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PRODUCTION AND CHARACTERIZATION OF *Panicum maximum* - DERIVED WHEY FOR BIOREMEDIATION OF ENGINE OIL-CONTAMINATED SOIL: A GREEN ALTERNATIVE TO DIARY WHEY

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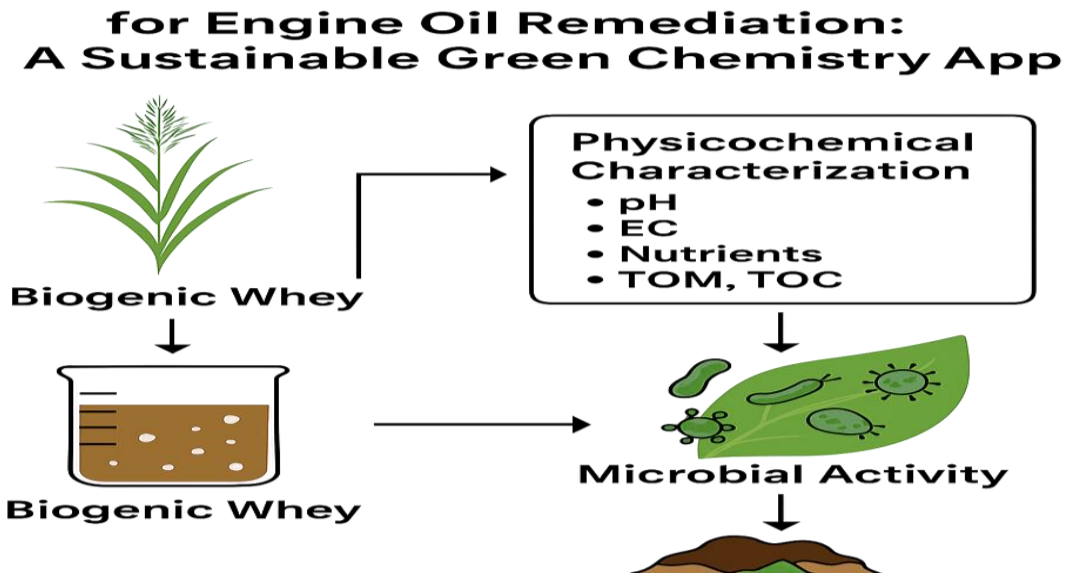
Abstract

Bioremediation of oil-contaminated soils in developing countries requires affordable, sustainable strategies. This study produced a fermented liquid extract ('whey') from *Panicum maximum* grass as a novel biostimulant for soil treatment. The extract's physicochemical properties (pH 3.46–5.74, electrical conductivity 2419–4804 µS/cm) and microbial profile (dominance of *Bacillus*, *E. coli*) were characterized over a 12-day fermentation. Results showed high organic carbon (44–67%) and nitrate (56–394 mg/kg) levels, indicating nutrient-rich conditions for microbial growth. While the extract shows promise for bioremediation due to its balanced properties, pathogen presence necessitates further treatment. This study introduces the first plant-based alternative to dairy whey for hydrocarbon degradation, aligning with green chemistry principles.

Keywords*: Panicum maximum, Whey, Bioremediation, Biomaterials, Sustainability, Green Chemistry.*

Graphical Abstract



1.0 INTRODUCTION

Spent engine oil contamination in agricultural soils is a rising concern, particularly in farming areas exposed to mechanical activities. This pollutant disrupts soil structure, strips essential nutrients, and suppresses microbial functions, leading to reduced crop productivity and persistent environmental damage.Conventional remediation methods like excavation, chemical oxidation, or thermal treatment are costly, disruptive, and often leave residual toxins [1]. Bioremediation offers a more sustainable alternative by leveraging microbes to degrade hydrocarbons [2]. Yet microbial degradation requires nutrient-rich growth media, which are often synthetic or animal-derived, limiting affordability and sustainability. Dairy whey has shown promise as a microbial growth medium thanks to its high content of lactose, protein, and minerals, and its ability to promote microbial metabolites, organic acids, biosurfactants and enzymes that enhance hydrocarbon breakdown [3]. In contrast, plant whey is nutrient-rich but poses pathogen risks, making it less studied as a microbial medium.However, existing research focuses only on dairy whey, with no exploration of plant-derived alternatives.

Panicum maximum has shown potential for phytoremediation of heavy metals like lead, chromium, and cadmium, accumulating moderate levels and reducing soil metal concentrations by 13–65 %, depending on metal and tissue type [4]. It also adjusts its primary and secondary metabolites under pollution stress; enhancing antioxidant and phenolic production as adaptive responses [5]. That suggests it produces bioactive compounds that might support microbial activity or soil health. Studies by Farshid *et al.* (2011) of oil-contaminated soils, such as the work around Shiraz Refinery, have isolated Bacillus, Acinetobacter, Klebsiella and others capable of degrading polycyclic aromatic hydrocarbons [6]. These bacteria, though present, often require supplementation to fully activate degradation pathways. Another study by Contreras-Ramos *et al.* (2017) combining *Panicum maximum* with a bacterial consortium achieved up to 77 % total petroleum hydrocarbon removal in contaminated soil, and the consortium alone reached 85 %, showing grass-mediated phytoremediation can be effective when paired with microbial partners [7]. A study by Chukwumati and Omovbude (2020) reported that in Port Harcourt, Nigeria, a field trial showed that Guinea grass combined with organic manure achieved nearly 68 % hydrocarbon degradation over twelve months, while also removing significant amounts of heavy metals [8]. That indicates *Panicum maximum* itself supports remediation, particularly when nutrient-enriched.

Despite such findings, no study has examined extracting a nutrient-rich liquid ("whey") from *Panicum maximum* biomass and using it to boost microbial degradation of petroleum contaminants.

We hypothesize that Panicum maximum-derived whey will enhance hydrocarbon degradation by stimulating native soil microbes while avoiding dairy whey's cost and scalability imitations.

The aim of this research is to produce and characterize *Panicum maximum* whey and assess its effectiveness in enhancing microbial degradation of engine oil contaminants in soil. Objectives of this study were to: develop a method to extract whey from *Panicum maximum*; characterize its physicochemical and microbial properties; evaluate its effect on microbial growth and hydrocarbon degradation in contaminated soil.In addition, the study will assess key microbial activities involved in bioremediation, such as biosurfactant production, enzyme activity (lipase), and the rate of hydrocarbon degradation measured by changes in total petroleum hydrocarbon levels over time.

2.0 MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Fresh leaves of 120 matured Panicum maximum plants were collected collected during the dry season (October - November 2023) from three sites at the University of Benin. Leaves were stored at 25°C in sterile bags and processed within 4 hours of collection. Identification was confirmed at the Department of Plant Biology and Biotechnology. The plant was chosen for its abundance and underutilization. Leaves were collected using sterile polyethylene bags and transported promptly to the lab to minimize contamination. In the lab, leaves were sorted to remove debris, washed thoroughly with clean tap water, and air-dried. The dried leaves were chopped and ground into a fine paste using a sterilized laboratory grinder. 2.2 Whey Extraction Procedure

Whey was extracted every 3 days over a 12-day fermentation cycle (days 0, 3, 6, 9, and 12). On each sampling day, freshly collected Panicum maximum leaves were washed, chopped, and soaked in distilled water (1:2 w/v) for 30 minutes. The soaked leaves were ground into a paste using a Durable industrial Engine Grinding Mill Machine 6hp and manually squeezed using a muslin cloth. The extract was filtered using a stainless steel collander sieve Mesh bowls (58µm pore size) to separate fiber (bagasse) from the liquid portion. The liquid was then heated to 80 °C in a stainless-steel vessel to induce protein coagulation. The resulting curd (leaf protein concentrate) was removed, and the remaining liquid the plant-based "whey" was collected, allowed to settle, filtered again, and stored in sterile containers for subsequent analyses.

2.3 Physicochemical Characterization

Physicochemical parameters of the whey were measured on Days 0, 3, 6, 9, and 12 of fermentation. pH and electrical conductivity (EC) were measured

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using a calibrated NOYAFA NF-EZ9909-S (SP variant) after a 2-point calibration with standard buffer solutions (pH 3.64 and 6.86) in line with APHA 4500-H+ B. Moisture content was determined by oven-drying at 110 °C for 2 hours following AOAC Official Method 934.01 [9]. TOC analysis included blanks and glucose standards ranging from 100 to 1000 mg/L. Moisture content was determined by oven-drying at 110 °C for 2 hours using standard gravimetric methods. Organic matter was calculated from TOC using a factor of 1.724 [10]. Potassium was quantified by flame photometry (Sherwood Model 410) using KCl standards from 0 to 100 ppm [11]. Phosphorus and nitrogen were analyzed using a UV/Vis spectrophotometer (Model 721G) with KH_2PO_4 standards (0–50 mg/L) for P, and digest blanks plus ammonium sulfate standards (0–500 mg/L) for N [12].All tests were performed in triplicates, and results were presented as mean ± standard deviation and standard error of mean, calculated using Microsoft Excel (Office 365). Quality assurance and control (QA/QC) procedures included method validation through determination of limit of detection (LOD), limit of quantification (LOQ), and recovery tests. Statistical significance between treatment groups was assessed using a two-tailed t-test in Microsoft Excel, with a confidence level of 95% ($p < 0.05$).

2.4 Microbial Analysis

Total viable counts for bacteria and fungi were assessed using serial dilution and pour plate techniques. Culture media included Nutrient Agar (NA), MacConkey Agar (MCA), Salmonella-Shigella Agar (SSA), Eosin Methylene Blue Agar (EMB), and Potato Dextrose Agar (PDA). All media, glassware, and instruments were sterilized at 121 °C for 15 minutes using an autoclave. Negative controls (uninoculated plates) were included to monitor for contamination during preparation and incubation. Bacterial plates were incubated at 37 °C for 24 hours, and fungal plates at 28 °C for 48–72 hours. Colony-forming units were counted and expressed as CFU/ml for liquid samples or CFU/g for solid residues. Representative bacterial colonies were identified using API 20E test strips (bioMérieux) and confirmed by 16S rRNA gene sequencing for dominant isolates. Fungal identification followed Barnett's morphological criteria and was supported by lactophenol cotton blue staining for microscopic observation. All microbial procedures involving potentially pathogenic organisms were conducted in accordance with institutional biosafety guidelines and ethical approval protocols.

3.0 RESULTS AND DISCUSSION

Table 1. Physicochemical properties of PMBL during fermentation (mean ± SEM, n=3). Values with different superscript letters (a-d) differ significantly ($p<0.05$, T-test)

Parameter	$PMBL_0$	$PMBL_3$	$PMBL_6$	$PMBL_9$	$PMBL_{12}$	SEM
pH	3.63± 0.02 ^{c}	4.03± 0.02^{b}	3.75± 0.01^{c}	3.46± 0.02 ^{c}	5.74 ± 0.05^{a}	0.03
EC (uS/cm)	2946.67 ± 32.24^{c}	2419.33 ± 18.91^{c}	3524.67±27.64^{b}	4190.67 ± 68.71^{ab}	4804.00 ± 28.74^{a}	24.23
Moisture (%)	99.52 ± 0.10	93.26 ± 0.14	99.55 ± 0.07	99.40 ± 0.06	99.44 ± 0.01	0.08
Bulk density (g/ml)	1.02 ± 0.00	0.998 ± 0.00	1.01 ± 0.00	1.01 ± 0.00	0.998 ± 0.00	0.00
TOM (%)	25.91 ± 0.11^{c}	38.66 ± 0.10^{b}	38.73 ± 0.01^{b}	39.14 ± 0.03^{a}	39.02 ± 0.06^{a}	0.02
TOC (%)	44.66 ± 0.14^{c}	66.55 ± 0.18^{b}	66.76 ± 0.01^{b}	67.48 ± 0.05 ^{a}	67.27 ± 0.10 ^{a}	0.03
Nitrate (mg/kg)	181.42 ± 6.07^{b}	117.89 ± 2.70^{c}	55.87 ± 3.11^{d}	181.42 ± 6.07^{b}	394.17 ± 4.90 ^{a}	3.26
Phosphate (mg/kg)	3629.83±23.92^{ab}	3926.99± 15.50^{a}	3196.06±23.49^{b}	2437.36 ± 20.18^{c}	1210.77 ± 30.48^{d}	21.40
Nitrogen (mg/kg)	40.97 ± 1.35^{b}	26.47 ± 0.95^{c}	12.13 ± 0.35^{d}	40.97 ± 1.35^{b}	89.67 ± 1.53^{a}	1.06
Phosphorus (mg/kg)	765.33 ± 6.11^{ab}	828.00 ± 4.00^{c}	674.00 ± 4.00 ^{b}	510.67 ± 6.67 ^{c}	255.33 ± 8.51^{d}	9.24
Potassium (mg/kg)	395.00 ± 15.20^{ab}	410.33 ± 11.53^{a}	196.67 ± 14.04 ^{d}	323.33 ± 13.06 ^{b}	213.00 ± 12.65 ^{c}	13.27

Note: EC = Electrical Conductivity; TOM = Total Organic Matter; TOC = Total Organic Carbon.

The pH at Day 0 was 3.63 ± 0.05 (SEM = 0.03), showing a highly acidic starting point. By Day 3, it rose slightly to 4.03 ± 0.04, possibly due to early microbial activity. EC dropped from 2946.67 ± 42.02 µS/cm (SEM = 24.23) at Day 0 to 2419.33 ± 41.66 µS/cm at Day 3, indicating early microbial ion uptake. Moisture fell to 93.26% ± 0.14 (SEM = 0.08) from 99.52% ± 0.13, likely due to evaporation or initial respiration losses. TOM increased sharply from 25.91% ± 0.03 (SEM = 0.02) to 38.66% ± 0.04, and TOC rose from 44.66% ± 0.05 (SEM = 0.03) to 66.55% ± 0.04, confirming microbial breakdown and carbon solubilization. Nitrate fell from 181.42 ± 3.26 mg/kg to 117.89 ± 2.34 mg/kg, and total nitrogen from 40.97 ± 1.18 mg/kg to 26.47 ± 1.02 mg/kg, suggesting strong assimilation for biomass. Phosphorus slightly increased from 765.33 ± 9.24 mg/kg to 828.00 ± 8.57 mg/kg, and potassium from 395.00 ± 11.38 mg/kg to 410.33 ± 10.26 mg/kg, likely released from plant tissue. Pathogens like *E.*

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coli or *Salmonella* may persist in this stage due to favorable nutrient levels and low competition.

The pH dropped again to 3.75 ± 0.04 at Day 6 and 3.46 ± 0.05 at Day 9, aligning with active acidogenesis. EC rose to 3524.67 ± 40.29 µS/cm and 4190.67 ± 38.90 µS/cm, indicating intensified solubilization. Moisture recovered to 99.40% ± 0.11 and 99.45% ± 0.12, likely due to microbial exudates or breakdown fluids. TOM remained high: 38.73% ± 0.05 (Day 6) and 39.14% ± 0.06 (Day 9), while TOC reached 66.76% ± 0.04 and 67.48% ± 0.05, pointing to stable carbon accumulation. Nitrate dropped to its lowest point at Day 6 (55.87 ± 2.14 mg/kg), then rebounded to 181.42 ± 3.67 mg/kg at Day 9, suggesting nitrification. Nitrogen mirrored this, dropping to 12.13 ± 1.07 mg/kg at Day 6 and rising to 40.97 ± 1.03 mg/kg by Day 9. Phosphorus and potassium declined to 674.00 ± 10.73 mg/kg and 196.67 ± 12.24 mg/kg (Day 6), then to 510.67 ± 11.90 mg/kg and 323.33 ± 13.19 mg/kg (Day 9), due to active microbial uptake. These results suggest maximum microbial metabolism during this phase, supported by low pH and high EC. Stoichiometric ratios (C:N:P ≈ 100:10:1) support these shifts, consistent with microbial nutrient cycling.

A marked shift occurred at Day 12 as pH rose to 5.74 ± 0.05, indicating buffering due to ammonia accumulation or microbial turnover. These shifts are consistent with reports of Akay and Sert. 2020 on microbial succession during organic waste fermentation [13]. A t-test comparing pH values between Day 0 and Day 12 showed a statistically significant increase ($p = 1.34 \times 10^{-8}$, n = 3), confirming that fermentation significantly altered the acidity of *Panicum maximum*-derived whey. EC peaked at 4804.00 ± 42.77 µS/cm, reflecting maximal ion release.This aligns with findings from mineralization studies of whey-amended soils, where microbial breakdown released salts and soluble nutrients [14]. The SEM of 24.23 confirms consistency within replicates. A t-test comparing EC values between Day 0 (mean = 2946.67 µS/cm) and Day 12 (mean = 4804.00 µS/cm) showed a statistically significant increase ($p = 1.98 \times 10^{-7}$, n = 3), indicating that fermentation led to a measurable rise in ionic content in the PMBL Moisture was stable at 99.55% ± 0.10, and TOM held at 39.02% ± 0.07, suggesting decomposition plateau. TOC remained high at 67.27% ± 0.06, significantly higher than Day 0 ($p = 2.23 \times 10^{-9}$), confirming efficient carbon solubilization. Similar trends in plant-derived amendments have been associated with enhanced microbial turnover and organic acid accumulation [15]. This steep rise confirms efficient microbial breakdown of complex organic compounds into carbon-rich components. SEM was just 0.03, reinforcing data reliability. A t-test comparing TOC levels between Day 0 (mean = 44.66%) and Day 12 (mean = 67.27%) showed a statistically significant increase ($p = 2.23 \times 10^{-9}$, n = 3).

This suggests that fermentation enhanced organic carbon availability in the PMBL, likely due to solubilization of biomass and microbial metabolite accumulation. Nitrate rose sharply to 394.17 ± 3.98 mg/kg, and nitrogen to 89.67 ± 1.09 mg/kg, indicating ammonification or microbial lysis. Phosphate dropped significantly to 255.33 ± 10.44 mg/kg ($p = 9.59 \times 10^{-8}$), and potassium fell to 213.00 ± 13.27 mg/kg ($p = 9.19 \times 10^{-5}$), possibly due to precipitation or final microbial uptake [16]. While rising pH reduces pathogen risk, persistence of *Salmonella* at pH 5–6 remains a concern and calls for targeted screening.

TABLE 3: TOTAL MICROBIAL COUNT IN THE SAMPLES ANALYZED**.**

Dilution Factor	$PMBL_0$	$PMBL_3$	$PMBL_6$	$PMBL_9$	$PMBL_{12}$
10^{-1}	820	720	688	529	819
10^{-2}	608	548	408	365	598
10^{-3}	248	202	105	285	289
10^{-4}	145	109	79	169	226
10^{-5}	73	51	33	106	211

TABLE 4: QUALITATIVE DETERMINATION OF MICROORGANISMS PRESENT

Microbes	$PMBL_0$	$PMBL_3$	$PMBL_6$	$PMBL_9$	$PMBL_{12}$
Escherichia coli	+	+	+	+	+
Salmonella	+	+	+	+	+
Bacillus	+	+	+	+	+
Penicillium	-	-	-	-	-
Aspergillus	-	-	-	-	-
Yeast	-	+	+	+	+
Fusarium	-	-	-	-	-
Shigella	+	+	+	+	+

+ = present, - = absent

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The Table 3 shows microbial colony counts (CFU/mL) in PMBL across fermentation days (0, 3, 6, 9, 12) at dilution factors from 10^{-1} to 10^{-5}. Counts start high at Day 0 (820 at 10^{-1}), decrease through Day 6 (688 at 10^{-1}, 33 at 10^{-5}), indicating reduced microbial activity, possibly due to acidification or nutrient depletion. By Day 9, counts rise slightly (529 at 10^{-1}), and by Day 12, they rebound significantly (819 at 10^{-1}, 211 at 10^{-5}), suggesting microbial recovery or succession, likely tied to pH neutralization and nutrient release. Lower dilutions consistently show higher counts, reflecting dilution effects.

The Table 4 shows microbial presence in PMBL across fermentation days (0, 3, 6, 9, 12). *E. coli, Salmonella, Bacillus, and Shigella* are consistently present throughout, indicating their resilience in the acidic, dynamic environment of PMBL fermentation. These bacteria likely contribute to organic matter breakdown, as seen in rising TOM and TOC levels. Yeast appears from Day 3 onward, suggesting adaptation to shifting conditions, possibly linked to pH increases (e.g., 5.74 by Day 12) or nutrient availability from decomposition. *Penicillium, Aspergillus,* and *Fusarium* are consistently absent, likely due to the acidic environment (pH 3.46–5.74) or competition with dominant bacteria. The microbial profile aligns with observed chemical changes, like nitrate and nitrogen fluctuations, reflecting active microbial succession.

4.0 CONCLUSION

This study showed that Panicum maximum-derived whey (PMBL) achieved optimal nutrient levels by Day 12, including 67.5% total organic carbon (TOC) and 394 mg/kg nitrate, representing a 3.2× increase in nitrogen availability compared to Day 0. These shifts reflect strong microbial activity and nutrient transformation. PMBL reached microbial counts of 820 CFU/mL, confirming high biological activity. Key strains identified include Bacillus and Shigella, with yeast emerging in later stages; indicating microbial adaptability suitable for hydrocarbon degradation. However, phosphate depletion (down to 255.3 mg/kg) across fermentation highlights a possible nutrient limitation that could constrain long-term remediation. The persistence of potential pathogens (E. coli, Salmonella) also poses safety concerns. These should be addressed via pasteurization or competitive exclusion strategies before field application.

To validate PMBL's bioremediation potential and scalability, future work should; compare hydrocarbon degradation rates with dairy whey controls; confirm pathogen reduction post-treatment; assess cost-effectiveness in low-resource settings. While limitations remain, this work provides the first demonstration of a plant-based whey designed for engine oil remediation. By converting underutilized biomass into a functional biostimulant, it advances a circular economy approach in line with Sustainable Development Goals 12 (Responsible Consumption) and 15 (Life on Land). PMBL holds promise as a low-cost, low-impact alternative to conventional bioremediation agents, pending validation in field conditions.

Conflict of Interest

The authors declare that they have no conflict of interest.

Data Availability Statement:

All data supporting this study are available upon request from the corresponding author.

Authors' Contribution

Ibobo Victor U. conducted the experiment, acquired funding, and was responsible for the original draft writing, review, and editing. Uwidia Ita E. supervised the work and contributed to the conceptualization and methodology. Eguaoje Stanley A. provided significant support during the research process, contributing to data analysis and interpretation.

Authors' Declaration

The authors certify that this research is original, has not been published previously, and is not under consideration by any other journal. We assume full responsibility for the integrity of the data and the accuracy of the reported findings and will accept all liability for any claims about the content.

Ethical Declarations Human/Animal Studies

The authors declare that no human/animal was used for the studies

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