

Bioremediation of marine oil spill using beeswax

Riya Ann Mathew^{1*}, V. Lavanya², S. Rasiga³, & Marykutty Abraham⁴

¹Centre for Environmental Studies, Anna University, Chennai, India

²Department of Civil Engineering, Jerusalem College of Engineering, Chennai, Tamil Nadu, India

³Department of Civil Engineering, Coimbatore Institute of Technology, Coimbatore, Tamil Nadu, India

⁴Centre for Remote Sensing and Geoinformatics, Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu, India

*[E-mail: riya.am@gmail.com]

Oil spills can have devastating environmental, economical, and social impacts. The use of bioremediation is a preferred technique for cleaning up oil spills because it facilitates the process of naturally converting oil into non-toxic byproducts without any further local environmental disruption. An experimental study was conducted to degrade marine oil spill using beeswax as a biostimulant for bioremediation and also to study the chemical variation. Beeswax, a nutrient source for proliferation of olephic (oil eating) bacteria, was added in intervals of five days and the corresponding dissolved oxygen (DO) was measured using Azide Winkler's method. The recuperation of the DO concentration from 3.1 mg/l to 7.4 mg/l is an indication of the reduction in oil content. An oxygen sag curve and a graph showing temporal variation of chemical parameters such as nitrite, nitrate, and ammonium were plotted. During the period of study, the oil content reduced from 10 ml to 5 ml. As the addition of biostimulant resulted in an increase in the rate of bioremediation, the use of beeswax as a biostimulant appears to be an effective tool for the clean-up of oil-contaminated marine waters without adverse environmental impact.

[**Keywords:** Bioremediation; Biostimulation; Beeswax; Oil spill; Dissolved oxygen]

Introduction

Bioremediation is the process of remediating the harmful effects of pollutants with the aid of biological clean-up technique. The following are the two main approaches:

- (i) *Bioaugmentation* – The introduction of bacteria to a spill site to supplement the indigenous microbial population for digestion of spilled oil; and
- (ii) *Biostimulation* – The introduction of nutrients or other growth-enhancing substances to a spill site to stimulate the growth of indigenous microbes required and thus aid in the faster degradation of oil¹.

On 28 January 2017, two ships, BW Maple carrying LPG, and MT Dawn Kanchipuram carrying spirit oil and diesel, respectively, collided at Kamarajar Port, Ennore, Chennai, India. It was reported that about 208 tonnes of oil mixed with sludge and 99,000 L of oil mixed with water were removed. Many sea turtles and fish died as a result of pollution caused by the spill. The local fishing communities were affected due to the decline in fish sales as the public were of the notion that fish was contaminated and was not fit to consume².

A method of bioremediation of a petrochemical spill involves providing microbial nutrients in the

form of spheres comprising soy wax or beeswax. The spheres are applied onto the petrochemical spill and indigenous microbes degrade the oil spill. The beeswax can also maintain a high population of hydrocarbon degraders for a long period of time³.

Aerobic microbes use dissolved oxygen (DO) in water for cellular respiration to oxidize hydrocarbons to carbon dioxide. After the oil is digested by olephilic bacteria, the DO content recuperates. Thus, copious presence of such bacteria is indicated by reduction in DO content. The olephilic bacteria become dormant when there is insufficient supply of nitrogen and phosphorus required for their profuse growth. The optimum amount of nitrogen was found to increase the cell growth rate, decrease the acclimation phase, and sustain fast activity of microorganisms^{4,5}. The excessive use of nutrients such as nitrate, phosphate, and sulfate can inhibit microbial metabolism⁶.

The oil is degraded by a consortium of bacteria present in the marine water. About nine bacterial strains, namely, *Pseudomonas fluorescens*, *P. aeruginosa*, *Bacillus subtilis*, *Bacillus* sp., *Alcaligenes* sp., *Acinetobacter lwoffii*, *Flavobacterium* sp., *Micrococcus roseus*, and *Corynebacterium* sp. were observed in polluted streams degrading crude oil⁷.

Mechanical methods cannot recover more than 10–15% of the oil spill. Whereas, bioremediation is beginning to emerge as a promising technology, particularly as a secondary treatment option for oil spill clean-up. Bioremediation is a simple and cost-effective technique over other techniques. Biostimulation can cause an acceleration of the natural biodegradation processes^{8,9}. Bioremediation technology is based on the premise that a large percentage of oil components is readily biodegradable in nature^{10,12}. Several studies are available for using microbial activities as a source of remediation of spilled oil^{13,15}. Experimental studies showed that beeswax (a biostimulant) is an efficient source of nutrient to the olephilic bacteria¹⁶. Bacterial consortium has been proven to be effective in the biodegradation of crude oil¹⁷. Biostimulation of indigenous microorganisms is viable, cost-effective, and less destructive *in situ* clean-up technique for the remediation of polluted soils^{18,21}.

Bioremediation of Exxon–Valdez oil spill using fertilizers containing nitrogen showed an increase in the rate of degradation of oil²². Biostimulation is essential for maximizing the oil removal and reducing the adverse environmental impact²³. A 129-day field trial of bioremediation of oil spill using PRP (Petroleum remediation product) containing mineral nutrients and non-pathogenic bacteria within spherical particles made from beeswax exhibited both bioaugmentation and biostimulation properties. The study further demonstrated that PRP relied on indigenous bacteria, not encapsulated ones, to perform the oil biodegradation²⁴. PRP was used for onsite remediation of fouling beaches and mangroves along the coast of Persian Gulf, Abu Dhabi, in January 1998 for the oil spill of 4,000 metric tons which resulted in marked improvement compared to untreated areas²⁵. In another study, PRP was used to treat the water surface and the shore where heavy oil was spilled into a lagoon in Mexico in 1994. After 26 days, it was estimated that approximately 75% of the original oil had been degraded based on visual examination of oil coverage on the lagoon surface²⁵.

Bioremediation is demonstrated as an environmentally benign approach unlike chemical methods as no chemicals are added to the site and also eliminates the need to remove and transport the toxic compounds to another site. It does not cause any disruption to the natural habitat which often occurs from physical and chemical methods of clean-up. There is also savings in cost and time put forth by workers to clean a contaminated site. In 2015, India had the largest

beeswax output of 22,000 tons, which is 33% of global production, followed by Ethiopia, Argentina, and South Korea²⁶. The reasons for selecting beeswax as a biostimulant for the present study were its ease of availability and convenient application to a spill in comparison to other methods of spill remediation¹³. The environmental threat posed by marine oil spill can be best mitigated by bioremediation technique. This study is carried out to assess the extent of oil removal from marine water using beeswax as a biostimulant.

Materials and Methods

A laboratory scale experimental set-up was arranged to study the effectiveness of beeswax in digesting marine oil spill. For the study, marine water sample was collected in prewashed plastic containers from Marina beach, Chennai. About 2 l of marine water sample was introduced to a glass tank and 10 ml of oil was added. To supplement nutrients for microbial proliferation, 25 g of beeswax was added as shown in Figure 1. Various chemical water quality parameters, such as nitrite, nitrate, ammonia, pH, DO, were analyzed and the results were tabulated. The DO of the sample was determined at intervals of five days and oxygen sag curve was plotted. The experiment was repeated with the addition of 25 g beeswax at intervals of five days for 26-day study period and the corresponding chemical water quality parameters were determined. The study was conducted during February–March when the average atmospheric temperature ranged between 22 °C and 33 °C.

The DO content was determined as per Azide Winkler method²⁷. Various chemical water quality parameters, such as nitrite, nitrate, and ammonia in marine water were measured as per American Public Health Association (APHA) procedure²⁷. The marine water sample was sacrificed to determine the oil content remaining after experiment by evaporation of



Fig. 1 — Experimental setup of marine water sample with beeswax

sample in a rotary evaporator. Beeswax was provided as spheres, which was capable of binding and absorbing and containing oil from water. Beeswax can absorb up to 20 times its weight of oil. This can be applied as a loose powder by spraying the spheres onto the spill. The powder floats on top of the water as it is less dense than marine water and binds with oil immediately. When the powder becomes saturated with oil, it is left in place and the oil is degraded by indigenous microorganisms contained in water. Subsequently, the beeswax is also degraded through the action of microorganisms.

Results and Discussion

The analysis of chemical water quality parameters for marine water is given in Table 1. The initial DO content in the marine water was obtained as 6.7 mg/l. The addition of 10 ml of oil to the marine water sample resulted in the reduction of DO content to 3.1 mg/l. By biostimulation using beeswax, the DO content was increased to 7.4 mg/l within 26 days under laboratory conditions. A minimum DO level of 4–5 mg/l is desirable for the survival of aquatic life²⁸. During the study period, the oil content reduced from 10 ml to 5 ml using 125 g of beeswax. The variation in DO content with time during the experiment is given in Table 2 and the corresponding oxygen sag curve is shown in Figure 2. The oil spilled in marine water caused sudden dip in the DO content. The addition of beeswax resulted in the proliferation of olephilic bacteria which in turn digested the oil thereby recuperating the DO content.

The observed reduction in pH from 8.0 to 7.1 is presented in Figure 3. The pH fluctuation is due to the

biological activity by microbes, changes in temperature, carbon dioxide concentration, salinity, and alkalinity². Marine water is faintly alkaline in nature. Slight reduction in pH does not have any adverse effects on microorganisms. Microbial activity will be more at higher temperature and hence it is a viable option for regions with tropical climate similar to India.

The temporal variations in concentrations of nitrite, nitrate, and ammonia during the experiment are shown in Figure 4. The concentrations of nitrite, nitrate, and ammonia ranged from 0.2 mg/l to 0.4 mg/l, 0.23 mg/l to 0.45 mg/l and 0.02 mg/l to 0.35 mg/l, respectively. Nitrite is due to the biological oxidation of ammonia by microbes through nitrification process². Ammonia is a nutrient which comes to the coastal waters through sewage discharge and agricultural runoff. Nitrate is a limiting nutrient in the marine waters and must be supplemented for microbial proliferation. Microbes consume nutrients for their growth and utilize oil as their carbon source.

The use of spheres of beeswax to clean-up an oil spill is preferable as it is biodegradable and environmentally benign. Bioremediation helps indigenous microbes to digest and destroy the toxic compounds

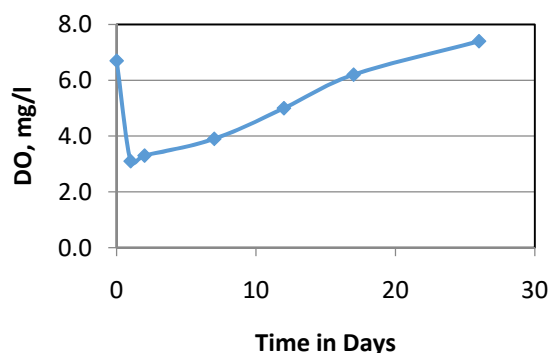


Fig. 2 — Oxygen sag curve representing the variation of DO

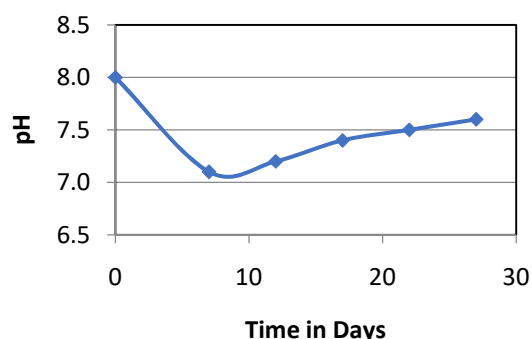


Fig. 3 — Temporal variation of pH during the study

Table 1 — Chemical water quality of raw marine water sample

Sl.No	Parameter	Value (mg/l)
1	DO	6.7
2	pH	8.0
3	Nitrite	0.42
4	Nitrate	0.50
5	Ammonia	0.45

Table 2 — Variation of DO during bioremediation

Sl. No.	Time (days)	DO (mg/l)
1	0	6.7
2	1	3.1
3	2	3.3
4	7	3.9
5	12	5.0
6	17	6.2
7	26	7.4

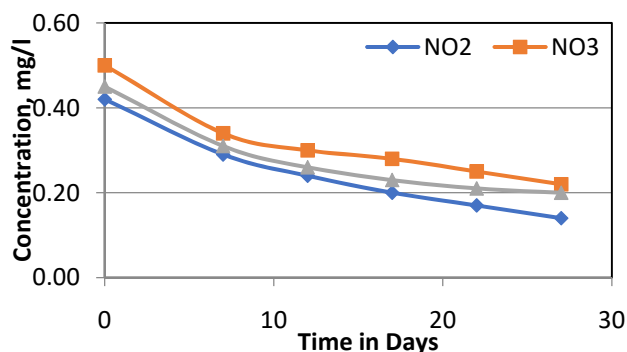


Fig. 4 — Temporal variation of nitrite, nitrate, and ammonia during the study

in the environment, without the need for further collection and removal. Physical containment using booms is not effective for the management of large spills, and flaring of spilled oil may contribute to greenhouse gases in the atmosphere and chemical dispersants may be toxic to the marine environment. Beeswax, a nutrient source, helped the oil-eating bacteria to proliferate and degrade oil. Hence it is a cheap, effective, and environmentally sound means to remediate marine oil spill.

The potential of field application includes a convenient *in situ* remediation of oil spill, low cost, and simpler treatment technique. On the other hand, the challenges include the slow action time which depends on the spill site, climatic conditions, and characteristics of oil. Tidal action can cause further dispersion of oil or may agglomerate to form tar ball which sinks to the bottom of sea. The oil-spilled marine water has to be isolated using booms to prevent further dispersion and primarily to have better control on treatment while applying biostimulant (beeswax).

Summary and Conclusion

There is a need to remediate accidental oil spill in a cost-effective and convenient manner without further pollution to the environment. Experimental study by adding beeswax to oil-spilled marine water sample indicated that the nutrient content of marine water reduced as it was utilized as nutrient source by oil-eating bacteria present in oil-spilled water. The oil content reduced from 10 ml to 5 ml and the DO recuperated from 3.1 mg/l to 7.4 mg/l using beeswax. Bioremediation of marine oil spill with biostimulation using beeswax is a technically and economically feasible practice. The study can be further extended to optimize the dosage of beeswax for oil digestion. The

use of other easily available and cheap biostimulants containing nitrate and phosphate can be studied.

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