Isolation and study of cyanobacterial cultures from oil refinery wastewaters

Madina Kazim, Aliyam Dauletova, Sandugash Sandybayeva, Zhanat Bukharbayeva, and Bolatkhan Zayadan

Abstract. Wastewater from oil refineries, as well as other chemical industries, has specific features. Phototrophic microorganisms are commonly used to treat wastewater containing toxic components, such as oil and petroleum products. Cultivating microalgae and cyanobacteria in nutrient-rich wastewater can yield high biomass for simultaneous biotreatment, making it a more cost-effective and environmentally friendly approach. This study investigated the species composition of microalgae in wastewater from oil refineries in the Atyrau region. The results showed that Cyanophyta representatives dominated the microalgae composition in the enrichment culture. The presence of cyanobacteria in the wastewater was attributed to the high nutrient and calcium content of the organic matter. An axenic culture of the cyanobacterium *Anabaena* sp. was isolated to study its ability to grow and degrade different concentrations of motor oil. The results indicate the potential of *Anabaena* sp. strain in remediating aquatic environments contaminated with petroleum products.

1 Introduction

The pollution of aquatic environments by crude and refined oil is a significant environmental problem that leads to unmanageable losses of biological life. This issue has a negative impact on the world economy, particularly in the areas of oil production and transport [1]. Unidentified hydrocarbons, such as petroleum, fuel oil, paraffin, oils, and their impurities, are the most common pollutants found in wastewater. UNESCO has identified these substances as among the top ten most hazardous environmental pollutants due to their high toxicity [2]. Bioremediation is a modern method used for the development of environmentally friendly technologies to protect the environment and restore natural resources [3]. The use of microalgae and cyanobacteria in bioremediation technology has several advantages over conventional cleaning methods. These include low cost, the ability to remove contaminants without leaving traces or residues, and the decomposition of contaminants into natural end products in the environment [4]. In particular, the use of cyanobacteria in the clean-up of sites contaminated with petroleum hydrocarbons is considered a process of stimulation and enhancement due to their ability to secrete organic exudates combined with their reported biodegradation potential [5]. For example, cyanobacterial species such as *Oscillatoria salina*, *Plectonema terebrans*, *Aphanocapsa* sp., and *Synechococcus* sp. have been successfully used in bioremediation of crude oil spills in different parts of the world [6]. According to Sood et al.’s study [7], bioremediation using cyanobacteria is a viable alternative for wastewater management due to the high nitrogen and phosphorus demand during cyanobacteria growth [8]. Additionally, wastewater bioremediation with cyanobacteria can adjust pH, reduce total dissolved solids, and eliminate both chemical oxygen demand and biological oxygen demand [9]. Ren et al. (2019) reported that cyanobacteria produce biomass containing lipids, carbohydrates, and other compounds that can be used for biofuel production during growth in wastewater. This technology can integrate wastewater treatment with biofuel production and water recycling for agriculture [10]. The study aimed to isolate phototrophic microorganisms from industrial wastewater of oil refineries, determine the species composition of the algalflora of the studied samples, and study the growth ability of promising strains in the presence of petroleum products at different concentrations.

2 Materials and methods

Cyanobacteria cultures were isolated from wastewater of oil refineries in Atyrau region. The biological mats from selected wastewaters were repeatedly washed with double distilled water and were transferred into 250 ml flasks containing BG11 medium [11]. Conventional microbiological methods were used to obtain and isolate allogologically pure cultures [12].

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Cyanobacterial isolates were identified morphologically using an optical light microscope (MicroOptix MX 300T, Austria) equipped with a digital camera and visualization system. To study the productivity of newly isolated cultures, cyanobacterial cells were continuously cultured in 500 ml glass vessels under laboratory luminostat. The cultures were continuously aerated under artificial light with an intensity of 50 μmol(photon)s−1 and a sterile gas-air mixture enriched with 1% CO₂. Growth was monitored to determine the stimulatory or inhibitory effects of pollutants on the tested cyanobacteria (their resistance or sensitivity) in order to identify the most resistant and promising species for bioremediation. Three replicates of each sample were collected aseptically from both a control culture (without contaminants) and three samples with added motor oil (5, 10 and 15 mg/L) at each exposure time. Cyanobacterial culture growth was monitored using a PD-303UV spectrophotometer (Japan) at a wavelength of λ=750 nm (OD750). Chlorophyll α content was determined by extracting pellets using the standard acetone extraction method [13]. The data were statistically analyzed and expressed as mean ± standard deviation. The relative standard error was 2-5% for major parameters and less than 10% for minor components.

3 Results and discussion

3.1 Study of cyanobacteria isolated from oil refinery wastewaters

The study focused on the wastewater of Atyrau oil refinery. A WTP operated by “Kaztransoil” CJSC in Atyrau city supplies drinking water to the residents of the city and Makat district through the main water pipeline “Atyrau-Makat” which sources from the Zhaiyk river. Additionally, “Kaztransoil” CJSC operates a WSS on Kigach river in Kurmangazy district which supplies water to residents of Isataisky and Zhylyoi districts through the “Kigash-Kulsary” main water pipeline. In Kurmangazinskiy, Inderskiy, and Makhambetskiy districts, domestic water is sourced from the Zhaiyk, Kigash, and Sharon rivers. Wastewater from petrochemical enterprises is stored in ponds, and the resulting oil sludge is then pumped into underground storage. Discharging wastewater that contains harmful substances such as sulphates, chlorides, nitrogen compounds, phenols, heavy metal salts, benzpyrene, Cd, Ni, Cu, and Pb into the environment poses a threat to both human health and the ecosystem [14]. The composition of cyanobacteria in water treatment facilities reflects the concentration of harmful substances. Several researchers have observed that the concentration of harmful substances in water and air near the GPP exceeds the MPC by tens and hundreds of times. The wastewater from the Atyrau Refinery is highly contaminated with benzpyrene, Cd, Ni, Cu, Pb, phenol, and other carcinogenic substances that accumulate in the soil [15].

In the studied water samples from the investigated area, a total of 32 species were identified, which belonged to 4 taxonomic groups. The dominant group was Cyanophyceae, accounting for 46.88% with 15 species. Chlorophyceae was the second most dominant group with 12 species, accounting for 37.5% of the identified species. Bacillariophyceae (diatoms) accounted for 12.5% with 4 species, while Euglenophyceae accounted for only 3.13% with 1 species (Fig. 1).

![Fig. 1. Abundance of various groups of algoflora in wastewater of Atyrau Refinery. CYA: Cyanophyceae, CHL: Chlorophyceae, BAC: Bacillariophyceae, EUG: Euglenophyceae.](image-url)

The following dominant genera of microalgae were recorded in all samples studied: Chlorella,
Ankistrodesmus, Euglena, Scenedesmus; as well as genera of cyanobacteria - Syneschossocus, Phormidium, Anabaena and others. The selected cultures of cyanobacteria generally grew as free-floating, floc-like, gelatinous, mucilaginous masses, while some grew as a suspension in liquid medium. The thalloms' colours ranged from pale green to bluish-green and dark green. Most of the forms were planktonic. The selected cultures displayed various growth forms on agar-based media, including small mucilaginous spots or balls, spreading types, or small disorderly flakes. The Anabaena culture exhibited filamentous growth of a spreading type, resembling a net-like layer on agar. To identify productive strains, algologically pure cyanobacterial cultures of the genera Anabaena, Synechococcus and Phormidium were initially tested for growth potential (Fig. 2). In laboratory conditions, all newly isolated cultures of cyanobacteria were grown for 14 days in different nutrient media - BG11, Zarruk and Gromov.

The experiment showed that the culture of cyanobacterium Anabaena had high growth dynamics. The growth profile of Phormidium genus cultures were similar to that of Anabaena for the first three days of cultivation, but the optical density values of cells were lower in the following days. The minimum values of growth rate coefficient were observed in cyanobacteria of Synechococcus genus.

Figure 3 shows that the cyanobacterium genus Anabaena had the highest maximum cell-specific concentration of chlorophyll (Chl) α (about 9.64 µg/ml on 12th day), which was almost twice as high as the minimum value of Chl α (4.83 µg/ml for Synechococcus on 12th day). Cyanobacterium culture Anabaena was selected for further study based on growth and dry biomass monitoring, as well as criteria such as chlorophyll accumulation potential. Anabaena sp. is a filamentous cyanobacterium with heterocysts that belongs to the family Nostocaceae (Fig. 4). The trichomes are often arranged chaotically, while single trichomes consist of spherical cells. Heterocysts are rare, but akinetes are ubiquitous. The trichomes have small, deep constrictions, and the walls are covered with mucous membranes. The cells are cylindrical, barrel-shaped or spherical, and are...
either whitish or light blue green. The heterocysts vary in shape from oval to globular or elongated barrel-shaped, with more vegetative cells.

![Cells image](image)

**Fig. 4.** Cells image (100x) of new isolated cyanobacterial strain *Anabaena* sp. VC- vegetative cell, GT-heterocyst cell

### 3.2 Evaluating the growth ability of *Anabaena* sp. strain in medium supplemented with petroleum products

A comparative assessment was conducted under laboratory conditions to analyze the growth of cyanobacterium *Anabaena* sp. in a medium with the addition of motor oil at concentrations of 5, 10, and 15 mg/L over a period of 14 days.

![Growth rate](image)

**Fig. 5.** Growth rate during bioremediation: *Anabaena* sp. in used motor oil with concentrations 5, 10, 15 mg/L

Figure 5 displays the growth rate of the cyanobacterium *Anabaena* sp. under motor oil contamination conditions. High growth was achieved on 3rd day of incubation at a concentration of 5 mg/L, followed by the results of control, 10 and 15 mg/L concentrations, respectively. On 12th day of incubation, the optical density values were higher at 5 mg/L motor oil concentration compared to the control sample, with an optical density of 0.73.
Fig. 6. Dry weight analysis during bioremediation: *Anabaena sp.* in used motor oil with concentrations 5, 10, 15 mg/L.

The dry mass of the cyanobacterium *Anabaena sp.* was measured with the addition of motor oil at concentrations of 5, 10, and 15 mg/L at the beginning of cultivation and after reaching the stationary phase of growth rate. Fig. 6 illustrates that the maximum amount of dry mass was obtained at a motor oil concentration of 5 mg/L. At higher concentrations of motor oil, 10 and 15 mg/L, minimum values were observed.

Table 1. Effect of different concentration of motor oil on Chl α of *Anabaena sp.* Results are mean of 3 replicates (=standard error)

<table>
<thead>
<tr>
<th>Motor oil concentrations, mg/L</th>
<th>Content of Chl α, µg/ml</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>3rd day</td>
</tr>
<tr>
<td>5</td>
<td>2.70±0.17</td>
</tr>
<tr>
<td>10</td>
<td>2.63±0.34</td>
</tr>
<tr>
<td>15</td>
<td>2.68±0.13</td>
</tr>
</tbody>
</table>

*Anabaena sp.* strain exhibited increased biomass when grown in a medium supplemented with 5 mg/L motor oil. The growth increase suggests that *Anabaena sp.* utilized hydrocarbon compounds as a source of nutrition, as evidenced by the treatment with motor oil. However, Table 2 shows a decrease in Chl α during the 14-day incubation period. At 14 days, the Chl α level of *Anabaena sp.* was 0.59±0.15 µg/ml when exposed to 5 mg/L of motor oil. The decrease in Chl α may have been influenced by the high amount of carbon as an energy source, causing the cyanobacteria to switch to a heterotrophic or mixotrophic type of nutrition, which resulted in a decrease in Chl α.

Previous studies have shown that cyanobacteria can degrade petroleum products in contaminated waters [16, 17]. Additionally, microalgae and cyanobacteria have been found to rapidly adapt to oil pollution and reproduce at low oil concentrations due to their adaptation to extreme environmental conditions [18].

4 Conclusion

In the conducted experiments, *Anabaena sp.* demonstrated resistance to the toxic compounds found in motor oil, successfully growing under extreme conditions. Although an increase in biomass was observed, the Chl α content in the *Anabaena sp.* culture decreased, which may indicate the development of an adaptation mechanism to high oil content. Further research will aim to investigate these mechanisms.

The data provided highlight the importance of addressing the issue of treating oil-contaminated water. Further research in this area can aid in optimizing the processes of self-treatment and bioremediation of wastewater contaminated with oil and oil products. It is anticipated that a consortium of algae and cyanobacteria will be developed in the near future for downstream wastewater treatment, bioremediation, bioenergy production, and CO2 capture. This will contribute to sustainable development and outstanding research achievements. The use of microalgae and cyanobacterial consortia can facilitate the development of integrated biotechnologies for treating and remediating polluted water bodies. Additionally, the biomass can be utilized for biofuel production.
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Authors' contribution

Bolatkhan Zayadan: Conceptualization, Resources, Supervision and Writing (Discussion, Conclusion); Sandugash Sandymbayeva: Editing and Final preparation of the manuscript; Madina Kazim: Writing (Original Draft and Results, Discussion), Resources, Methodology; Aliyam Dauletova: Writing (Introduction, Methods); Zhanat Bukharbayeva: Editing and Visualization. All authors have read and agreed to the published version of the manuscript.

References