ; 3 – horizontal synthetic ropes (5-20 m length) with added thalli of F.vesiculosus ; 4 – vertical lead ropes (5-20 m length) with added thalli of Saccharina latissima or contested ; 5 – a cargo for holding the leads in a vertical position ; 6 – a float ).

Staging the SAP is performed according to the scheme neared to staging the laminaria plantation. The features of technology of SAP are: 1) in technology of SAP in horizontal ropes (the surface layer of water) on its length of 10-20 m there are added plants (F.vesiculosus, at the age of 3-4 years) selected at the litoral. Adding is made in groups of 3-4 plants every 5 sm. Usually the horizontal ropes in the surface layer of water are set in two parallel lines. 2) In bioremediation of deep layers of water (up to 20 m), during preparing SAP there used a method of interweaving the young thalli of S.latissima (10-20 sm) in vertical substrates – the leads up to 20 m length. The selection of thalli of S.latissima is conducting in the upper horizont of sublitoral it is used specially planted seedlings. Interweaving the selected plants in the leads prepared in advance is made in groups of 2-3 plants every
3) In case of using fucus algae in bioremediation of layers of water to the deep of 8 m, specially staging SAP at desalinated coastal regions, the selected thalli of F. vesiculosus are interweaving into substrates in groups of 3-4 plants every 5 sm. 4) Frequently staging plantation is to be proceeded on a long distance from the place of preparing substrates. In this cases there is a point to make all the preparatory works at a place of the main basement, and a delivery to the place of standing the plantation is to be proceeded for substrates with already interweaved algae. It's recommended to have some “planting areas” to provide storing in the sea of ready for staging at plantation substrates with algae. Substrates with fucus algae are better to be placed at the litoral, in usual for algae place of habitat (Figures 3-4). Substrates with thalli of S.latissima, future vertical ropes of plantation, are to be placed for overexposure under water on the deep not less than 0,5-1 m.

![Figures 3-4. The creation of “planting areas” for overexposure of substrates with biomaterial.](image)

The delivery of substrates of algae to the place of staging of plantation is made only after the skeleton of plantation is ready: the anchors, placed along the bottom, fixing vertical ropes with floats on the surface.

9. The aprobation of the SAP.
To aprobate the technology of SAP there was chosen an area at the Olenya bay of the Kola bay of the Barents sea – the waters of ship repairing factory “Nerpa” (SRF “NERPA”), which have specialized in ship repairing and utilization of submarines.

![Figure 5. The sanitary algae plantation in the Olenya bay. Horizontal ropes (two lines) with fucus algae.](image)
In the Olenya bay there are several sources of oil pollution: SRF “Nerpa”, a pier, an oil base and a parking of ships.

At the Olenya bay the sanitary algae plantation have existed for 2.5 years (Figure 5). It has survived after several storms and the spills of oil products. The monitoring of plantation has showed the correctness of the staging technology. At the plantation here was observed a growth of fucus algae, appearing of young seedlings of laminaria, may be, as the result of spontaneous release of plant spores. This way, the SAP can be considered as self-replicating system. According to analogic scheme a sanitary algae plantation was staged at the Kandalakshskaya bay of the White Sea in the waters of an oil base of Belomorsk, where there was a constant leak of oil products. Firstly staging the plantation have been taken place parallely with staging the floating barrier. The bonnes have prevented the spreading of oil products on the surface of water, and the SAP has had a goal of cleaning the limited by bonnes from oil products. Plantation-biofilter functionted in the area of the oil base of Belomorsk for 4 months till the liquidation of the source of pollution. This work had a great significance because of closeness of oil base of Belomorsk to the coastal area of the state national park of Kandalaksha.

The suggested technology scheme of SAP is multipurpose either for the north seas or for the Far eastern and southern. A difference is a bio component to be selected according to features of a region. So in the Far Eastern seas there can be Sargassum, Laminaria, and in the southern seas – Cystoseira. Naturally this leads to a change of a content of epiphytic microorganisms.

**Acknowledgments**
The researches were made with a financial support of programm of RFFI “Dangerous situation”, grant N 18-05-80058. informacionnye i biologicheskie tekhnologii v osvoenii resursov shel'fovых morej. M.: Nauka, 2005. P. 256-273.

**References**
[1] Voskoboinikov, G.M., Matishov, G.G., Bykov, O.D., Maslova, T.G., and Usov, A.I. Resistance of marine macrophytes to oil pollution // Doklady Biol. Sci., 2004, vol. 397, N 6. P. 340–341.
[2] Voskoboinikov, G.M., Il’inskii, V.V., Lopushanskaya, E.M. and Pugovkin, D.V. Possible role of marine macrophytes in water surface purifying of oil pollution // Oil and Gas of Arctic Shelf - 2008. Proceedings of International Conference: Murmansk, November 12-14. Murmansk: MMBI KSC RAS, 2008. P. 66-67.
[3] Voskoboinikov G.M., Pugovkin D.V. O vozmozhnoj roli Fucus vesiculosus v ochistke pribrezhnyh akvatorij ot neftyanogo zagryazneniya // Vestnik MGTU, 2012, vol. 15, N. 4, P. 716-720.
[4] Il’inskij V.V., Voskoboinikov G.M., Pugovkin D.V., Komarova T.I., Adeikina A.A. Vliyanie neftyanogo zagryazneniya sredy na sostav i chislennost' geterotrofnych ehpiifitnyh bakterij buroj vodorosli Fucus vesiculosus // Vestnik Yuzhnogo nauchnogo centra RAN, 2010, vol. 6, N 2, P. 98–100.
[5] Voskoboinikov G.M., Il’inskij V.V., Lopushanskaya E.M., Makarov M.V., Pugovkin D.V., Ryzhik I.V., Liaimer A., Jensen J.B. Sanitarnaya vodoroslevaya plantaciya dlya ochistki pribrezhnyh akvatorij ot nefteproduktov: ot teorii k praktike // Voprosy sovremennoj al'gologii. 2017. № 3 (15). P. 160-186
[6] Voskoboinikov G. M., Matishov G. G., L. O. Metelkova, Z. A. Zhakovskaya, and E. M. Lopushanskay Participation of the Green Algae Ulvaria obscura in Bioremediation of Sea Water from Oil Products // Doklady Biol. Sci. vol. 481, N 1, P. 139-141
[7] Semenov A. M., Fedorenko V. N., Semenova E. V. Microorganisms on the surfaces of marine macrophytes in the northern seas of Russia and prospects for their practical application // Biosphere, 2014. Т. 6, № 1. P.60-70
[8] Pilatti, F., Ramlov, F., Schmidt, E., Kreusch, M., Pereira, D., Costa, Ch., de Oliveira, E., Bauer, Cl., Rocha, M., Bouzon, Z., and Maraschin, M., In vitro exposure of Ulva lactuca Linnaeus
(Chlorophyta) to gasoline - Biochemical and morphological alterations // Chemosphere, 2016, N. 156. P. 428–437.

[9] Voskoboinikov G.M. Mekhanizmy adaptacii, regulyacii rosta i perspektivy ispol'zovaniya makrofitov Barentseva morya // Avtoref. soisk. uch. step. dokt. biologich. nauk. Murmansk, 2006. 46 p.

[10] Makarov M.V. Adaptaciya vodoroslej Barentseva morya k usloviyam osveshcheniya. // Avtoref. soisk. uch. step. dokt. biologich. nauk. Murmansk, 2006. 46 p.

[11] Pugovkin D. V., Liaimer A., Jensen J. B. Epiphytic bacterial communities of the alga Fucus vesiculosus in oil-contaminated water areas of the Barents Sea // Doklady Biol. Sci. 2016, vol. 471, N 1 P. 269-271.

[12] Pugovkin D.V. Ehphitnye bakteriocenozy Fucus vesiculosus L. Barenceva morya i ih rol' v degradacii neftyanych zagryaznenij // Avtoref. soisk. uch. step. kand. biol. Nauk. Murmansk, 2017. 23 p.

[13] Makarov V.N., Dzhus V.E., Matishov G.G., Hohryakov K.B., Voskoboinikov G.M., Denisenko N.V., Shoshina E.V. Nauchno-prakticheskie aspekty kul'tivirovaniya laminarii saharistoj v Barencevom more: Prepr. Apatity: Izd. KNC RAN, 1987. 44 p.

[14] Voskoboinikov G.M., Makarov M.V., Panteleeva N.N. Problemy i perspektivy biotekhnologii kul'tivirovaniya buryh vodoroslej v Barentsevom more // Sovremennye informacionnye i biologicheskie tehnologii v osvoenii resursov shel'fovyh morej. M.: Nauka, 2005. P. 256-273.