Original paper

The Ability to Accumulate and Transform Diesel Fuel by Green Algae *Ulva lactuca* of the Barents Sea

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Abstract

The article presents the results of experiments on the ability of the green alga Ulva lactuca to absorb and transform diesel fuel from marine water for 5 and 10 days. The original marine water contained 0.62 mg/L of petroleum hydrocarbons, which is about 12 maximum permissible concentrations (MPC). During the experiment with the addition of 20 mg/L of diesel fuel (400 MPC) to the water, the absorption of the introduced hydrocarbons was observed in the experimental tanks without algae. Apparently, they were absorbed by water microorganisms. On the 5th day of the experiment, the petroleum hydrocarbons concentration in the water decreased by 40% and amounted to 12 mg/L (240 MPC). When ulva thalli were added to the water, the total content of petroleum hydrocarbons in the water on the 5^{th} day decreased by 86% (to 2.8 mg/L), and on the 10th day, it increased (to 4.2 mg/L). A slight increase in the concentration of diesel fuel hydrocarbons in water indicates a reverse process of releasing hydrocarbons absorbed by ulva into water. In the experiment with the addition of diesel fuel to the water at a concentration of 10 mg/L, the content of hydrocarbons in algae tissues on the 5th and 10th days was recorded at the level of 0.6 mg/g. The marker ratio of \sum n-alkanes / \sum petroleum products in ulva during the experiment was 0.2. A decrease in this indicator to 0.18 on the 10^{th} day of the experiment indicates the beginning of the transformation of the hydrocarbons chemical structure. When 20 mg/L of diesel fuel (400 MPC) were added to the water, this indicator on the 5th and 10th days was 0.25 and 0.28, respectively, indicating an active process of hydrocarbon absorption by the algae surface, which was not yet complete by the 10^{th} day. The experiment results allow us to conclude that U. lactuca is able to absorb and transform petroleum hydrocarbons and participates in the bioremediation of coastal waters.

Keywords: *Ulva lactuca*, Barents Sea, diesel fuel, petroleum product accumulation, petroleum product destruction, pollution tolerance

Acknowledgements: The work was funded by the Russian Science Foundation under grant 22-17-00243 "Radiation oceanology and geoecology of the coastal shelf of the Barents and White Seas. Bio-abiotic interactions in the system: bottom sediments–water–macroalgae–microorganisms, their role in remediation of the marine coastal ecosystem under radiation and chemical contamination in the Arctic conditions".

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Способность к аккумуляции и трансформации дизельного топлива у зеленой водоросли *Ulva lactuca* Баренцева моря

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Аннотация

Оценена способность зеленой водоросли Ulva lactuca к поглощению и трансформации дизельного топлива из морской воды в ходе экспериментов длительностью 5 и 10 сут. Исходная морская вода содержала 0.62 мг/л нефтяных углеводородов (около 12 ПДК). В ходе эксперимента с добавлением в воду дизельного топлива 20 мг/л (400 ПДК) в опытных емкостях без водорослей наблюдался процесс поглощения введенных углеводородов, по-видимому, микроорганизмами, обитающими в воде. На пятые сутки опыта концентрация нефтяных углеводородов в воде снизилась на 40 % и составила 12 мг/л (240 ПДК). При добавлении в воду талломов ульвы валовое содержание нефтяных углеводородов в воде на пятые сутки уменьшилось на 86 % (до 2.8 мг/л), а на десятые сутки увеличилось (до 4.2 мг/л). Незначительное увеличение концентрации углеводородов дизельного топлива в воде говорит об обратном процессе высвобождения поглощенных ульвой углеводородов в воду. В опыте с добавлением в воду дизельного топлива в концентрации 10 мг/л содержание нефтяных углеводородов в тканях водорослей на пятые и десятые сутки было зарегистрировано на уровне 0.6 мг/г. Маркерное соотношение Σ н-алканов/ Σ нефтепродуктов у ульвы в течение эксперимента равнялось 0.2. Снижение этого показателя до 0.18 на десятые сутки опыта свидетельствует о начале трансформации химической структуры углеводородов. При добавлении в воду дизельного топлива 20 мг/л (400 ПДК) этот показатель на пятые и десятые сутки составил 0.25 и 0.28 соответственно, что указывает на активное поглощение углеводородов поверхностью водорослей, которое к десятым суткам еще не завершилось. На основании результатов экспериментов делается вывод о способности U. lactuca к поглощению и трансформации нефтяных углеводородов и ее участию в биоремедиации прибрежных акваторий.

Ключевые слова: *Ulva lactuca*, Баренцево море, дизельное топливо, аккумуляция нефтепродуктов, деструкция нефтепродуктов, толерантность к загрязнению

Благодарности: работа выполнена при финансовой поддержке гранта РНФ 22-17-00243 «Радиационная океанология и геоэкология прибрежного шельфа Баренцева и Белого морей. Биокосные взаимодействия в системе: донные отложения – вода – макроводоросли – микроорганизмы, их роль в ремедиации морской прибрежной экосистемы при радиационном и химическом загрязнении в условиях Арктики». Для цитирования: Способность к аккумуляции и трансформации дизельного топлива у зеленой водоросли *Ulva lactuca* Баренцева моря / Г. М. Воскобойников [и др.] // Экологическая безопасность прибрежной и шельфовой зон моря. 2024. № 4. С. 95–105. EDN VCUXQT.

Introduction

In recent years, interest in the possible role of macrophyte algae in bioremediation of coastal waters from petroleum products (PP) has increased. This is caused by the information obtained about the ability of algae not only to accumulate PP on the surface of the thallus, but also to absorb and further transform and incorporate PP into the metabolism of cells. The described processes are carried out largely due to hydrocarbon-oxidising bacteria (HOB), which are present in large numbers on the surface of macroalgae, especially in petroleum-contaminated coastal waters [1, 2]. The objects of research on the effect of PP on algae as well as the role of algae in environmental bioremediation were mainly representatives of brown algae, such as laminaria and fucus algae, which constitute the main phytomass in the coastal area of the Barents Sea¹⁾ [3, 4]. A smaller amount of data on the aforementioned subject has been obtained for green algae, although the literature suggests that they also have some tolerance to petroleum contamination [5–7]. Earlier in our experiments on the influence of diesel fuel (DF) on the early stages of Ulva lactuca development it was shown that at DF concentration of 5 mg/L (100 MPC) in water, a slowdown of seedlings development on the 20th day of the experiment was observed, at 25 mg/L (500 MPC) – on the 10th day and at 50 mg/L (1000 MPC) toxicant content, the death of seedlings was observed on the 5th day of the experiment [8]. In experiments on the effect of DF on the species U. intestinalis, closely related to Ulva lactuca, it was observed that the addition of DF to the medium at a concentration of 1-5 mg/L (20-100 MPC) did not result in the death of algae, but rather caused a decrease in photosynthetic activity and content of photosynthetic pigments. The addition of DF in the range of 50 to 150 mg/L (1000-3000 MPC) to the environment has been observed to induce gradual and irreversible changes in algae, ultimately resulting in plant death. It has been demonstrated that at a concentration of 150 mg/L, the mortality of algae occurs within three days of the experiment [9]. To date, the literature contains no information on the range of tolerance of adult Ulva lactuca thalli to DF, nor on the ability of Ulva lactuca to absorption and transform the toxicant.

Ulva lactuca is a species of green algae that is cosmopolitan in distribution. It was relatively rarely found in the Barents Sea until recently, now being observed to spread actively along the Eastern Murman littoral zone [10]. Diesel fuel is one of the most common marine toxicants due to the fact that it is used by marine transport as well as by coastal heating plants (CHP) [11].

¹⁾ Stepanyan, O.V., 2003. [Morpho-Functional Changes in Algal Macrophytes of the Barents Sea under the Influence of Oil and Oil Products]. Doctoral Dissertation. Murmansk, 146 p. (in Russian).

The aim of our study is to obtain information on the absorption and transformation of PP by *U. lactuca* tissues, changes in the algae occurring at the cellular level at 10 and 20 mg/L DF content in seawater and *U. lactuca* possible role in bioremediation. The article uses materials of the paper abstracts of the White Sea Student Scientific Session of SPbU².

Material and methods

In August 2023, *Ulva lactuca* thalli without any indications of reproduction and water were sampled on the littoral of the Zelenetskaya Bay of the Barents Sea in the area of the seasonal biostation of Murmansk Marine Biological Institute of RAS (69°07′09″ N, 36°05′35″ E). Experiments were performed in a thermostated space at 8–10 °C with constant illumination of 150 µmol m⁻²·s⁻¹, 24L:0D photoperiod (polar day period), and aeration of the aquatic environment. The water used for the experiment with a salinity of 33 ‰ was subjected to filtration through a cottongauze filter and subsequently cooled to a temperature of 8–10 °C. A total of 18 experimental tanks were utilised in the study (three control tanks with algae, three control tanks without algae, six experimental tanks with algae and six experimental tanks without algae (three for each concentration)). Each tank contained 2 L of prepared water, to which 10 and 20 mg/L of summer diesel fuel (200 and 400 MPC, respectively) were added. Adult thalli of *U. lactuca*, with a mass of 5 g each, were placed in the tanks where the algae were to be located, three specimens per one experimental tank. The tanks were aerated by an air compressor.

All experimental tanks were tightly closed with a lid in order to avoid loss of DF volatile fractions. DF concentrations were chosen to determine tolerance to the toxicant and to analyse morphological changes in U. lactuca. At the very beginning (day 0), on the 5th and 10th days of the experiment, algae samples were collected and examined by gas chromatography/mass spectrometry for the PP content. Throughout the experiment, samples of algae not exposed to PP were studied as a control sample. Water was analysed for DF content in the initial sample, control one and after the DF addition in the presence and absence of algae in the water. Each water and algae analysis was carried out in three repetitions. The sample and instrumental analysis process preparation was carried out based on the EPA 8270 methodology detailed in previous studies [12]. In order to analyse changes in algae under the influence of PP occurring at the cellular level, 1 cm² cuttings were made from thalli and placed in Eppendorf tubes with fixative. Prefixation was carried out with 2.5% glutaric aldehyde on a cacodylate buffer (c-c-b) with 1.5% tannin added to the fixative, and postfixation was carried out with 1% OsO4 (osmium oxide (VIII)) on a similar buffer. The osmotic pressure of both fixatives was adjusted to the osmotic pressure of seawater in the environment (1100 mOsm) using sucrose. Fixation took place at a temperature of 0...+5 °C according to the following scheme: prefixation with glutaric aldehyde for 18 h, washing of c-c-b in two shifts

²⁾ Kudryavtseva, E., Salahov, D. and Voskoboynikov, G.M., 2024. The Ability of the Green Algae Ulva Lactuca to Purify Seawater from Diesel Fuel. In: SPbU, 2024. [White Sea Student Scientific Session. Paper Abstracts. 1–2 February 2024, Saint Petersburg]. Saint Petersburg: Svoe Izdatelstvo, p. 50 (in Russian).

of 6 h, postfixation with OsO_4 for 18 h. Then the material was prepared for viewing in the electron microscope JEM-100C (manufactured by JEOL) according to generally accepted methods³⁾.

Results and discussion

Light-optical and electron-microscopic observations revealed that the thalli of U. lactuca, collected from a habitat where the PP concentration was 0.62 mg/L (12 MPC), exhibited no significant differences in cell ultrastructure when compared to previously studied thalli of ulva algae inhabiting waters with PP content ranging from 0.1–0.2 mg/L (2–4 MPC) observed in most of the studied bays of the Kola Bay and Eastern Murman coasts of the Barents Sea. All selected thalli were viable under control conditions until the conclusion of the experiment. No destructive changes in cell morphology were observed. Chloroplasts contained single-channel pyrenoids (letter P in Fig. 1, a), predominantly immersed; a large number of starch granules were detected (letter K in Fig. 1, a), indicating that photosynthesis was well underway (Fig. 1, a).

This was also confirmed by the bright green colour of thalli of the control variant which remained until the end of the experiment. At the same time, heterogeneity was observed in the cell structure on the control variant ulva cuttings at all experimental periods: the cells differed in the degree of photosynthetic apparatus development, partial volume of reserve substance. It should be noted that the same heterogeneity was observed when studying the ultrastructure of ulva cells in natural and experimental conditions in our earlier studies [13]. On the fifth day in both variants of the experiment with DF at concentrations of 10 and 20 mg/L, about 90% of cells in the ulva thalli showed no signs of damage, the thalli retained a homogeneous green colour. However, if in the control variant and in the variant with introduced DF at a concentration of 10 mg/L pyrenoids were present in the majority of chloroplasts, then under DF exposure at a concentration of 20 mg/L, pyrenoids were detected only in chloroplasts in only 30-40% of the cells (Fig. 1, b), which can indicate a decrease in the activity of photosynthetic apparatus functioning in the majority of ulva cells. On the 10th day of the experiment, following the introduction of DF (10 mg/L) into the water, no alterations were observed in the structure of the majority of thalli cells (up to 70%). However, a small proportion of cells at this concentration, as well as the majority of the ulva cells (up to 80%) at 20 mg/L, exhibited a minimal amount of organised structures: cytoplasm remnants were localised near the envelope and chloroplasts were not detected (Fig. 1, d). Starch grains were present in the cells, but in much smaller amounts than in the cells of the control variant and at the previous stage (5 days) of the experiment. A large number of bacteria were detected on the outside of the cell envelope (Fig. 1, c, d). In addition, a small proportion of the thalli cells were characterised by an intact structure.

³⁾ Weakley, B., 1972. *A Beginner's Handbook in Biological Electron Microscopy*. London, Edinburg: Churchill Livingstone Publ., 240 p.

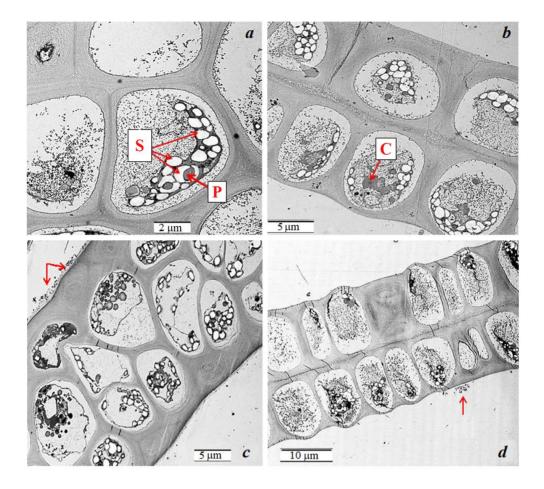


Fig. 1. Changes in *Ulva lactuca* cells in the experiment: a - control for 0 days; b - 5 days of the experiment at 10 mg/L (200 MPC) diesel fuel (DF); c - 5 days of the experiment at 20 mg/L (400 MPC) DF; d - 10 days of the experiment at 20 mg/L (400 MPC) DF. Notations: P - pyrenoid, S - starch granules, C - core. Arrows indicate bacteria on the thallus periphery

The results of the water samples demonstrated that during the experiment with the addition of DF to water at a concentration of 20 mg/L (without ulva), destruction and absorption of the introduced hydrocarbons (IH) occurred, apparently by microorganisms present in the water. On the fifth day of the experiment the PP concentration in water decreased by 40% and made 12 mg/L. The bioremediation ability of microorganisms was noted earlier in studies with other algae species [1]. In the case of the ulva sample addition to the water, the gross PP content in the water decreased by 86% to 2.8 mg/L on the 5th day and increased slightly (to 4.2 mg/L)

on the 10th day. An increase in the PP concentration in water on the 10th day is indicative of a reverse process, i.e. the release of absorbed IH into water. This can be reflected in the change (partial destruction) of the cell membrane system observed in electron microscopic photographs. A similar result was recorded in earlier studies on the effect of crude oil hydrocarbons on the green algae *Acrosiphonia arcta* [14]. The values of the index reflecting the degree of transformation of hydrocarbons (Σ n-alkanes/ Σ PP) remained high enough (> 0.2) throughout the experiment, i. e. the processes of absorption/release of such a quantity of IH (20 mg/L) take place without significant transformation. When DF is added at a concentration of 20 mg/L, a time interval of 10 days is likely to be inadequate for observing substantial alterations in the structure of petroleum IH, both with and without the involvement of microalgae and ulva.

Fig. 2 shows changes in the concentration of alkanes and gross content of petroleum products in *Ulva lactuca* tissues.

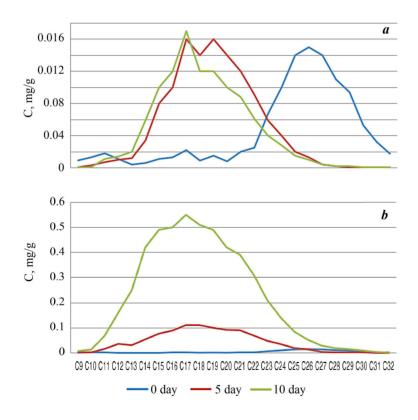


Fig. 2. Mass fraction of n-alkanes in the tissues of the *Ulva* lactuca during the experiment with the addition of DF: a - 10 mg/L (200 MPC); b - 20 mg/L (400 MPC)

Initially, the algal sample (control, day 0) contained 0.540 mg/g PP, of which C_9-C_{30} n-alkanes were 0.1 mg/g. The sample was characterised by an increased content of petroleum IH in a range of C_{23} - C_{30} (Fig. 2). It is important to note the absence of any expressed endogenous n-alkanes in ulva compared to algae living in polluted water areas [15, 16]. On the 5th day of the experiment without DF addition (control, day 5), gross IH content in ulva tissues decreased to 0.49 mg/g, on the 10^{th} day – to 0.34 mg/g. The amount of n-alkanes in algal tissues also decreased fourfold, from 0.1 $\mu g/g$ (day 0) to 0.026 mg/g (day 10). The value of the marker indicator \sum n-alkanes/ \sum PP also underwent a significant decrease from 0.19 (on the first day) to 0.08 (on days 5 and 10), indicating active IH transformation during the initial five days. The value of this indicator at the level of less than 0.1 is characteristic of algae growing in relatively clean water areas. The process of petroleum IH absorption by the surface of the algae is initiated upon the DF addition. In the experiment with the addition of DF at a concentration of 10 mg/L, the PP content in ulva tissues on the 5th and 10^{th} days was recorded at 0.6 mg/g (Fig. 2, a). The marker ratio of Σ n-alkanes/ Σ PP was established at 0.2 during the course of the experiment. A slight decrease in this index to 0.18 on the 10th day signifies the onset of a transformation in the chemical structure of hydrocarbons. In the ulva sample where DF was added in the amount of 20 mg/L, this index on the 5th and 10th day was recorded at 0.25 and 0.28, respectively, indicating an active process of hydrocarbon absorption which was not yet completed on the 10th day. The maximum PP content in the algae was recorded on the 10th day of the experiment when 20 mg/L was added, and was found to be 18 mg/g (Fig. 2, *b*).

Table demonstrates generalised results of the experiment conducted with *Ulva lactuca*.

The processes of PP absorption and transformation are characteristic for ulva as well as for all previously studied macrophyte algal species [12, 14]. In comparison with another green alga, acrosiphonia [14], ulva exhibits a higher rate of IH absorption/transformation, which can be attributable to the disparity in thalli structure, with ulva exhibiting a lamellar configuration and acrosiphonia displaying a siphonal structure. It can be assumed that the thallus lamellar structure is more favourable for the location of epiphytic hydrocarbon-oxidising bacteria.

Gross content of petroleum hydrocarbons (PH) in water (mg/L) and in algae (mg/g) during the experiment with the addition of 20 mg/L (400 MPC) DF

Time, days	PH in water	PH in algae
0	20	0.54
5	2.8	4
10	4.2	18

Conclusion

The experiments demonstrated that the impact of summer DF at a concentration of 10 mg/L (200 MPC) for 10 days and at a concentration of 20 mg/L (400 MPC) for 5 days at a temperature of $8-10^{\circ}$ C was not lethal for the littoral species of green algae *Ulva lactuca*. It has been demonstrated that, under natural conditions, long-term growth at PP concentrations of approximately 0.6 mg/L (12 MPC) enables ulva cells to develop adaptive reactions and withstand single PP discharges (leaks). It has also been demonstrated that *U. lactuca* incorporates itself rapidly into the process of bioremediation in instances where seawater has been contaminated with petroleum products. It can be assumed that a relatively wide range of tolerance to petroleum products is one of the factors that has enabled *U. lactuca* to occupy a certain ecological niche on the littoral zone of the Murmansk coast of the Barents Sea, which is currently characterised by a high degree of petroleum pollution. This hypothesis is supported by the experimental evidence and existing literature data.

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Submitted 03.06.2024; accepted after review 23.06.2024; revised 18.09.2024; published 20.12.2024

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Contribution of the authors:

Grigory M. Voskoboinikov – ideology and statement of the study problem, electron microscopic work, writing the article, editing the text

Larisa O. Metelkova – analytical studies of the content, transformation of diesel fuel in water and algae, analysis and discussion of the results, participation in the preparation of the article for publication

Dmitry O. Salakhov – selection of algae and water for the study, setting up the experiment, analysis of the literature on the study topic, analysis and discussion of the results, preparing the article for publication

Ekaterina O. Kudryavtsteva – participation in setting up and conducting the experiment, fixing and preparing material for light and electron microscopy, participation in preparing the article for publication

All the authors have read and approved the final manuscript.