RESEARCH ARTICLE

Development of technology for biological treatment of wastewater contaminated with oil and oil products using aquatic plants and algae

Zhanat Bukharbayeva[1*], **Bolatkhan Zayadan**[2], **Gulzira Yernazarova**[2], **Lyailya Anuarova**[3], **Nurken Aimukhanov**[4], **Assiya Maimatayeva**[1]

[1] Department of Biology, Abai Kazakh National Pedagogical University, Almaty 050010, Republic of Kazakhstan
[2] Department of Biotechnology, Al-Farabi Kazakh National University, Almaty 050040, Republic of Kazakhstan
[3] Department of Biology, Kazakh National Women's Teacher Training University, Almaty 050000, Republic of Kazakhstan
[4] Nazarbayev Intellectual School of Physics and Mathematics, Uralsk 090014, Republic of Kazakhstan

Abstract: In the context of advancing "green" development and global environmental standards, there is a pressing need to focus on biological methods of wastewater treatment in Kazakhstan, particularly concerning oil pollution. This study aims to develop an optimal technological model to minimise destructive pollution of wastewater by oil products using algae and aquatic plants on the example of artificial biopondsi within the Ozen field owned by "Ozenmunaigas" JSC in the Mangistau region. Through a comprehensive set of scientific methods including induction and deduction, abstraction, system analysis, synthesis, concretisation, formalisation, and generalisation, the study assesses the ecological state of water resources, identifies pollution dynamics, and evaluates the impact of algae and aquatic plants on oil-contaminated effluents. The research culminates in the development of an effective biotreatment technology to mitigate the negative effects of wastewater on water quality and enhance Kazakhstan's overall environmental landscape. Furthermore, the study explores innovative technological and management approaches to reduce pollutant concentrations, emphasizing the importance of ecosystem-based solutions and modern bio-treatment technologies. The findings hold practical significance for modernizing water treatment system, informing future preventive measures for water resource protection, and promoting the adoption of biological treatment as a viable alternative to traditional methods.

Keywords: Oil film, toxicant, ecosystem, eutrophication, biocoenosis, bioremediation

Correspondence to: Zhanat Bukharbayeva, Department of Biology, Abai Kazakh National Pedagogical University, Almaty 050010, Republic of Kazakhstan; E-mail: zhanatbuk@hotmail.com

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1 Introduction

Oil and its derivatives are the substances with the maximum toxicological hazard for the aquatic ecosystems of Kazakhstan. Their water-soluble and colloidal fractions, which consist of aromatic hydrocarbons by 90%, are monitored in water bodies in significant concentrations (0.5-40 mg/l) (Ergozhin et al., 2021; Manasa et al., 2020; Singh and Singh, 2019). The current situation actualises the optimisation of wastewater treatment systems from oil-containing pollutants as one of the first-priority topical environmental problems of Kazakhstan.

The main sources of large-scale pollution of the water environment by oil and oil products in the republic are oil terminals and oil depots, extractive enterprises, river and sea oil tankers, oil products transportation and storage systems, as well as railway transport and petrol stations. Actual volumes of accumulated waste oil products amount to hundreds of thousands of cubic metres, accumulating at individual facilities and forming a source of permanent oil ingress into the aquatic environment. The predominant number of outdated oil sludge and waste storage has turned from a means of counteracting oil pollution of the environment into an active source of such pollution. There is a lack of targeted programmes and initiatives to address this problem. Most destructive processes in aquatic ecosystems occur directly under the action of pollutants contained in oil products. Irrational application of morally and physically outdated methods of wastewater treatment often leads to secondary pollution or turns out to be ineffective. At the same time, there is a lack of strict liability measures for violations of environmental legislation. Optimisation of water treatment from oil products should be as prompt, efficient and economically accessible as possible.

The consequences of uncontrolled discharges of oily effluents into natural water bodies were studied by modern researchers, such as Zhylkybayev et al. (2023). Scientists assess the current state of the wastewater treatment system in Kazakhstan and its urgent problems in the conditions of the transition economy. Representatives of another scientific school Abdukodirova and Abduganiev (2023) developed technology for the use of bio-treated wastewater in agriculture. Ayatkhan and Kaliyeva (2020), modern Kazakhstani researchers in the industry under study, proposed progressive methods of wastewater treatment to intensify the process, including the use of membrane bioreactor and preventive resource-saving technologies. At the same time, Umbetova et al. (2022) emphasise the aspects of toxic-carcinogenic properties of oil and its producers, which as components of effluents get into water bodies. Scientists make ecological substantiation of biological treatment of sewage, convincing in the method's non-alternativeness. Modern scientist Myrzabaeva et al. (2021), concluded that most destructive processes in aquatic ecosystems occur directly under the action of pollutants contained in petroleum products. The researchers' conclusions are developed by Murzabekova (2023), studies the specifics of the process of development and application of biological methods of wastewater treatment from oil products, which are difficult to be treated by traditional treatment even with satisfactory conditions of complete wastewater treatment systems.

Despite significant scientific achievements in the studied branch, there are no practical developments of algorithms for the implementation of biotreatment technology, there is a deficit of experimental research in the field. The question of the feasibility of wastewater treatment with the help of aquatic plants and algae remains unexplored. Thus, the special urgency of the development and application of biological methods of wastewater treatment from oil products, which are difficult to treat by conventional treatment even with satisfactory conditions of complete wastewater treatment systems, is identified. Such a concept is a basis for the creation of an effective system of preventive protection from the negative impact of oily wastewater and therefore requires an innovative scientific approach.

The study aims to develop a promising technology for the biotreatment of wastewater containing oil and petroleum products using aquatic plants and algae, with subsequent experimental verification in practice. Within the set goal the following tasks of the research study were defined:

1. Release of hydrocarbon-oxidising microalgae from the artificial biopond of the Uzen field belonging to "Ozenmunaigas" JSC in the Mangistau region.

2. Identification of microalgae cultures isolated by molecular genetic analysis.

3. Determination of resistance to oil and oil products of hydromacrophytic plants, subsequent selection of aquatic plants with phytoremediation potential.

4. Creation of a consortium of microalgae and higher aquatic plants, development of technology for biological treatment of water sources contaminated with oil and oil products through modelling experiments.

2 Materials and Methods

In the course of research implementation, general scientific methods of cognition were applied, as well as the experimental study was reproduced. The deduction method was used to determine the vectoriality of the process of impact of toxic pollutants in the composition of effluents on aquatic ecosystems of Kazakhstan, as a multicomponent process with a wide range of destruction. The abstraction method was used to form an algorithm for the monitoring process and operational control of indicators of quantitative and qualitative composition of oil-containing effluents. The generalisation method was used to effectively identify the characteristic features of the current research problems, the possibilities of optimising the situation and selecting the most beneficial technological and management solutions. The concretisation method was used to identify the factors of economic feasibility and environmental effectiveness of technological and managerial measures for biological wastewater treatment. Synthesis was used to determine the feasibility of innovative solutions in the biological wastewater treatment industry. An empirical modelling method was used in the current study to develop an optimal system of biological wastewater treatment in Kazakhstan, containing oil and oil products, considering regional peculiarities and specifics.

To determine the characteristics, advantages, and expected performance of certain solutions for wastewater biotreatment, a systematic approach was used in the current study. At the same time, the variability of a combination of technological and managerial solutions, the importance of timely risk forecasting and modelling of situation development in the process of practical implementation of innovations were emphasised. The practical part of the study is an experiment implemented on artificial bioponds in the areas of the Ozen field belonging to "Ozenmunaigas" JSC in the Mangistau region. After the extraction of hydrocarbon-oxidising microalgae from the artificial biopond, the microalgae culture was identified using the method of molecular genetic expertise. For this purpose, the obtained material was cultured in special flasks with a sterilised liquid nutrient medium. Tamiya and Gindaka media, as well as concentrated nutrient media of Gromovoy and Fitzgeralds, were used. The microalgae suspension after one or more secondary inoculations was then transferred to Petri dishes containing solid nutrient agar. Petri dishes were exposed to light until colonies were formed. Special substrates were used to obtain pure algal cultures of microalgae.

Identification of microalgae cultures was carried out using the molecular genetic analysis method. Several methods were used in the process implementation: DNA extraction method, polymerase chain reaction (PCR) amplification method, and phylogenetic analysis. Extraction of genomic DNA was performed by hot phenol extraction method (0.1% sodium disulphate) was added to phenol concentration. RNA was removed from the nucleic acid mixture using RNase A. Purified genomic DNA was extracted with phenol-chloroform mixture (1:1), and DNA was precipitated with ethanol. The resulting PCR product was analysed by electrophoresis. PCR amplification method was also used. Phylogenetic analysis was performed using the Kimurim method model and MEGA software version 6.06. Following this, the level of resistance to oil and oil products of hydromacrophytic plants was determined and aquatic plants with phytoremediation potential were selected. To select resistant hydromacrophytes, obvious signs of reaction to excessive amounts of oil and petroleum products were analysed. Limit concentrations of petroleum hydrocarbons for the plants and morphological characteristics of the effects of petroleum products were determined.

At the final stage of the experimental part of the study, a consortium of microalgae and higher aquatic plants was created using the modelling method, based on which the technology of biological treatment of water sources polluted by oil and oil products was proposed. The information obtained as a result of the experiment was processed using the method of grouping, statistical analysis, and mathematical processing.

3 Results

One of the most urgent environmental problems of Kazakhstan is to solve the problem of destruction and pollution of water resources. The most widespread impurities of effluents in Kazakhstan are such oil products as petrol, oils, and paraffin (Zhylkybayev et al., 2023). Their solubility in water is low and decreases from petrol products to heavier products – paraffin and oils. They are kinetically unstable and are kept in suspension by the dynamic forces of water flow (Abdukodirova and Abduganiev, 2023). It should be noted that complex processes of ecological and chemical transformation occur in the aquatic environment of a natural object after a certain time after ingestion with runoff, forming a synergetic toxic effect. According to the research results, about 30.2 km^3 of the river flow resources of Kazakhstan are required to achieve the goal of sufficient dilution of wastewater to fully neutralise toxic pollution (Ayatkhan and Kaliyeva, 2020; Ergozhin et al., 2021). The destructive impact of wastewater on the environment consists mainly of oxidation processes, which significantly reduce the content of dissolved oxygen in the aquatic environment while increasing the level of biochemical need for it. Once in the aquatic environment, oil-containing pollutants form a film that increases the thickness of the natural surface level. According to observations, one tonne of oil, spreading, can cover 20 km^2 of water surface for 6-7 days (Manasa et al., 2020; Singh and Singh, 2019).

Oil-containing components of effluents, getting into a water body, are distributed into different aggregate fractions, one of which is a thin film, which localises on the water surface and provokes the disruption of exchange processes between the atmosphere and hydrosphere. Such destructive processes negatively affect the physical, chemical and hydrobiological conditions of the aquatic environment, and also take part in the process of destructive climate change (Abdukodirova and Abduganiev, 2023). Contaminating hydrocarbons are in the aquatic environment, except for film, also in dissolved form, with heavy fractions settling to the bottom (Manasa et al., 2020; Umbetova et al., 2022). Being in an aqueous medium, oil-containing compounds spread by spreading, drifting, and sedimentation. At the same time, several transformations occur due to evaporation, dispersion, emulsification, dissolution, oxidation, and biodegradation, in the process by which oil changes its physical and chemical properties (Myrzabaeva et al., 2021). It should be noted that the intensity of these processes depends on the concentration of oil, its composition, and structural characteristics, as well as on external factors of the aquatic environment (Murzabekova, 2023). The peculiarities of the distribution of oil-containing substances in the aquatic environment are the heterogeneity of its content and maximum localisation at the interface between water and different media (Abdukodirova and Abduganiev, 2023; Sanghamitra et al., 2021; Singh and Singh, 2019).

For the treatment of oily wastewater from dissolved and colloidal impurities, in most cases, sedimentation and flotation methods, and chemical and biological methods are used (Chesnokova and Shalay, 2019; Qachach et al., 2021). Such measures do not always make it possible to purify wastewater up to the limit of normative values. In addition, the applied methods operate in suboptimal modes, with outdated materials and technologies of water treatment, not corresponding to the principles of rational water use. Under the current conditions, the development and application of innovative biological technologies to increase the efficiency of wastewater treatment from soluble and dispersed oil products are particularly relevant (Kuyukina et al., 2020).

The main reason for the high oil content in the aquatic environment is imperfect methods of wastewater treatment, physical and moral deterioration of wastewater disposal systems, and ineffective management decisions. Currently, about 50% of wastewater flows into the water bodies of Kazakhstan without treatment. In many regions, for example, in Kyzylorda, Shymkent, and Aktau for a long-time water treatment facilities have been put into operation without proper periodic modernisation and technological renewal (Ergozhin et al., 2021). The problems of oily effluent discharge into the environment should be levelled by using the purification potential of various types of biological processes. In developed countries, methods of using algae and higher plants for wastewater treatment are popularised, as well as the use of algae for cleaning water bodies from nutrients that lead to eutrophication. In addition, the study of the possibilities of using algae in biological ponds and evaporation ponds in oily wastewater treatment systems is of considerable scientific and technical interest (Mitra et al., 2022).

Biological treatment of wastewater containing oil and oil products is the most universal method for the neutralisation of organic pollutants. The basic principle of biological treatment is the ability of living organisms to utilise various organic substances contained in wastewater, including as a source of nutrients for life processes. The main objective of biological treatment is positioned as the transformation of organic pollutant compounds into neutral safe oxidation products (Qachach et al., 2021; Umbetova et al., 2022). The process of biochemical destruction of organic impurities is realised through the use of functional features of bacteria and microorganisms living in the aquatic environment. The processes of biochemical treatment of effluents containing petroleum products are usually carried out in biofilters, bioponds and aeration tanks (Abdukodirova and Abduganiev, 2023; Chesnokova and Shalay, 2019). Among the advantages of the biological wastewater treatment method are the ability to remove from the aquatic environment various organic compounds with toxic effects, as well as low operating cost. It is worth emphasising the fact that the application of biological treatment technologies implies compliance with the technological regime of treatment and control of the concentration of some pollutants that can have a toxic effect on microorganisms.

The technology of the wastewater treatment process using a shallow biopond with marsh vegetation and peat substrate implies a mandatory preliminary stage of mechanical treatment in a settling pond (Singh and Shikha, 2019). The treated wastewater is discharged from the biopond into the general wastewater disposal system, where disinfection measures are implemented according to the method defined in the design process of the local wastewater treatment system. After the disinfection process, the treated wastewater is released into the water body (Ayatkhan and Kaliyeva, 2020; Kuyukina et al., 2020). The presence of a large amount of hard-to-oxidise oil products and mechanical impurities in the composition of wastewater makes it necessary to treat oiliest wastewater in three stages (Mitra et al., 2022; Sanghamitra et al., 2021). Initially, free-dripping oil and suspended solids are separated at mechanical treatment facilities. Implementation of flotation treatment before biological treatment is an important factor in increasing the efficiency of the process because as a result of its effective implementation the concentration of hard-to-oxidise compounds in the composition of wastewater is minimised.

Analyses of the functioning of biological wastewater treatment ponds show that the maximum efficiency of biodegradation of oil products is demonstrated by oil-oxidising microorganisms obtained by auto-selection. Such microbial communities are extracted from existing sites of oil contamination of soil and water environments, where they take an active part in the biodegradation of petroleum products under natural conditions (Mitra et al., 2022; Murzabekova, 2023). In particular, periphyton develops on flooded dams, the specific composition of which includes blue-green and diatom algae, as well as a community of bacteria capable of oxidising oil-containing compounds. Materials such as expanded clay, activated carbon, and chalk-like limestone are used as carriers. The development of green algae is also characteristic. The peculiarity of the described process is positioned natural stable support of high concentrations of individual cells of microorganisms and hydrobionts in the purification zone (Kuyukina et al., 2020; Umbetova et al., 2022).

Under conditions of constant water flow in biotreatment facilities, a specific biocoenosis is formed. At that, the higher aquatic vegetation fulfils the role of periphyton carrier, which includes a community of various algae, microflora, and protozoa. The root system of plants accumulates mineral substances necessary for life support, acting as a filter for the aquatic environment. Water quality after passing through a multi-stage wastewater biotreatment system complies with generally accepted standards (Mitra et al., 2022). This type of system is not difficult to operate and has a simple design. They can provide stable support of proper water quality for discharge into open water bodies, which is especially important in the hot and arid climate of Kazakhstan (Ayatkhan and Kaliyeva, 2020; Ergozhin et al., 2021). Water purification using microalgae is a method based on biocatalysis technology. Microalgae can reduce concentrations of toxic substances and also use phosphorus and nitrogen as energy sources. Bioremediation is positioned as a cost-effective and promising biotechnological method that can neutralise petroleum hydrocarbons.

In the course of the experiment, hydrocarbon-oxidising microalgae were isolated from the artificial biopond of the Uzen field belonging to “Ozenmunaigas” JSC in the Mangistau region. The material obtained for mass cultivation (several cubic centimetres of water, green shoots, slime) was cultivated in special flasks with a sterilised liquid nutrient medium. The flasks were placed on special shelves with fluorescent lamps or in front of a window (with natural light, but not direct sunlight) so that the illumination was about 6-10 thousand lux.

Tamiya and Gindaka media, as well as Gromovoy and Fitzgerald concentrated nutrient media were used to obtain mother cultures of unicellular protococcal algae. Algologically and bacteriologically pure forms were used to assess algal activity. Then 1 ml or more of microalgae suspension after one or more secondary inoculations, depending on the purity of the collection cultures, was transferred into Petri dishes containing solid nutrient agar using simple microbiological techniques and spread over the agar surface using a sterilised cup. Petri dishes were exposed to light until colony formation. To obtain pure algal cultures of microalgae, special substrates were used as nutrient media. It should be noted that the factors of environmental acidity, aeration, temperature, light, and humidity are very important for the growth of microalgae, so the quality of the nutrient medium often determines the results of the whole study. At the same time, the development of microalgae is possible only within certain limits of each factor, and these limits are often not the same for different groups of microalgae.

The next stage of the experiment was the identification of microalgae cultures isolated by molecular genetic expertise. Several methods were used during the realisation of the process. The DNA extraction method involved the isolation of genomic DNA from microalgae cells by hot phenol extraction for molecular analysis. For efficient cell disruption, 0.1% sodium disulphate was added to the phenol concentration. RNA was removed from the nucleic acid mixture using RNase A. Purified genomic DNA was extracted with a phenol-chloroform mixture (1:1), DNA was precipitated with ethanol, and the resulting precipitate solution was dissolved in a small amount of sterilised distilled water. The quality and quantity of the obtained PCR product were analysed by electrophoresis on 1% agarose gel on Tris-acetate buffer using MiniHorizontal, Electrophoresissystem (VWR International, USA). GeneJET™ GelExtractionKit (Fermentas) was used to extract DNA fragments from agarose gel.

PCR amplification method was also used. Polymerase chain reaction was performed using GenAmp 2720 DNA amplifier (Applied Biosystems, USA). The PCR scheme consisted of an initial denaturation at 95°C for 3 min, followed by 40 cycles with the following parameters: denaturation at 95°C for 30 s, annealing at 54°C for 20 s, and elongation at 72°C for 30 s. This was followed by a final elongation step at 72°C for 10 min. Phylogenetic analysis was performed using the Kimurim method model using MEGA software version 6.06. Screening of hydrocarbon-oxidising microalgae isolated from oil-contaminated water, cultures growing in diesel fuel and motor oils, aromatic hydrocarbons (xylene), polycyclic aromatic hydrocarbons (naphthalene) Chlorella sp. Anabena.sp strain allowed selection. The isolated active cultures were identified for taxonomic taxonomy of selected

Table 1. Characterisation of an identified culture of *Chlorella sp.* BZh-1

No.	Indicator	Peculiarities
1	Author(s) and year of description	B.K. Zayadan, G.I. Ernazarova, J. Bukharbaeva (2023)
2	Method of obtaining/extracting strain (association)	Found in natural conditions, isolated by repeated sowing in nutrient media / Zh. Bukharbaeva
3	Identification location	Biotechnology Laboratory of Al-Farabi Kazakh National University, Almaty, 71 Al-Farabi Ave.
4	Culture-morphological features and physiological-biochemical properties of the strain (association)	Strain cells are globular, thin shells, chromatophores are broadly lenticular, and vacuoles are visible. Cell diameter 1.7-6.7 µm, sometimes up to 15, diameter of mother cell with daughter cells up to 28 µm. Cells are uninucleated with a single ribbon-shaped postenucleated chloroplast. No pyrenoid. It is isolated on BG-11 nutrient medium. Optimal growth is also observed on media 04, L2-min, at 25-280C. The main pigment is chlorophyll a, b. Some contain special pigments such as phycocyanin, siphonoxanthin, astaxanthin, loroxanthin, zeinoxanthin, neoxanthin, and allene and acetylene carotenoids such as diatoxanthin and diadinoxanthin. Are photosynthetic and therefore autotrophic.
5	Scope of the strain (association)	As an industrial strain of the whole variety of microalgae, Chlorella belongs to the most valuable ones due to the high content of all essential amino acids in the complete protein, carbohydrates, vitamins and lipid compounds, including carotenoids and other valuable plastid pigments. Based on previously conducted studies the possibility of using the culture of green microalgae Chlorella in the treatment of aqueous media from petroleum hydrocarbons was confirmed. The application of Chlorella accumulating culture was investigated in water media polluted with oil and oil products (diesel fuel), as well as in oil-contaminated water media.
6	Activity (productivity) of the strain (association) with an indication of cultivation conditions, methods, media, and production indicators	The growth rate was determined using the Sirenko method. Culture purity, dry biomass weight (g/l), and titre (CFU/ml) were determined: for Chlorella T>M=B, (1.4-1.8)±0.1. The culture titre was in the range of 106-107 CFU/ml.
7	Method, conditions and composition of media for long-term storage of strain (association)	The strain is stored in BG-11 nutrient medium at +5°C and cells can be stored at 20°C.
8	Hazard class (pathogenicity)	The microalgae *Chlorella sp.* BZh-1 strain has no pathogenicity or toxicity.
9	Deposition form	Storage, security deposit, national patent deposit.

microalgae (Table 1 and 2).

Table 2. Composition of Tamiya nutrient medium for fermentation of *Chlorella sp.* BZh-1 strain (association)

Composition element	Stock	ml/l
$NaNO_3$	150 g/l	10ml
$K_2HPO_4 \cdot 3H_2O$	40 g/l	1ml
$MgSO_4 \cdot 7H_2O$	75 g/l	1ml
$CaCl_2 \cdot 2H_2O$	36 g/l	1ml
Citric acid	6 g/l	1ml
$Na_2EDTA \cdot 2H_2O$	1 g/l	1ml
Na_2CO_3	20 g/l	1ml
H_3BO_3	1.81 g	
$MnCl_2 \cdot 4H_2O$	0.222 g	
$ZnSO_4 \cdot 7H_2O$	0.39 g	
$Na_2MoO_4 \cdot 2H_2O$	0.079 g	
$CuSO_4 \cdot 5H_2O$	0.0494 g	
$Co(NO_3)_2 \cdot 6H_2O$	2.86 g	
Vitamin B12 (cyanocobalamin)		5mg/5ml (solution)
Biotin		1mg/10ml (solution)

The next stage of the experiment was to determine the resistance to oil and oil products of hydromacrophytic plants and the selection of aquatic plants with phytoremediation potential, as well as the creation of a consortium of microalgae and higher aquatic plants for the development of technology for biological treatment of water sources polluted by oil and oil products. Initially, the composition of the microbiocenosis of the artificial biota of the Ambar in the areas of the Ozen field belonging to "Ozenmunaigas" JSC in the Mangistau region was assessed.

It should be noted that in nature and laboratory conditions algae growth is accompanied by the development of microorganisms. The intensity of self-purification processes occurring in these water bodies largely depends on the relationships of all these organisms. This testifies to the fact that in algobacterial communities there are complex interactions between microorganisms and microalgae, with the latter often playing a leading role. That is, there are elements of both symbiosis and antagonism between the components of the algobacterial community, and symbiont interaction occurs through trophic and functional relationships. In the process of life, algal extracellular secretions (carbohydrates, lipids, polypeptides, organic acids and amino acids, vitamins) can be a source of nutrition for symbiotic bacteria or, on the contrary, a limiting factor.

The mass development of accompanying bacterioflora was found to be caused by high-molecular metabolites released by algae, and algal cells cannot break down these compounds into low-molecular compounds without bacterial exoenzymes. Moreover, during the exponential growth phase, more than 70% of the excretions are low molecular weight organic acids. It was also found that bacterial growth was inhibited, creating optimal conditions for algal nutrition. At the same time, companion bacteria also act as antagonists towards symbiotic algae. The inhibitory effect of bacteria on algae is due to bacterial metabolites, mainly antibiotics, lysozymes. Mass development of bacteria is observed in culture under unfavourable conditions for the growth and development of algae, such as reduction or acidification of the nutrient medium, accumulation of metabolic products, and change of temperature regime.

The available data on the effect of petroleum hydrocarbons on aquatic plants are quite contradictory. The diversity of aquatic plants resistant to oiled water is poorly understood. It is erroneous to judge habitat quality by the presence or absence of a species during one-off surveys. Therefore, the study of anatomy-morphological and physiological features of aquatic plants under oil pollution is one of the most urgent problems due to the lack of information about them. At the same time, knowledge of the degree of plant resistance to hydrocarbon pollution is necessary for the possibility of their cultivation in oil-contaminated water, as well as for solving problems related to water purification (phytoremediation). In particular, the most effective ways to preserve, control and restore anthropogenically damaged aquatic ecosystems and their biodiversity are the study and prevention of the impact of modern pollutants on hydromacrophytic plants.

To select stable hydromacrophytes, the analysis of obvious signs of HCV reaction to excessive amounts of oil and oil products was carried out on the example of highly active aquatic plants *Ludwigia palustris*-Super Red, *Bacopa monnieri*. The intensive development of aquatic plants is influenced by many factors: water transparency and temperature, the value of biogenic macro- and microelements in water, the gas content of water, pH value, successional exchange of phytocenosis, displacement of one plant by another and many others. The factors were optimised in laboratory conditions. The ability of plants to clean the aquatic environment from organic pollutants depends on their enrichment of water with oxygen, absorption of substances formed as a result of biochemical processes, and resistance and adaptation of plants to a particular environment. For this purpose, dissolved oxygen in water is used, as well as the decomposition of complex organic substances by oxidation, after which there is a decrease in the index of nitrogenous compounds and the continuation of normal plant life.

In the course of the experiment, the possibilities of using highly active aquatic plants *Ludwigia palustris*-Super Red, and *Bacopa monnieri* in phytoremediation of aquatic environment from oil are considered. According to the results, the effect of oil on aquatic plants depends on the concentration. The dose limit was found to be 5% for aquatic plants and 7% for aquatic plants such as *Bacopa monnieri*. They adapt well to water bodies contaminated with petroleum hydrocarbons by intensively accumulating oil from the aquatic environment. Thus, the use of this plant for phytoremediation is possible at oil hydrocarbon concentrations of 5%. The resistance of *Ludwigia palustris* to a 3% concentration of petroleum hydrocarbons was estimated to be 20-50% in all morphological parameters. Chlorophyll formation was impaired in leaves, photosynthesis activity decreased, and pink colour changed from green to brownish yellow, white powdery patina appeared. It was found that a 5% concentration of petroleum hydrocarbons is the limiting dose for the aquatic plant *Ludwigia palustris*. At low concentrations of petroleum hydrocarbons (3%), *Bacopa monnieri* restored leaf plate turgor, and new leaves and roots appeared, creating opportunities for the resumption of active normal life. The changes induced in *Bacopa monnieri* at 7% oil concentration, i.e. loss of turgor, necrosis, leaf and root die-back, and reduced photosynthetic activity, ranging from 30-70%. Colouring, i.e. appearance of chlorosis, changed from yellow-green to brownish. It was found that a 7% concentration of petroleum hydrocarbons is the limiting dose for the aquatic plant *Bacopa monnieri*. Thus, *Bacopa monnieri* can better absorb petroleum hydrocarbons from aquatic environments.

According to the results of the experimental study of oil's influence on hydromacrophytic plants at the ultrastructural level, it becomes obvious that all morphometric indices of plants are high. In the studied species under oil pollution, the leaf plate had a loose structure. The size of parenchyma cells increased significantly, and their order decreased. As a result of tissue damage, chlorophyll was destroyed, membrane permeability increased in the stem, membranes became brittle, and cell protoplast concentrated in the centre of each cell. Conducting bundles are "compressed", deformed, and destroyed by cells of the primary sheath parenchyma. The cells of the nucleus of the central cylinder were friable, without starch inclusions. The study results are of practical value for finding out the degree of aquatic plant resistance to hydrocarbon pollution, as well as for the implementation of biodiagnostics and monitoring of complex anthropogenic impact, which are necessary steps in solving phytoremediation problems. Aquatic plants with phytoremediation potential are resistant to the toxic effects of high concentrations of pollutants and participate in their absorption or destruction, and macrophytes are destructors of xenobiotics. In this connection, algae and higher aquatic plants are expedient to use in the practice of water treatment from oil products.

Theoretical data on the effect of petroleum hydrocarbons on aquatic plants are quite contradictory. The diversity of aquatic plants resistant to oil-contaminated water is insufficiently studied. Therefore, the study of anatomy-morphological and physiological features of aquatic plants under oil pollution is one of the most urgent problems. At the same time, knowledge of the degree of plant resistance to hydrocarbon pollution is necessary to be able to grow them in oil-contaminated water, as well as to solve problems related to water purification (phytoremediation). In particular, the most effective ways to preserve, control and restore anthropogenically damaged aquatic ecosystems and their biodiversity are to study and prevent the impact of modern pollutants employing hydromacrophytic plants and algae.

4 Discussion

The results of modern scientific research substantiate the highly negative consequences of the impact of pollution of natural water bodies with wastewater containing oil and petroleum products. Deng et al. (2021) assure in the process of scientific research that there is a global problem of non-compliance of water environment quality standards with the parameters of wastewater discharged into natural water bodies after treatment facilities. Thus, wastewater, even in compliance with discharge standards, always carries a load of varying degrees on the quality parameters of the aquatic environment.

Discharge of oily wastewater, according to the studies of Remmas (2022), maximum deterioration of water quality in a natural water body in the discharge location, contributing to the increase in the temperature of the aquatic environment, minimisation of dissolved oxygen, and increase in the concentration of organic substances. The author draws attention to the fact of prolongation of the consequences of the above processes, primarily in the form of the destruction of the composition of local biodiversity and, the replacement of intolerant species of biota with more adapted ones. In the researcher's opinion, the biological method of wastewater treatment is characterised by the principles of environmental friendliness and sustainability of the natural environment, which corresponds to global trends. He foresees the important fact that in the process of biological treatment, no compounds alien to the natural environment are formed and, at the same time, the destruction of organic pollutants to natural compounds of carbon dioxide and water, without requiring the use of chemical reagents. The results of the current study concur with the findings of the researcher, identifying biotreatment as an effective promising method for optimising wastewater treatment systems in a sustainable resource management strategy.

Kumar et al. (2022) emphasise that due to physical and chemical factors of self-purification of the aquatic environment, it is possible to achieve insignificant changes in the composition of effluents, without the destruction of oil products. Scientists are convinced that the predominant importance of self-purification of natural water resources is assigned to biological interaction processes, among which hydrocarbon-oxidising microorganisms show maximum activity. They are convinced that due to their activity, oil-containing compounds are transformed into simple compounds, and new organic compounds are accumulated, as well as their inclusion in the carbon cycle in water bodies. In the study of the researchers as well as in the current study, it is proposed to maximise the synergy potential of hydrocarbon-oxidising microorganisms, algae, and higher plants, so similar conclusions can be stated. To achieve the effect of intensification of biological wastewater treatment, the researchers foresee the expedient organisation of preliminary aeration of water with the help of modern installations, as well as the use of microorganisms that destroy sulphate compounds. In addition, the scientists consider it necessary to continuously monitor the activated sludge used for treatment.

Many modern scientists, for instance, Makut et al. (2022), to effectively purify and disinfect water as an alternative to chemical methods (ozonation or ultraviolet disinfection), propose using algae and higher aquatic plants. The researchers insist that biopurification technologies involve active use of the potential of natural self-purification processes, the maximum effect of which is demonstrated in the synergy of mechanical, physicochemical purification processes and biological processes of pollutant neutralisation. As an example, scientists cite the combination of the sedimentation method with biocoagulation and photosynthesis. It should be noted that such results of scientific research only partially coincide with the conclusions of the current study, as the latter positions the promising advantage of biotreatment as a priority independent wastewater treatment.

Among the advantages of methods of treatment of wastewater containing oil products employing algae and higher plants, Zhu et al.(2021) emphasise efficiency, cost-effectiveness, environmental safety, and absence of secondary pollution. This fact is also emphasised by Hamdhani et al. (2020). The researchers' conclusions are identical to the results of the current study, where water treatment using the potential of biocoenosis is positioned as a priority prospect for the development of technologies for the neutralisation of oil products in wastewater. The results of the studies by Wen et al. (2017) emphasise the expediency of the application of aquatic organisms-filtering organisms, hydrocarbon-oxidising microorganisms, and aquatic plants (eichhornia, water moss, elodea, cassava, urutia, rudist, cattail) in the process of biotreatment. Also, scientists propose to apply the potential of microbial-plant interactions to purify the aquatic environment. The present study, as well as in the study of Y. Wen et al. also emphasises the need to effectively use the purification functionality of plants and microorganisms.

In the development of these scientific searches, Jones et al. (2022) and also Van Vliet et al. (2021) believe that bacteriocoenoses of epiphytic hydrocarbon-oxidising bacteria *Pseudomonas fluorescens*, *P. guinea*, *Ochrobactrum anthropi*, *Rhodococcus fascians* and *fucus* algae create conditions for effective utilisation of petroleum hydrocarbons, successfully cope with their significant concentrations in the aquatic environment, significantly optimising the processes of oil pollution destruction. It is difficult to disagree with the researchers' conclusions. At the same time, researchers Comber et al. (2022) focus on the method of wastewater treatment from oil products through the process of the symbiosis of algae *fucus*, *Laminarya*, *Phyllariella* and hydrocarbon-oxidising microorganisms. The method proposed by the scientists involves the use of a filter, which is a system of synthetic ropes planted with algae and populated with oil-oxidising microorganisms. The symbiosis of hydrocarbon-oxidising bacteria with brown algae *fucus vesiculosus* , *Saccharina latissima* is positioned as the basis of sanitary algae plantation. The method, which in the study by the researchers positioned as a priority, in the current study is also seen as promising for use in specific conditions and in those cases where it is inappropriate to use classical methods of biopurification.

Hossain et al. (2022) determined the predominance of bacterial microflora in the biofilm at the beginning of the cleaning process, as well as the advantage of the decomposition processes of organic compounds under conditions of oxygen access. At the same time, blue-green algae (e.g. *Oscillatoria tenuis* and *Phormidium fovealarum*) are usually present among algae. Scientists emphasise the fact that algae have a developed mucus cover that protects them from the toxic effects of oil, as well as the ability to absorb dissolved organic matter directly from the water column. The researchers insist that the species composition is transformed during the wastewater treatment process, with the prioritisation of algae *p. Euglena* and *p. Chlomidamonas*. Such a trend, according to scientists, is an indicator of the dynamics of the quality of the water that is treated and also indicates the proper efficiency of pollutant removal. To confirm or refute the scientists' conclusions is foreseen to be a difficult task, as it requires long experimental studies. In the theoretical aspect, they do not contradict the results of the current study.

Fan et al. (2022) emphasise the fact that even in the case of the application of a perfect water treatment system, inorganic substances that are in an undissolved state, as well as more than 10% of organic pollutants cannot be neutralised, remaining in the composition of treated wastewater. This indicates, according to scientists' conviction, the actualisation of priorities of water use strategy following the requirements of a sustainable natural environment. The concept of "green" water use, from their point of view, implies, in particular, isolation of the anthropogenic water cycle from the natural one, i.e. transition to closed water supply. The introduction of innovative environmental monitoring capabilities, the implementation of objective assessment to minimise the impact of wastewater on aquatic ecosystems, the priority of preventive protection and the regeneration of problematic locations are also, according to the researchers, necessary methods for achieving the goals of sustainable water use. The above priorities are fully synergistic with the main message of the results of the current study.

The considered methods of biological treatment from oil hydrocarbons have characteristic advantages and disadvantages. The selection of the priority method is determined by the local specificity of the process of oil pollution of the aquatic environment. The conclusions obtained as a result of the experiment in the course of the current study unequivocally confirm the expediency of using the cleaning potential of plants and algae in the fight against oil pollution. The most promising is the integrated use of several methods, which will minimise the destructive load on the environment. Innovative solutions in the field of biological methods of oily wastewater treatment require further research with maximum use of the purification potential of algae and higher plants. Development of Kazakhstan's water treatment system in this direction corresponds to the goals of "green" development, ecologization of production processes and sustainable habitat.

5 Conclusions

The study of plant adaptability under conditions of increased anthropogenic load on aquatic ecosystems is of great theoretical interest and has significant practical value as a scientific basis for biomonitoring of polluted water bodies, their phytoremediation, as well as for increasing the stability of hydrocoenoses in general and preserving their biological diversity.

The study succeeded in analysing the role of aquatic plants *Ludwigia palustris* and *Bacopa monnieri* employing comparative anatomy-morphological studies as biosorbents of oil compounds and adaptation reactions at the ultrastructural level. The phytoremediation role of aquatic plants in water purification from oil and oil products was studied. In the course of the study, it was proved that wastewater biotreatment maximally uses the natural potential of higher plants, algae, and microorganisms in the aspect of the ability to oxidise and regenerate organic compounds that are in the aquatic environment in suspended form or the form of colloidal solutions. Based on this, the process of biological treatment of wastewater from oil and oil products is positioned as the most environmentally friendly promising technology of water treatment.

The research results obtained by the method of experiment, modelling and statistical processing of morphometric indicators are positioned as useful for determining the degree of resistance of plants and algae to hydrocarbon pollution, for biodiagnostics and monitoring the state of complex anthropogenic impacts, solving complex problems of phytoremediation. The information, which was synthesized and actualized in the course of the study, should be used in the process of development and modernization of the water use system of Kazakhstan and, the formation of vector programmes of preventive protection of water resources from oil-containing effluents. The possibilities of practical implementation of biological treatment of oily wastewater as a priority and effective alternative to the traditional treatment system require further research.

Conflict of Interest

None of the authors have reported a conflict of interest.

Author Contributions

Z.B., B.Z., and G.Y.: conceptualization, methodology, data curation, writing-original draft preparation. N.A.: visualization, investigation, and supervision. A.M. and L.A.: software, validation, writing-reviewing, and editing. All authors read and approved the final manuscript.

References

Abdukodirova, M.N. and Abduganiev, M.M., 2023. Biotreated wastewater for use in agriculture. Eurasian Journal of Academic Research, 3(10): 294-302.
https://zenodo.org/records/10056982

Ayatkhan, M. and Kaliyeva, G.K., 2020. Advanced wastewater treatment methods. Bulletin of the Kokshetau Technical Institute, 40(4): 54-57.
http://kti-tjm.kz/public/uploads/OBNOVLENIE_SAITA_2015/OONIiRIR/VESTNIKI/Vestnik_4_40.pdf♯page=54

Chesnokova, M.G. and Shalay, V.V., 2019. Prediction test of active silt druming on the biological cleaning unit of waste water at oil refining enterprise. AIP Conference Proceedings, 2141(1): 020029.
https://doi.org/10.1063/1.5122048

Comber, S.D.W., Gardner, M.J., Ansell, L., Ellor, B., 2022. Assessing the impact of wastewater treatment works effluent on downstream water quality. Science of The Total Environment, 845: 157284.
https://doi.org/10.1016/j.scitotenv.2022.157284

Deng, S., Wang, B., Zhang, W., Su, S., Dong, H., Banat, I.M., Sun, S., Guo, J., Liu, W., Wang, L., She, Y., Zhang, F., 2021. Elucidate microbial characteristics in a full-scale treatment plant for offshore oil produced wastewater. PLoS ONE, 16(8): e0255836.
https://doi.org/10.1371/journal.pone.0255836

Ergozhin, E.E., Chalov, T.K., Kovrigina, T.V., Melnikov, Ye.A., 2021. Modern state of the refining industry and oil products market in Kazakhstan. Chemical Journal of Kazakhstan, 67(3): 6-38.
https://www.chemjournal.kz/index.php/journal/article/view/200/178

Fan, Q., Lu, T., Deng, Y., Zhang, Y., Ma, W., Xiong, R., Huang, C,. 2022. Bio-based materials with special wettability for oil-water separation. Separation and Purification Technology, 297: 121445.
https://doi.org/10.1016/j.seppur.2022.121445

Hamdhani, H., Eppehimer, D.E., Bogan, M.T., 2020. Release of treated effluent into streams: A global review of ecological impacts with a consideration of its potential use for environmental flows. Freshwater Biology, 65(9): 1657-1670.
https://doi.org/10.1111/fwb.13519

Hossain, M.R., Khalekuzzaman, M., Kabir, S.B., Islam, M.B., Bari, Q.H.B., 2022. Production of light oil-prone biocrude through co-hydrothermal liquefaction of wastewater-grown microalgae and peat. Journal of Analytical and Applied Pyrolysis, 161: 105423.
https://doi.org/10.1016/j.jaap.2021.105423

Jones, E.R., Bierkens, M.F.P., Wanders, N., Sutanudjaja, E.H., van Beek, L.P.H., van Vliet, M.T.H., 2022. Current wastewater treatment targets are insufficient to protect surface water quality. Communications Earth & Environment, 3: 221.
https://www.nature.com/articles/s43247-022-00554-y♯citeas

Kumar, L., Chugh, M., Kumar, S., Kumar, K., Sharma, J., Bharadvaja, N., 2022. Remediation of petrorefinery wastewater contaminants: A review on physicochemical and bioremediation strategies. Process Safety and Environmental Protection, 159: 362-375.
https://doi.org/10.1016/j.psep.2022.01.009

Kuyukina, M.S., Krivoruchko, A.V., Ivshina, I.B., 2020. Advanced bioreactor treatments of hydrocarbon-containing wastewater. Applied Sciences, 10(3): 831.
https://doi.org/10.3390/app10030831

Makut, B.B., Goswami, G., Das, D., 2022. Evaluation of bio-crude oil through hydrothermal liquefaction of microalgae-bacteria consortium grown in open pond using wastewater. Biomass Conversion and Biorefinery, 12, 2567-2581.
https://doi.org/10.1007/s13399-020-00795-x

Manasa, R.L. and Mehta, A., 2020. Wastewater: Sources of pollutants and its remediation. In: Environmental biotechnology (pp. 197-219). Cham: Springer.
https://doi.org/10.1007/978-3-030-38196-7_9

Mitra, S., Campo, R., Bhowmick, S., Biswas, A., 2022. Membrane bioreactors for the treatment of oily wastewater: Pros and cons. In: Advances in Oil-Water Separation (pp. 469-487). Amsterdam: Elsevier.
https://doi.org/10.1016/B978-0-323-89978-9.00007-0

Murzabekova, Zh., 2023. Ecological state of the environment of the West-Kazakhstan fields. Bulletin of West Kazakhstan Innovative and Technological University, 26(2): 237-241.
https://wkitu.kz/wp-content/uploads/2023/08/sbornik-vestnik-2-26-23.pdf♯page=237

Myrzabaeva, Zh., Syzdykova, M., Isaeva, A., 2021. Laboratory research of phytomeliorative method of municipal wastewater treatment. In: Technical-Scientific Conference of Undergraduate, Master and PhD Students. (pp. 579-582). Chisinau: Technical University of Moldova.
https://ibn.idsi.md/vizualizare_articol/133711

Qachach, H., Abriak, N., El Mahrad, B., Souabi, S., Tahiri, M., 2021. Biological treatment of fuel wastewater generated from a thermal power plant by continuous and discontinuous aeration. Desalination and Water Treatment, 222: 145-155.
https://www.deswater.com/DWT_articles/vol_222_papers/222_2021_145.pdf

Remmas, N., 2022. Biotreatment potential and microbial communities in aerobic bioreactor systems treating agro-industrial wastewaters. Processes, 10(10): 1913.
https://doi.org/10.3390/pr10101913

Sanghamitra, P., Mazumder, D., Mukherjee, S., 2021. Treatment of wastewater containing oil and grease by biological method – A review. Journal of Environmental Science and Health, 56(4): 394-412.
https://doi.org/10.1080/10934529.2021.1884468

Singh, R.L. and Singh, R.P., 2019. Advances in biological treatment of industrial waste water and their recycling for a sustainable future. Singapore: Springer.
https://doi.org/10.1007/978-981-13-1468-1

Singh, S. and Shikha., 2019. Treatment and recycling of wastewater from oil refinery/petroleum industry. In: Advances in Biological Treatment of Industrial Waste Water and Their Recycling for a Sustainable Future (pp. 303-332). Singapore: Springer.
https://link.springer.com/chapter/10.1007/978-981-13-1468-1_10

Umbetova, Sh.M., Otarbayev, B.S., Shegenbayev, A.T., Abdikerova, U.B., 2022. The current state of the wastewater disposal system and ecological justification of biological wastewater treatment in the city of Kyzylorda. Bulletin of the Korkyt Ata Kyzylorda University, 61(2): 157-164.
https://vestnik.korkyt.kz/wp-content/uploads/2022/06/journal2022_1bolim.pdf♯page=158

Van Vliet, M.T.H., Jones, E.R., Flörke, M., Franssen, W.H.P., Hanasaki, N., Wada, Y., Yearsley, J.R., 2021. Global water scarcity including surface water quality and expansions of clean water technologies. Environmental Research Letters, 16(2): 024020.
https://doi.org/10.1088/1748-9326/abbfc3

Wen, Y., Schoups, G., van de Giesen, N., 2017. Organic pollution of rivers: Combined threats of urbanization, livestock farming and global climate change. Scientific Reports, 7: 43289.
https://doi.org/10.1038/srep43289

Zhu, Y., Schmidt, A.J., Valdez, P.J., Snowden-Swan, L.J., Edmundson, S.J., 2021. Hydrothermal liquefaction and upgrading of wastewater-grown microalgae: 2021 state of technology. Richland: Pacific Northwest National Laboratory.
https://doi.org/10.2172/1855835

Zhylkybayev, T., Zolotov, A., Ospanov, Ye., Zhanuzakov, Ye., Algazinov, N., 2023. The current state and problems of wastewater treatment in Kazakhstan. The Bulletin of KazATC, 127(4): 514-523.
https://doi.org/10.52167/1609-1817-2023-127-4-514-523