सी एस आई आर-राष्ट्रीय समुद्र विज्ञान संस्थान
(वैज्ञानिक एवं औद्योगिक अनुसंधान परिषद्)
दोना पौला 403 004 गोवा, भारत
CSIR - National Institute of Oceanography
(Council of Scientific & Industrial Research)
Dona Paula 403004 Goa, India

NIO/TSP-02/2025 (TSP003732) 08.09.2025

TO WHOMSOEVER IT MAY CONCERN

This is to certify that "ECO RED" oil spill dispersant manufactured by Environmental A1 Cleaning Solutions and provided by Adithiyaa Agency, Tamil Nadu, was tested for dispersion efficiency and biodegradability using standard protocols as described in the report herein and has been approved for use in Indian waters for efficiently combating oil spills by CSIR- National Institute of Oceanography, Dona Paula, Goa. The product complies with the criteria specified in the Policy and Guidelines for the Use of Oil Spill Dispersants in Indian Waters (Coast Guard Manual 2025), confirming its suitability for a shelf life extension of up to three years for use in marine oil spill response operations.

The validity of the test report is for a period of three years from the date of issue of test certificate. These test results are applicable only to the particular batch of oil spill dispersant sample supplied by the company to CSIR- National Institute of Oceanography and will not be valid for any other similar samples or for the same sample after shelf life period.

Dona Paula
08.09.2025

08/09/2025
Dr. Sandhya S V

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Annexure I

Test Parameters	Recommended Criteria[1]	Test Results of "ECO RED" Oil Spill Dispersant Type II/III	
Dispersant efficiency	>60 %[1]	OSD liquid concentrate Type-III; 1:25 v/v OSD: oil Ratio	80.28 %
		1:10 dilution Type-II; 1:2.5 v/v OSD: oil Ratio	84.2 %
Biodegradability	Should be biodegradable[1]	Biodegradable by indigenous marine bacteria	

[1] Policy and Guidelines for the Use of Oil Spill Dispersants in Indian Waters (Coast Guard Manual 2025).

Dona Paula

08.09.2025

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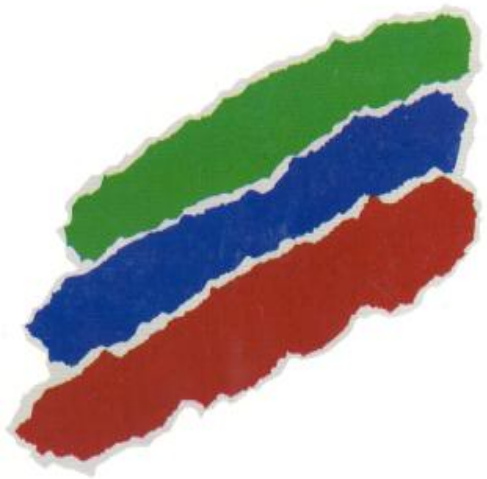
NIO/TSP-02/2025
(TSP 003732)

Title

Testing the efficiency and biodegradability of Oil Spill Dispersants (OSD) for its use in the marine environment

Sponsored by:

Adithiyaa Agency
Phase-III, No. L-114, Anna Nagar P.O,
Tiruchirappalli
Tamil Nadu, 620026



SEPTEMBER 2025

सीएसआईआर – राष्ट्रीय समुद्र विज्ञान संस्थान
CSIR-NATIONAL INSTITUTE OF OCEANOGRAPHY
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सीएसआईआर
CSIR
भारत का नवाचार इंजन
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DESCRIPTION	**PAGE**

PROJECT TEAM

Dr. Sandhya S V	**Project Leader**
Dr. Noyel V	**Team Member**
Amal Santhosh	**Team Member**

EXECUTIVE SUMMARY

A product- labelled "ECO RED" manufactured by Environmental A1 Cleaning Solutions has been supplied by Adithiyaa Agency, Tamil Nadu, for the purpose of evaluation. As per the manufacturer's claim, the said product was approved for the use in marine environment as per the certificate issued by CSIR-NIO (NIO/TSP-02/2019 (TSP 3275) dated on 11-03-2019 for a period of five years. As per Policy and Guidelines for Use of OSD in Indian Waters (Coast Guard Manual 2025), the shelf life of the product may be extended up to three years based on the efficiency test results conducted by CSIR-NIO. The Oil Spill Dispersant (OSD) for testing was provided to CSIR-NIO in clean polyethylene containers for testing and subsequent approval prior to its application in marine environments.

The given product was evaluated for its efficiency following standard protocols. Testing at CSIR-NIO was conducted for both liquid concentrate (Type III) and its 1:10 seawater-diluted diluted forms (Type II). Bombay High Crude Oil (BHCO) was utilized as the reference oil in all the tests.

The oil spill dispersant was tested for both Type-III and Type-II forms.

- OSD Type-III is a concentrate to be used in the concentrated form without dilution.
- OSD Type-II is dilutable concentrate (1:10 dilution with sea water) with a similar formulation to Type-III product.

The efficiency index of OSD, provided by Adithiyaa Agency, Tamil Nadu, proved to be quite efficient in removing oil with dispersion efficiency index (EI) as follows:

- OSD liquid concentrate (Type-III) when used in the ratio of 1:25 (OSD: Oil v/v) was 80.28 %
- 1:10 dilution (Type-II) of OSD: Oil in the ratio of 1:2.5 v/v was 84.2 %

The OSD used in the sea should be biodegradable. Microbial activity in test flasks containing the given OSD as the sole carbon source confirmed the biodegradability of the OSD by the indigenous marine bacteria and support its suitability for environmentally safe use in oil spill remediation.

Based on the results of the conducted evaluations, the OSD provided by Adithiyaa Agency, Tamil Nadu exhibited favorable characteristics in terms of dispersion efficiency in both its concentrated (Type III) and diluted (1:10, Type II) forms and was found to be biodegradable by indigenous marine bacteria. These findings collectively suggest that the given OSD is effective, and biodegradable, meeting the criteria for extending the shelf life of the product up to three years for application in marine environments.

1. INTRODUCTION

Each year, millions of gallons of crude oil and refined petroleum products are spilled into marine environments. The escalating global demand for oil and the associated increase in seaborne petroleum transport have significantly heightened the risk of environmental contamination through accidental spills. It is estimated that approximately 6.1 million metric tons of oil enter the oceans annually from various sources (McCarthy et al. 1978). While major spills are often attributed to oil tanker accidents or offshore drilling operations, smaller yet frequent contributions to oil spills may occur due to water sports or water vehicles such as motorboats and jet skis and leaking fuel. It can also occur from human activities, where petroleum products or various forms of oils used on land are eventually transported into the nearby water bodies. The transportation of oil via waterways remains a leading contributor to spill incidents, and offshore drilling operations release petroleum substances either directly or indirectly into the marine ecosystem. Among these, oil tanker accidents remain the most well-known sources of major oil spills, often receiving widespread media coverage.

Regardless of the source, oil spills are a major contributor of hydrocarbons to marine systems and are recognized as a significant form of pollution capable of causing severe ecological damage, health risk to people, seabirds and air-breathing marine species. These impacts are especially pronounced near oil extraction sites, major shipping lanes, and terminals such as ports and refineries (Martínez-Gómez et al. 2010). The hydrocarbons in petroleum-based oils negatively impacts marine life at concentration as low as 1ppb. The heavier components of crude oil, such as polycyclic aromatic hydrocarbons, are non-volatile, persistent and posing long-term environmental risks. However, they are less toxic than the lighter fractions such as benzene or toluene. Both

acute and chronic exposure to oil spills negatively affect marine and coastal biodiversity, disrupt trophic interactions, and impair broader ecological functions (Martínez-Gómez et al., 2010). The severity of oil spill impacts depends on various factors including the chemical nature of the oil, volume and location of the spill, meteorological and oceanographic conditions, response time, and the methods employed for containment and cleanup (Adofo et al. 2022). Hence, all possible efforts should be made to reduce the risk that spilled oil poses to aquatic life and shorelines (Liu 1983; Heintz et al. 1999). The overarching objective of oil spill response efforts is the rapid containment, dispersion, and mitigation of oil to prevent ecological harm, particularly in sensitive coastal zones. A systematic and efficient risk assessment approach is essential to establish the method of most effective mitigation. Though mechanical response is the preferred means to combat an oil spill, relying solely on this approach is often impractical due to its inherent limitations, such as low recovery efficiency and inadequacy in addressing large-scale spills. Even for calm conditions, it may not be feasible to use mechanical equipment alone to deal with large spills wherein oil may spread rapidly over large areas (Coast Guard Manual 2025). For these reasons, use of chemical dispersants is considered a complementary option in responding to an oil spill emergency. Dispersants change the fate of oil at sea by facilitating the breakdown of an oil slick into tiny droplets, which are suspended and disseminated in water mass, thus enhancing the penetration of oil into the water column. These dispersants, typically composed of surfactant-solvent mixtures, can be applied from vessels or aircraft at the surface or injected subsea at the spill site. The resultant fine oil droplets are more bioavailable to indigenous microorganisms, facilitating faster biodegradation and sedimentation. However, dispersants do not reduce the total volume of spilled oil; rather, they only change the properties of the spilled oil so that it gets broken down from the surface making them more readily bioavailable for break down.

In the present study, a product termed “ECO RED” manufactured by Environmental A1 Cleaning Solutions has been supplied by Adithiyaa Agency, Tamil Nadu to the CSIR-National Institute of Oceanography for evaluation. The product, described as a fully biodegradable, non-hydrocarbon-based liquid concentrate, was supplied in chemically inert polyethylene containers for assessment of its suitability as a marine OSD.

2. SCOPE OF STUDY

The scope of this study was to evaluate

- The effectiveness of the given OSD- "ECO RED" provided by Adithiyaa Agency, Tamil Nadu for dispersion efficiency
- To test the biodegradability of OSD- "ECO RED" provided by Adithiyaa Agency, Tamil Nadu by indigenous marine bacteria

These tests were carried out in combination with the given OSD and Bombay high crude oil (BHCO). CSIR- NIO has been recognized as one of the authorized institutions for oil spill response for conducting the tests of OSD for its toxicity, efficiency, stability and bio-degradability by the regulatory agency i.e. Indian Coast Guard.

3. OBJECTIVES OF THE STUDY

The objectives of the proposed work included:

a) To test the efficiency index of oil spill dispersant

The oil spill dispersant efficiency index is defined as the percentage by weight of the test oil spill dispersant that dissolves oil and transfers it as small droplets into the water column under test conditions, assuming complete and even distribution at the time of sampling.

b) To assess the biodegradability of oil spill dispersants

The oil spill dispersant used in the sea should be biodegradable. Hence, untreated seawater was used as a source of microorganisms and added to mineral medium containing Oil Spill Dispersant, as a source of nutrients for microbial metabolism. Primarily, turbidity formed in the flask indicated degradation of the OSD with good growth of the indigenous marine bacteria and was further confirmed by determining the total viable count through the spread plate technique.

4. OIL SPILL DISPERSANT EFFICIENCY

The efficiency index of an oil spill dispersant is defined as the percentage, by weight of the test oil which has been transferred, as small droplets into the water phase under the conditions of the test, assuming complete and even distribution at the time of sampling. Every OSD has an optimum capacity for dispersing oil. The present report measures the optimum quantity of OSD provided by Adithiyaa Agency, Tamil Nadu, that can be used to efficiently remove the reference test oil (BHCO) under the study conditions. Increasing the quantity or concentration of OSD, does not necessarily increase its efficiency in removing the oil. It may cause the OSD to remain in the marine environment causing harmful effects on the marine flora and fauna in the long run.

4.1 Protocol for the OSD efficiency test

- Three clean 1L separating flasks were filled with 250ml seawater at room temperature (26 ± 1°C).
- Using the syringe, transferred 5ml, by weight, of BHCO to the surface of the seawater in all the separating flasks. The weight of the syringe before and after oil addition gave the weight of the oil added. The experiment was carried out in triplicates.
- To the first separating flask, only oil was added which acted as a control.
- To the second separating flask, in addition to oil, 0.2ml of OSD provided by Adithiyaa Agency, Tamil Nadu (Type-III) was added. [Final OSD: oil ratio was adjusted to 1:25 v/v].
- To the third separating flask, 2 ml of Type-II OSD (1:10 dilution) was added, in addition to oil [Final OSD: oil ratio was adjusted to 1:2.5 v/v].

- After the addition of OSD to the separating flask, the contents were well agitated manually for 2 minutes and then left undisturbed for 1 minute.
- An aliquot of 50ml oily water was withdrawn from the flask into another 100ml separating flask and repeatedly extracted in a suitable organic solvent (methylene chloride). The organic extracts were pooled together, dried over anhydrous sodium sulfate and made to a known volume (100ml) using a measuring cylinder.
- The absorbance of the methylene chloride extract was measured against a methylene chloride blank on a Shimadzu UV-VIS (UV-1800) Spectrophotometer at 580nm using a 10 mm glass cell. The weight of the oil in the 50ml sample was determined by calibrating it with the standard reference oil (WSL 2007).

Calculation of OSD efficiency index

Efficiency Index (EI) was calculated using the formula:

$$EI\% = \frac{\text{Concentration of oil in 50 ml oily water}}{\text{Total weight of oil added to the 250 ml seawater}} \times 500$$

Fig. 4.1. Determination of the efficiency index of OSD

4.2 Results of the OSD efficiency test

Reference oil was first calibrated as calibration helped to yield a factor which proved useful for calculating the amount of oil in the samples. The efficiency index of OSD, provided by Adithiyaa Agency, Tamil Nadu as Type-III when used in the ratio of 1:25 (OSD: Oil v/v) was 80.28 % while for 1:10 dilution (Type-II) of OSD: oil in the ratio of 1:2.5v/v was 84.2 % as shown in the Tables below (Table 4.1 & 4.2).

Table. 4.1. Efficiency index of oil spill dispersant for Type-III: oil (1:25 v/v) sample

S. No.	Ratio of OSD: Oil	Vol. of OSD Added (ml)	Weight of oil added (g)	Wt. of oil in 50ml oily water (g)	Efficiency index (%)	Mean (%)
1	1:25	0.2	4.86	0.775	79.77	**80.28**
2	1:25	0.2	4.59	0.752	81.94	
3	1:25	0.2	4.7	0.744	79.13	

Table. 4.2. Efficiency index of oil spill dispersant for Type-II: oil (1:2.5 v/v) sample

S. No.	Ratio of OSD: Oil	Vol. of OSD Added (ml)	Weight of oil added (g)	Wt. of oil in 50ml oily water (g)	Efficiency index (%)	Mean (%)
1	1:2.5	2	4.86	0.854	87.8	**84.2**
2	1:2.5	2	4.86	0.798	82.2	
3	1:2.5	2	4.95	0.817	82.5	

Table 4.3. The efficiency criterion adopted for evaluation as per "Policy and Guidelines for Use of OSD in Indian Waters (Coast Guard Manual 2025)"

Type II	50% (minimum)
Type III	60% (minimum)
Type II/III	60% (minimum for both)

5. BIODEGRADABILTY

It is essential that the OSDs should be biologically degradable. Biodegradation refers to the microbial-mediated breakdown of chemical substances into simpler, non-toxic forms. While all living organisms—including microorganisms, plants, and animals—possess enzymatic systems capable of degrading various compounds, the most significant and extensive biodegradation processes in the environment are primarily carried out by microorganisms such as bacteria, fungi, and microalgae. Primary degradation involves the transformation of the parent compound into intermediate metabolites, whereas complete degradation results in the conversion of the compound into water and carbon dioxide. Biodegradation can occur under aerobic (in the presence of oxygen) or anaerobic (in the absence of oxygen) conditions. In order to ensure that the OSDs do not persist in the environment, it is important that the OSDs possesses the important characteristics of biodegradability. In general, most OSDs are readily biodegradable under aerobic conditions, particularly by indigenous marine bacteria (Lee et al. 1985; Liu 1983; Una & Garcia 1983). The rate and extent of biodegradation are influenced by multiple physicochemical and biological factors, including compound solubility, molecular structure, temperature, oxygen availability, sediment characteristics, and microbial population density. Water-soluble compounds tend to undergo more rapid degradation due to improved dispersion in aquatic systems and enhanced oxygen availability for microbial respiration. However, while high biodegradability is a desirable property for oil spill remediation agents, it is not the sole determinant of environmental safety. Rapid microbial degradation can lead to increased biological oxygen demand (BOD), potentially depleting dissolved oxygen in the local environment and adversely affecting marine biota. Primary biodegradation was assessed by monitoring the growth of indigenous marine bacteria from seawater in mineral media (Bushnell Haas Broth) devoid of carbon source. Carbon source in the media was substituted by the OSD to be tested. This leaves the microorganisms with no option but to utilize the OSD as the sole carbon source for growth.

5.1 Experimental protocol

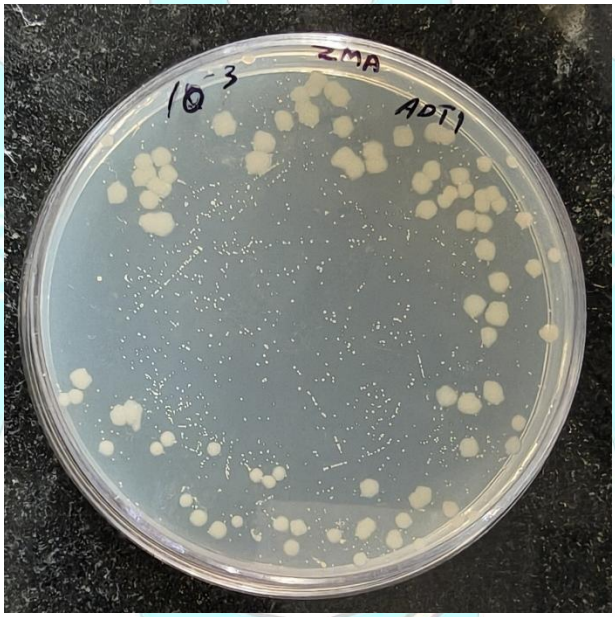
All glassware used in the experiment were thoroughly washed and sterilized, and each experiment was performed in duplicates.

- Two flasks containing 100 ml Bushnell Haas Broth (BHB) media (Hi-Media, India) were prepared.
- Five ml of untreated seawater was inoculated into the sterile BHB media. Untreated seawater acted as a source of microorganisms
- Two ml OSD to be tested was added in test flask as a source of nutrients for microbial metabolism. Control flasks were devoid of carbon source.
- Flasks were incubated for 48-72 hours at 28 ± 2 ºC on a shaker at 120rpm.
- Following incubation, microbial growth in the flasks was assessed by visualizing turbidity in test flasks and by determining the total viable count through the spread plate technique.

5.2 Results of biodegradability study

An increase in turbidity was observed in the test flasks over the incubation period, indicating microbial proliferation when the given OSD was provided as the sole carbon source. Correspondingly, Zobell Marine Agar plates inoculated from the test flasks showed visible bacterial growth with a total bacterial count of 7.2 X 10^6 $CFUmL^{-1}$, confirming the presence and metabolic activity of viable microbial populations. These observations suggest that the test OSD supported microbial growth and could be considered as biodegradable under the given experimental conditions.

Fig. 5.1. Zobell Marine Agar plates inoculated from test flasks showing visible bacterial growth following incubation



6. CONCLUSION AND RECOMMENDATIONS

As per the information provided by Adithiyaa Agency, Tamil Nadu, the Oil Spill Dispersant (OSD) - "ECO RED", supplied to CSIR-NIO for testing and approval for its use in the marine environment is a fully biodegradable liquid formulation, free from hydrocarbons. It is specifically developed to efficiently emulsify and disperse oil spills from various surfaces. According to the manufacturer, the product meets the necessary environmental safety standards. The scope of this study was to evaluate the performance of the given OSD based on its dispersion efficiency and biodegradability. Dispersion efficiency testing was conducted at CSIR-NIO using both the concentrated form of the dispersant (Type III) and a 1:10 dilution (Type II), with natural seawater. Bombay High Crude Oil (BHCO) was used as the reference oil for all experimental procedures.

From the results it is seen that the efficiency index of the given OSD, as Type-III, when used in the ratio of 1:25 (OSD: Oil v/v) was 80.28 % while for 1:10 dilution (Type-II) of OSD: Oil in the ratio of 1:2.5 v/v was 84.2 %.

Biodegradability was assessed by monitoring microbial activity in test flasks containing the OSD as the sole carbon source. An increase in turbidity over the incubation period, along with visible bacterial growth on Zobell Marine Agar plates, confirmed that indigenous marine bacteria were able to utilize the dispersant. These results demonstrate the biodegradability of the product and support its suitability for environmentally safe use for combating oil spills.

Overall, the evaluation results indicate that the Oil Spill Dispersant, "ECO RED" provided by Adithiyaa Agency, Tamil Nadu performs effectively in both concentrated (Type III) and diluted (Type II) forms with respect to dispersion efficiency, and was found to be biodegradable by indigenous marine bacteria. The product complies with the criteria specified in the Policy and Guidelines for the Use of Oil Spill Dispersants in Indian Waters (Coast Guard Manual 2025), confirming its suitability for a shelf life extension of up to three years for use in marine oil spill response operations.

***The validity of the test report is for a period of Three Years from the date of issue of test certificate.**

Disclaimer:

***These test results are applicable only to the particular batch of Oil Spill Dispersant sample supplied by the company to CSIR-NIO and will not be valid for any other similar samples or the same sample after shelf life period.**

***The results reported in the study are based on laboratory test/conditions and may be indicative of its probable performance in natural sea condition.**

7. REFERENCES

- Adofo, Y. K., Nyankson, E., & Agyei-Tuffour, B. (2022). Dispersants as an oil spill clean-up technique in the marine environment: A review. Heliyon, 8(2022), e10153. https://doi.org/10.1016/j.heliyon.2022.e10153
- Heintz RA, Short JW, and Rice SD. (1999). Sensitivity of fish embryos to weathered crude oil: Part II. Increased mortality of pink salmon (Oncorhynchus gorbuscha) embryos incubating downstream from weathered Exxon Valdez crude oil. Environ Toxicol Chem 18:494–503.
- Lee, K., C. S. Wong, W. J. Cretney, F. A. Whitney, T. R. Parsons, C. M. Lalli and J. Wu. (1985). Microbial response to crude oil and Corexit 9527: SEAFLUXES enclosurestudy. Microbial Ecology 11: 337-351.
- Liu, D. (1983). Fate of oil dispersants in aquatic environment. Science of the Total Environment 32: 93- 98.
- Martínez-Gómez, C., Vethaak, A. D., Hylland, K., Burgeot, T., Köhler, A., Lyons, B. P., Thain, J., Gubbins, M. J., & Davies, I. M. (2010). A guide to toxicity assessment and monitoring effects at lower levels of biological organization following marine oil spills in European waters. Journal of Marine Systems, 79(3–4), 177–190. https://doi.org/10.1016/j.jmarsys.2009.10.012
- McCarthy L.T., Lindblom G.P., Walter H.F. (1978). Book on Chemical Dispersant for the control of oil Spills: A symposium. www.asrm.org
- Policy and Guidelines for the Use of Oil Spill Dispersants in Indian Waters (Coast Guard Manual 2025).
- Una, G. V. and M. J. N. Garcia. (1983). Biodegradation of non-ionic dispersants in seawater. Journal of Applied Microbiology and Biotechnology 18: 315-319.
- Warren Spring Laboratory Report (WSL). ANNEX 1 TO APPENDIX A, LR 448 (OP) (revised February 2007) (original print April 1983), Warren Spring Laboratory, Stevenage, England, United Kingdom.

8. ACRONYMS/ABBREVIATIONS

ACRONYM	FULL FORM
BHCO	Bombay High Crude Oil
CSIR	Council of Scientific and Industrial Research
EI	Efficiency Index
ICG	Indian Coast Guard
NIO	National Institute of Oceanography
OD	Optical Density
OSD	Oil Spill Dispersant