

Genotypic Diversity and Lipolytic Potency of Indigenous Microbial Consortia Isolated from Diverse Oily Niches for Enhanced Bioremediation

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ABSTRACT

The escalating discharge of effluents laden with fats, oils, and greases (FOGs) represents a formidable challenge to global aquatic ecosystems, qualifying these substances as Emerging Pollutants of Concern (EPC). This investigation aligns with Sustainable Development Goal (SDG) 6 (Clean Water and Sanitation) and SDG 12 (Responsible Consumption and Production) by evaluating the degradative potential of indigenous microflora. 55 microbial strains were isolated from specialized niches, including kitchen greywater, bathwater, and industrial drainages from Ambernath MIDC, Dombivli MIDC, and Ulhasnagar. Taxonomic characterization identified five predominant genera: *Corynebacterium*, *Staphylococcus*, *Lactobacillus*, *Micrococcus*, and *Pseudomonas*. Quantitative screening via microtiter plate assays utilizing p-iodonitrotetrazolium (INT) indicator revealed high metabolic specificity; *Pseudomonas* spp. demonstrated maximal potency for Groundnut oil (0.517), while *Corynebacterium xerosis* and *Micrococcus luteus* exhibited superior degradation for Almond (3.149) and Palm oils (3.185), respectively.

These results delineate a biological framework for developing high-efficiency microbial consortia for industrial wastewater treatment.

Keywords: effluents, greywater, microtiter, oils, INT

INTRODUCTION

Environmental degradation through lipidic contamination is a critical byproduct of the global edible oil industry, which in India alone generates approximately **7 million liters of wastewater daily**. FOGs are characterized by their low density and immiscibility, forming surface films that preclude atmospheric oxygen transfer and light penetration, thereby decimating **neustonic (surface dweller)** and aquatic communities. These pollutants interfere with traditional aerobic treatment by reducing oxygen transfer rates and clogging vital infrastructure.

Bioremediation emerges as the most sustainable paradigm for mitigating such impacts, utilizing the catalytic versatility of living organisms to transform hazardous lipids into innocuous metabolites. The efficacy of this process is predicated on **microbial adaptation**; communities previously exposed to hydrocarbons exhibit significantly higher biodegradation rates due to selective enrichment and genetic shifts. While hydrocarbon-degrading microbes typically constitute less than 1% of total microflora, their population can surge to 10% in contaminated environments. This study focuses on isolating these "adapted" agents from domestic and industrial sites to optimize secondary biological treatment.

Vegetable Oils and Lipid Chemistry

Vegetable oils are primarily composed of **triacylglycerols**—glycerol esters of fatty acids. Naturally occurring fats contain a variety of fatty acids differing in chain length and degree of saturation, such as **Lauric (12:0)**, **Myristic (14:0)**, and **Palmitic (16:0)**. Oils like Groundnut and Sunflower contain high proportions of unsaturated fatty acids, such as **Oleic (18:1)** and **Linoleic (18:2)**. In India, oils like Coconut and Palm are significant pollutants; Coconut oil is dominant in Lauric acid (40–60%), which is known for its antimicrobial properties in certain contexts but represents a high organic load in wastewater.

Table 1: Fatty Acid Composition of Fats and Oils of Plant Origin.

Plant Oils	Relative Proportion of Fatty Acyl groups											
	4:0-10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	Others
Coconut Oil	15	47	18	9	-	3	6	2	-	-	-	-
Palm Oil	8	48	16	8	-	3	15	2	-	-	-	-
Groundnut Oil	-	-	-	11	-	2	48	32	-	1	2	22:0+1 2:0 5%
Sunflower Oil	-	-	-	7	-	5	19	68	1	-	-	-
Soyabean oil	-	-	-	11	-	4	24	54	7	-	-	-

Degradation Of Vegetable Oils

Most natural fats, such as those in vegetable oils, are complex mixtures of simple and mixed triacylglycerols. These contain a variety of fatty acids differing in chain length and degree of saturation. Vegetable oils such as Corn (Maize) and Olive oil are composed largely of triacylglycerols with unsaturated fatty acids and thus are liquids at room temperature. They are converted industrially fatty acids and thus are liquids at room temperature. They are converted industrially into solid by catalytic hydrogenation, which reduce some of their double bonds to single bonds (Nelson D. et al, 2002)

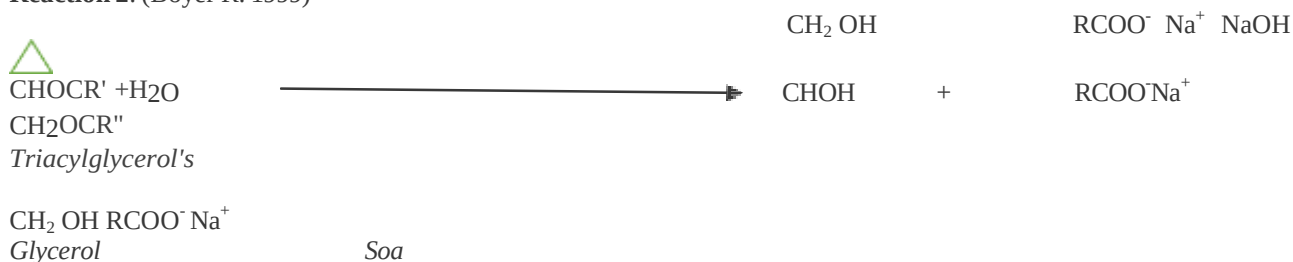
The principal route for fatty acid degradation in all cells--- microbial, plant and animal ---is by the β - oxidation cycle. The β - oxidation cycle yields a succession of acyl-CoA units as the fatty acid is progressively shortened by C_2 units. An odd-chain-length fatty acid will produce at the final step an acetyl-CoA esters can be detected during the oxidation of fatty acid by cell free extracts indicating that this is not as tightly a coupled sequence of reactions as had originally been thought (Ratledge C., 1992).

Reaction 1: Commercial hydrogenation of vegetable oils often leads to trans acids. (Boyer R. 1999)



Triacylglycerols undergo hydrolysis when boiled with acid or bases.

Reaction 2: (Boyer R. 1999)



Microbial Catabolism of FOGs

The biodegradation of lipids is initiated by **extracellular lipases (ester hydrolases)**, which catalyze the hydrolysis of ester bonds between glycerol and acyl moieties. This reaction releases free fatty acids, which are subsequently processed via the **-oxidation cycle**. This cycle shorten the fatty acid chain by two-carbon units (), yielding **Acetyl-CoA** for energy production. Literature confirms that genera such as *Pseudomonas*, *Bacillus*, and *Micrococcus* are among the most efficient hydrocarbon utilizers in crude and vegetable oil-contaminated sites

1. Aims and Objectives of Study

The primary objective is the systematic isolation and characterization of lipolytic microorganisms from diverse oil-stressed environments.

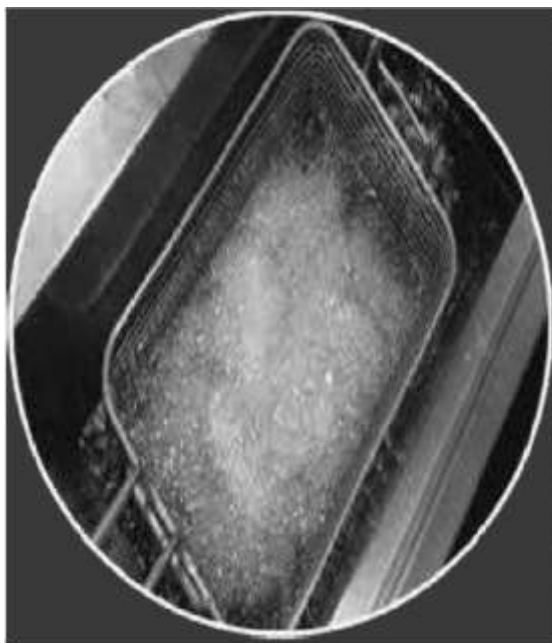
- **Physicochemical and Microbiological Characterization:** To evaluate the baseline pollution load of wastewater from MIDC industrial sectors and domestic sources.
- **Enrichment and Isolation:** To selectively cultivate vegetable oil- degrading strains using specialized **Bushnell Hass enrichment protocols**.
- **Taxonomic Identification:** To identify isolates to the species level through rigorous morphological, cultural, and biochemical assays

- **Quantitative Potency Mapping:** To measure the biodegradation potential of each strain against five specific vegetable oils using high- throughput **microtiter plate analysis**.
- **Consortium Development:** To establish the biological foundation for a microbial consortium capable of 80–100% removal efficiency.

MATERIALS AND METHODS

1.1 Environmental Sampling and Site Selection

Effluents and soil samples were harvested from five distinct anthropogenic niches: **Kitchen Greywater** (the highest contributor of FOGs), **Bathwater** (rich in cosmetic lipids), and three industrial drainage sites—**Ambernath MIDC**, **Dombivli MIDC**, and **Ulhasnagar**. Samples were stabilized at 4°C in sterile containers to preserve microbial integrity.



B) Kitchen Graywater



A)

B) Bath Water



C) Ambernath MIDC



D) Dombivli MIDC

1.2 Culture Media and Enrichment Strategy

Enrichment was conducted in **Bushnell Hass (BH) Medium**, which contains essential nutrients except for a carbon source. The BH medium composition included:

Table 2: Chemical Composition of Bushnell-Hass Medium

Component	Chemical Formula	Quantity (per 1,000 ml)
Magnesium sulphate	$MgSO_4 \cdot 7H_2O$	0.20 g
Calcium chloride	$CaCl_2 \cdot 2H_2O$	0.02 g
Monopotassium phosphate	KH_2PO_4	1.00 g
Ammonium nitrate	NH_4NO_3	1.00 g
Ferric chloride	$FeCl_3$	0.05 g
Dipotassium phosphate	K_2HPO_4	1.00 g
Distilled Water	H_2O	1,000 ml
Final pH		7.2

Vegetable oil used as a carbon source are:

- Groundnut oil
- Almond oil
- Coconut oil
- Palm oil
- Sunflower oil

1.3 Isolation and Taxonomic Identification

Purified colonies were obtained via the **four-quadrant streak plate technique** on BH agar supplemented with 10% oil. Diagnostic identification protocols included:

- Morphological:** Gram staining and macroscopic examination of colony raise and color.
- Biochemical:** Catalase, Oxidase, Indole, Citrate utilization, Urease, Gelatin hydrolysis, Starch hydrolysis, and Triple Sugar Iron (TSI) tests.
- Enzymatic:** Lipase activity was specifically validated using tributyrin and vegetable oil agar.
- Scheme for Microbial Identification

Preliminary Tests (Morphological & Cultural)	Physiological Activities
Macroscopic Examination: (Colony Characteristics: size, shape, color, margin, opacity)	Lipase Activity, Gelatine hydrolysis (Gelatinase Test), Nitrate Reduction, Sugar Fermentation (Glucose, Sucrose, Lactose)
Microscopic Examination (Gram Reaction/Staining)	Oxidase Test, Catalase Test, Indole test, Starch Hydrolysis, Citrate utilization
	Triple Sugar Iron (TSI) (H_2S production, gas, and slant/butt reaction)

1.1 Quantitative Potency Assay (Microtiter Analysis)

Isolates were resuspended in BH medium and matched to the **first tube of Brown's opacity standard**. Aliquots were placed in microtiter wells with BH medium and specific oil substrates. Plates were incubated for **21 days at 26°C**. Post-incubation, **p-iodonitrotetrazolium (INT)** indicator was added; a red or brown precipitate confirmed positive hydrocarbon catabolism. Biomass growth was quantified via spectrophotometry at **600 nm**.

RESULTS AND DISCUSSION

1.2 Taxonomic Profiling and Distribution

From the 55 successful isolates, a representative subset of 10 was rigorously identified to the species level. The genera distribution (Table 4) revealed a high prevalence of **Lactobacilli** (30%), followed by **Staphylococcus** (20%), **Micrococcus** (20%), and **Corynebacterium** (20%).

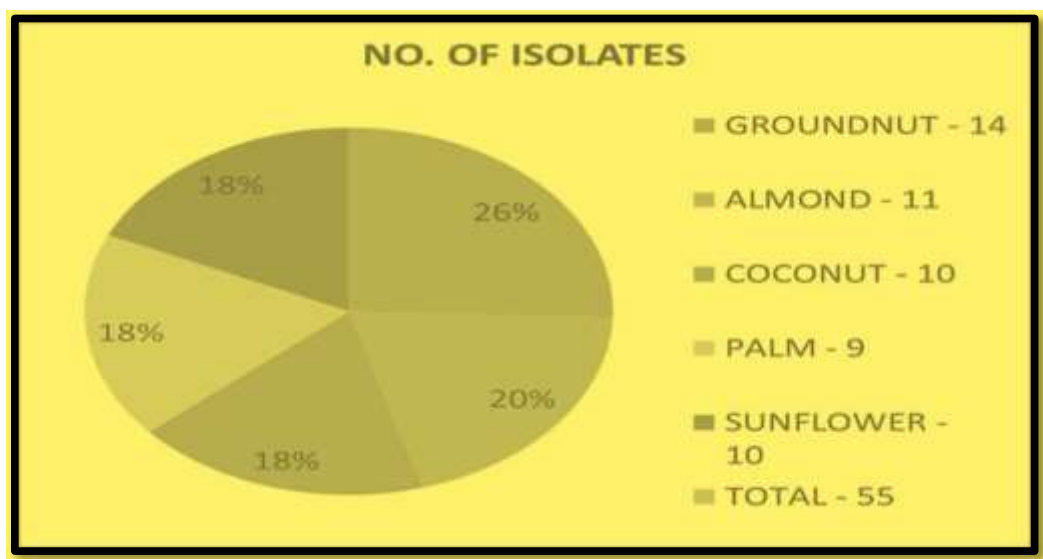
Table 4: Identification of High-Potency Bacterial Isolates

Isolate Number	Taxonomic Identification
1	<i>Corynebacterium kutscheri</i>
2	<i>Staphylococcus aureus</i>
3	<i>Lactobacilli spp.</i>
4	<i>Micrococcus luteus</i>
5	<i>Micrococcus SPP.</i>
6	<i>Lactobacillus casei</i>
7	<i>Corynebacterium xerosis</i>
8	<i>Pseudomonas spp.</i>
9	<i>Staphylococcus spp.</i>
10	<i>Lactobacillus dulbruecki</i>

Table 5: Distribution of isolates

Genus	No. of isolates	Percentage distribution (%)
<i>Pseudomonas spp.</i>	1	10
<i>Staphylococcus spp.</i>	2	20
<i>Micrococcus spp.</i>	2	20
<i>Lactobacilli spp.</i>	3	30
<i>Corynebacterium spp.</i>	2	20

Total 55 isolates were isolated from all sample. Number of isolates for each oil degraders are shown in below pie diagram:



1.3 Quantitative Screening of Biodegradation Potency

The absorbance data at 600nm provided a clear hierarchy of metabolic efficiency across different substrates:

Table No. 5: Quantitative screening of isolates for oil degradation (Optical density at 600nm. For each oil)

Isolate No.	Groundnut oil	Almond oil	Coconut oil	Palm oil	Sunflower oil
1	0.279	1.085	1.189	1.303	1.969
2	0.454	1.775	2.352	1.938	2.066
3	0.445	1.825	2.417	2.216	1.868
4	0.406	2.687	2.365	3.185	1.525
5	0.384	1.998	2.094	1.311	2.608
6	0.459	2.309	2.229	2.062	2.421
7	0.372	3.149	2.645	2.578	1.514
8	0.517	1.929	1.685	2.605	1.379
9	0.111	0.109	0.110	0.111	0.104
10	0.088	0.088	0.088	0.088	0.086

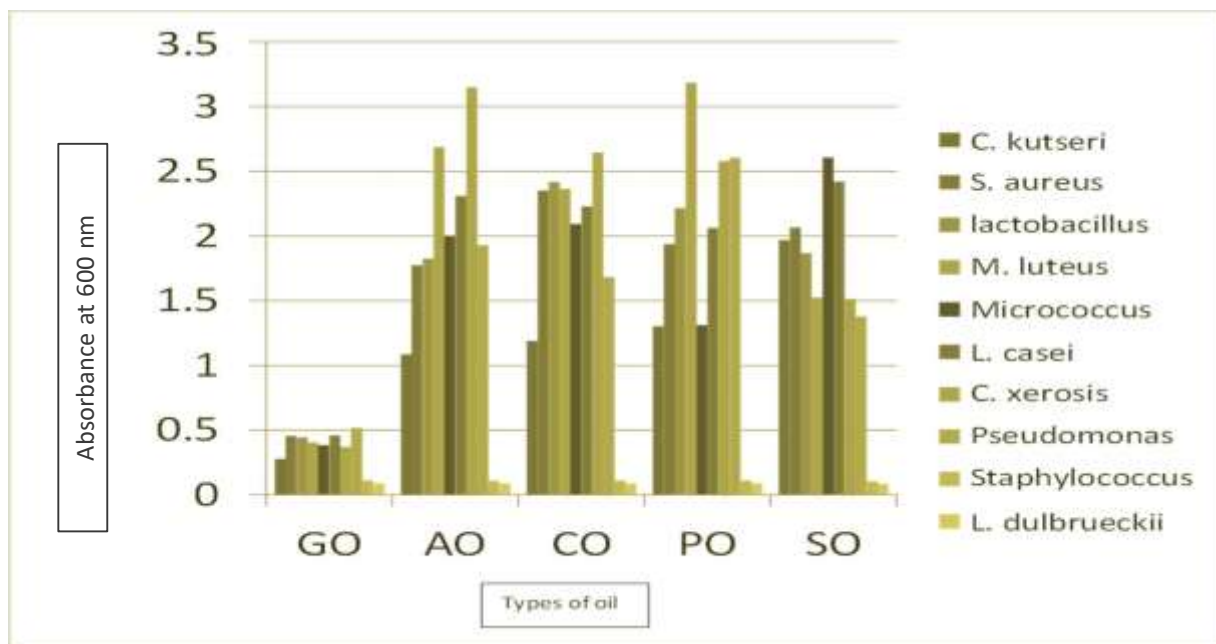
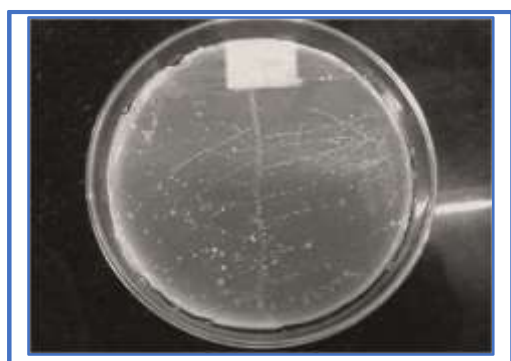


Figure: Potential isolates for degradation of Vegetable oil

DISCUSSION OF METABOLIC MECHANISMS

The superior performance of isolates from **Ambernath MIDC** and **kitchen greywater** validates the principle of environmental adaptation. *Pseudomonas spp.* was identified as the most active utilizer of Groundnut oil, which aligns with literature stating that the *Pseudomonas* genus is the most efficient among hydrocarbon-degrading microorganisms. The variation in OD values suggests that while some microbes are generalists, many exhibit a high degree of substrate specificity based on the fatty acid chain lengths present in the oils (e.g., the high Lauric acid content in Coconut oil)



Isolation of Oil degrading Organisms

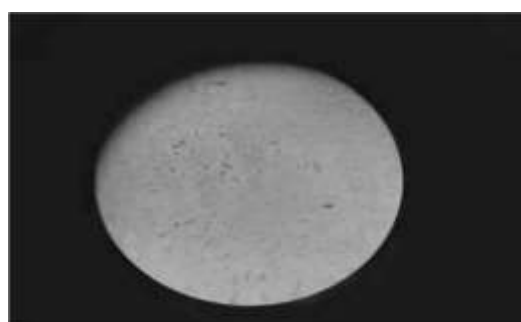


Figure: Gram staining

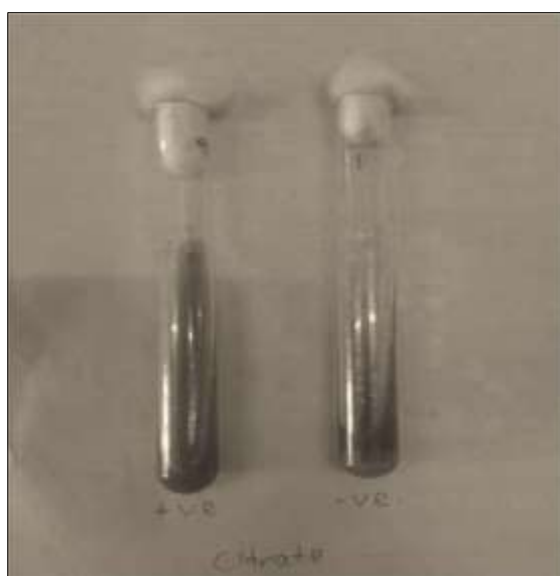
1.1 Morphological characteristic if isolates

Biochemical characteristics

Isolate No.	Size (mm)	Shape	Color	Margin	Consistency	Opacity	Elevation	Gram character
1	1-2	Circular	White	Entire	Smooth	Translucent	SR	Gm+ve rods
2	Pinpoint	Circular	White	Entire	Smooth	Opaque	SR	Gm+ve cocci
3	1	Circular	White	Entire	Smooth	Opaque	SR	Gm+ve rods
4	Pinpoint	Circular	Golden yellow	Entire	Smooth	Translucent	SR	Gm+ve cocci
5	1	Circular	White	Entire	Smooth	Translucent	SR	Gm+ve rods
6	Pinpoint	Circular	Golden Yellow	Entire	Smooth	Translucent	SR	Gm+ve cocci
7	1	Circular	White	Entire	Smooth	Opaque	SR	Gm+ve rods
8	Pinpoint	Circular	Golden yellow	Entire	Smooth	Translucent	SR	Gm+ve rods
9	1-2	Circular	White	Entire	Smooth	Opaque	SR	Gm+ve cocci
10	1	Circular	White	Entire	Smooth	Opaque	SR	Gm+ve rods

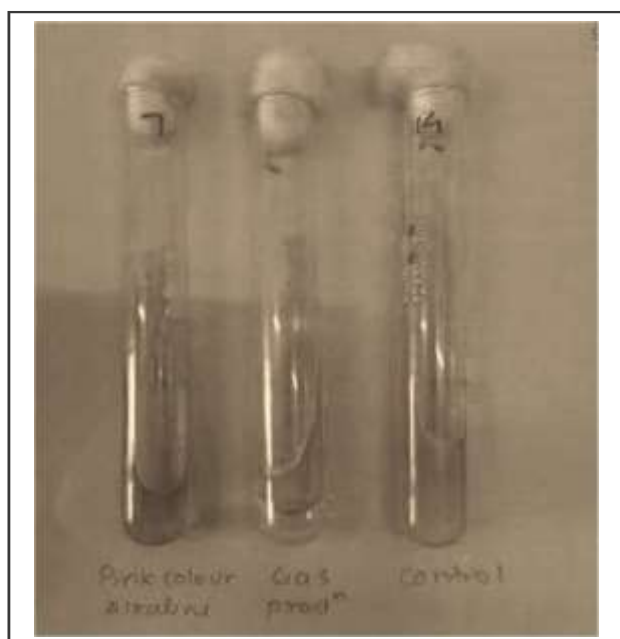
Isolate no.	Gram reaction	Sugar Fermentation A G A G A G A G	Ca	Oxi	Cit	Gh.	Ind	Nit	TSI S B	U	Sh
1	Gm +ve	- + + - - + + +	+	+	+	+	+	+	P Y	10.5	+
2	Gm +ve	+ - + + + - + +	+	-	+	-	+	+	P Y	13.5	+
3	Gm +ve	- - - - - - - -	-	-	+	-	-	-	P P	-	+
4	Gm +ve	- - - - - - - -	+	+	+	-	-	-	P P	9.5	+
5	Gm +ve	+ - + - + - + -	-	-	-	+	-	+	Y Y	8.0	-
6	Gm +ve	+ + - - + + + +	+	+	+	-	-	+	P Y	-	+
7	Gm +ve	+ + + - + + + +	+	+	+	-	-	+	P Y	7.5	-
8	Gm -ve	- - - - + + + +	-	+	+	-	-	+	P Y	14.0	-
9	Gm +ve	- - - - - - - -	+	+	+	-	-	+	Y Y	9.0	-
10	Gm +ve	- - - - - - - -	-	-	+	-	-	+	P Y	13.0	-

Key for 5.4 and 5.5:		
Gm +ve : Gram-positive	Oxi: oxidase	Glu: Glucose
Gm - ve : Gram-negative	Ca: Catalase	Cit: Citrate
Oxi : Oxidase	Man: Mannitol	Gh: Gelatin hydrolysis
A : Acid	G: Gas	S: Slant
B : Butt	Ind: Indole	Nit: Nitrate
TSI: Triple Sugar Iron	Lip: Lipase activity	+: Positive
- : Negative	P: Pink	Y: Yellow
SR : Slightly raised		

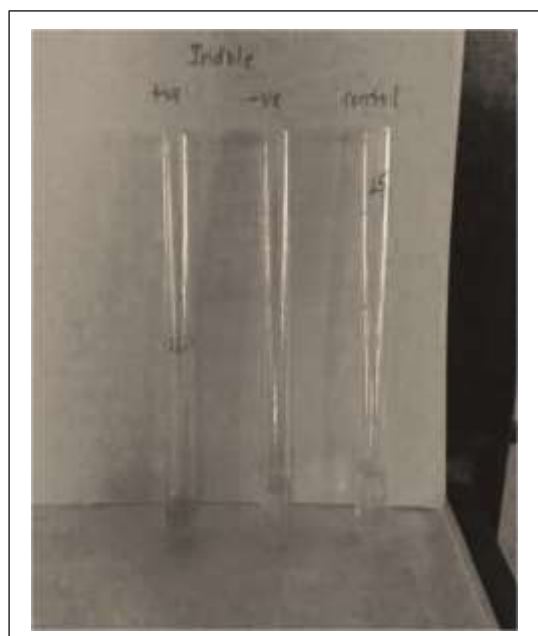


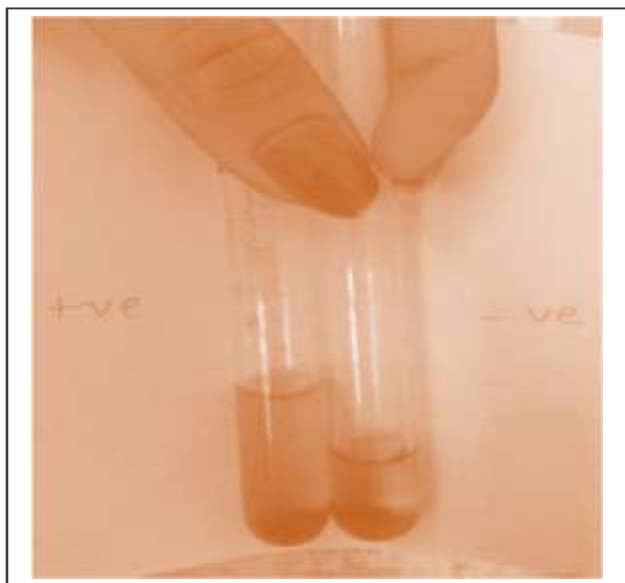
Result of citrate test

Result of TSI test

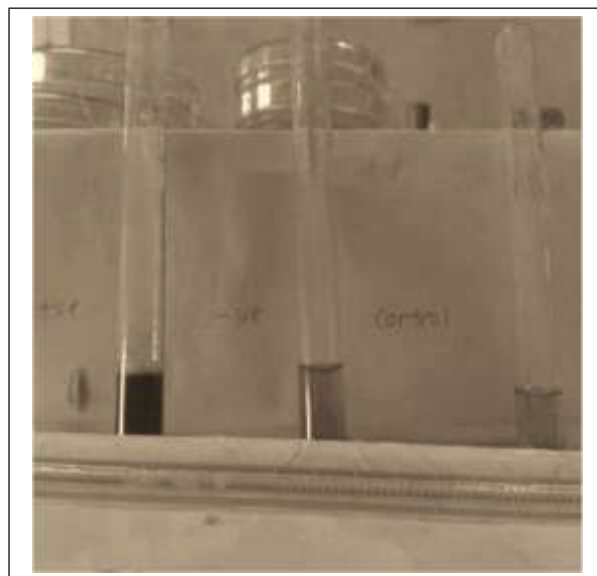


Result of Indole test

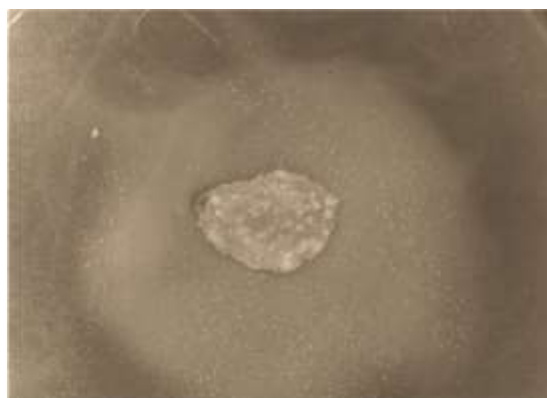




Result of Nitrate test



Result of Starch hydrolysis test



Result of gelatinase Test



Result of lipase test



Result of catalase test

CONCLUSION

This research establishes that anthropogenic oily niches are reservoirs of highly specialized lipolytic bacteria. A diverse suite of microorganisms capable of significant vegetable oil degradation successfully identified, addressing the critical need for biological treatment of EPC-laden wastewater. The identification of ***Pseudomonas***, ***Micrococcus***, and ***Lactobacillus*** species as potent degraders confirms that adapted indigenous microflora are superior to non-adapted strains for bioremediation.

Future Prospects

Future studies should explore the **synergistic effects** of a multi-species consortium to manage mixed lipidic wastes more effectively. Additionally, research into the optimization of environmental parameters, specifically **pH and temperature**, will be essential for scaling these laboratory findings into industrial-scale bioreactors.

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