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RESEARCH ARTICLE
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Efficacy of *Chlorella Sp.* In Diesel Fuel Degradation in a Model Experimental Study

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ABSTRACT

This study aims to assess the degradation of petroleum products using Chlorella strain algae. To assess the level of petroleum product decomposition by algae based on mass spectrometric detection gas chromatography and to determine the algae's resistance to various concentrations of petroleum products, mineral culture media with different concentrations of diesel fuel (1:10, 1:100, 1:1000) were used for cultivating green algae. Based on the research results, when diesel fuel was diluted with water at a ratio of 1:10, the amount of hydrocarbons detected by gas chromatography was nearly 2.5 times lower than when diluted under the same conditions in the presence of photosynthetic algae. This suggests potential biological degradation. When diluted at a ratio of 1:100, the quantity of these substances did not significantly differ between the control and experimental groups. However, when diluted at a ratio of 1:1000 in the experimental group with algae, the amount of identified hydrocarbons was 17 times higher. Thus, these findings indicate an acceleration in the degradation of petroleum products. The research results under in vitro conditions, focusing on studying the resistance of certain algae to petroleum products at the cellular level, may be utilized in the development of biotechnology for the remediation of water bodies and/or the purification of wastewater from petroleum contamination, particularly diesel fuel. Additionally, these findings could contribute to developing technologies aimed at conducting rehabilitation efforts in freshwater ecosystems contaminated with petroleum products using microscopic algae.

Keywords: Oil; Petroleum products; Algae; Phytoremediation; Phytoextraction

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INTRODUCTION

Oil pollution of water sources seriously threatens aquatic ecosystems and human health, requiring effective remediation strategies (Asadulagi et al., 2024). Microalgae, due to their unique metabolic capabilities, are being utilised as a promising research subject in bioremediation (Yernazarova et al., 2023). The research considers the potential of microalgae to break down oil and purify water. Aquatic life and the surrounding ecosystem are harmed by oil pollution of water sources. Oil is a mixture of hydrocarbons that contains thousands of compounds of different structures. Oil hydrocarbons have four components: alkanes, polyaromatic hydrocarbons, resins and asphaltenes. Alkanes are the primary components of oil that have the lowest toxicity and are biochemically easily degraded. Polyaromatic hydrocarbon compounds are characterized by solid materials due to their high melting and boiling points, low solubility, low and high molecular mass, and low vapour pressure. Increasing the amount of the polyaromatic hydrocarbon ring reduces its solubility in the aqueous phase, but it is well soluble in organic solvents because they are hydrophobic. During sediment deposition and division, these compounds are

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precipitated, diffused, and sorbent to organic matter in sediments. Although they are stable and easily destroyed in the environment, they persist for an extended long time. Both asphalt and resin are hydrocarbon compounds that include oxygen, sulfur, and nitrogen, as well as non-carbon compounds and are identified by their polar compounds (Hazaimeh & Ahmed, 2021).

The processed and extracted oil from the "Tengiz" oil field located in the western part of the country contains a high concentration of hydrogen sulfide, as well as radioactive barium and thorium (Neverov et al., 2023). Nickel and vanadium, which are commonly found in petroleum products, can induce ecological toxicity when entering the soil along with petroleum products. Additionally, contamination with crude oil can lead to alterations in soil microbial populations and enzymatic systems (Yavari et al., 2015; Niyazbekova & Nazarenko, 2018; Shaikhutdinov & Kasanova, 2020; Tang & Chai, 2020).

Petroleum hydrocarbons undergo biochemical degradation in deep reservoirs, which naturally enhances oil recovery from the reservoir. However, in relatively stable small lakes in certain oil-producing regions with low temperatures, oxygen deficiency, and limited photodegradation conditions, the degradation of oil and petroleum products occurs very slowly. As a result, the ecological and recreational value of organisms living in these water bodies is completely lost (Perminova et al., 2022).

Numerous techniques exist today for processing petroleum products, which fall into the categories of physical and chemical techniques. Chemical techniques include chemical oxidation, such as the Fenton process, dispersants (mainly surface-active chemicals used for oil spills), and others. The adsorption of petroleum products using adsorbent materials is one of the most popular techniques. Although it is an economical and environmentally beneficial technology, it is not very effective (Kosheleva et al., 2022; Simion et al., 2022).

Compared to some physical and chemical methods, biological remediation has a much lower environmental impact and is considered an economical method. Biological remediation uses microorganisms or plants to remove pollutants. The advantage of this method lies in lower labor costs and the potential for complete mineralization of oil into CO_2 and H_2O (Shaikhutdinov & Kasanova, 2020). However, the application of this method in field conditions is not always successful, as weather changes may prevent microorganisms from completely eliminating pollutants. Additionally, microbiological treatment is influenced by factors such as nutrient and oxygen deficiency, biodegradability, moisture content, oil concentration, pH, and sometimes temperature. Consequently, phytoremediation has become the most sought-after method of remediation in the past twenty years (Abdullah et al., 2020).

Phytoremediation is a method of bioremediation in which selected plant species are used to remove, transform, store, and/or eliminate pollutants in soil and groundwater (Anekwe & Isa, 2022). Phytoremediation has been applied since the early 1990s, primarily for the remediation of soils contaminated with less hazardous substances that may pose a threat to public health (Tang & Angela, 2019; Suchshikh et al., 2023).

Phytoremediation of wastewater - technology of pollution treatment with aquatic plants and algae. For example, it is known that various macrophytes, differing in structure and taxonomy, are capable of sorbing petroleum products, further breaking them down, and absorbing them into the plant tissues (Voskoboinikov et al., 2021a). Plants used for phytoremediation should have the ability to absorb, immobilize, and transport heavy metals into different parts of their biomass (roots, stems, and leaves). Many studies have established that different plant species employ varying mechanisms for the accumulation, excretion, and distribution of metals (Tang & Chai, 2020). Microalgae have enzymatic pathways that allow them to break down carbohydrates into simpler compounds. Metabolic activity is provided by photosynthetic processes that aid in the conversion of oil pollutants into biomass and harmless by-products. In addition, microalgae can improve the dispersion and solubility of oil and increase the rate of degradation. Xenobiotic pollution levels, including oil products and heavy metals, can be detoxified and transformed through the metabolic pathways of algae. Algae can remove pollutants by two processes: bioaccumulation and biodegradation. Bioaccumulation is a process that allows living organisms to accept and store certain organic and inorganic substances within cells, and biodegradation is a natural process in which organic substances decay into simple substances, such as carbon produced by algae or other organisms (bacteria, fungi) (Zainith et al., 2021).

The mechanism of phytoremediation can be divided into the decomposition of pollutants, extraction, and suppression or a combination of these three methods. Additionally, it can also be classified based on mechanisms of pollutant removal or neutralization. These mechanisms include the sequestration of pollutants from soil or groundwater, the concentration of toxicants in plant tissues, decomposition through various biotic or abiotic processes, evaporation or transpiration of volatile pollutants from the plant into the air, and immobilization of pollutants in the root zone (Abdullah et al., 2020).

Oswald's studies have shown that they can protect algae from toxic compounds in wastewater by increasing the destruction of harmful pollutants through a symbiotic habitat between microalgae and bacteria. The symbiotic life base between microalgae and bacteria is used by microalgae to produce CO_2 in O_2 through photosynthesis, which can be used by heterotrophic bacteria to assimilate and decompose carbon, nitrogen and phosphorus organically. Moreover, the release of CO_2, inorganic nitrogen, and phosphorus from bacteria's aerobic metabolism can be utilised as nutrients for further photosynthesis through microalgae. There are significant benefits in treating polluted water with algae. For example, polluted waters are the first to be treated at low prices, and algae can live in limited habitats with additional nutrients, so collected trace elements can be converted into value-added products, such as biogas, biofuels, fertilizers, animal feed, and others (Li et al., 2019; Ansabayeva & Akhmetbekova, 2024).

Numerous studies have investigated how petroleum hydrocarbons biodegrade on Chlorella vulgaris, a green algae species (Ramazanova et al., 2021). When cultivated for 7 and 10 days in a 20 g/L oil concentration, C. vulgaris had a high ability not only to significantly resist microalgae but also to recover crude oil hydrocarbons. The dry weight of C. vulgaris increased with the increase in crude oil concentration, suggesting that crude oil has a positive impact on algae growth (Kalhor et al., 2017).

In a study of the role of microalgae in refinery wastewater, various physicochemical parameters were studied, including pH wastewater treated with Scenedesmus obliquus and pH wastewater without Edescensmus obliquus (control), alkalinity, hardness, heavy metals, and free ammonia. It has been observed that Scenedesmus obliquus has the ability to eliminate a variety of physicochemical parameters (Rajasulochana et al., 2009).

Metabolic engineering, enzyme engineering, omics technology, and other systems biology are rapidly evolving. Optimization of the metabolic network of the bacteria that separate oil hydrocarbons can ensure shorter and more accurate bioremediation through metabolic engineering strategies; biocatalysts with more stable and excellent catalytic activity can accelerate biodegradation by enzyme engineering; omics technology not only provides additional components for engineering bacterial structures but can also obtain the structure and composition of the microbial community in the contaminated environment. Comprehensive information on the microbial community provides some theoretical basis for creating artificial mixed microbial communities for the bioremediation of oil contamination.

The development of genetic engineering strains and the study of mixed microbial degradation systems can be promoted by utilizing various systems biology methods for simultaneous bioremediation (Dashkevich et al., 2022). The assessment of degradation efficiency and rate is still used in research on bioremediation of oil pollution because accurate degradation has not been achieved yet (Alekseev et al., 2022). Therefore, to choose a specific plan for two methods of generating genetically engineered strains and creating mixed microbial systems, it is necessary to first assess the oil-contaminated environment. For example, the use of genetically engineered strains in a polluted environment has a natural advantage, as the fewer species of microorganisms there are, the less they affect the biological community in the environment. To contaminate complex components near refineries, oil spill areas, and other areas, it is possible to use appropriate mixed microbial flora to speed up the bioremediation process (Yang et al., 2021).

Microalgae are considered one of the most likely raw materials for biofuel production, and a study has been conducted on the ability of Chlorella vulgaris, blue-green algae Synechococcus sp and its consortium to grow and degrade hydrocarbons in various concentrations (0, 0.5, 1 and 1.5%) such as kerosene. Overall, the algae consortium shows that it can absorb and remove kerosene from water, as well as produce biofuels such as biodiesel and petroleum-based fuels (Hamouda et al., 2023).

The use of photosynthesis of microalgae is an effective method of biofixation of CO_2. Microalgae are often used in the biofixation of CO_2 due to their photosynthetic productivity, high lipid accumulation, high biomass productivity and valuable non-fuel by-products. Autotrophic or photosynthetic microalgae possess numerous CO_2 fixation abilities and are more effective than terrestrial plants. Microalgae offer a more sustainable treatment option. Microalgae are the dominant group of micro-organisms in biological treatment due to their ability to biosorb toxic heavy metals and use organic and inorganic nutrients from wastewater, which are the main polluters of the environment. In addition, microalgae contribute to the bio-sequestration of CO_2 and provide valuable by-products that can be used for many purposes, such as fuel. Specific methods of carbon biofixation and bioprocessing can be implemented towards environmental sustainability and economic facility (Jalilian et al., 2020).

Research in the field of phytoremediation often focuses on regional plant species capable of removing specific pollutants. However, the effectiveness of phytoremediation is limited by the bioavailability of petroleum hydrocarbons. This is because oil and petroleum products are characterized by low solubility in water and high soil sorption (Liao et al., 2016).

Most plants used for the degradation of petroleum hydrocarbons are terrestrial plants, as most research has been concentrated on phytoremediation of hydrocarbon-contaminated soils. Therefore, the use of aquatic plants is relatively rare. Various plant species, including Scirpus (bulrush) and Eichhornia (water hyacinth), as well as perennial plants like Typha and Phragmites (common reed), are widely employed due to their widespread distribution, high biomass, and resistance to toxicants (Abdullah et al., 2020).

Aquatic plants play a key role in freshwater ecosystems as they provide food, structure and habitat for a wide range of terrestrial and aquatic animals (Nugmanov et al., 2022; Temreshev et al., 2022). The main characteristics of aquatic plants include morphophysiological and physiological adaptations necessary for development in different aquatic environments, leaves with morphological changes, and a special structure that allows them to float on the surface of water. Aquatic plants can be classified as follows: Surface water plants. These are plants that live on the surface of water and their structure consists of stems, roots, leaves and free-floating flowers. Their main function in wastewater treatment is to purify or filter the water by trapping pollutants. Such plants include: *Scirpus validus, Typha latifolia, Ceratophyllum demersum, Potamogeton pectinatus, Maranta arundinacea, Lemna spp, Eichhornia crassipes* and *Pistia stratiotes*. *Submerged* aquatic plants (Ramazanova et al., 2021).

The biological characteristics and evolutionary aspects of Salix plants, such as fast growth, resistance to sources of pollution, high water consumption, and an extensive root system, have been studied for a long time. These properties allow the use of Salix plants for the phytoremediation of industrial areas. It is known that Salix species are hyperaccumulators of heavy metals. They accumulate more pollutants in their leaves and stems than in their roots. The biofiltration properties of willow plants remove heavy metals from the environment, such as from soil, wastewater, etc., and are also based on absorption and binding mechanisms. The ability of plants to accumulate heavy metals directly depends on the properties of cell walls (Terebova et al., 2014).

Paspalum vaginum Sw. is a primary target for the phytoremediation of hydrocarbons. It is known that various macrophytes, differing in structure and taxonomy, absorb and subsequently degrade petroleum products. For example, research has shown the significant role of live and dried algae, such as Fucus vesiculosus, in cleaning coastal waters from petroleum products. It has been found that these algae can be effectively used for daily preventive cleaning of seawater when the amount of diesel fuel does not exceed 3 mg/L (60 SHEEN) (Takáčová et al., 2022; Read et al., 2023).

Recent studies have shown that, macroalgae such as *Ascophyllum nodosum*, *Fucus vesiculosus*, F. *distichus, F. Serratus, Saccharina latissima, Palmaria palmatai,* and *Ulvaria obscura* are able to neutralize toxic effects of diesel fuel (Voskoboinikov et al., 2021b). Even with the growing use of renewable energy sources like solar and wind, oil and gas still have a big impact on the global economy. (Apergis et al., 2023). Hydrocarbons are still widely used in various forms of transportation, including internal combustion engines, diesel and gasoline generators, as well as in the production and use of lubricants for various mechanisms and in the petrochemical industry for the production of polypropylene pellets and plastics (Ilyushin & Afanaseva, 2020). However, despite all safety measures taken, the extensive use of hydrocarbons ultimately leads to various leaks of this valuable and highly toxic raw material. Currently, there is an escalating global environmental crisis associated with pollution from anthropogenic substances, particularly hydrocarbons and petroleum products, which are adversely affecting aquatic ecosystems. In Kazakhstan, the most pressing environmental issues are associated with the contamination of water ecosystems by hydrocarbons (petroleum products) in regions where hydrocarbons are extracted, processed, and transported. This is particularly evident in the Caspian Sea region, as well as in various small lakes, rivers, and streams within the Caspian lowlands. (Mazina et al., 2022; Ramazanova et al., 2022).

Water used for industrial, domestic, or municipal purposes can be restored to its original state through wastewater treatment (Shilnikova, 2023). Classic chemical, chromatographic, and IR spectroscopic methods are used for the qualitative determination of petroleum products in water. Diesel fuel, due to its hydrophobic properties, can form a layer on the water's surface, making it easier to remove. However, in the sea, under the influence of wind and currents, petroleum products evaporate, spread, and settle in sediment. If the oil spill zone is not cleaned in a timely manner, diesel fuel emulsifies with water, requiring complex cleanup methods.

In this regard, our research focused on studying the petroleum-degrading ability of Chlorella microalgae specifically grown for different concentrations of diesel fuel (1:10, 1:100, and 1:1000), used as a model experiment.

The purpose of the research work is to determine the activity of Chlorella strain algae grown in different concentrations of diesel in a model experiment to break down oil products.

MATERIALS & METHODS

The objects of the study included collection strains isolated from soil contaminated with oil and petroleum products. Additionally, new species of microalgae isolated from the soil of the Atyrau region were also studied.

The microalgae were cultivated in a Voroshilov-Dianov medium with the following composition g/L: NH_4NO_3 – 1.0g, K_2HPO_4 – 1.0 g, KH_2PO_4 – 1.0g, $MgSO_4$ – 0.2g, $CaCl_2{\cdot}6H_2O$ – 0.02g, $FeCl_3$ – traces, NaCl – 1.0g, pH = 7.0-7.2. To assess the level of resistance of the algae at the cellular level, one species of green algae, *Chlorella sp*., was selected (Fig. 1).

Fig. 1: Study of algae growth and destructive activity

In vitro conditions, 10 ml of agarized nutrient VD medium were poured into sterile Petri dishes, and after the medium had cooled down, a disk was created using a drill. Different concentrations of diesel fuel were applied to the resulting hole. Three dilutions of diesel fuel with distilled water were prepared: 1:10, 1:100, 1:1000, and a control was maintained with a medium without any diesel fuel. Simultaneously, experiments were conducted using liquid medium with the same diesel fuel concentrations and compared to a control with liquid medium without diesel fuel. The experiments were conducted in triplicate. The work was carried out under aseptic conditions in a laminar flow hood. The cultivation period was 10 days. Data analysis was performed using Microsoft Excel 2010.

Gas Chromatography Method

The biomass of *Chlorella* obtained was extracted with 2mL of hexane, and then 1mL of the extract was taken and analyzed using gas chromatography-mass spectrometry (7890A/5975C).

Analysis conditions: Sample volume: 1.0μL, injection temperature: 250°C, split flow: 10:1. Separation was carried out using a DB-35MS capillary chromatographic column, 30m in length, 0.25mm internal diameter, and 0.25μm film thickness, at a constant flow rate of the carrier gas (helium) at 1 ml/min. The chromatographic temperature was programmed from 40°C (held for 5min) at a heating rate of 7°C/min to 150°C, then at a heating rate of 5°C/min to 300°C (held for 5min). The total analysis time was 55.7min. Detection was performed in SCAN mode with a mass range of m/z 34-750. The Agilent MSD ChemStation software (version 1701EA) was used for controlling the gas chromatography system, recording, and processing the obtained results and data. Data processing included determining retention times, peak areas, as well as processing of spectral information obtained using the mass spectrometric detector. To interpret the obtained mass spectra, Wiley 7th edition and NIST'02 libraries were used (with a total of over 550,000 spectra in the libraries).

RESULTS

The study of hydrocarbon content—the breakdown products of diesel fuel—conducted using gas chromatography in the variants with diesel fuel dilution with water at ratios of 1:10, 1:100, and 1:1000 during Chlorella cultivation showed that with dilution at 1:10 - 1:100, the number of detected substances decreased from 30 to 21.

The number of detected hydrocarbon substances decreased from 30 in the control group to 21 in the presence of Chlorella algae (Table 1, Fig. 2). This substantial reduction indicates that the algae contributed to the degradation of hydrocarbons. The data suggest that Chlorella sp. actively metabolizes hydrocarbon compounds, particularly at this higher concentration of diesel, leading to a significant reduction in detectable substances.

In the experimental variant with a 1:100 dilution (Fig. 3), the number of detected substances further decreased to 17. This continued reduction underscores the effectiveness of Chlorella algae in degrading hydrocarbons, even at lower concentrations of diesel fuel. The ability of the algae to maintain high degradation efficiency across varying dilutions highlights its potential for scalable bioremediation applications.

Interestingly, when diesel fuel was diluted at a ratio of 1:1000, the number of identified hydrocarbons increased to 17 (Table 1, Fig. 4). This was contrary to expectations, as the presence of algae typically reduces hydrocarbon content. This increase could indicate the formation of intermediate degradation products or incomplete metabolism by the algae at this dilution level. It suggests that while Chlorella sp. is effective at degrading hydrocarbons, certain conditions may lead to the accumulation of intermediate compounds, necessitating further research to optimize conditions for complete degradation).

Table 1: Presence of diesel degradation products in samples of algae (Chlorella strain.) at different dilutions

Holding time, min			Identified substances	Percentage content, %		
1:10	s100	s1000		1:10	s100	s1000
10,16	-	10,93	Mesitylene	1,69	-	3,11
10,98	10,93	-	Benzene, 1,2,4-trimethyl-	2,48	3,15	-
-	13,17	-	Benzene, 1-ethyl-2,4-dimethyl-	-	1,00	-
11,73	11,63	11,60	Undecane	3,41	5,55	6,42
13,13	-	-	Undecane, 2-methyl-	2,46	-	-
14,19	14,06	14,03	Dodecane	3,70	5,70	6,18
-	-	17,52	Dodecane, 2,6,10-trimethyl-	-	-	2,70
16,39	16,25	16,22	Tridecane	5,92	7,21	8,39
18,12	-	-	Naphthalene, 1,2,3,4-tetrahydro-6-methyl-	3,65	-	-
18,43	18,27	18,24	Tetradecane	5,22	8,59	10,76
18,87	18,83	-	Naphthalene, 1,2,3,4-tetrahydro-5-methyl-	1,93	2,11	-
19,07	-	-	Naphthalene, 1,2,3,4-tetrahydro-2,3-dimethyl-	3,53	-	-
-	-	19,14	Hexadecane, 2,6,10,14-tetramethyl-	-	-	6,14
19,36	19,31	-	Benzocycloheptatriene	2,19	2,84	-
19,64	19,02	-	Naphthalene, 1,2,3,4-tetrahydro-2,6-dimethyl-	2,99	3,06	-
19,76	-	-	1H-Indene, 1-ethylidene-	3,67	-	-
20,00	-	-	Naphthalene, 6-ethyl-1,2,3,4-tetrahydro-	2,72	-	-
20,31	20,16	20,12	Pentadecane	5,69	7,85	9,48
20,79	-	-	Naphthalene, 1,2,3,4-tetrahydro-6,7-dimethyl-	2,54	-	-
21,48	-	-	Naphthalene, 1,7-dimethyl-	3,01	-	-
21,96	-	-	Naphthalene, 2,6-dimethyl-	2,72	-	-
22,12	21,97	21,93	Hexadecane	5,07	7,93	9,56
-	-	22,52	Pentadecane, 2,6,10-trimethyl-	-	-	5,60
22,67	-	-	*1-Decanol, 2-hexyl-*	2,95	-	-
23,67	23,52	23,49	Pentadecane, 2,6,10,14-tetramethyl-	5,48	5,74	6,72
24,01	23,84	23,81	Heptadecane	3,95	8,28	8,57
24,45	-	-	Naphthalene, 1,6,7-trimethyl-	2,60	-	-
25,66	25,53	24,52	Hexadecane, 2,6,10,14-tetramethyl-	3,45	6,12	1,32
25,90	25,75	25,72	Octadecane	4,99	5,68	5,04
27,80	27,67	27,64	Nonadecane	5,29	6,61	4,15
29,68	29,56	29,54	Eicosane	2,92	5,10	3,28
31,52	31,41	31,40	Heneicosane	3,13	3,75	2,57
-	33,21	-	Docosane	-	2,12	-
-	34,96	-	Tricosane	-	1,20	-
36,69	36,65	-	Tetracosane	0,69	0,40	-
-	33,21	-	Docosane	-	2,12	-

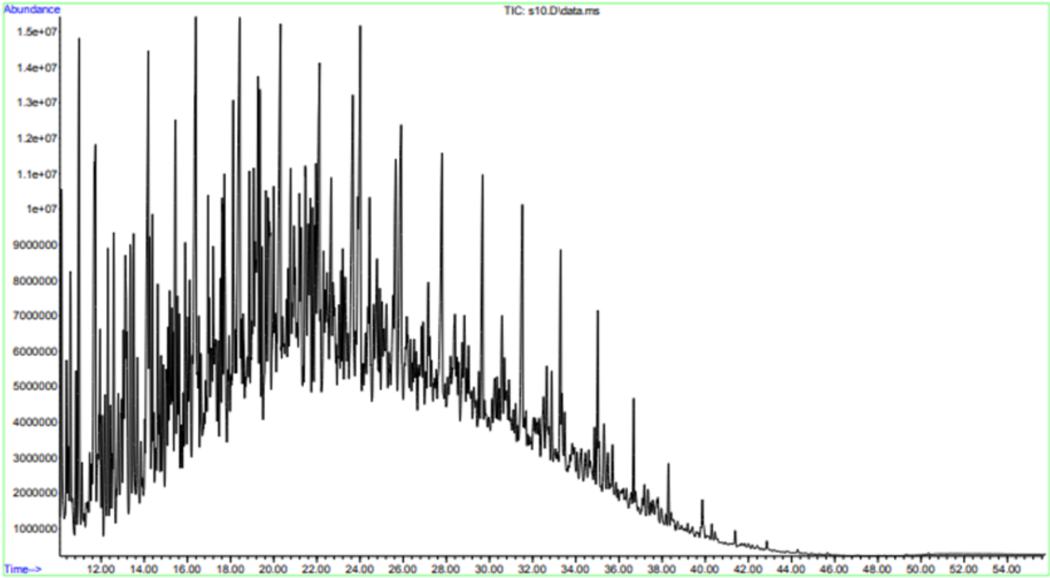

Fig. 2: Chromatogram of the *1:10 extract*

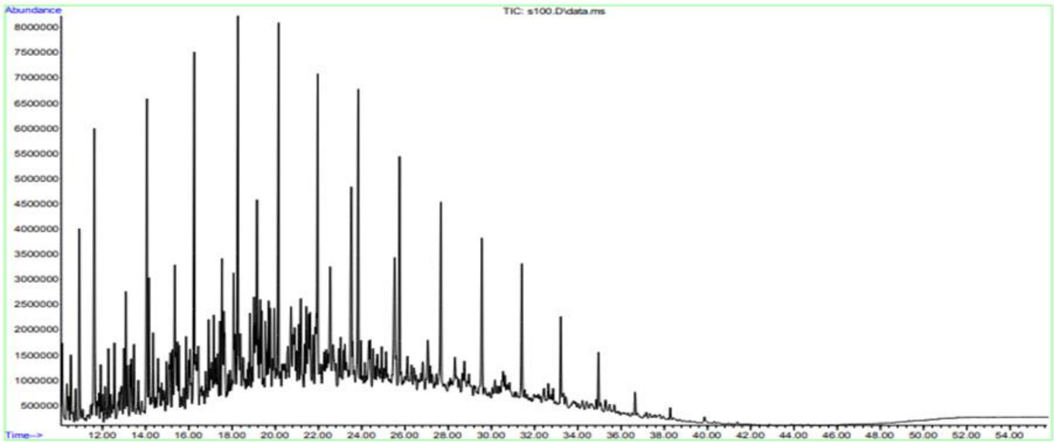

Fig. 3: Chromatogram of the *1:100 extract*

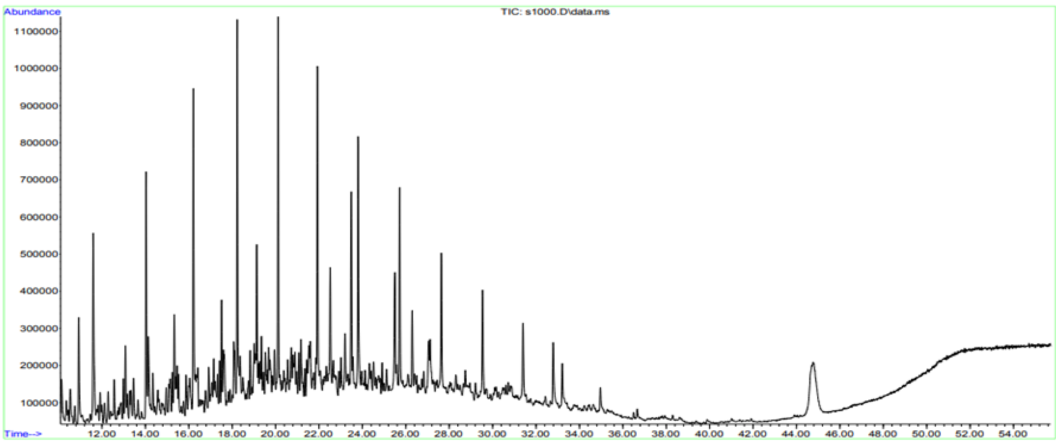

Fig. 4: Chromatogram of the *1: 1000 extract*

Compared to the control experiments (without algae), when diluting the petroleum product (diesel) with water at a 1:10 ratio, the number of hydrocarbon substances detected by gas chromatography was almost 2.5 times lower in the absence of algae than in their presence. This suggests biological degradation. This result highlights the dual role of Chlorella sp. in both reducing hydrocarbon concentration and potentially facilitating the breakdown of these compounds into less complex forms. When diluted at 1:100, the quantity of such substances did not significantly differ between control and experimental groups. However, when diluted at 1:1000 in the presence of algae, the number of detected hydrocarbon substances was 17 times higher. Thus, the obtained data indicate: 1) accelerated degradation of petroleum products and 2) dilution also contributes to the acceleration of hydrocarbon and hydrocarbon-derived product degradation, especially diesel, in the presence of certain aquatic photosynthetic organisms, such as green algae like Chlorella. Overall, the study demonstrates that Chlorella sp. algae are effective in degrading hydrocarbons, particularly at higher concentrations of diesel fuel. However, the unexpected increase in detectable hydrocarbons at the highest dilution suggests that additional factors, such as exposure time, algal density, and environmental conditions, must be optimized to ensure complete degradation.

DISCUSSION

This study demonstrates the efficiency of Chlorella sp. strain algae in degrading petroleum products, particularly diesel fuel, under controlled conditions. The result obtained agrees with the findings of (Song et al., 2022), who studied the Effects of Chlorella vulgaris Enhancement on Endogenous Microbial Degradation of Marine Oil Spills and Community Diversity and noticed an increase in the degradation efficiency of crude oil in seawater when C. vulgaris LH-1 was added.

The results show that the microalgae chlorella's effectiveness is strongly dependent on the concentration of diesel fuel as there was a definite reduction in the total hydrocarbon count (THC) from 30 at 1:10 to 17 at 1:1000; this suggests that Chlorella algae not only contribute to the breakdown of hydrocarbons but do so more efficiently as the diesel concentration decreases. The data obtained from the incomplete removal of THC are consistent with the results of the authors (Ugboma et al., 2023), who studied the Bioremediation Potential of Algae (Chlorella vulgaris) in Crude Oil Contaminated Sediment, and this calls for more research before chlorella sp. It can be adopted as a means of bioremediation (Zhang et al., 2023; Nasiyev et al., 2024).

From our results in Table 1, we observe a uniform increase in the percentage volume of identified hydrocarbons, except octadecane and below, we notice a reduction in percentage as the dilution increases. From this, we can conclude that the degradation of hydrocarbons above C18 was optimal at 1:100. This calls for more research to understand and increase the degradation of heavy molecular weight hydrocarbons (Kussainova et al., 2023; Isina et al., 2024). According to the review of (Radice et al., 2023), there may be a need to increase the experiment time from 10 days as they concluded that the removal of heavy molecular weight hydrocarbons was 78% in 14 days at 10g/L using chlorella vulgaris and 100% for low molecular weight with the same parameters.

From our results in Fig. 1-2, we notice that analytes stop being detected at the 40-44-minute mark, but on close examination of Fig. 3, we notice a ghost peak at the 44-minute mark. Further research is required to study the elution rate at 1:1000.

The implications of these findings are significant for bioremediation (Nasiyev et al., 2023; Dukenov et al., 2023). Chlorella's ability to degrade diesel fuel suggests its potential application in cleaning up oil spills or treating petroleum-contaminated wastewater (Sapanov et al., 2024; Naliukhina et al., 2024). The study also highlights that dilution can enhance algae-based bioremediation by optimizing the concentration levels for maximum degradation efficiency.

Future research should focus on exploring different algal strains, environmental conditions, the effect of different growth parameters such as pH, temperature, oxygen level, etc, and their effectiveness on other petroleum products (Makenova et al., 2023). Additionally, combining algae-based bioremediation with other methods, such as microbial consortia or nutrient enrichment, could further improve the degradation of petroleum products in contaminated environments (Zhao et al., 2024; Makenova et al., 2023).

Conclusion

The data obtained during our study on the resistance of certain algae to petroleum products at the cellular level under in vitro conditions can be used in the development of biotechnology for the remediation of water bodies and/or the purification of wastewater from petroleum contamination, specifically diesel. Additionally, it can be utilized in the development of technologies aimed at conducting reclamation activities in freshwater bodies polluted with petroleum products using microscopic algae.

Conflict of Interest

The authors have no conflict of interest to declare.

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