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New *Pusillimonas thiosulfatoxidans* 1/8an strain isolated from oil-contaminated soil for oil biodegradation in Kazakhstan

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ABSTRACT

The oil-oxidizing 1/8an strain was isolated from the oil-contaminated soil in the Atyrau region, Kazakhstan. Based on the analysis of 16S rRNA sequences, the strain was identified as *Pusillimonas thiosulfatoxidans.* The strain effectively decomposed crude oil at 10 and 30 ℃ and showed the ability to degrade oil at 50 ℃. Gas chromatographic analysis showed that the strain degraded medium- and long-chain n-alkanes. The strain degraded oil in high salinity (up to 10%). The study of the influence of the medium's pH on oil degradation showed that the strain degraded oil in both acidic (pH 5) and alkaline (pH 9) environments. The strain degraded both light and heavy oils. Thus, the *P. thiosulfatoxidans* 1/8an strain can be used in the bioremediation of oil-contaminated soils under extreme conditions.

Keywords: Bioremediation, Biodegradation, Oil-oxidizing microorganisms, Oil, Temperature, Salinity.
Article type: Research Article.

INTRODUCTION

Environmental pollution caused by the uncontrolled use of natural resources is a global problem (Ajona & Vasanthi 2021; Martirosyan *et al.* 2022; Nasiyev *et al.* 2022). The widespread use of oil and petroleum products often leads to environmental disasters in the form of runoff from refineries and emergency oil spills. While some crude oil compounds decompose easily, long-chain alkanes and especially polycyclic aromatic hydrocarbons (PAHs) are relatively resistant to biodegradation. Due to their carcinogenic and mutagenic properties, some oil components are classified as high-priority pollutants that pose a danger to humans and wildlife (Li *et al.* 2022; Imam *et al.* 2021; Ghorbannezhad *et al.* 2022; Truskewycz *et al.* 2019). The process of complete natural destruction of oil takes at least 25 years. Thus, the periods of self-healing of ecosystems disrupted as a result of oil pollution are long (Bociu *et al.* 2019). Bioremediation methods based on the use of microorganisms capable of decomposing oil and petroleum products have been developed as an alternative to chemical and physical methods of cleaning contaminated media They offer a comparative advantage, since they are economically beneficial and less harmful to the environment (Lješević *et al.* 2020; Morales Guzmán *et al.* 2017). Bioremediation includes the use of endemic or artificially introduced autochthonous microorganisms for detoxification and degradation of pollutants (bioaugmentation) (Gillespie & Philp 2013). Currently, more than 100 genera of bacteria, yeast, and mycelial fungi are capable of decomposing hydrocarbons. The proportion of oil undergoing biodegradation ranges from 6 to 82% for soil fungi and from 0.13 to 100% for bacteria (Stepanova *et al.* 2022). However, bioremediation of oil-contaminated soils in extreme environmental conditions is difficult. Lack of nutrients, elevated temperatures, and high mineralization are among the main environmental factors affecting the diversity and activity of soil microorganisms. The efficiency of oil biodegradation is influenced by both abiotic factors (temperature, water availability, aeration, pH, mineralization) and biotic factors (microbial species; Venosa

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& Zhu, 2003; You *et al.* 2018; Xu *et al.* 2021). Thus, Yaling (Gou *et al.* 2019) showed that temperature is one of the most important factors in bioremediation. On the one hand, temperature affects the physical and chemical state of petroleum hydrocarbons. On the other hand, it affects the growth and metabolic activity of soil microbes. As a rule, at elevated temperatures, the viability of many bacterial species decreases due to damage to their cellular structures. Various studies indicate a range of 30-40 °C as the optimal temperature for biodegradation of oil in the soil (Chatterjee *et al.* 2008) This is higher than the summer soil temperature in temperate latitudes, which ranges from 25 to 30 °C, but acceptable in the sharply continental climate in the oil-producing regions of Kazakhstan.
Low temperatures affect the bioavailability of oil, changing its solubility, viscosity, and fluidity, thereby hindering the access of microorganisms to hydrocarbons. Temperatures below the optimal range lead to a slowdown in cellular metabolism and a decrease in the rate of degradation (Lofthus *et al.* 2018; Lofthus *et al.* 2020). An important factor affecting the biodegradation of oil is the degree of salinity in the environment. Qin *et al.* (2012) suggested that salinity has a great influence on bioremediation and biodegradation and affects the growth and diversity of microbes. Salinity harms the activity of some key enzymes involved in the degradation of hydrocarbons (Hua *et al*. 2010; Ebadi *et al.* 2017). The acidity of the medium also plays an important role: in natural conditions, bio-destructor microorganisms are active in a narrow pH range (neutral or close to neutral values). The pH level can vary greatly and must considered when developing biological purification methods. The pH of the environment affects processes such as transport through the cell membrane and the balance of catalytic reactions, as well as the activity of enzymes. Most heterotrophic bacteria tend to grow at a pH from neutral to alkaline, whereas soil acidity can be in the range of 2.5-11 (Al Hawash *et al.* 2018) The sharp continental climate in the oil-producing regions of Kazakhstan predetermines the search for new microorganisms capable of oxidizing oil hydrocarbons both at elevated and lower temperatures. The purpose of this work was to study the oil-oxidizing potential of a new *Pusillimonas thiosulfatoxidans* 1/8an strain isolated from the oil-contaminated soil in Kazakhstan.

MATERIALS AND METHODS

Chemicals. Reagent grade (RG) mineral salts were produced by AppliChem. Hexane, o-xylene, naphthalene, fluorene, anthracene, and phenanthrene were obtained from Sigma-Aldrich (purity 98-99%).

Nutrient media. Nutrient agar (Titan Biotech Ltd, India), agar-agar (Himedia, India), mineral medium of the following composition (g L^{-1}): NH_4NO_3: 1.0, K_2HPO_4: 1.0, KH_2PO_4: 1.0, $MgSO_4$: 0.2, $CaCl_2 \times 6H_2O$: 0.02, $FeCl_3$: traces, NaCl: 10.0, pH 7.0-7.2.

Oil. The oil was provided from the oil fields in the Atyrau region, Kazakhstan.

Oil from the Dossor oil field: density: 0.84 g cm^{-3}, paraffin content: 0.31-2.07%, sulfur: 0.22%, resin: 7%.

Oil from the Zhanatalap oil fields: density: 0.814 g cm^{-3}, paraffin content: 0.41-1.51%, sulfur: 0.12%, asphaltenes: 0.02%, silica gel resins: 0.63-1.05%, sulfuric acid resins: 6%.

Oil from the Zapadnaya Prorva field: density: 0.899 g cm^{-3}, paraffin content: 2.05-4.22%, sulfur: 0.33-1.46%, asphaltenes: 1.12%, resins: 15.6%.

Oil from the Karsak oil field: density: 0.933 g cm^{-3}, paraffin content: 0.17-2.19%, sulfur: 0.1-0.6%, wood resins: 9-52%.

Isolation and identification of the strain

Isolation of the microbial strain was carried out using the method of accumulative cultures. Into a flask with 100 mL mineral medium containing 1% vol. Dossor oil, 10 g oil-contaminated soil collected in the Atyrau region was added. The accumulative culture was incubated on an orbital shaker at 30 ℃ and 180 rpm. After 14 days, 10 mL of the culture fluid was transferred to a flask with a fresh mineral medium, and 1% by volume of oil was added and cultivated for another cycle. To isolate the oil-oxidizing strain, 1 mL of suspension was taken and serial dilutions were made (10^{-1}-10^{-7}), then sown in Petri dishes with nutrient agar. The grown individual colonies were thinned out with a depleting streep method to check the purity. The pure cultures were transplanted onto mown nutrient agar. To identify and characterize the strain, including Gram staining, colony size, morphology, and basic pigment formation, oxidation/fermentation, catalase and oxidase measurement, acid formation from carbohydrates, and other biochemical tests were carried out based on the Manual of Methods for General Bacteriology (Methods of general bacteriology 1984). For the taxonomic characteristics of the strain, 16S rRNA was analyzed using Sanger's method (Sanger *et al.* 1977). For this purpose, daily cultures of microorganisms were

used. Genomic DNA from bacteria was isolated using PureLink® Genomic DNA Kits (Invitrogene, USA) according to the manufacturer's protocol. A pair of universal primers were used in the work: 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and 806R (5'-GGACTACCAGGGTATCTAAT-3'). The reaction mixture (30 mL) contained 3 mL of 10x reaction buffer (Fermentase), 2.5 mm $MgCl_2$, 0.2 mM of each deoxyribonucleoside triphosphate (dNTP), 10 pmol of each of the primers, and 1 unit of Taq polymerase Maxima Hot Start Taq DNA Polymerase (Fermentas). Polymerase chain reaction (PCR) was performed in a Mastercycler proS thermal cycler (Eppendorf). The reaction was started by incubating the mixture at 95 °C for 7 min, followed by 30 cycles consisting of 95 °C (30 seconds), 55 °C (40 seconds), and 72 °C (1 min). The final elongation was performed at 72 °C for 10 min. The amplified product was separated in 1.5% agarose gel, the strips were stained with ethidium bromide and visualized in an UV transilluminator. A 1x TVE buffer was used as an electrode buffer. The PCR product was purified using CleanSweep™ cleaning reagent (Thermo Fisher Scientific, USA). The sequencing of fragments of the 16S rRNA gene of bacteria was carried out using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) according to the manufacturer's protocol (BigDye® Terminator v3.1 Cycle Sequencing Kit Protocol Applied Biosystems, USA), followed by the separation of fragments on an automatic genetic analyzer 3500 DNA Genetic Analyzer (Applied Biosystems, Hitachi, Japan). The sequencing results were processed in the SeqA software (Applied Biosystems). The search for homologous nucleotide sequences of 16S rRNA genes was carried out using the BLAST software (Basic Local Alignment Search Tool) in the International Gene Bank database of the National Center for Biotechnological Information of the USA [23]. Phylogenetic analysis was performed using MEGA6 software. The alignment of nucleotide sequences was performed using the ClustalW algorithm. To construct phylogenetic trees, the Neighbor-Joining (NJ) method was used.

Determination of the spectrum of used substrates of the strain

The strain was grown on agarized mineral medium in Petri dishes in hexane, o-xylene, and naphthalene vapors. Fluorene, anthracene, and phenanthrene had previously been dissolved in chloroform and applied in a thin uniform layer to the surface of the medium. After the chloroform had evaporated, a thin film formed on the surface. The ability of the studied strain to consume hydrocarbons was assessed visually by the intensity of growth (no growth, slight, moderate, good).

Biodegradation of oil

In Erlenmeyer flasks with 100 mL of mineral medium, 5 mL suspension of daily culture was introduced. As the only source of carbon and energy, 1% vol. of Dossor oil was added. The flasks were incubated in thermostatically controlled shakers at a temperature of 30 ºC for 14 days. The mineral medium with oil served as a control sample.

Biodegradation of oil at different temperatures

In Erlenmeyer flasks with 100 mL mineral medium, 5 mL suspension of daily culture and 1% vol. of Dossor oil were added. The flasks were incubated in thermostatically-controlled shakers at temperatures of 10 and 50 ºC for 14 days.

Biodegradation of oil with different NaCl content in the medium

To study the destructive ability of the strain at different salinity values of the medium, a mineral medium was used, to which 2, 5, and 10% NaCl were added. In Erlenmeyer flasks with 100 mL medium, 5 ml suspension of daily culture was introduced. Oil was added in an amount of 1% by volume. The flasks were incubated in a thermostatically-controlled shaker at 30 ºC for 14 days.

Biodegradation of oil at different pH values of the medium

A mineral medium with different acidity was used. pH 5 and 6 were established by acidification of the medium with sulfuric acid, and pH 8 and 9 by alkalinization with 10% NaOH solution. In flasks with 100 mL medium, 5 mL suspension of daily culture and 1% vol. of Dossor oil were added. The flasks were incubated in thermostatically-controlled shakers at a temperature of 30 ºC for 14 days.

Biodegradation of oil from different fields in Kazakhstan

In Erlenmeyer flasks with 100 mL mineral medium, 5 mL of suspension of daily culture was introduced. Oil from the Zhanatalap, Zapadnaya Prorva, and Karsak fields were used as the only source of carbon and energy. Oil was

added in the amount of 1% vol. The flasks were incubated in thermostatically-controlled shakers at a temperature of 30 ºC for 14 days. The mineral medium with oil served as a control sample. The quantitative determination of the residual oil content in the medium was carried out using the gravimetric method.

Determination of residual oil content in the medium

The quantitative determination of the residual oil content in the medium was carried out by the gravimetric method. The residual oil was extracted with chloroform in a dividing funnel. The chloroform extract was placed in cups with a constant weight, dried at room temperature in a fume hood, and weighed on OHAUS ExplorerEX 124 analytical scales (USA). The amounts of consumed oil and petroleum products were calculated based on the formula:

$$A = \frac{A1 - A2}{A1} \times 100\%$$

where A is the amount of oil consumed (%);
A1 is the amount of oil introduced;
A2 is the amount of residual oil.

The concentration of petroleum hydrocarbons was measured using a 7890 gas chromatograph combined with a mass spectrometer (Agilent, USA). The DB-35MS column (30 m × 0.25 mm, film thickness 0.25 microns, J&W Scientific Inc., USA) was used as a stationary phase, and helium (> 99.995%, Orenburg-Techgas, Russia) as a mobile phase (1.0 mL/min). The injected volume was 1.0 µL with a division ratio of 10:1. The temperature of the gas chromatography (GC) furnace was initially set at 40 °C for 5 min, then increased to 150 °C at a speed of 7°C/min and then heated to 300 °C at a speed of 5 °C/min for 5 min. The temperatures of the injector and the transport line were set at 250 and 280 °C, respectively. Mass spectrometry (MS) detection was performed at 70 eV with a scanning mass range of m/z 34-850. Agilent MSD ChemStation software (version 1701EA) was used to control the GC system for recording and processing results and data. Data processing included the determination of retention times, peak areas, and the processing of spectral data obtained by an MS detector. To decipher the obtained mass spectra, the Wiley 7th edition software and the NIST'02 library were used (the total number of spectra in the library exceeds 550 thousand).

RESULTS

Characteristics of the strain

The strain was isolated from oil-contaminated soil sampled in the Atyrau region. Its cultural-morphological and physiological-biochemical signs were studied. On nutrient agar, the strain formed round pale pink colonies, convex with an even edge and with a diameter of up to 3 mm (Fig. 1). The cells were represented by Gram-negative mobile rods, aerobic, catalase- and oxidase-positive. Once growing on a nutrient broth, the strain formed hydrogen sulfide and ammonia. It did not restore nitrates, assimilated lactose, sucrose, and mannitol and also formed acid from glucose. The strain grew at a temperature from 4 to 50 ℃ (the optimal temperature was 28-30 ℃) and was salt-resistant (up to 10% NaCl).

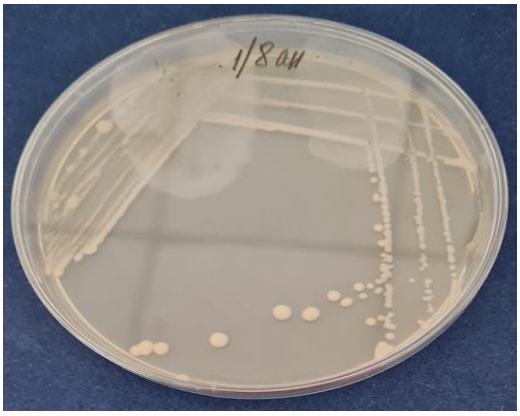

Fig. 1. Colonies of the 1/8an strain on nutrient agar.

A phylogenetic tree based on the 16S rRNA gene was calculated using the extended Maximum Likelihood method. A comparative analysis of the 16S rRNA sequence in the GenBank database was carried out. The similarity with the nearest strain NR 171398.1:61-785 *P. thiosulfatoxidans* YE3 was 100.0% (Fig. 2), which allowed us to attribute the strain to this species.

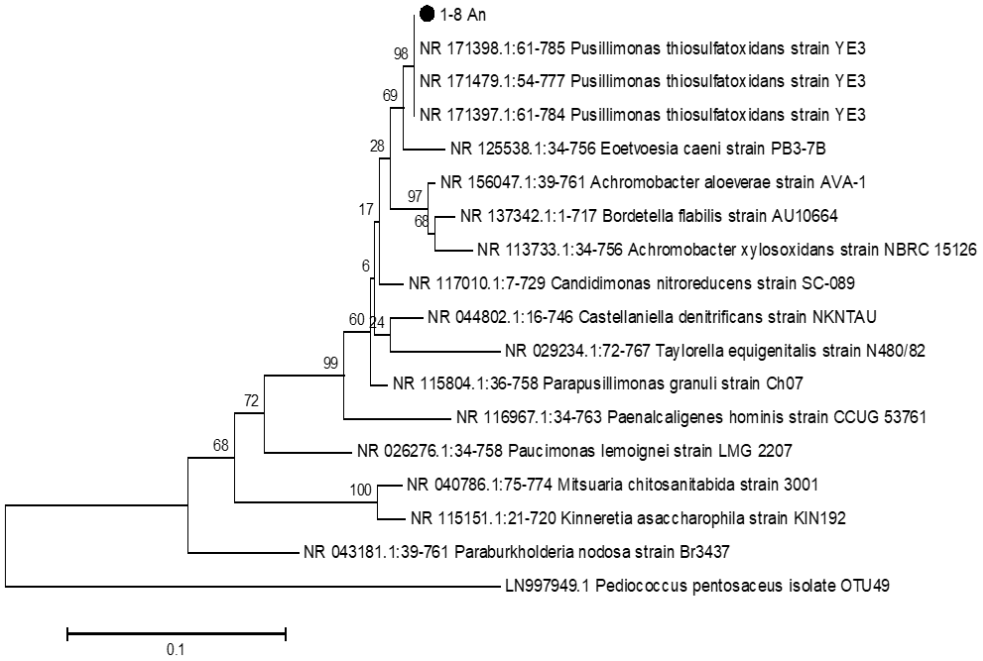

Fig. 2. A dendrogram showing phylogenetic relationships based on 16S rRNA gene sequencing.

The substrate specificity of the strain was determined. We found that the most easily degraded substrates were hexane and o-xylene (Table 1). PAHs underwent degradation to a lesser scale. The strain showed moderate growth on naphthalene, fluorene, and anthracene and weak growth on phenanthrene.

Table 1. Growth of the 1/8an strain on various hydrocarbons.

hexane	o-xylene	naphthalene	fluorene	anthracene	phenanthrene
+++	+++	++	++	++	+

Note: +: slight growth, ++: moderate growth, +++: good growth

Biodegradation of oil by the strain

Degradation of Dossor oil by the strain in a liquid mineral medium was studied. The initial oil content was 1% vol. Cultivation was carried out at a temperature of 30 ℃ for 14 days. Gravimetric analysis showed that the oil content decreased by 56.1% compared to the control variant (Fig. 3). The abiotic loss of oil was 17.5%.

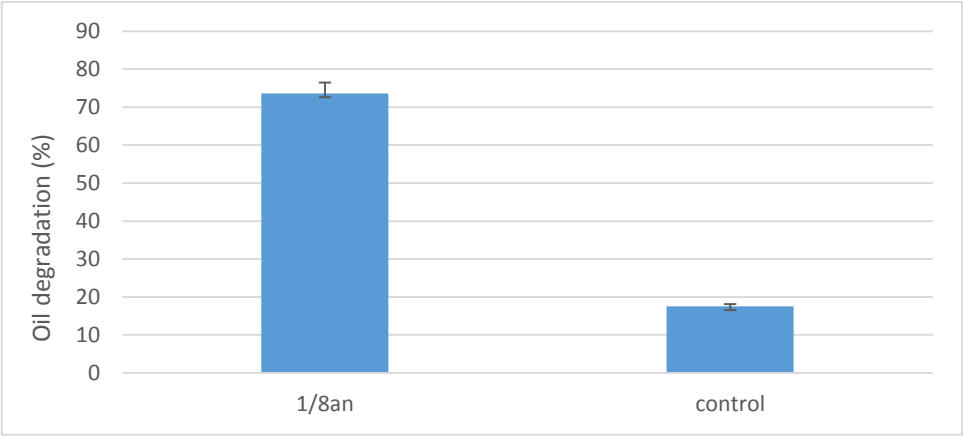

Fig. 3. Degradation of Dossor oil by the *P. thiosulfatoxidans* 1/8an strain.

The content of n-alkanes in oil after cultivation with the strain is presented in Table 2. The results showed that the strain decomposed n-alkanes C11 and C22-C28 more efficiently. After 14 days, they were completely degraded. The biodegradation of n-alkanes C12-C21 was 63.9-92.9%.

Table 2. The peak area of n-alkanes after cultivation of the *P. thiosulfatoxidans* 1/8an strain in a liquid mineral medium with 1% oil at 30℃

n-Alkanes	Peak area (10^{-6})	
	Control	1/8an
Undecane	4.1 ± 0.13	Not detected (n/d)
Dodecane	28.3 ± 2.15	2.0 ± 0.16
Tridecane	58.7 ± 5.40	12.6 ± 1.73
Tetradecane	58.9 ± 5.74	17.8 ± 4.18
Pentadecane	54.4 ± 5.25	18.8 ± 5.35
Hexadecane	51.0 ± 6.01	16.2 ± 4.43
Heptadecane	44.6 ± 3.42	16.1 ± 3.37
Octadecane	35.6 ± 2.99	10.2 ± 2.01
Nonadecane	35.0 ± 5.56	n/d
Eicosane	31.4 ± 3.18	7.9 ± 0.14
Heneicosane	28.5 ± 5.20	9.0 ± 1.59
Docosane	24.6 ± 3.70	n/d
Tricosane	19.5 ± 3.51	n/d
Tetracosane	15.5 ± 2.02	n/d
Pentacosane	13.4 ± 1.81	n/d
Hexacosane	13.0 ± 1.82	n/d
Heptacosane	11.3 ± 1.92	n/d
Octacosane	8.5 ± 1.26	n/d

Biodegradation of Dossor oil by the strain at different temperatures

The degradation of the Dossor oil by the strain in a liquid mineral medium at low and elevated temperatures was studied. The initial oil content was 1% vol. Fig. 4 shows the degradation of oil after 14 days of cultivation at temperatures of 10 and 50 ℃. Gravimetric analysis showed that the degradation of oil was most effective at 10 ℃. The strain degraded 50.2% of oil relative to the control variant. The biodegradation of oil was slower at 50 ℃. An amount of 20.7% of oil was degraded in 14 days. The abiotic loss of oil was 15.6 and 18.4% at 10 and 50 ℃, respectively.

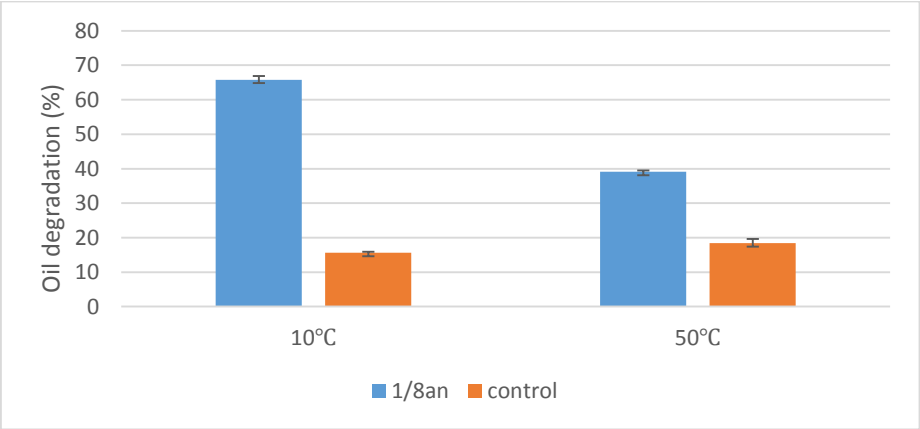

Fig. 4. Degradation of Dossor oil by the *P. thiosulfatoxidans* 1/8an strain at different temperatures.

Similarly, to its behavior at 30 ℃, at a temperature of 10 ℃, the strain completely decomposed n-alkanes C22-28 (Table 3). In addition, n-Alkanes with a shorter chain (C12-21) were degraded by 61.0-79.6% under the influence of the strain.

Table 3. The peak area of n-alkanes after cultivation of the *P. thiosulfatoxidans* 1/8an strain in a liquid mineral medium with 1% oil at 10℃.

n-Alkanes	Peak area (10^{-6})	
	Control	1/8an
Undecane	53.1 ± 0.51	n/d
Dodecane	58.9 ± 0.56	12.0 ± 0.4
Tridecane	73.0 ± 1.59	25.4 ± 1.1
Tetradecane	63.3 ± 1.24	23.0 ± 1.4
Pentadecane	71.0 ± 19.27	20.7 ± 1.9
Hexadecane	53.1 ± 4.64	20.7 ± 5.0
Heptadecane	44.3 ± 3.25	16.5 ± 3.6
Octadecane	37.0 ± 1.06	10.3 ± 2.5
Nonadecane	34.8 ± 0.58	11.2 ± 1.2
Eicosane	28.6 ± 0.25	8.0 ± 0.0
Heneicosane	29.2 ± 0.44	6.5 ± 0.2
Docosane	24.4 ± 1.56	n/d
Tricosane	19.6 ± 1.71	n/d
Tetracosane	15.5 ± 0.91	n/d
Pentacosane	13.2 ± 0.84	n/d
Hexacosane	12.7 ± 0.99	n/d
Heptacosane	10.2 ± 1.11	n/d
Octacosane	7.5 ± 1.38	n/d

Biodegradation of Dossor oil by the strain at different NaCl content in the medium

The effect of NaCl concentration in the medium on the degradation of oil by the strain was studied. The initial oil content was 1% vol. Cultivation was carried out at 30 °C for 14 days. The results showed that at 2% NaCl, the strain degraded 53.2% of oil relative to the control variant (Fig. 5). By an increase in the concentration of salt, the degree of degradation of oil upraised. At 5 and 10% NaCl, the strain degraded oil at the same level (60.1 and 61.2%, respectively).

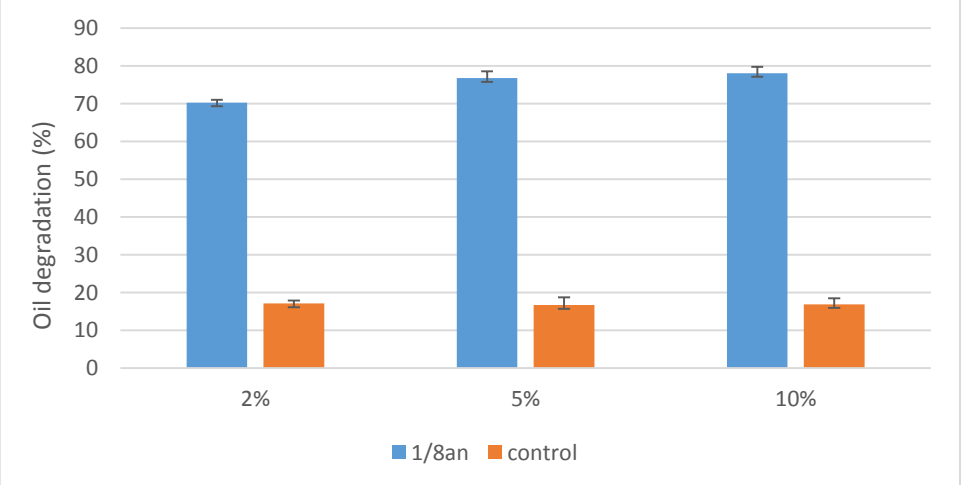

Fig. 5. Degradation of Dossor oil by the *P. thiosulfatoxidans* 1/8an strain at different concentrations of NaCl in the medium.

Biodegradation of Dossor oil by the strain at different pH values of the medium

The pH of the environment is a factor affecting the bioremediation of oil-contaminated ecosystems. The destruction of oil by the strain at different pH values of the medium was studied. The strain was cultivated in flasks with a mineral medium containing 1% by volume of oil at 30 ℃ for 14 days. Table 2 presents data on the biodegradation of oil at pH 5-9.

Table 2. Degradation of Dossor oil by the *P. thiosulfatoxidans* 1/8an strain at different pH levels of the medium.

	Degree of oil degradation (%)				
	pH 5	pH 6	pH 7	pH 8	pH 9
1/8an	56.2	60.4	71.2	83.3	73.1
control	15.7	16.3	15.9	16.2	16.3

In a neutral environment, the loss of oil under the influence of the strain was 55.3% relative to the control variant. At pH 5 and 6, the degree of destruction of oil decreased and amounted to 40.5 and 44.1%, respectively. By increasing pH values, the destructive activity also exhibited elevation, reaching a maximum of pH 8 (64.1%). At pH 9, the degree of oil degradation by the strain was 56.8%.

Degradation of oil from different fields in Kazakhstan by the strain

The ability of the strain to degrade oil from different fields in Kazakhstan (Zhanatalap, Zapadnaya Prorva, and Karsak) was studied. The strain was cultivated in flasks with a mineral medium containing 1% by volume of oil at 30 ℃ for 14 days.

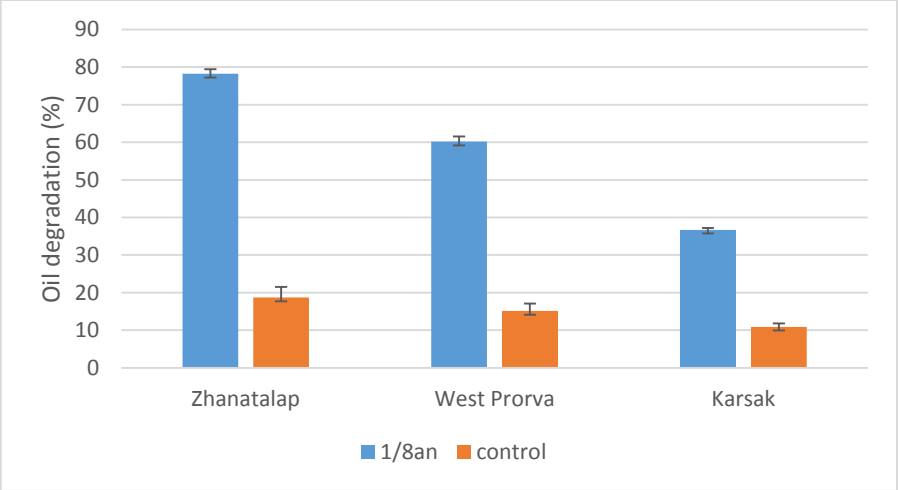

Fig. 6. Degradation of oil from different fields by the *P. thiosulfatoxidans* 1/8an strain.

We found that Zhanatalap oil underwent biodegradation better (Fig. 6). The strain degraded 59.5% of oil relative to the control variant. Under the same conditions, 45.1% of Zapadnaya Prorva oil was degraded. Karsak oil was more resistant to microbial decomposition. After 14 days, its decrease under the action of the strain was 25.8%.

DISCUSSION

Oil is the most common fossil fuel in the world. It is a complex multicomponent system, mainly consisting of various hydrocarbons, such as alkanes, cycloalkanes, mono-, bi-, and polyaromatic compounds, resins, and asphaltenes. Despite humanity's need for oil, it negatively affects the environment due to its toxicity. The environmental problem is particularly acute at oil production sites or during its transportation. Since it is impossible to replace oil with less toxic fuel, it is necessary to develop methods to reduce the toxic effects of petroleum hydrocarbons on the environment (Stepanova *et al.* 2022). Among the available methods, biological methods are widely used to reduce the harmful effects of petroleum compounds on contaminated objects. Bioremediation, mainly produced by local bacteria, is considered the most promising way to clean the environment from crude oil pollution (Tanzadeh *et al.* 2020). We isolated the 1/8an strain capable of decomposing petroleum hydrocarbons from oil-contaminated soil. Phylogenetic analysis using the 16S rRNA gene exhibited

100% similarity with the strain NR 171398.1:61-785 *P. thiosulfatoxidans* YE3. Representatives of the genus *Pusillimonas* are gram-negative, oxidase- and catalase-positive bacteria belonging to the family *Alcaligenaceae*. The strain *P. thiosulfatoxidans* YE3^T, capable of oxidizing thiosulfate, was isolated from activated sludge obtained at a municipal wastewater treatment plant in Daejeon, Republic of Korea (Koh *et al.* 2019). There is no information about the possibility of oil degradation by this type. However, the *Pusillimonas* sp. T7-7 strain can use diesel fuel (alkanes C_5-C_{30}) as the only source of carbon and energy (Li *et al.* 2013). The effect of microorganisms on hydrocarbons and the possibility of their biodegradation are different. The tendency to decrease the stability of hydrocarbons during biodegradation is as follow: asphaltenes > resin > polycyclic aromatic compounds > cyclic alkanes > small aromatic compounds > branched alkanes > linear alkanes (Shahebrahimi *et al.* 2020). Saturated alkanes are the most degradable fraction in crude oil. As a rule, medium-length N-alkanes (C10-C25) decompose most easily. Alkanes with a longer chain (C25-C40) are hydrophobic solids and are difficult to decompose due to their poor solubility in water and bioavailability (Unimke *et al.* 2018). *Pseudomonas aeruginosa* SJTD-2 strain has been isolated, effectively decomposing medium- and long-chain n-alkanes (C10-C26) and oil as the only carbon sources. It tends to consume long-chain n-alkanes (C18-C22), and n-docosan is considered the best carbon source for its growth (Xu *et al.* 2015). Tanzadeh *et al.* (Tanzadeh *et al.* 2020) reported that the *Bacillus cereus* (J3) strain has a better ability to degrade n-alkanes with short (C9-C13) and medium (C13-C25) chain lengths (degradation rate > 95%) than the long-chain ones (C25-C36). We found that the *P. thiosulfatoxidans* 1/8an strain completely decomposed n-alkanes C22-C28. The degree of degradation of n-alkanes C12-C21 was 63.9-92.9%. Among the physical factors, temperature plays an important role in the biodegradation of hydrocarbons, having a direct impact on the chemical composition of pollutants, as well as on the physiology and diversity of microbial flora. Although the biodegradation of hydrocarbons can occur over a wide temperature range, the rate of biodegradation usually declines by a decrease in temperature, which is the result of a drop in the rate of enzymatic activity (Das & Chandran 2011; Al Hawashet al. 2018). The study of the degradation of Dossor oil by the new *P. thiosulfatoxidans* 1/8an strain on a mineral medium for 14 days at 10, 30, and 50 ℃ showed that the strain worked most effectively at 30 ℃. It also showed high activity at 10 ℃. High or low NaCl content in the medium display different effects on the decomposition of oil. The maximum biodegradation of crude oil is usually observed at lower mineralization and drops by elevating NaCl concentration. However, (Thavas *et al.* 2008) have reported that *P. aeruginosa* is capable of hydrocarbon decomposition in high salinity. In the study conducted by (Khanpour Alikelayeh *et al.* 2020), the effect of NaCl concentration on the biodegradation of crude oil, in general, was insignificant, and an elevation in the salinity of the medium led to a slight increase in biodegradation. The *Bacillus licheniformis* Y-1 strain grew well and showed high hydrocarbon-degrading ability in an environment with 50 g L^{-1} NaCl and could grow in an environment with 70 g L^{-1} NaCl (Liu et al. 2016) We showed the ability of the *P. thiosulfatoxidans* 1/8an strain to decompose oil in high salinity of the medium. The strain degraded 60.1 and 61.2% of oil at a NaCl concentration of 5 and 10%, respectively.

The pH of the environment is a factor affecting the bioremediation of oil-contaminated ecosystems. Most heterotrophic bacteria prefer to grow at a pH from neutral to alkaline. The *P. thiosulfatoxidans* 1/8an strain grew and degraded oil at a pH of the medium from 5 to 9. The maximum removal of oil was observed at pH 8. In an acidic environment, the activity of the strain decreased. Our data are consistent with those obtained by (Khanpour Alikelayeh *et al.* 2020) In the case of *B. licheniformis* strain isolated by them, the maximum percentage of biodegradation was recorded at pH 8.5 and the minimum at pH 5.5. (Thavasi *et al.* 2007) studied the biodegradation of crude oil by *P. aeruginosa* strain in the pH range from 5.0 to 9.5. The observed maximum growth and biodegradation occurred at pH 8.0 with 85% decomposition of crude oil. Pawar (Pawar 2015) also noted that soil pH 7.5 was most suitable for the decomposition of all PAHs. The biodegradation of oil is a complex process mainly influenced by the composition of hydrocarbons (Ajona & Vasanthi 2021). Analysis of the chemical composition of the studied oils exhibited that the dense oil or, so to say, difficult for biodegradation is the oil of the Karsak field whose density reaches 0.933 g cm^{-3} and resin content reaches 52%. The less-dense oil of the Zapadnaya Prorva field underwent biodegradation more successfully. The oil of the Zhanatalap deposits, which belongs to light, low-resinous, and low-aromatic oils, was the easiest for decomposition by microorganisms.

CONCLUSION

The new *P. thiosulfatoxidans* 1/8an strain was isolated from the oil-contaminated soil of the Atyrau region, Kazakhstan. There is information in the literature about the alkane-monooxygenase system in the *Pusillimonas*

sp. strain T7-7, which indicates the ability of *Pusillimonas* sp. to oxidize alkanes. We showed the ability of the *P. thiosulfatoxidans* 1/8an strain to degrade oil. It effectively decomposed crude oil at low and moderate temperatures. The strain degraded medium- and long-chain n-alkanes. The strain showed resistance to the high salinity of the medium. The study of the influence of the medium's pH on oil degradation showed that the strain degraded oil in both acidic and alkaline environments. We found that the strain was also capable of degrading both light and heavy oils. Thus, our study showed that *Pusillimonas* sp. can be used to eliminate oil pollution.

Conflict of Interest

The authors declare that there is no conflict of interest.

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