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PHYSICOCHEMICAL STABILITY AND EMULSIFYING ACTIVITY OF A BIOSURFACTANT OF SALINE ORIGIN

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Abstract

Biosurfactants produced by microorganisms from saline environments have attracted increasing interest due to their potential to remain functional under conditions that limit the efficiency of chemical surfactants. The aim of the present study was to evaluate the physicochemical stability and emulsifying activity of a crude biosurfactant produced by a bacterial isolate from the Techirghiol saline lake. The experimental design included testing the biosurfactant at different pH values, NaCl concentrations, and temperatures, by determining the emulsification index at 24 h. Emulsifying performance was further evaluated in a simplified diesel-contaminated saline-water microcosm at different salinities. This study examines how the biosurfactant performs under high-salinity conditions and indicates potential value for hydrocarbon dispersion and microbial activity in saline environments.

Key words: *bacteria, biosurfactants, diesel, physicochemical stability, saline environments.*

INTRODUCTION

Saline and hypersaline environments, from coastal salterns and salt lakes to industrial brines, are increasingly exposed to hydrocarbon contamination associated with oil extraction, transportation, and refining activities. In such ecosystems, high ionic strength and reduced water activity may limit the effectiveness of conventional chemical surfactants and hinder the natural attenuation of petroleum hydrocarbons. Therefore, halophilic and halotolerant microorganisms represent promising sources of surface-active compounds capable of retaining functionality under high salinity, as well as under pH and temperature conditions that inhibit many traditional surfactant-producing organisms (Edbeib et al., 2016).

Microbial biosurfactants decrease surface and interfacial tension and facilitate the emulsification of hydrophobic compounds, including petroleum hydrocarbons. This process enhances dispersion in aqueous environments and can increase substrate bioavailability for microbial degradation. Biosurfactants comprise a structurally diverse group of molecules, predominantly glycolipids, lipopeptides, and phospholipids, whereas high molecular weight bioemulsifiers primarily contribute to emulsion stabilization rather than lowering surface tension (Silva et al., 2022).

Many biosurfactants are biodegradable and exhibit relatively low toxicity. Moreover, they can remain active under environmental conditions in which certain synthetic surfactants show reduced performance. These features support their potential application in bioremediation, enhanced oil recovery, and the treatment of petroleum hydrocarbon-contaminated water (Sun et al., 2018; Ali et al., 2021; Lima et al., 2024).

Biosurfactants produced by isolates obtained from petroleum-impacted environments (e.g., soils and related sites) have been described to retain surface activity and emulsification capacity under high-salinity conditions (Sun et al., 2018; Ali et al., 2021; Silva et al., 2022). Similarly, investigations of biosurfactants derived from halophilic microorganisms indicate that these compounds can remain functional in saline conditions, although performance may vary depending on the producing strain and the biosurfactant class (Gomes et al., 2018; Barakat et al., 2017; Waghmode et al., 2019; Wu et al., 2022). Recent studies on diesel-contaminated saline soils further demonstrate that the addition of

biosurfactants can improve hydrocarbon removal under salinity stress (Barzegar et al., 2025). Together with similar reports, these findings highlight the biotechnological potential of halophilic or halotolerant microorganisms from saline environments, which are comparatively less explored than marine ecosystems.

Despite progress in this field, stability is not always evaluated using approaches that reliably predict performance under application-relevant conditions. In several studies, surface tension reduction is considered the primary indicator of stability, whereas assays involving petroleum-derived substrates are performed separately or with limited methodological integration. Consequently, establishing a direct link between reported physicochemical stability and actual functional performance in saline systems remains challenging.

In this context, the present study investigates a selected halotolerant isolate and its crude biosurfactant. The specific objectives were: (i) to evaluate the stability of the crude biosurfactant under different pH values, temperatures, and NaCl concentrations using the emulsification index, and (ii) to assess its diesel emulsification performance in simplified saline-water microcosm systems.

MATERIALS AND METHODS

Sampling and bacterial isolation

Water and mud samples used in this study were collected in summer 2024 from Lake Techirghiol (Latitude: 44.05318° N, Longitude: 28.60330° E), located in Constanța County, Romania. Surface water was sampled in sterile containers, and bottom mud was collected from a depth of 10 cm using a sterile spatula. Samples were kept in an icebox during transport and transferred directly to the laboratory for further analysis.

The isolation medium used for the bacteria included in this study was prepared according to Ventosa et al. (1982), with minor modifications, and had the following composition (g/L): $MgSO_4$ 1.0, $CaCl_2 \cdot 2H_2O$ 0.36, KCl 2.0, $NaHCO_3$ 0.06, NaCl 100, peptone 5.0, glucose 1.0, yeast extract 10.0, and agar 20.0.

Serial decimal dilutions (10^{-1}-10^{-3}) of the water and mud samples were prepared in sterile saline solution (5% NaCl, w/v) and inoculated onto the same agar medium (10% NaCl) by the pour plate method (Sanders, 2012). Briefly, 1.0 mL of each dilution was transferred to sterile Petri dishes and mixed with molten agar medium. The plates were incubated aerobically at 30°C for 5-7 days. The number of viable microorganisms was expressed as CFU/mL for water and CFU/g for the mud sample. Representative colonies were purified by repeated streaking on solid isolation agar until morphologically uniform growth was obtained. Isolates with distinct colony morphologies were routinely cultivated on Luria-Bertani (LB) (Sezonov et al., 2007) medium supplemented with 5% (w/v) NaCl. Stocks were prepared in glycerol and stored at −80°C.

Chloride concentration was determined by argentometric titration using the Mohr method (Hauser, 2002).

Biosurfactant screening and assays

Isolates were cultivated in LB broth supplemented with 5% (w/v) NaCl at 30°C under agitation (150 rpm) for 72 h. Following incubation, cultures were centrifuged (7500 rpm for 10 min), and the resulting cell-free supernatant (CFS) was collected for subsequent analyses as crude biosurfactant extract. Emulsifying capacity was evaluated by determining the 24 h emulsification index (E_{24}), in accordance with the protocol described by Stancu (2024). CFS was mixed with diesel fuel, vortexed (3 min), and left to stand at room temperature for 24 h. Positive and negative controls were included. Biosurfactant production was also analyzed using the CTAB (cetyltrimethylammonium bromide) agar plate method as described by Neagu & Stancu (2025). Cultures were spot-inoculated (20 µL) onto the CTAB agar, air-dried, and incubated at 30°C for 3-5 days. A dark blue halo around the colony was considered a positive reaction.

Characterization of halotolerant bacteria

Phenotyping features and rep-PCR fingerprinting

The selected isolates were preliminarily characterized based on colony morphology on saline agar (cell shape, pigmentation, colony margin), Gram reaction, motility, growth temperature, growth on LB agar supplemented

with 0-20% (w/v) NaCl, as well as the basic biochemical traits, including catalase and oxidase activities. Growth on eosin methylene blue (EMB) agar was recorded as part of differential phenotypic profiling.

Diesel tolerance was evaluated using the hydrocarbon overlay agar method (Stancu, 2025). Cultures were spot-inoculated (20 µL) onto LB and minimal salt (MSM) agar (Stancu, 2023), air-dried, and overlaid with diesel for 2 h. After removal of the diesel, plates were incubated at 30°C for 1-5 days. Tolerance was assessed by comparing colony growth with the control (plates not exposed to diesel).

Genomic DNA was extracted from bacterial isolates using the PureLink Genomic DNA Kit (Invitrogen, Carlsbad, CA, USA). Repetitive extragenic palindromic polymerase chain reaction (REP-PCR) was performed using genomic DNA, the $(GTG)_5$ primer, and GoTaq G2 Flexi DNA polymerase (Promega, Madison, WI, USA) according to the method previously described (Stancu, 2015). PCR was performed in a Mastercycler pro S (Eppendorf, Germany) with initial denaturation at 94°C for 10 min, 35 cycles (94°C for 1 min, 52°C for 1 min, 65°C for 8 min), and a final extension at 65°C for 16 min. PCR products were separated by electrophoresis on 2.0% (w/v) agarose gels stained with SYBR Safe DNA Gel Stain (Invitrogen).

Salt tolerance and physicochemical stability of the biosurfactant produced by T3 isolate

For the salt tolerance assay, a fresh overnight culture was grown in LB broth with or without 1% (v/v) diesel, and HM broth (Ventosa et al., 1982) with 1% (v/v) diesel. Then, cultures were spot-inoculated (20 µL) onto the LB agar with 0, 5, 10, 15, and 20% NaCl (w/v) and incubated at 30°C for 72 h.

The physicochemical stability of the crude biosurfactant produced by isolate T3, was evaluated by measuring the E_{24} after exposure to different temperature, pH, and salinity conditions. Thermal stability was assessed by incubating CFS aliquots at 20-100°C for 60 min, followed by cooling to room temperature. pH stability was tested over a range of pH 4-9, adjusted using 0.1 N HCl or 0.1 N NaOH. Salinity stability was examined by adjusting NaCl to final concentrations of 0-20% (w/v). After each treatment, emulsification activity (E_{24}) was determined. A negative control was prepared under identical conditions without CFS.

Diesel emulsification in simplified saline-water microcosms

Diesel emulsification was evaluated in simplified saline-water microcosms at different salinity levels. Microcosms were prepared by mixing CFS, sterile saline solution (0, 5, 10, 15, or 20% NaCl, w/v), and diesel fuel at a 2:2:1 (v/v/v) ratio. Samples were vortexed for 3 min and left for 24 h at room temperature, after which E_{24} was recorded. A negative control was prepared under identical conditions without CFS.

RESULTS AND DISCUSSIONS

Lake Techirghiol is located in Constanța County, southeastern Romania, in the coastal zone of the Black Sea. It is described as a unique Romanian ecosystem due to its hypersaline character and the high productivity associated with the formation of therapeutic sapropelic mud. Long-term records indicate pronounced variations in water level and salinity, while the lake remains highly mineralized overall (Barbulescu & Barbes, 2013).

The physicochemical data obtained indicate that Lake Techirghiol is a salt-rich environment with high ionic strength, providing favourable conditions for the growth of halophilic and halotolerant microorganisms. The pH of the water was 8.35, consistent with the alkaline profile reported for this lake (Maftei et al., 2020). The chloride ion concentration in the water and mud samples was 60 g/L and 25 g/L, respectively. Bacterial abundance in the water sample was 9.3×10^3 CFU/mL, in line with previous reports for Techirghiol surface water (Aonofriesei, 2007). The higher viable count in the mud sample (1.1×10^5 CFU/g) suggests that sediment provides a more favorable environment for bacterial growth, likely due to its greater organic matter content and protective conditions against environmental stress.

Biosurfactant screening was first performed on thirty-six isolates obtained from water and mud samples using the diesel emulsification assay. The results showed that six (T1-T6) isolates

produced extracellular compounds with high emulsification capacity, as indicated by E_{24} values ranging from 90% to 100% (Table 1, Figure 1a). The E_{24} index reflects the stability of the hydrocarbon-water emulsion after 24 h; high values therefore indicate strong biosurfactant-producing ability. While other halophilic and halotolerant bacteria have been reported to produce emulsifying compounds, their E_{24} values are generally lower and depend on the strain, hydrocarbon type, and assay conditions. For example, haloalkaliphilic *Bacillus* strains showed E_{24} values of approximately 56-57% (Barakat et al., 2017), while similar values (~56%) were reported for halophilic isolates from saltern environments (Berberov et al., 2026). The high E_{24} values obtained in the present study suggest that the halotolerant isolates produce extracellular surface-active compounds capable of stabilizing the water-diesel interface. In saline environments, the secretion of extracellular products can contribute to stress adaptation (e.g., biofilm formation, exopolysaccharide matrix development), while also facilitating interaction with hydrophobic compounds (Bhat & Roach, 2025; Berberov et al., 2026). In contrast, none of the isolates produced a detectable halo on CTAB agar, consistent with the absence of detectable rhamnolipid production.

Characterization of halotolerant bacteria
Phenotyping features

Six representative halotolerant isolates (T1-T6) were selected from Lake Techirghiol water and mud samples and subjected to preliminary phenotypic characterization, including some biochemical assays. The results are summarized in Table 1.

Table 1. Characterization of halotolerant bacterial isolates

Characteristics	Isolate					
	T1	**T2**	**T3**	**T4**	**T5**	**T6**
Biosurfactant						
Emulsification index (E_{24}, %)	90	90	100	100	100	100
CTAB agar	-	-	-	-	-	-
Phenotypic						
Source of isolation - Techirghiol	water	water	Mud	water	water	water
Gram reaction	-	+	+	+	+	+
Cell shape	ND	ND	Rod	ND	ND	ND
Margin	regular	regular	Irregular	regular	regular	regular
Pigmentation	cream	cream	Cream	beige	beige	beige
Motility	ND	ND	+	ND	ND	ND
NaCl tolerance (0-20%)	0-10%	0-10%	0-10%	0-10%	0-10%	0-10%
Temperature growth (°C)	30	30	30	30	30	30
Catalase	-	+	+	-	-	-
Oxidase	+	+	+	+	+	+
Lactose fermentation (EMB)	-	-	-	-	-	-
Diesel tolerance						
LB agar-diesel	+	+	+	+	+	+
MSM agar-diesel	-	+/-	+/-	-	-	-
REP-PCR fingerprinting (bp)	200-3500	300-2000	200-1700	200-2800	200-3100	200-3100

(+) Positive reaction; (–) Negative reaction. Diesel tolerance (+) growth comparable to control; (+/-) reduced growth; (–) no growth

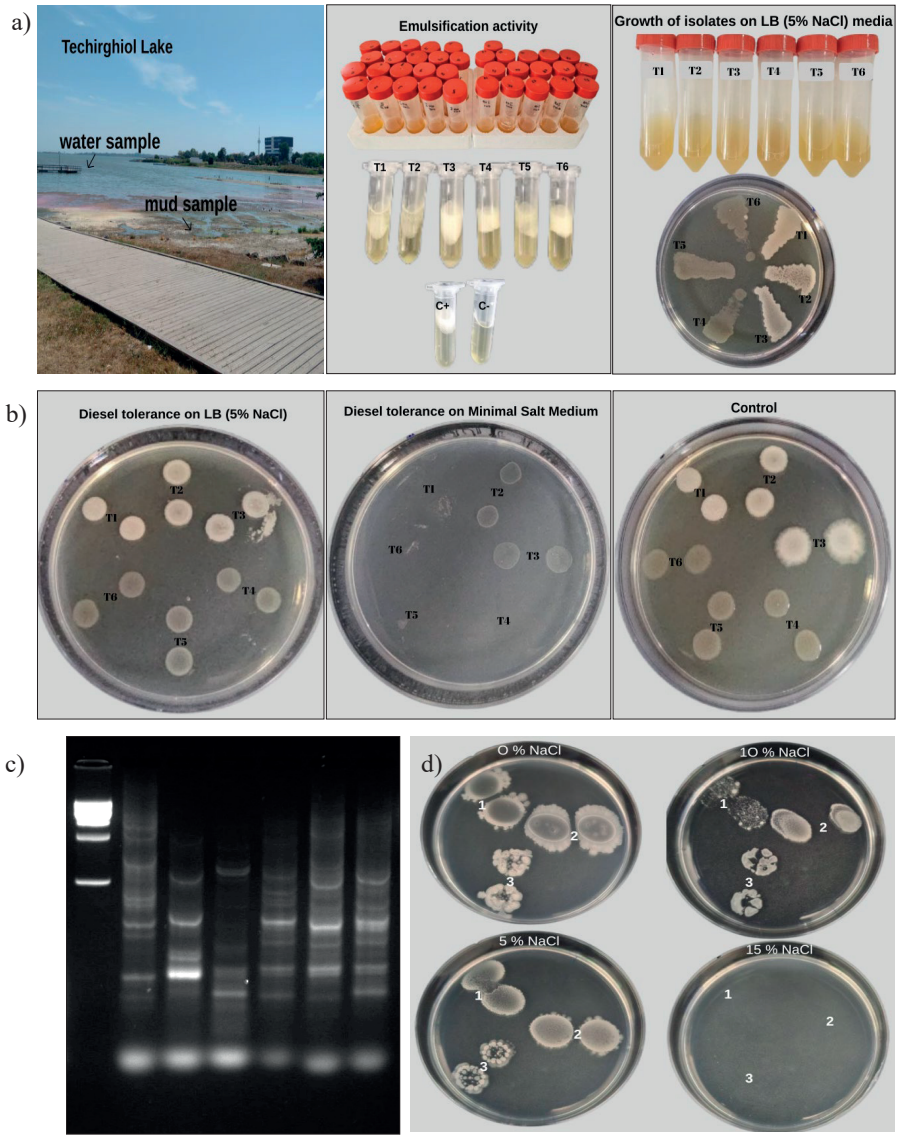


Figure 1. Isolation, screening and characterization of halotolerant bacteria
a) water and mud samples from Lake Techirghiol; emulsification activity of the CFS; isolates growth on LB-NaCl 5%; b) diesel tolerance of T1–T6 isolates on LB and MSM agars, as compared with control (LB agar); c) $(GTG)_5$ rep-PCR fingerprinting of T1–T6 isolates; 1 kb DNA ladder (M). d) Salt tolerance of T3 grown in LB broth (1), LB-diesel 1% (2), HM-diesel 1% (3)

T3 was isolated from Techirghiol mud, whereas T1, T2, T4, T5, and T6 were obtained from surface water. Gram staining revealed a differentiated profile among the isolates: T2, T3, T4, T5, and T6 were Gram-positive, whereas T1 was Gram-negative. Based on visual inspection, colony pigmentation was grouped into two categories: cream (T1-T3) and beige (T4-T6). Colony margins were predominantly regular, except for T3, which showed irregular margins. This isolate consisted of rod-shaped, motile cells. Salinity tolerance on LB agar was evaluated at 0%, 5%, 10%, 15%, and 20% (w/v) NaCl. Growth was robust up to 10% NaCl, whereas no colonies were observed at 15% or 20% NaCl, consistent with a halotolerant profile. All isolates grew at 30°C. On EMB agar, T2, T3 and T4 isolates showed no lactose-fermentation phenotype, indicating a lactose-negative reaction. In contrast, T1, T5, and T6 did not form colonies on this medium.

Diesel tolerance was observed for all six isolates on LB agar, as indicated by growth comparable to the control. In contrast, on MSM in the presence of diesel, only isolates T2 and T3 exhibited reduced growth, whereas T1 and T4-T6 showed no growth (Table 1, Figure 1b). Notably, isolates T1, T4, T5, and T6 failed to grow following hydrocarbon overlay, indicating strain-dependent differences under nutrient-limited conditions.

$(GTG)_5$ REP-PCR fingerprinting revealed distinct profiles for all selected isolates (Table 1, Figure 1c). The water-derived isolates (T1, T2, T4, T5, and T6) exhibited banding patterns ranging from approximately 200 to 3500 bp, whereas T3 displayed a narrower size range (approximately 200-1700 bp) and a clearly distinct fingerprinting. This distinct pattern indicates genetic variability among the isolates and highlights the divergent genomic profile of T3.

Overall, these findings suggest that biosurfactants produced by these halotolerant isolates exhibit properties that could be exploited for hydrocarbon bioremediation and the restoration of saline or contaminated soils, although further studies are needed to confirm their efficacy *in situ*.

Salt tolerance and physicochemical stability of the biosurfactant produced by T3 isolate

Based on its maximal E_{24} and overall screening performance, T3 was selected for subsequent assays. Before evaluating the physicochemical stability of its biosurfactant, the salinity tolerance of T3 was assessed by spot growth on LB agar containing 0-20% (w/v) NaCl. The bacterium exhibited robust growth at 0-10% NaCl, whereas no growth was observed at 15% or 20% NaCl (Figure 1d). Colony morphology varied with the pre-culture medium, consistent with phenotypic adaptation (e.g., changes in cell-surface traits and extracellular polymers) rather than alteration in halotolerance.

The stability of the cell-free culture supernatant from T3 was subsequently evaluated under different temperature, pH, and salinity conditions after 60 minutes of exposure, using E_{24} to quantify emulsification activity (Figure 2).

Thermal stability was tested between 20 and 100°C. Emulsification remained high at moderate temperatures, with E_{24} values of 90-100% at 20-30°C and 80% at 40°C. Activity was still substantial at 50°C and 60°C (~73%), indicating notable heat tolerance. A sharp decline occurred at 80°C (~23%), and no emulsification was detected after exposure to 100°C, suggesting that prolonged heating severely impairs the biosurfactant's activity.

pH stability was evaluated from 4 to 9. The emulsification index remained high under acidic conditions, with E_{24} values of ~93% at pH 4 to 5 and ~83.3% at pH 6. Activity decreased markedly at pH 7 (~27%) and remained low at pH 8 to 9 (~10%), indicating strong sensitivity around neutral to slightly alkaline pH values.

Salinity stability was tested by adjusting NaCl to final concentrations of 0-20% (w/v). E_{24} values were maximal at 0-5% NaCl (100%) and decreased at 10% NaCl (80%), with complete loss of emulsification at 15-20% NaCl. These data suggest that the CFS from T3 is highly effective under moderate salinity, whereas higher salt concentrations significantly compromise its emulsifying performance.

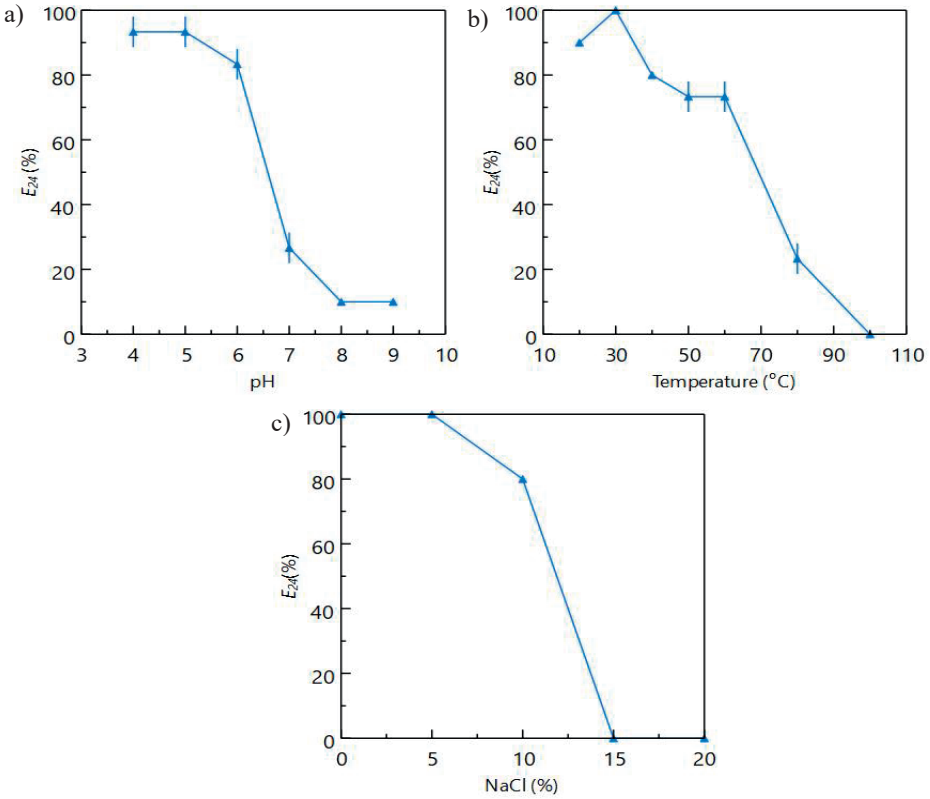


Figure 2. Physicochemical stability of the biosurfactant produced by T3 isolate
Biosurfactant stability under different (a) pH values (4-9); (b) temperatures (20-100°C); and (c) NaCl (5-20%) concentrations; error bars represent the standard deviation of n = 3 biological replicates

Our results further indicate that E_{24} values obtained from crude CFS are strongly influenced by the producing strain and assay-specific parameters. Consequently, the apparent stability of CFS may vary substantially between studies, reflecting the influence of assay-specific parameters such as the nature of the hydrophobic phase, phase ratio, and exposure time (Qi et al., 2025).

Diesel emulsification in simplified saline-water microcosms

The E_{24} stability assay showed how pH, temperature, and salinity affected the crude biosurfactant. To link these physicochemical stability results with functional behavior in saline systems, we further tested diesel emulsification in simplified saline-water microcosms at increasing NaCl concentrations (5-20%, w/v). The results showed that diesel emulsification by T3 CFS was strongly dependent on salinity. At 5% and 10% NaCl, emulsification was maximal (E_{24} = 100%) (Figure 3).

In contrast, at 15% and 20% NaCl, no stable emulsion was detected (E_{24} = 0%). Although the mechanism was not investigated, we did not observe visible precipitation in the 15% and 20% NaCl treatments, suggesting that the loss of emulsification was not associated with macroscopic precipitation of the active fraction. The obtained results are broadly consistent with the halotolerant growth profile of isolate T3, which showed robust growth up to 10% NaCl but not at 15-20% NaCl.

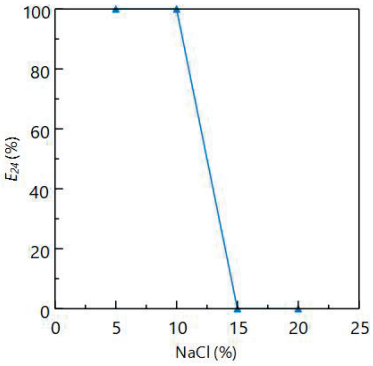


Figure 3. Diesel emulsification in simplified saline-water microcosms
Microcosm assay using diesel emulsification by T3 CFS at different NaCl concentrations (0-20%); data are presented as mean standard deviation of n = 3 biological replicates

Although biosurfactant production and emulsifying performance are not identical traits, the similar limits suggest adaptation to moderate salt levels. Thus, the crude biosurfactant is likely more suitable for low to moderately saline systems.

CONCLUSIONS

The present work indicates that saline environments such as Lake Techirghiol host halotolerant bacteria capable of producing biosurfactants with strong diesel emulsification capacity. From the screened isolates, the cell-free supernatant of T3 showed an emulsifying activity profile that varied with pH and temperature, with high activity at moderate temperatures and acidic pH, but reduced activity at neutral to alkaline pH and after high-temperature exposure. Salinity-limited emulsification: activity remained maximal at 0-5% NaCl, decreased at 10% NaCl, and was lost at ≥15% NaCl. Importantly, the microcosm model showed the same overall trend, with stable diesel emulsification at 5-10% NaCl and no stable emulsion at 15-20% NaCl after 24 h.
In conclusion, these preliminary results suggest that the biosurfactant produced by the halotolerant isolate T3 may be useful for hydrocarbon dispersion in low to moderately saline waters or soils, as it may enhance diesel phase dispersion and potentially support the bioavailability of diesel-range hydrocarbons.

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REFERENCES

Ali, F., Das, S., Hossain, T. J., Chowdhury, S. I., Ali, F., Das, S., Hossain, T. J., Chowdhury, S. I., Zedny, S. A., Das, T., Ahmed Chowdhury, M. N., & Uddin, M. S. (2021). Production optimization, stability and oil emulsifying potential of biosurfactants from selected bacteria isolated from oil-contaminated sites. *R. Soc. Open Sci.*, *8*, 211003.

Aonofriesei, F. (2007). Abundance and activity of heterotrophic bacterial community in the water column and sediments of Tekirghiol Lake. *Proceedings of the Romanian Academy, Series B*, (1), 3–10.

Barakat, K. M., Hassan, S. W. M., & Darwesh, O. M. (2017). Biosurfactant production by haloalkaliphilic *Bacillus* strains isolated from Red Sea, Egypt. *Egyptian Journal of Aquatic Research*, *43*(3), 205–211.

Barbulescu, A., & Barbes, L. (2013). Assessment of Techirghiol Lake surface water quality using statistical analysis. *Revista de Chimie (Bucharest), 64*(8).

Barzegar, G., Jorfi, S., Sanaei, D., Rezaei, M., Jokar, R., Sharifan, H., Jafari, K., & Biswas, J. K. (2025). Bioremediation of diesel-contaminated saline soil and enhancement of microbial salinity tolerance by a biosurfactant-producing *Bacillus subtilis* AHVKH11 and external surfactant application: bio-toxicity assessment. *Scientific Reports*, *15*, 20673.

Berberov, K., Boyadzhieva, I., Yakimova, B., Petkova, H., Stoineva, I., Nacheva, L., & Kabaivanova, L. (2026). Exploring biosurfactant production from halophilic bacteria, isolated from Burgas salterns in Bulgaria. *Marine Drugs, 24*(1), 53.

Bhat, S. P., & Roach, D. J. (2025). Bioactivity of microbial biofilms in extreme environments. *Frontiers in Microbiology, 16*, 1602583.

Edbeib, M. F., Abdul Wahab, R., & Huyop, F. (2016). Halophiles: Biology, adaptation, and their role in decontamination of hypersaline environments. *World Journal of Microbiology and Biotechnology*, *32*, 135.

Gomes, M. B., Gonzales-Limache, E. E., Sousa, S. T. P., Dellagnezze, B. M., Sartoratto, A., Silva, L. C. F., Gieg, L. M., Valoni, E., Souza, R. S., Torres, A. P. R., Sousa, M. P., De Paula, S. O., Silva, C. C., & Oliveira, V. M. (2018). Exploring the potential of halophilic bacteria from oil terminal environments for biosurfactant production and hydrocarbon degradation under high-salinity conditions. *International Biodeterioration & Biodegradation*, *126*, 231–242.

Hauser, B. A. (2002). Chloride. In B. A. Hauser (Ed.), *Drinking water chemistry: A laboratory manual* (pp. 93–95). Lewis Publishers, Boca Raton, FL.

Lima, B. G. A., Santos, J. C. V., Silva, R. R., Caldas, M. C. F., Meira, H. M., Rufino, R. D., Sarubbo, L. A., &

Luna, J. M. (2024). Sustainable production of biosurfactant grown in medium with industrial waste and use for removal of oil from soil and seawater. *Surfaces*, *7*, 537–549.

Maftei, C., Buta, C., & Carazeanu Popovici, I. (2020). The impact of human interventions and changes in climate on the hydro-chemical composition of Techirghiol Lake (Romania). *Water, 12*, 2261.

Neagu, S., & Stancu, M. M. (2025). Novel halotolerant bacteria from saline environments: Isolation and biomolecule production. *BioTech, 14*, 49.

Qi, G.-N., Qin, W.-Q., Li, G.-J., Ma, T.-T., Liu, Y.-F., Zhou, L., Liu, J.-F., Gang, H.-Z., Yang, S.-Z., & Mu, B.-Z. (2025). A new bacterial strain producing both of the surfactin and fengycin lipopeptide biosurfactant with strong emulsifications on crude oil. *Applied Biochemistry and Biotechnology, 197*, 1192–1208.

Sanders, E. R. (2012). Aseptic laboratory techniques: plating methods. *Journal of Visualized Experiments,* 63*,* e3064.

Sezonov, G., Joseleau-Petit, D., & D'Ari, R. (2007). *Escherichia coli* physiology in Luria-Bertani broth. *Journal of Bacteriology, 189*(23), 8746–8749.

Silva, T. A., Santos, R. B., Silva, R. C., & Campos-Takaki, G. M. (2022). Production and functional stability of the biosurfactant isolated from *Stenotrophomonas maltophilia* UCP 1601. *Research, Society and Development*, *11*(11), e87111132995

Stancu, M. M. (2015). Investigation of the organic solvent resistance mechanisms in *Rhodococcus* and *Lysinibacillus* using several molecular forensic tools. *Environmental Forensics, 16*(3), 242–256.

Stancu, M. M. (2023). Characterization of new diesel-degrading bacteria isolated from freshwater sediments. *International Microbiology, 26*, 109–122

Stancu, M. M. (2024). Characterization of a new *Pseudomonas aeruginosa* strain isolated from petroleum-polluted soil. *Waste and Biomass Valorization, 15*, 1–10.

Stancu, M. M. (2025). Investigating the potential of native soil bacteria for diesel biodegradation. *Microorganisms, 13*, 564.

Sun, W., Cao, W., Jiang, M., Saren, G., Liu, J., Cao, J., Ali, I., Yu, X., Peng, C., & Naz, I. (2018). Isolation and characterization of biosurfactant-producing and diesel oil degrading *Pseudomonas* sp. CQ2 from Changqing oil field, China. *RSC Advances, 8*(69), 39710–39720.

Ventosa, A., Quesada, E., Rodriguez-Valera, F., Ruiz-Berraquero, F., & Ramos-Cormenzana, A. (1982). Numerical taxonomy of moderately halophilic Gram-negative rods. *Journal of General Microbiology, 128*(9), 1959–1968.

Waghmode, S., Suryavanshi, M., Dama, L., Kansara, S., Ghattargi, V., Das, P., Banpurkar, A., & Satpute, S. K. (2019). Genomic insights of halophilic *Planococcus maritimus* SAMP MCC 3013 and detail investigation of its biosurfactant production. *Frontiers in Microbiology, 10*, 235.

Wu, B., Xiu, J., Yu, L., Huang, L., Yi, L., & Ma, Y. (2022). Biosurfactant production by *Bacillus subtilis* SL and its potential for enhanced oil recovery in low permeability reservoirs. *Scientific Reports, 12*, 7785.