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Biosurfactants and bioemulsifiers: from contaminated soils to remediation via food-waste valorization.

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Abstract

Hydrocarbon-contaminated soils serve as rich ecological niches for the isolation of biosurfactant (BS) and bioemulsifier (BE) - producing microorganisms. This study adopted a waste valorization approach by converting food and agricultural residues into biosurfactants, which can be applied in soil remediation techniques, such as soil washing, soil flushing, and in situ biostimulation.

Real contaminated soil was used as inoculum for BS and BE production under anaerobic conditions, with nitrate and sulfate as electron acceptors. A microemulsion composed of waste frying oil and chickpea powder served as the carbon source, while hydrogen peroxide was added to stimulate microbial oxidative activity.

The effectiveness of the produced biosurfactants was evaluated using oil displacement and emulsification index (EI24h) tests on various hydrocarbons, including gasoline, diesel, fuel oil, and n-hexadecane. Biodegradation and stimulation assessments are ongoing, employing either freshly or aged contaminated soils treated with crude or microfiltered biosurfactant mixtures as biostimulants. Phytotoxicity tests on *Zea mays*, *Lepidium sativum*, and *Sorghum bicolor* to assess the safety of these mixtures, are in progress.

The overarching goal is to demonstrate that waste-derived biosurfactants can enhance sustainable and cost-effective soil remediation strategies, without the need for microbial bioaugmentation.

Keywords: Biosurfactants, bioemulsifiers, waste-to-resource strategies, soil remediation.

1. Introduction

It is well established in scientific literature that certain hydrocarbon-degrading microorganisms also produce biosurfactants (BS) and bioemulsifiers (BE), which enhance the bioavailability of hydrophobic carbon sources and accelerate remediation processes. Consequently, hydrocarbon-contaminated soils represent valuable reservoirs for the isolation of microbial strains capable of BS and BE production.

Our “Boosting remediation one waste at a time” approach focuses on transforming waste matrices—such as food and agricultural residues—into valuable BS or BE. These compounds can be applied in soil remediation strategies, including soil washing, soil flushing, and in situ biostimulation, via injections aimed at enhancing the bioavailability of contaminants. This study integrates the principles of waste valorization and sustainable remediation, effectively “feeding two birds with one seed”.

2. Materials and methods

To simulate BS and BE production under anaerobic, amended conditions, a real hydrocarbon-contaminated surface soil (sieved to 4 mm) was used as the inoculum. This approach aimed to investigate the native microbial community’s potential for BS and BE biosynthesis under oxygen-deprived and static conditions over a period of nine months. A total of 60 microcosms were prepared, 12 for each experimental condition. Each microcosm had a total volume of 250 mL (40 g dried soil and 200 mL microemulsion, electron acceptor and oxidant solutions, according to **Table 1**).

Table 1.: Experimental conditions

	Concentration
NaCl	0.0 or 0.5 g/L
$MgSO_4$	0.1 or 0.5 g/L
KNO_3	5 or 40 mg/L
Urea	0.1%
Chickpea Powder	6.0%
Waste Frying Oil	1.5%
H_2O_2 3%	0.35g/1g TPH
Bromoethanesulfonate	50 mM

Electron acceptor (EA) solutions were added to establish either nitrate-reducing or sulfate-reducing conditions. Potassium nitrate (KNO_3) was supplied at concentrations ranging from 5 to 40 mg/L, whereas magnesium sulfate ($MgSO_4$) was added at concentrations between 0.1 to 0.5 g/L. Following amendment, the systems were sealed to prevent reoxygenation.

To induce tenside biosynthesis, a microemulsion consisting of waste frying oil, chickpea powder, and deionized water was used, with or without the addition of 0.5 g/L NaCl to modulate salinity and osmotic pressure. To enhance oxidative stress and stimulate microbial peroxidase and catalase activity, a 3% hydrogen peroxide solution (corresponding to 0.35 g H_2O_2 per 1 g of total petroleum hydrocarbons TPH) was directly injected into

dry soil. Methanogenesis was inhibited in all microcosms by the addition of 50 mM of 2-bromoethanesulfonate (BES).

Crude supernatants, either microfiltered (at 0.45 µm) or containing suspended cells, were used for qualitative and semi-quantitative assays, including oil displacement tests and 24-hour emulsification index (EI24h) measurements. In oil displacement tests a layer of oil was deposed on a 10 mL DI water volume and a drop of crude extract is then added to the center of the oil layer and the diameter of the clear zone was measured. EI24h tests conducted by mixing equal volumes (5 mL each) of biosurfactant solution (either filtered or crude extract) and fuel oil in a 15 mL Falcon tube. After 24 hours of static incubation, the EI values (as ratio of Emulsion height to Total liquid height in the Falcon-tube), were recorded. These assays evaluated the efficacy of BS and BE on various hydrocarbon-rich substrates (gasoline, diesel, fuel oil, and n-hexadecane).

Microcosms were established using either weathered or freshly contaminated soils to evaluate the desorption, long-term emulsification, and biostimulatory capabilities of the crude biosurfactant mixtures. Prior to application, the mixtures were microfiltered to reduce purification costs, while preserving their biostimulatory properties and avoiding the need for bioaugmentation.

Ongoing biodegradation assessments aim to evaluate the effectiveness of the produced biosurfactants in remediating either recent or aged hydrocarbon contamination in unsaturated soils.

Finally, phytotoxicity tests on microfiltered BS/BE mixtures are ongoing; these include germination index measurements, secondary root elongation, and outdoor growth assessments, using three standard model plant species: *Zea mays, Lepidium sativum,* and *Sorghum bicolor*.

3. Results

Preliminary screening results (**Table 2**) indicate that, according to oil displacement tests performed with fuel oil, the initial availability of terminal electron acceptors in a complex medium (waste frying oil + chickpea powder + deionized water) and adequate salinity conditions promoted more frequent production of surface-active agents. Nonetheless, the lack of EA did not entirely inhibit the biosurfactant synthesis. Further investigations will focus on assessing the biosynthesis kinetics and composition of productive biomass.

Salinity was found to influence the emulsion stability. The average stability in saline and non-saline tests after 24 h of stasis resulted in more efficient emulsification non-saline conditions. Although the EI24h mean value was higher in saline tests, non-saline conditions yielded a greater number of positive emulsification results. EI24h assays showed no significant coalescence over a period of up to one month.

Table 2: Results of Oil displacement and Emulsification index tests performed with fuel oil, 12 tests for each condition . For oil displacement tests, the percentage of tests with no detected oil displacement (NEG), with oil displacement <1 cm (in diameter) (PART), and oil displacement ≥1 cm (in diameter) (POS) is reported. For the emulsification index at 24 h, the percentage of tests exceeding the cut-off of 50% at 24 h is reported, as well as the mean ± standard deviation of EI24h in tests exceeding cut-off 50%.

Test condition	EAs	Oil displacement test			Emulsification index at 24 h			
		NEG	PART	POS	Crude extract		Microfiltered supernatant	
					Cut-off$_{50}$	Mean EI ± St.Dev.	Cut-off$_{50}$	Mean EI ± St.Dev.
0.5 g/l NaCl ("saline")	$MgSO_4$ 0.5 g/L + KNO_3 40 mg/L	25%	33%	42%	33%	58% ± 6%	25%	60% ± 10%
	$MgSO_4$ 0.1 g/L + KNO_3 5 mg/L	25%	25%	50%	17%	61% ± 1%	0%	-
No added NaCl ("non saline")	$MgSO_4$ 0.5 g/L + KNO_3 40 mg/L	75%	0%	25%	25%	54% ± 4%	17%	54% ± 4%
	$MgSO_4$ 0.1 g/L + KNO_3 5 mg/L	33%	33%	33%	33%	56% ± 4%	17%	62% ±10%
Control	No added EAs	92%	8%	0%	0%	-	0%	-

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