• ARTICLE — VOL. 9

Activating microbes in oil bioremediation — the biocatalyst (live catalyst) —

Verified by laboratory culture testing — oxygen supply, microbial activity, and the effectiveness of a biocatalyst

BioRangers

Overview

In oil bioremediation, degradation depends on the microbes coming into contact with the oil and obtaining water, oxygen, and nutrients (nitrogen, phosphorus, potassium). Because oil degradation is oxidative degradation, an aerobic environment is preferable, and the oxygen supply tends to become the rate-limiting factor. We therefore focused on the oxygen supply and used laboratory testing to examine whether an oxygen-release agent and a biocatalyst (live catalyst) can activate microbes.

Focus **The oxygen supply that governs microbial activity**	Items compared **Oxygen-release agent (calcium-based) / biocatalyst**
Indicators **pH, dissolved oxygen, total microbial count, oil sheen**	Conclusion **The catalyst activated the microbes most**

Oxygen **Essential to aerobic oxidative degradation; its supply readily becomes rate-limiting**	Water **The medium for metabolism and mass transfer**
Nutrients **Nitrogen, phosphorus, potassium (N·P·K)**	Contact between microbes and oil **The precondition for the degradation reaction to occur**

• Background

The oxygen-release agent and the biocatalyst

Oil degradation is oxidative degradation, so the environment must be arranged so that the oxygen supply does not become the rate-limiting factor (in theory, about 3 kg of oxygen is required for the microbial degradation of 1 kg of benzene).

Oxygen-release agents come in magnesium-based and calcium-based forms, and in both cases care must be taken because the pH shifts toward alkaline. In this test we used a non-hazardous product composed of calcium peroxide (content around 30%), calcium hydroxide, and calcium sulfate dihydrate. The biocatalyst (live catalyst) is an oxygen-rich water refined from naturally derived organic matter; through catalytic action it separates oxygen atoms from water molecules while also taking oxygen up onto the cell surfaces of the microbes. Because it is not in free form, it cannot be measured as dissolved oxygen.

• Method

The laboratory test method

In a preliminary test, we added a nutrient agent and the multi-microbial agent “Terrazyme” to 1 L of tap water, and measured the changes in pH and dissolved oxygen while adding the oxygen-release agent in stages. Based on those results we determined the dosages and carried out a culture test using diesel oil. We measured pH, dissolved oxygen, and total microbial count, and finally observed the oil sheen.

Test series	Water	Diesel oil	Oxygen-release agent	Terrazyme	Nutrient agent (N·P·K)	Essential-element solution
① Blank	Tap water 400mL	0.4g	None added	0.8g	0.8g	0.2mL
② Catalyst	Catalyst 400mL	0.4g	None added	0.8g	0.8g	0.2mL
③ Oxygen-release agent 1.5g/L	Tap water 400mL	0.4g	0.6g	0.8g	0.8g	0.2mL
④ Oxygen-release agent 3.0g/L	Tap water 400mL	0.4g	1.2g	0.8g	0.8g	0.2mL

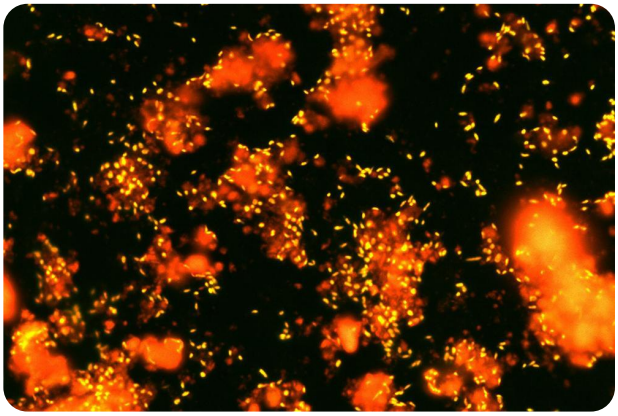
Test results

In the preliminary test, the pH exceeded 9.0 once 3.0 g of oxygen-release agent had been added to 1 L of tap water, so the culture test was set up with two series: 3.0 g/L and half that, 1.5 g/L. In the culture test, in ③ and ④ — to which the oxygen-release agent was added — the pH rose as the test progressed, and ④ at 3.0 g/L exceeded pH 10.0 after 5 days. Dissolved oxygen was somewhat higher in ③ and ④, but it did not reach double.

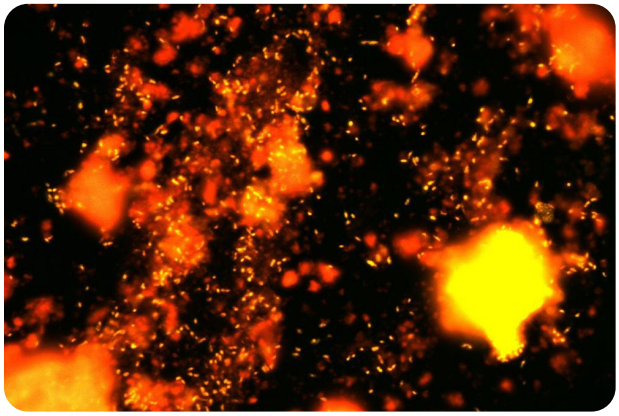
Series	Item	Immediately	After 1 day	After 3 days	After 5 days
① Blank	pH	6.31	6.62	6.72	6.34
① Blank	Dissolved oxygen (mg/L)	4.87	4.39	5.36	5.23
② Catalyst	pH	6.40	7.10	7.13	6.70
② Catalyst	Dissolved oxygen (mg/L)	7.63	8.87	9.02	8.94
③ Oxygen-release agent 1.5g/L	pH	5.56	4.10	7.78	5.30
③ Oxygen-release agent 1.5g/L	Dissolved oxygen (mg/L)	5.89	4.29	7.78	5.30
④ Oxygen-release agent 3.0g/L	pH	8.25	9.51	9.83	10.09
④ Oxygen-release agent 3.0g/L	Dissolved oxygen (mg/L)	6.44	5.09	5.90	5.70

For the total microbial count, ② — cultured with the catalyst — grew the most markedly, reaching about 8.8 times after 1 day and nearly 100 times after 3 days. Its growth rate was 10 times faster than that of ①, cultured in tap water; the slowing after day 3 is thought to be because the diesel oil had been fully degraded. By contrast, in ③ and ④, to which the oxygen-release agent was added, no growth was observed and the count declined, with ③ reaching 0 cells/ml after 5 days.

Series	Immediately	After 1 day	After 3 days	After 5 days
① Blank	1.01E+06	1.39E+06	1.02E+07	2.39E+08
② Catalyst	1.31E+06	1.16E+07	1.19E+08	2.43E+08
③ Oxygen-release agent 1.5g/L	6.25E+05	2.56E+05	2.72E+05	0.00E+00
④ Oxygen-release agent 3.0g/L	6.41E+05	1.60E+05	2.88E+05	3.21E+04

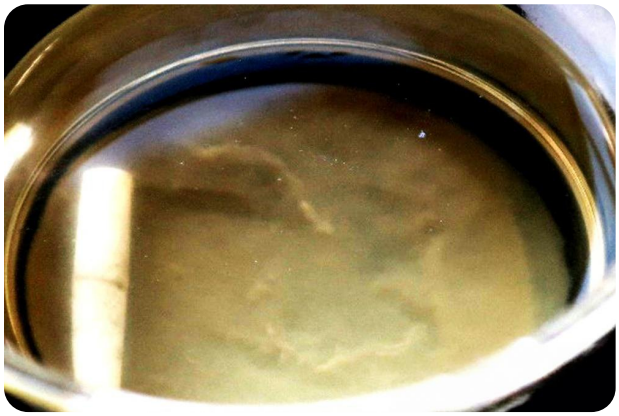


① Blank (fluorescence microscope)

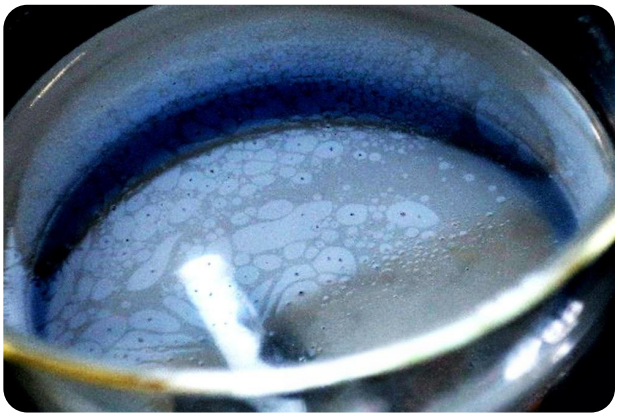


② Catalyst: microbes growing densely

① Blank: oil sheen remaining in spots



② Catalyst: no oil sheen, water clear



③ Oxygen-release agent 1.5g/L: oil covers the entire water surface

④ Oxygen-release agent 3.0g/L: oil covers the entire water surface

• Discussion

Discussion and conclusion

The highest microbial activity was in ②, the catalyst. Its total microbial count was high, the oil was degraded rapidly, and the oil sheen disappeared. The reason its dissolved oxygen was lower than the other series is thought to be that the active microbes consumed correspondingly more oxygen. As the dosage of the oxygen-release agent was increased, the pH exceeded the optimal range for the microbes, so no advantageous dissolved-oxygen level could be secured. The cause of the decline in microbial count in ③ and ④ may have been the pH or the effect of the calcium sulfate dihydrate, among others, but this test did not pin it down. ① the blank can also be said to have sufficient activity, although it takes more time than ②.

Raising microbial activity leads to greater speed and reliability of remediation. This test showed that the biocatalyst is an effective means: it creates an environment that helps microbes grow, prevents the oxygen depletion that accompanies growth, and does not greatly change the pH. In Japan, even just to remove oil odour and oil sheen, high-cost methods with a heavy environmental burden — such as excavation removal or lime mixing — tend to be adopted; we hope that bioremediation using a biocatalyst can challenge this reality.