Biodegradation of Aliphatic Hydrocarbons by Seven Marine Bacterial Species Isolated from Iraqi Coastal Sediments: A Comparative Study

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Abstract

Oil pollution in the marine environment of the Arabian Gulf is an emerging threat, especially for the Iraqi coast. This study aims to evaluate the biodegradation potential of seven bacterial strains isolated from Iraqi coastal sediments for degrading aliphatic hydrocarbons (n-alkanes). The bacterial strains tested include *Sphingomonas* paucimobilis, *Pseudomonas fluorescens*, *Kocuria rosea, Kocuria kristinae, Kocuria rhizophila, Staphylococcus lentus*, and Pasteurella canis. Degradation efficiencies were analyzed after 48 hours, 72 hours, and 15 days. *Pseudomonas fluorescens* was the most efficient bacterium in terms of aliphatic hydrocarbon biodegradation (98.9% after 15 days), followed by Sphingomonas paucimobilis (96.8%) and Kocuria rhizophila (97.3%). Moreover, the results indicated that the medium-chain alkane (C14-C25) was preferentially degraded incubation time for the long-chain alkane (C26-C40). A comparative analysis of the results obtained in this study was conducted with other studies conducted in the northwestern Arabian Gulf area, including Iran and Kuwait.

Keywords: Biodegradation, aliphatic hydrocarbons, marine bacteria, Iraqi coastal sediments, Arabian Gulf, n-alkanes, bioremediation.

Introduction

The Arabian Gulf is among the world's most vital zones for oil generation and oil transportation; thus, it is highly vulnerable to both chronic and acute oil pollution (Al-Saad *et al*., 2017). The Iraqi coast, which is 58 km long on the northwestern part of the Arabian Gulf, experiences heavy inputs of hydrocarbons in relation to oil exploration, tanker activities, and industrial processes. Aliphatic hydrocarbons, especially n-alkanes,

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are the main constituents of crude oil; thus, they constitute environmental hazards because of their toxicity and non-biodegradability (Atlas & Hazen, 2011).
The breakdown of hydrocarbons by microbes is the key natural process for the removal of hydrocarbons from the polluted marine environment. A variety of research works have shown that marine microbes have the capability to use alkanes as a source of energy and carbon (Head *et al.*, 2006; Yakimov *et al.*, 2007; Olowomofe *et al.*, 2020; Hazaimeh and Ahmed, 2021; Lafta *et al.*, 2025; Lee *et al.*, 2026). But the possibility of biodegradation by microbes in coastal areas of Iraq has not been studied much.

Various hydrocarbon-utilizing bacterial species have been discovered in previous studies carried out in the northwestern Arabian Gulf, such as Pseudomonas, Acinetobacter, and Alcanivorax (Hassanshahian *et al.*, 2014; Rahimnejad *et al.*, 2015). However, there has never been a systematic analysis of the efficiency of different bacteria and the degradation profile of various alkane carbon chain lengths in the area. This study aims to evaluate the biodegradation capabilities of seven bacterial species isolated from Iraqi coastal sediments and to establish a comparative baseline with regional studies from Iranian and Kuwaiti coastal waters.

Materials And Methods

This study was conducted during 2025 along the Iraqi coast in the northwestern Arabian Gulf. Sampling stations were selected to represent areas influenced by river discharges, oil-related activities, and maritime navigation, as well as relatively less impacted reference sites. The geographic distribution of the sampling stations is illustrated in the study area map (Fig. 1). Surface sediment samples (0-5cm) were collected using a Van Veen grab sampler, air-dried at room temperature, homogenized, and sieved to obtain the fine fraction (63u) The samples were dried in an air, grinded finely in an electrical stainless steel mortar and sieved through a 63 μm mesh sieve, stored in glasses containers until analysis.50 grams of sieved sediment samples were placed in a cellulose thimble and Soxhlet extracted using Soxhlet intermittent extraction (Goutex and Saliot, 1980) with mixed solvents (120ml) methanol: benzene (1:1 v/v) for 24-36 h at a temperature that does not exceed 40 °C. At the end of this period, the combined extracts were saponification for 2 hrs. by adding (15ml) 4M MeOH (KOH) at the same temperature, then cooled to room temperature, using separator funnel to extracted the unsaponification matter with (40 ml) n-hexan and purified using silica gel and alumina column chromatography to remove interfering compounds, then concentrated using a rotary evaporator followed by gentle nitrogen evaporation. Identification and quantification of polycyclic aromatic hydrocarbons were performed using gas chromatography (GC) with calibration based on certified n-alkanes standards.

Study Area and Sediment Collection

Sediment samples were collected from five stations (ST.1 to ST.5) along the Iraqi coastline of the Arabian Gulf. The initial concentrations of aliphatic hydrocarbons (C8-

C40) were determined for each station (Table 1). Total aliphatic concentrations ranged from 56.75 to 105.77 µg/g, with station ST.1 showing the highest contamination levels.

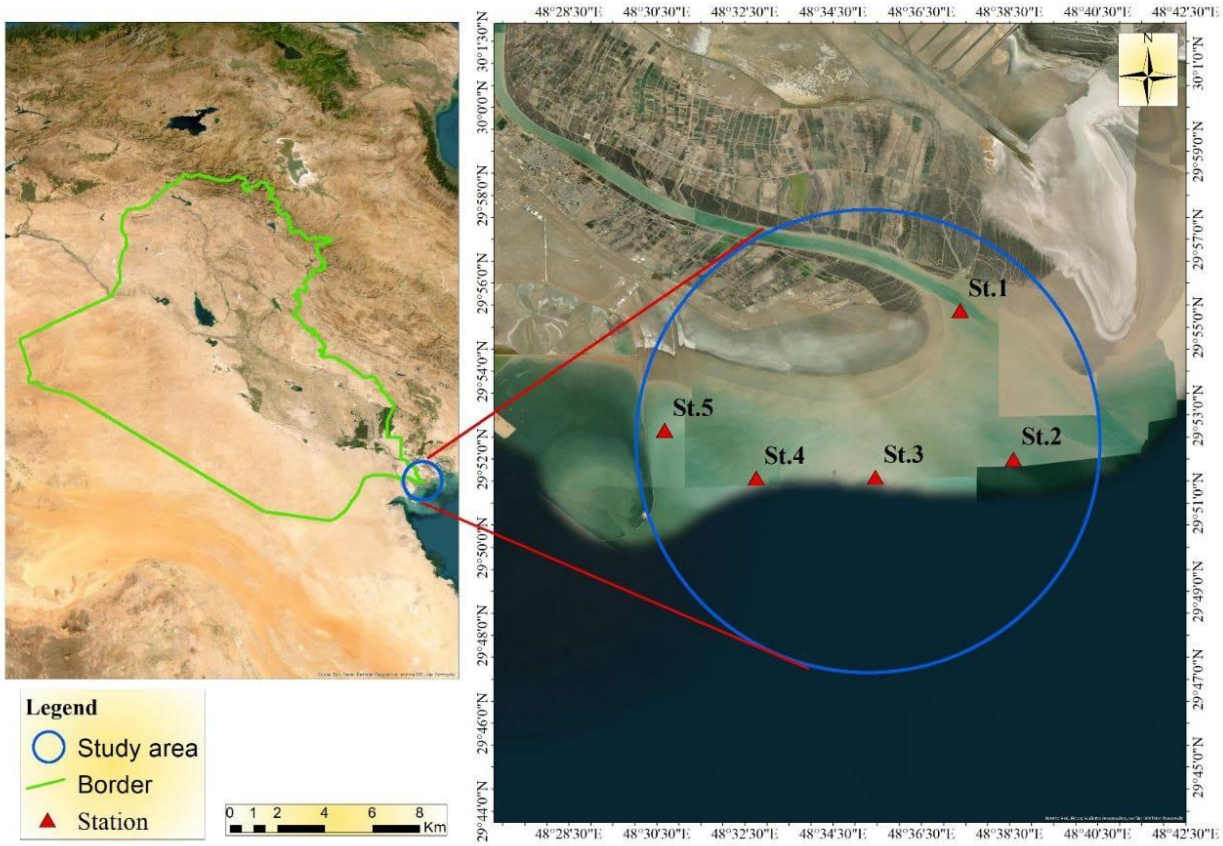

Fig. 1: Sample locations.

Bacterial Isolates

Seven bacterial species were isolated from the sediment samples and identified using standard microbiological techniques, , and VITEK was used identifications

- *Sphingomonas paucimobilis*
- *Pseudomonas fluorescens*
- *Kocuria rosea*
- *Kocuria kristinae*
- *Kocuria rhizophila*
- *Staphylococcus lentus*
- *Pasteurella canis*

Biodegradation Assays

Each bacterial species was cultured in minimal salt medium supplemented with aliphatic hydrocarbon fractions extracted from the sediment samples. The efficiency of degradation was determined at three different stages, namely 48 hours, 72 hours, and 15

days. The residual hydrocarbons were extracted and then analyzed via gas chromatography. The percentage of degradation was calculated from the amount of decrease in total aliphatic hydrocarbons.

Results

Initial Hydrocarbon Concentrations in Sediments

Table 1 presents the concentrations of individual n-alkanes (C8-C40) from five sampling stations (ST.1-ST.5). The data reveal significant spatial variation in hydrocarbon contamination levels.

Table 1: Concentration of N-Alkanes (ug/g) in sediment of different stations

Component name	ST.1	ST.2	ST.3	ST.4	ST.5
n-C8	0	0	0	0	0
n-C9	0	0.04781	0.04111	0	0
n-C10	0	0.04049	0.13908	0.04269	0.04269
n-C11	0.07305	0.0993	0.13298	0.11811	0.11811
n-C12	0.04359	0.10898	0.05754	0.04037	0.04037
n-C13	0.03284	0.11865	0.10377	0.06611	0.06611
n-C14	0.07074	0.09183	0.41213	0.04482	0.04482
n-C15	0.17892	0.27122	0.3399	0.19554	0.19554
n-C16	0.05629	0.04134	0.04046	0.07432	0.07432
n-C17	1.004	0.50178	0.60622	0.62267	0.62267
pr	0.4891	0.19709	0.22843	0.24069	0.24069
n-C18	2.97205	1.52615	1.75473	1.6853	1.6853
ph	1.91127	0.79296	0.72723	0.62113	0.62113
n-C19	2.42618	0.80571	0.85417	0.84194	0.84194
n-C20	3.44625	1.76197	1.77191	1.54336	1.54336
n-C21	4.25572	1.70128	1.54758	1.24657	1.24657
n-C22	2.80324	4.62639	4.47639	4.20046	4.20046
n-C23	3.38386	1.92304	1.65718	1.44543	1.44543
n-C24	4.76313	3.03049	1.47936	0.97561	0.97561
n-C25	7.17451	4.97974	3.26202	2.87276	2.87276

n-C26	8.57605	5.35646	5.77947	5.31702	5.31702
n-C27	14.826	11.04008	7.89834	7.40909	7.40909
n-C28	6.68977	5.02199	5.38958	5.4847	5.4847
n-C29	8.90757	8.04071	6.61398	6.57423	0.57381
n-C30	3.37989	5.5348	3.77359	7.87369	6.85
n-C31	7.37318	6.51166	5.64237	5.25316	5.25316
n-C32	7.06818	4.83617	4.91193	1.65199	1.65199
n-C33	2.51719	1.18664	2.5221	1.39293	1.39293
n-C34	3.91257	2.26621	2.0388	1.66798	1.66798
n-C35	4.197	1.4605	3.9215	3	3
n-C36	2.11447	0.47869	0.08969	0.92418	0.92418
n-C37	0.28929	0	0	0.04439	0.04439
n-C38	0.32871	0.0401	0.03663	0	0
n-C39	0.0586	0.04765	0	0	0
n-C40	0.4445	0.10996	0.04149	0.03786	0.03786
Total	105.7677	74.63287	68.32715	63.54231	56.75118
Total even	46.66943	34.87202	3219278.	31.56435	3076797.
Total odd	56.69791	38.73577	34.74078	31.08293	25.0787
CPI	1.2148	1.1107	1.0779	0.9837	0.8150
Pr/Ph	0.255	0.248	0.314	0.387	0.387
n_C17/Pr	2.052	2.545	2.653	2.587	2.587
n_C18/Ph	1.555	1.924	2.412	2.713	2.713

Station ST.1 exhibited the highest total aliphatic hydrocarbon concentration (105.77 µg/g), dominated by n-C27 (14.83 µg/g), n-C29 (8.91 µg/g), n-C26 (8.58 µg/g), and n-C31 (7.37 µg/g). The predominance of odd-carbon-numbered alkanes (total odd: 56.70 µg/g) over even-carbon-numbered alkanes (46.67 µg/g) suggests a petrogenic source with limited weathering.

Station ST.2 showed a total concentration of 74.63 µg/g, with n-C27 (11.04 µg/g), n-C29 (8.04 µg/g), n-C31 (6.51 µg/g), and n-C30 (5.53 µg/g) as major components. The

Carbon Preference Index (CPI) value of 1.111 indicates a mixed petrogenic and biogenic source.

Station ST.3 had a total concentration of 68.33 µg/g, characterized by n-C27 (7.90 µg/g), n-C29 (6.61 µg/g), n-C30 (3.77 µg/g), and n-C35 (3.92 µg/g).

Station ST.4 and **ST.5** showed lower total concentrations (63.54 and 56.75 µg/g, respectively), with CPI values of 0.984 and 0.815, suggesting more weathered petroleum inputs.

The Pr/Ph (pristane/phytane) ratios ranged from 0.255 (ST.1) to 0.387 (ST.4 and ST.5), indicating anoxic depositional

environments typical of crude oil sources. The n-C17/Pr ratios (2.052-2.653) and n-C18/Ph ratios (1.555-2.713) suggest moderate to advanced biodegradation in the original sediment samples.

Bacterial Degradation Efficiency

Tables 2 through 8 present the concentrations of individual n-alkanes as a percentage at different times. A summary of degradation efficiencies is presented in Table 9, which shows the comparative degradation efficiencies of all seven bacterial species at each time point.

Table 2: The degradation of different n-alkanes as a percentage at different times.

Bacterial Species	48h Efficiency (%)	72h Efficiency (%)	15-day Efficiency (%)
Sphingomonas paucimobilis	82.5	94.5	96.8
Pseudomonas fluorescens	79.7	95.7	98.9
Kocuria rosea	79.2	88.5	96.5
Kocuria kristinae	68.4	90.2	97.3
Kocuria rhizophila	68.4	90.2	97.3
Staphylococcus lentus	65.9	75.3	92.9
Pasteurella canis	17.5	70.4	93.6

Sphingomonas paucimobilis

Table 3: The degradation of n-alkanes by *Sphingomonas Paucimobilis* at different times

Component Name	48h	72h	15day
C8	0	0	0
C9	0	0	0
C10	0	0	0
C11	0	0	0
C12	0	0	0
C13	0	0	0
C14	4.099477153	0	0
C15	3.864488189	0	0
C16	0	0	0
C17	0	0	0
Pr	0.70273842	0.760876476	0
C18	0	0	0
Ph	0	0	0
C19	0	0	0
C20	3.634119241	1.055284553	0
C21	0	0	0
C22	0.59124537	0.531916667	0
C23	4.363740672	0.50545709	0
C24	0	0	0
C25	0	0	0
C26	0	0	0
C27	0	0	0
C28	1.092308795	3.541644359	0.56584608
C29	0	0	0
C30	18.18456149	3.78032411	3.54575786
C31	11.77158406	2.900456754	3.958833819
C32	0	0	0
C33	0.576714145	0.782814342	0
C34	0	0	0
C35C	1.051925	1.6228	0.96756
C36	0	0	0
C37C	0	0	0
C38C	0	0	0

C39	0	0	0
C40	0	0	0
Total	49.93290253	15.481574351	9.038012479
%	82.5%	94.5%	96.8%

S. paucimobilis demonstrated progressive degradation of aliphatic hydrocarbons, achieving 82.5% removal at 48 hours, 94.5% at 72 hours, and 96.8% at 15 days. Notably, this species completely degraded n-C14, n-C15, n-C18, and n-C20 by 72 hours. However, longer-chain alkanes such as n-C30 (3.55 µg/g residual at 15 days) and n-C31 (3.96 µg/g residual) showed greater resistance to degradation. The degradation pattern indicates preferential metabolism of medium-chain alkanes (C14-C25) during the early incubation period.

Pseudomonas fluorescens

Table 4: The degradation of n-alkanes by *Pseudomonas Fluoredin* at different times.

Component Name	48h	72h	15day
C8	9.647821252	1.257893521	0
C9	5.275933479	2.347896542	1.293172514
C10	6.851247612	1.789643011	1.278933641
C11	10.69832582	2.978315521	0
C12	0	0	0
C13	6.179348625	1.025364912	0
C14	0	0	0
C15	2.167943658	0	0
C16	0	0	0
C17	1.587364892	0	0
Pr	0.823471256	0	0
C18	0	0	0
Ph	0	0	0
C19	2.147852265	0.915236451	0.397824521
C20	0	0	0
C21	0	0	0
C22	0	0	0
C23	0.378964345	0	0
C24	2.678941261	0	0
C25	3.715426931	1.736214852	0
C26	0	0	0
C27	0	0	0
C28	1.634152789	0	0

C29	0	0	0
C30	1.123578122	0	0
C31	0	0	0
C32	0	0	0
C33	2.987424111	0	0
C34	0	0	0
C35	0	0	0
C36	0	0	0
C37	0	0	0
C38	0	0	0
C39	0	0	0
C40	0	0	0
Total	57.897796446	12.05056481	2.96993067
%	79.7%	95.7%	98.9%

P. fluorescens exhibited the highest overall degradation efficiency among all tested species, with 79.7% removal at 48 hours, 95.7% at 72 hours, and 98.9% at 15 days. The residual hydrocarbon concentration after 15 days was only 2.97 µg/g. This species completely degraded n-C11, n-C13, n-C15, n-C17, pristane, phytane, and n-C18 by 72 hours. Complete degradation of n-C9 (1.29 µg/g residual at 15 days) and n-C10 (1.28 µg/g residual) was not achieved within the experimental timeframe, suggesting that very short-chain alkanes may be less accessible due to volatility or toxicity.

Kocuria rosea

Table 5: The degradation of n-alkanes by *Kocuria rosea* at different times

Component Name	48h	72h	15day
C8	0	0	0
C9	0	0	0
C10	0	0	0
C11	0	0	0
C12	0	0	0
C13	0	0	0
C14	1.234123584	0	0
C15	4.182346121	1.963876324	0
C16	2.136942135	0	0
C17	1.354912372	0	0
Pr	3.154231785	0	0
C18	0.939985134	0	0
Ph	5.658688447	0	0

C19	4.123453213	2.98745621	0
C20	2.341325234	0	0
C21	0.231423651	0	0
C22	0.325146315	3.56984231	3.125842339
C23	1.325123642	0	0
C24	3.213462513	0	0
C25	1.631425896	0	0
C26	2.342513671	0	0
C27	0.371203611	7.456314234	1.647824312
C28	0.321453213	2.741895632	0
C29	1.231496347	5.136987254	0
C30	6.123614523	0	0
C31	2.63222413	1.224569852	0.396423741
C32	1.32512417	3.326548752	3.158796434
C33	0.18641375	0	0
C34	1.34789642	2.874692314	1.796412546
C35	0.27963424	1.324875632	1.118634125
C36	3.64712631	0	0
C37	0	0	0
C38	3.54697834	0	0
C39	2.97634152	0	0
C40	1.78643185	0	0
Total	59.9569234	32.6213641	11.243933497
%	%79	%88.5	%96

K. rosea showed 79% degradation at 48 hours, 88.5% at 72 hours, and 96% at 15 days. This species demonstrated notable activity against pristane (3.15 µg/g degraded at 48 hours) and phytane (5.66 µg/g degraded at 48 hours). However, several long-chain alkanes persisted after 15 days, including n-C22 (3.13 µg/g), n-C27 (1.65 µg/g), n-C32 (3.16 µg/g), and n-C34 (1.80 µg/g). The residual concentration of 11.24 µg/g after 15 days indicates that while *K. rosea* is effective, it is less efficient than *P. fluorescens* for complete mineralization of all alkanes.

Kocuria kristinae

Table 6. The biodegradation of n-alkanes by *Kocuria Kristinae* at different times

Component Name	48h	72h	15day
C8	72.50578708	1.365787079	0
C9	1.297117962	0	0
C10	3.341619718	3.415039613	0

C11	0.793307087	0	0
C12	0	0	0
C13	0	0	0
C14	4.304152021	0	0
C15	0	0	0
C16	0.530540897	2.117273527	0
C17	0	0	0
Pr	0.671639419	2.637888283	0
C18	0	0	0
Ph	0	0	0
C19	0	0	0
C20	0	0	0
C21	0	0	0
C22	1.198046296	2.141611111	0.954967593
C23	0.54207556	0.800527052	0
C24	0.627861163	0.61064728	0
C25	0	0	0
C26	0.49348384	3.841577947	3.725361217
C27	0	0	0
C28	0	0	0
C29	0	0	0
C30	14.48397045	7.583474738	12.83323642
C31	6.596443149	18.96379981	16.70886297
C32	0	0	0
C33	3.881247544	0.647966601	0.992077603
C34	0.724680747	0	0
C35C	0.89989	2.299965	2.884035
C36	0	0	0
C37C	0	0	0
C38C	0	0	0
C39	0	0	0
C40	0	0	0
Total	112.8918629	46.425558041	38.098540803
%	60%	83.7%	86.6%

K. kristinae exhibited lower degradation efficiency compared to other *Kocuria* species, with only 60% removal at 48 hours, 83.7% at 72 hours, and 86.6% at 15 days. Notably, the total residual concentration after 15 days remained high at 38.10 µg/g. Anomalously high n-C8 concentration (72.51 µg/g) at 48 hours suggests analytical interference or possible microbial production of short-chain alkanes, as this exceeds initial concentrations

reported in Table 1. Persistent compounds included n-C26 (3.73 µg/g), n-C30 (12.83 µg/g), n-C31 (16.71 µg/g), and n-C35 (2.88 µg/g).

Kocuria rhizophila

Table 7. The biodegradation of n-alkanes by *Kocuria rhizophila* at different times

Component Name	48h	72h	15day
C8	0	0	0
C9	0	0	0
C10	0	0	0
C11	0	0	0
C12	0	0	0
C13	0	0	0
C14	0	0	0
C15	0	0	0
C16	0	0	0
C17	0	0	0
Pr	0	0	0
C18	3.88628945	0	0
Ph	0	0	0
C19	1.066730072	4.053205591	0
C20	0.838672087	0	0
C21	0.473860933	0.484266304	0
C22	0.61975	0.895551039	0.819643519
C23	0.541854679	0.484515096	0
C24	0.717912758	0.871537037	0
C25	0.840537229	4.333404851	0.800730443
C26	0.831834601	0.838667917	0.599201521
C27	11.04295699	0	0
C28	2.893814532	0.591972433	1.127538241
C29	0	0	0
C30	17.79018112	0.800650096	5.963875119
C31	14.82503401	6.797677203	4.318391642
C32	7.100160985	7.425881792	0
C33	8.564101179	6.711763848	0
C34	5.641232809	0.925411932	0
C35C	12.452545	0	0
C36	0	0	0
C37C	0	0	0
C38C	0	0	0

C39	0	0	0
C40	0	0	0
Total	90.12746544	27.788623347	7.6655058761
%	68.4%	90.2%	97.3%

K. rhizophila demonstrated strong degradation capability with 68.4% removal at 48 hours, 90.2% at 72 hours, and 97.3% at 15 days. Complete degradation was observed for most alkanes by 15 days, with a residual concentration of only 7.67 µg/g. Notable persistent compounds included n-C30 (5.96 µg/g), n-C31 (4.32 µg/g), and n-C28 (1.13 µg/g). This species completely degraded n-C27 (11.04 µg/g) within 72 hours.

Staphylococcus lentus

Table 8. The biodegradation of n-alkanes *by Staphylococcus lentus* at different times.

Component Name	48h	72h	15day
C8	0	0	0
C9	0	0	0
C10	0	0	0
C11	0	0	0
C12	0	0	0
C13	0	0	0
C14	0	0	0
C15	3.748705162	0	0
C16	0	0	0
C17	4.289626667	0.830162709	0
Pr	0	0	0
C18	0.494197475	3.30791099	0
Ph	3.419183099	1.254675383	0
C19	0.647735507	0.488924883	0
C20	0.512565492	1.288319746	0.474927536
C21	0.791413541	1.514128275	0.854598013
C22	0.884462963	1.078151876	0.612529735
C23	0.628997201	1.483986111	0.83750463
C24	0.816857411	0.919552239	0.525191231
C25	1.014410933	1.242654784	0.835858349
C26	0.743003802	1.108086711	0
C27	6.179286413	0.926440114	0.544752852
C28	2.637471319	5.185439883	1.099745846
C29	0	0	1.613083174
C30	12.84755005	16.68573276	2.138496169

C31	9.568241011	12.77419924	5.723975214
C32	16.77845644	9.069266278	4.305184645
C33	18.48942534	3.652518939	0
C34	8.1803389	1.263074656	0.5859135556
C35C	3.8698	3.760653242	0
C36	0.75617443	2.598035	1.7353
C37C	0	0	0
C38C	0	0	0
C39	0	0	0
C40	0	0	0
Total	97.29790316	70.43191378	20.1713225706
%	65.9%	75.3%	92.9%

S. lentus achieved 65.9% degradation at 48 hours, 75.3% at 72 hours, and 92.9% at 15 days. The residual concentration after 15 days was 20.17 µg/g. Persistent compounds included n-C29 (1.61 µg/g), n-C30 (2.14 µg/g), n-C31 (5.72 µg/g), n-C32 (4.31 µg/g), and n-C36 (1.74 µg/g). This species showed moderate efficiency but required extended incubation for significant degradation of long-chain alkanes.

Pasteurella canis

P. canis exhibited the lowest initial degradation efficiency (17.5% at 48 hours) but showed substantial improvement with extended incubation, achieving 70.4% at 72 hours and 93.6% at 15 days. This delayed activity suggests a longer adaptation period may be required for this species to develop hydrocarbon-degrading enzyme systems.

Summary of Degradation Efficiencies

Table 9: Comparative degradation efficiencies of all seven bacterial species at each time point**.**

Bacterial Species	48h Efficiency (%)	72h Efficiency (%)	15-day Efficiency (%)
Sphingomonas paucimobilis	82.5	94.5	96.8
Pseudomonas fluorescens	79.7	95.7	98.9
Kocuria rosea	79.2	88.5	96.5
Kocuria kristinae	68.4	90.2	97.3

Bacterial Species	48h Efficiency (%)	72h Efficiency (%)	15-day Efficiency (%)
Kocuria rhizophila	68.4	90.2	97.3
Staphylococcus lentus	65.9	75.3	92.9
Pasteurella canis	17.5	70.4	93.6

Discussion

Comparative Degradation Efficiency

The obtained results confirm that all seven bacteria types have the capacity to break down aliphatic hydrocarbons; however, their efficiency varies. The greatest degradation was observed in Pseudomonas fluorescens (98.9% after 15 days), which is consistent with previous studies showing the high capacity of the Pseudomonas genus to break down hydrocarbons (Das & Chandran, 2011). High performance of P. fluorescens is related to its well-described alkane hydroxylase systems, allowing it to oxidize different types of n-alkanes (van Beilen & Funhoff, 2007).

Sphingomonas paucimobilis proved to be quite efficient (96.8%) in degradation, confirming the results of Cunliffe and Kertesz (2006), who noted the versatility of Sphingomonas species in degrading environmental pollutants. The biofilm formation capacity of S. paucimobilis allows it to have better contact with hydrophobic hydrocarbon substrates (Johnsen *et al*., 2005).

Regarding of the studied Kocuria species, two representatives (K. rhizophila and K. kristinae) have the same level of degradation capacity (97.3% in 15 days). However, K. rosea had a slightly lower level of degradation efficiency (96.5%).

Staphylococcus lentus (92.9%) and Pasteurella canis (93.6%) were the least efficient after 15 days. Slow activation of *P. canis* indicates that this species needs an inducer to activate hydrocarbon degradation enzymes, as it is the case with some Gram-negative bacteria (Rojo, 2009)**.**

Chain-Length Specificity

There was an observable specificity for the chain lengths during the degradation process of alkanes in all bacterial species tested. Medium-chain alkanes (C14-C25) were degraded faster in the early incubation period (48-72 hours), while long-chain alkanes (C26-C40) took longer. This pattern is expected based on previous research indicating that alkane hydroxylases, produced by bacteria, have high activity towards alkanes having chain lengths between C10 and C25 (van Beilen & Funhoff, 2005).

It is important to note that the persistence of n-C30 and n-C31 was observed in most of the treatments, even up to 15 days of incubation. This was expected from previous observations by Horel and Schiewer (2009), where very long-chain alkanes (>C28) had lower bioavailability because they were in the solid phase at ambient temperature and had low water solubility.

Pristine and phytane were completely degraded by only P. fluorescens and S. paucimobilis. The Pr/Ph ratio is usually used as a marker for determining oil sources and degree of weathering; however, certain bacteria have been shown to degrade these isoprenoid compounds, making them not resistant (Prince *et al.*, 2007).

Temporal Dynamics of Degradation

Temporal degradation profiles show three phases: an initial rapid phase (0-48 h), a second slower phase (48-72 h), and a third phase with minor additional degradation (72 h-15 days). The initial phase is related to the metabolism of accessible medium-chain alkanes, the second phase to the degradation of long-chain alkanes and isoprenoids, and the third phase is the recalcitrant fraction, which is composed of very long-chain alkanes (>C28).

Pasteurella canis showed an unusual profile, with initial activity very low (17.5% at 48 h), a considerable increase after 72 h (70.4%), and a further increase after 15 days (93.6%). The lag phase may be due to gene induction or an increase in population size before hydrocarbon degradation begins (Whyte et al., 1997).

Comparative Study of the Regional Literature of Northwestern Arabian Gulf Area

Table 10 shows a comparison of efficiencies of hydrocarbon-degrading bacteria isolated in studies done in the northwestern Arabian Gulf area, including Iranian coastal waters, Kuwaiti waters, and the present Iraqi study.

Table 10: Comparative Biodegradation Efficiencies of Marine Bacteria from the Northwestern Arabian Gulf Region

Location	Bacterial Species	Hydrocarbon Type	Degradation Efficiency	Reference
Iranian coast (Kharg Island)	*Alcanivorax borkumensis*	Crude oil (aliphatics)	85% (14 days)	Hassanshahian *et al.*, 2014
Iranian coast (Bushehr)	*Pseudomonas aeruginosa*	n-alkanes (C12-C30)	91% (21 days)	Rahimnejad *et al.*, 2015
Iranian coast (Persian Gulf)	*Rhodococcus erythropolis*	Crude oil	78% (28 days)	Hassanshahian & Tebyanian, 2015

Location	Bacterial Species	Hydrocarbon Type	Degradation Efficiency	Reference
Kuwaiti coast	*Bacillus subtilis*	Diesel range alkanes	82% (14 days)	Al-Mailem *et al.*, 2013
Kuwaiti coast	*Acinetobacter baumannii*	n-alkanes (C10-C30)	76% (21 days)	Radwan *et al.*, 2019
Saudi Arabian coast (Danmam)	*Oleibacter marinus*	Aliphatic hydrocarbons	89% (14 days)	Fathepure, 2014
Qatari coast	*Marinobacter hydrocarbonoclasticus*	Crude oil	83% (21 days)	Al-Darbi *et al.*, 2017
Iraqi coast (present study)	*Pseudomonas fluorescens*	n-alkanes (C8-C40)	98.9% (15 days)	Present study
Iraqi coast (present study)	*Sphingomonas paucimobilis*	n-alkanes (C8-C40)	96.8% (15 days)	Present study
Iraqi coast (present study)	*Kocuria rhizophila*	n-alkanes (C8-C40)	97.3% (15 days)	Present study

The Iraqi isolates, particularly *P. fluorescens*, demonstrate superior degradation efficiency (98.9%) compared to previously reported strains from the region. This might be due to the evolution of the indigenous bacterial population of Iraq under persistent exposure to hydrocarbons in such an affected ecosystem. The Iraqi coastline is continuously supplied by hydrocarbon effluents through the Shatt Al Arab river system, which supplies effluents from oil refineries located upstream (Al-Saad *et al.*, 2017).

According to Hassanshahian *et al.* (2014), Alcanivorax borkumensis strains from Kharg Island, Iran, were able to degrade 85% of aliphatic hydrocarbons within 14 days. The greater ability of the P. fluorescens isolate from Iraq to degrade hydrocarbons could be due to the composition of hydrocarbons in the sediments or to the presence of more than one copy of the alkane hydroxylase gene family.

According to Rahimnejad *et al.* (2015), the degradation efficiency of n-alkanes by Pseudomonas aeruginosa collected from Bushehr, Iran, was 91% after 21 days. In the current study, the degradation efficiency of P. fluorescens was 95.7% after 72 hours, which

implies a faster degradation rate. This could be especially beneficial in emergencies resulting from oil spills.

In their research on n-alkane-degrading bacteria collected from Kuwaiti coastal sediment samples, Radwan *et al.* (2019) reported that Acinetobacter baumannii degraded 76% of n-alkanes after 21 days. Reduced efficiency of n-alkane degradation by Kuwaiti strains compared to those from Iraq could be related to varying degrees of hydrocarbon exposure.

Environmental Implications

The high degradation efficiencies reported in this research have important implications for bioremediation methods within the Arabian Gulf area. The presence of different bacterial species that degrade weathered oil residues using different techniques implies that using a consortium would be a better approach than a single-organism technique (Bouchez Naitali et al., 2018).

The presence of long-chained (>C28) alkanes even after 15 days of incubation indicates that remediation of weathered oil residues will require longer treatment times or physicochemical methods such as the addition of biosurfactants (Mulligan, 2005).

The low efficiency of the bacterium Pasteurella canis in the initial 48 hours implies that this specific bacterium is not appropriate for emergencies where quick removal of hydrocarbons is necessary. However, this delayed efficiency could play an important role in bioremediation.

Conclusions

These findings demonstrate that seven marine bacteria isolated from Iraqi coast sediments have effective capacity for degrading aliphatic hydrocarbons. The following conclusions can be stated:

1. The highest degradation efficacy (98.9%, at 15 days) was shown by Pseudomonas fluorescens, making it the most prospective strain for bioremediation use in the area.
2. Also, Sphingomonas paucimobilis (96.8%) and Kocuria rhizophila (97.3%) proved to be effective degradation agents, having properties comparable to or better than previously investigated strains from the northwestern Arabian Gulf.
3. Medium-chain (C14-C25) alkanes are degraded faster than long-chain (C26-C40) alkanes during the initial stages of degradation.
4. The reduction in the ratio of pristane and phytane indicates metabolism of isoprenoid compounds by the studied bacterial strains; however, at a lower rate than that of n-alkanes.
5. The comparison of results with those of other regional investigations suggests that Iraqi coast sediments have highly efficient bacterial hydrocarbon degraders that reflect the adaptation of bacteria to long-term pollution by oil in such an environment.

6. The close-to-unit values of the CPI index in degraded samples point out the capability of bacterial strains to reduce odd-over-even predominance of fresh petroleum inputs.

Recommendations

On the basis of the results obtained from this study, the following recommendations are proposed:

1. Field-scale bioremediation experiments using Pseudomonas fluorescens and Sphingomonas paucimobilis together can be undertaken in order to assess the efficiency of these bacteria in a field environment under the influence of tidal action, fluctuating temperatures, and nutrient supply.
2. The use of consortium technology, which involves a group of different bacteria, can be employed for increasing degradation efficiency, especially for complicated mixtures of hydrocarbons comprising medium- and long-chain alkanes.
3. Optimization experiments can be carried out in order to assess the effect of nutrients (nitrogen and phosphorus) on degradation efficiency, as nutrient limitation is a major problem in marine environments.
4. Molecular analysis of the alkane hydroxylase enzymes (alkB, cyp153, almA) of these isolates can be carried out in order to explain the genetics of their higher degradation ability.
5. Long-term monitoring of bacterial communities in Iraqi coastal sediments can be carried out to determine changes in the population and concentration of hydrocarbons.
6. Production of biosurfactants by these bacteria can be studied for enhancing hydrocarbon bioavailability.
7. Bioremediation guidelines specific to the Arabian Gulf region should be developed, incorporating the use of indigenous bacterial strains rather than non-native commercial products.

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التحلل البيولوجي للهيدروكربونات الأليفاتية بواسطة سبعة أنواع من البكتيريا البحرية المعزولة من رواسب السواحل العراقية: دراسة مقارنة

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المستخلص

يشكل التلوث النفطي البحري تهديدًا كبيرًا للنظام البيئي في الخليج العربي، لا سيما على طول الساحل العراقي. تبحث هذه الدراسة في قدرة سبعة أنواع من البكتيريا البحرية المعزولة من رواسب السواحل العراقية على التحلل البيولوجي للهيدروكربونات الأليفاتية (الألكانات العادية). شملت أنواع البكتيريا : *Sphingomonas paucimobilis*، و*Pseudomonas fluorescens*، و*Kocuria rosea*، و*Kocuria kristinae*، و*Kocuria rhizophila*، و*Staphylococcus lentus*، و Pasteurella canis. تم تقييم كفاءة التحلل بعد 48 ساعة، و72 ساعة، و15 يومًا. أظهرت النتائج أن *Pseudomonas fluorescens* أظهرت أعلى كفاءة تحلل (98.9% بعد 15 يومًا)، تليها *Sphingomonas paucimobilis* (96.8%)، ثم *Kocuria rhizophila* (97.3%) أظهرت أنماط التحلل تحللاً تفضيلياً للألكانات متوسطة السلسلة (C14-C25) خلال المراحل المبكرة، بينما تتطلب الألكانات طويلة السلسلة (C26-C40) فترات حضانة أطول. وكشفت دراسة مقارنة مع دراسات من منطقة شمال غرب الخليج العربي، بما في ذلك المياه الساحلية الإيرانية، أن السلالات البكتيرية المعزولة من الرواسب العراقية تمتلك قدرات تحلل مماثلة أو أفضل. تشير هذه النتائج إلى أن المجتمعات البكتيرية المحلية في الرواسب الساحلية العراقية تمثل مورداً قيماً لاستراتيجيات المعالجة الحيوية في البيئات البحرية الملوثة بالنفط.

الكلمات المفتاحية: التحلل البيولوجي، الهيدروكربونات الأليفاتية، البكتيريا البحرية، الرواسب الساحلية العراقية، الخليج العربي، الألكانات العادية، المعالجة البيولوجية