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Integrating enzymatic catalysis and microbial stimulation: Garbage enzymes for hydrocarbon degradation and Total Organic Carbon / Chemical Oxygen Demand reduction in well water stations

Adnan H. Abbas *

Scientific Research Commission, Baghdad, Iraq.

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Abstract

Hydrocarbon contamination in water resources poses a significant threat to environmental and public health due to its persistence, toxicity, and potential for bioaccumulation. Accidental leaks, industrial discharges, and crude oil spills contribute to the widespread presence of hydrocarbons in aquatic and terrestrial ecosystems, necessitating the development of efficient and sustainable remediation strategies. This study evaluates the effectiveness of a biological treatment approach using crude Garbage Enzymes (GE), produced through household food waste, for the remediation of hydrocarbon-polluted well water (HPWW) collected from two stations in the Diyala region of Iraq. Crude GE was applied at concentrations of 10% and 25% (GE: HPWW at 1:10 vol/vol), followed by a 24 h incubation period at ambient temperature. Gas Chromatography (GC) analysis demonstrated substantial hydrocarbon degradation, achieving removal efficiencies of 89.66% and 99.61% at Station 1, and 96.24% and 98.62% at Station 2, respectively. In addition to hydrocarbon breakdown, significant reductions in Total Organic Carbon (TOC) were observed, with removal rates of 74.4% and 80.8% at Station 1 and 90.7% and 94.4% at Station 2. Chemical Oxygen Demand (COD) values also declined markedly, decreasing by 73.4% and 81.1% at Station 1 and by 84.6% and 90.7% at Station 2 under the same treatment conditions. The findings highlight the potential of crude GE as a low-cost, eco-friendly, and efficient biocatalyst for hydrocarbon remediation. Compared with conventional microbial treatments, enzyme-based approaches offer enhanced safety, operational simplicity, and reduced ecological risk, without the need to introduce foreign or genetically modified microorganisms into the environment. Overall, this study underscores the promise of crude GE as a scalable and sustainable tool for mitigating hydrocarbon pollution and supports further research into enzyme-driven bioremediation technologies

Keywords: Eco-enzymes; Hydrocarbon pollutants; Biotreatment; Total Organic Carbon; Chemical Oxygen Demand

1. Introduction

Oil spills originating from underwater pipelines, ruptured storage tanks, and unintended offshore exploration activities represent a major environmental hazard to marine ecosystems. The severity of these incidents increases when spilled oil reaches coastal zones, which are typically more biodiverse and therefore more vulnerable to long-term hydrocarbon contamination (1). Petroleum hydrocarbons consist predominantly of linear alkanes, accompanied by smaller fractions of cycloalkanes and aromatic hydrocarbons. Within the aromatic fraction, trace quantities of metals such as iron, copper, nickel, and vanadium are often present, along with heteroatoms including nitrogen, oxygen, and sulfur. Furthermore, oil tank mixtures frequently harbor live bacterial populations, adding biological complexity to the contamination (2). Human exposure to petroleum hydrocarbons (PHCs), volatile organic compounds (VOCs), and polyaromatic hydrocarbons (PAHs) has been shown to pose significant health risks, as evidenced by studies of fuel station workers (3). Beyond direct human impacts, oil pollution has profound ecological consequences: the formation of a thin

* Corresponding author: Adnan H. Abbas

hydrocarbon film on the water surface restricts oxygen diffusion, thereby suffocating aquatic organisms, impairing photosynthesis, and disrupting insect and wildlife populations. This cascade of effects destabilizes food chains and undermines the integrity of the ecosystem (4).

In recent decades, the flow of the Tigris River has declined, a trend largely attributed to the increasing volume of wastewater coupled with insufficient treatment infrastructure (5). The unchecked discharge of hazardous pollutants from anthropogenic sources, including domestic sewage, hospital effluents, and industrial outputs, has further exacerbated this decline, introducing contaminants that disrupt the ecological balance of plants and aquatic organisms. The direct release of untreated wastewater into the river not only degrades water quality but also undermines the resilience of its ecosystems (6).

Researcher Rosukon Poompanvong dedicated three decades (1976-2006) to Garbage enzyme (GE), a product derived from the fermentation of fruit and vegetable waste (7). This fermentation process yields a complex mixture containing active enzymes and diverse microbiological flora (8). While GE has been explored for potential applications, its presence also raises environmental concerns, particularly regarding contamination of ponds and domestic wastewater systems (9).

Park et al. (10) describe Total Organic Carbon (TOC) as a key analytical parameter that quantifies both dissolved and particulate organic carbon within a given sample. This measurement serves as an indicator for assessing wastewater treatment systems, detecting contaminants, and ensuring the quality of water intended for sensitive applications in the pharmaceutical, semiconductor, and healthcare sectors.

Chemical Oxygen Demand (COD) is a fundamental parameter for assessing water pollution, offering a relatively rapid means of estimating biodegradation requirements through the dichromate oxidation method. Conventional colorimetric COD analysis has been widely applied in wastewater characterization, serving both risk assessment purposes and the development of advanced treatment technologies such as oxidation processes (11). Traditional procedures, however, demand large sample volumes and rely on substantial quantities of hazardous reagents, including potassium dichromate (K_2CrO_7), sulfuric acid (H_2SO_4), and mercuric sulfate ($HgSO_4$) (12). Although modifications to COD methodologies have been introduced to enhance sustainability and align with green chemistry principles, these approaches require reagent use and remain susceptible to matrix interferences. Consequently, COD determination is often costly, labor-intensive, and environmentally hazardous (13).

Against this backdrop, the present study seeks to investigate the effectiveness of biosynthesis derived from GE extract in treating hydrocarbon-contaminated well water in the Diyala region, with particular emphasis on its impact on TOC and COD concentrations.

2. Materials and Methods

2.1. Biosynthesis of Garbage Enzyme

Several investigations have demonstrated that GE can be synthesized by fermenting household food waste using a straightforward formulation of organic kitchen residues, brown sugar, and water (3:1:10) (14, 15). The prepared mixture is typically stored in sealed plastic containers in cool and dry conditions to facilitate natural decomposition. After a fermentation period of more than three months, the solution is filtered to yield the crude GE extract. From this extract, two experimental working concentrations, 10% and 25%, were subsequently prepared for application in laboratory studies.

2.2. Biological Treatment of Hydrocarbon Pollutant Well Water

Water samples collected from HPWW in the Diyala region (Stations 1 and 2) were treated with crude GE at working concentrations of 10% and 25% (GE: HPWW 1:10 vol/vol). The efficiency of biodegradation was subsequently evaluated using Gas Chromatography (GC) analysis, complemented by measurements of TOC and COD reductions following a 24 h incubation period.

2.3. Determination of Hydrocarbon Polluted Well Water Degradation by Gas Chromatography Analysis

Gas chromatography (GC; Dani Master 1000, Italy) was employed to quantify hydrocarbon residues present in the contaminated water samples. The analysis was conducted using a DN-5 capillary column (30 m × 0.32 mm) with nitrogen as the carrier gas at a flow rate of 1 mL/min. Detection was performed with a flame ionization detector (FID) maintained at 320 °C. The oven temperature program was set as follows: initial temperature of 50 °C, ramped at

10 °C/min to 200 °C with a (2 min) hold, followed by an additional increase at 10 °C/min to 280 °C with a final hold of (7 min). Water samples collected from hydrocarbon-contaminated wells in the Diyala region (Stations 1 and 2) were treated with crude GE at concentrations of 10% and 25% (GE: HPWW 1:10 vol/vol). Hydrocarbon degradation was assessed after 24 h at room temperature, and hexane and GC analysis were used to determine the extent of biodegradation.

2.4. Analyzing the Reduction of Total Organic Carbon

TOC analysis was conducted using a DRB200 reactor. For each measurement, 0.4 mL of buffer solution (pH 2.0) was added to 10 mL of HPWW at stations 1 and 2, with crude GE at concentrations of 10% and 25% (GE: HPWW 1:10 vol/vol). The prepared samples were incubated at room temperature for 1 h. Subsequently, TOC sulfate powder was used as the sample, and reagent blank acid vials were mixed using a stir plate for 10 min. From each prepared solution, 1.0 mL was transferred into the sample vial, while 1.0 mL of organic-free water was added to the reagent blank. Both vials were thoroughly mixed to ensure homogeneity.

Two MR/HR indicator ampoules were rinsed with deionized water, and one unopened ampoule was placed in each acid digestion vial. After snapping off the ampoule tip, it was allowed to drop into the vial, which was then tightly sealed. The vials were placed in the DRB200 reactor for 2 hours at 103-105 °C. Following digestion, the vials were removed and cooled for 1 h at room temperature to stabilize measurements. A deep blue coloration in the reagent blank confirmed digestion. TOC quantification was then performed according to Method 10173 using colorimetric measurement.

2.5. Measurement of the Reduction of Chemical Oxygen Demand

The COD in the HPWW (mg/L) was determined using a DRB200 reactor, applying the digestion and color-spectrophotometric method described by Ma et al. (16). A 100 mL aliquot of water collected from wells at Diyala stations 1 and 2, previously treated with crude GE at concentrations of 10% and 25% (GE: HPWW 1:10 vol/vol), and incubated for 24 h at room temperature, was homogenized for 30 sec. The DRB200 reactor was activated and preheated to 150 °C before analysis. Two COD digestion reagent vials were prepared, with one vial held at a 45° angle during setup.

Both vials were placed in the reactor; the first received 2.0 mL of the homogenized sample, while the second (blank control) received 2.0 mL of deionized water. Digestion was carried out for 2 h, after which the vials were cooled to 120 °C or below and gently inverted several times while still warm to ensure mixing. The vials were then transferred to a rack and allowed to cool to ambient temperature. COD quantification was subsequently performed using Method 8000 colorimetric determination.

3. Results and Discussion

3.1. Bioactivity of Garbage Enzyme

GE was prepared by combining brown sugar, fruit or vegetable residues, and water in a 1:3:10 ratio within sealed plastic containers. During the initial month of fermentation, gas evolution was observed, indicating active microbial activity. The mixture was maintained in a cool, dry environment for three months, after which the crude enzyme solution was obtained and filtered to remove solid residues. The final product is a light brown liquid characterized by low pH and enriched with diverse enzymatic and microbial components. According to Toppo and Maity (7), GE derived from fermented food waste and spoiled fruits and vegetables contains enzymes such as amylase, lipase, cellulase, urease, protease, alcohol, and organic acids. These biological constituents not only exhibit long-term stability but also increase in effectiveness over time. When diluted, GE demonstrates multifunctional enzymatic activity suitable for livestock applications, with its functions broadly categorized into degradation, conversion, and catalytic processes (17).

These compounds are generated through microbial enzymatic degradation, oxidation, release of carbon dioxide and water, energy generation, and the formation of secondary metabolites (18). Collectively, these biochemical transformations highlight the complex composition of GE and its potential as a sustainable biocatalyst for environmental and agricultural applications.

Ramirez et al. (19) emphasize that microbial growth and reproduction are sustained by the acquisition of carbon and energy generated during degradation processes. Each stage of these metabolic pathways is facilitated by a distinct enzyme, which may act extracellularly or be produced by the degrading microorganism itself (20).

3.2. Analyzing the Biodegradation of Hydrocarbon Pollutant Well Water

Table 1 summarizes the analytical results obtained from the application of crude GE concentrates at 10% and 25% (GE: HPWW 1:10 vol/vol), followed by GC evaluation at two monitoring stations in the Diyala region. The treatment demonstrated notable degradation efficiencies, achieving 89.66% and 99.61% at station 1, and 96.24% and 98.62% at station 2. These outcomes align closely with the reference protocol established by Zhu et al. (21), underscoring the reliability of GE in hydrocarbon remediation.

Table 1 Degradation of hydrocarbon pollutant well water stations 1 and 2 inoculated with a crude GE concentration (10 and 25 %), incubated for 24 h at room temperature

Concentration of GE (%)	Station 1	Station 2
	Degradation of Hydrocarbon pollutant well water treated with crude GE incubated for 24 h at room temperature (%)	
10	89.66	96.24
25	99.61	98.62

During aerobic fermentation, organic substrates undergo extensive biochemical conversion, resulting in the synthesis and accumulation of extracellular enzymes such as lipases, amylases, and proteases, and the capacity to degrade complex organic pollutants (22). Complete fermentation elevates acetic acid and hydrolytic enzymes while reducing pH, thereby creating favorable conditions for GE activity. Such properties extend their applicability across diverse environmental contexts, including composting, waste-activated sludge treatment, landfill leachate management, soil remediation, and wastewater purification (23).

Sambaraju and Lakshmi (24) investigated the application of GE in dairy wastewater treatment at concentrations of 5% and 10%. Their study demonstrated that GE is a cost-effective and efficient alternative to solubilizing complex organic matter. At a pH of 6.5, treatment with 10% GE achieved 26% reduction in suspended solids, highlighting its potential utility in industrial wastewater management and reinforcing its role as a sustainable bioremediation agent.

Beyond its relevance in wastewater treatment, hydrolytic enzymes are used for plant protection and pathogen suppression. Biocontrol bacteria produce a range of enzymes, including chitinase, cellulase, protease, lipase, glucanase, and amylase, which act on structural components such as cell walls, proteins, and nucleic acids. This enzymatic activity establishes an effective and environmentally sustainable biocontrol mechanism, offering significant promise for agricultural applications (25).

Crude GE acts through a synergistic set of pathways that explain the rapid hydrocarbon degradation observed in the experimental data. Hydrolytic and oxidative enzymes within GE cleave long-chain hydrocarbons into smaller, more biodegradable fragments, thereby reducing pollutant persistence. At the same time, GE supplies cofactors and nutrients that stimulate indigenous microbial communities, accelerating the consumption of hydrocarbon intermediates. In addition, enzymatic redox catalysis enhances the oxidative conversion of recalcitrant hydrocarbons into simpler compounds such as CO_2 and water. These mechanisms are consistent with genome-resolved studies that highlight the diversification of hydrocarbon-degrading enzymes across bacteria and archaea (26), further supported by the HADEG database, which confirms the central role of aerobic degradation enzymes in petroleum bioremediation (27).

The near-complete degradation achieved with GE underscores its potential as a low-cost, eco-friendly alternative to conventional chemical treatments for hydrocarbon-polluted water. The slightly higher efficiency observed at station 2 suggests that local water chemistry and microbial composition may influence enzymatic activity, a factor emphasized in recent reviews on microbial hydrocarbon degradation (28). Taken together, these findings provide strong experimental support for the growing body of literature advocating enzyme-based approaches to environmental cleanup, positioning GE as a sustainable pathway for rapid hydrocarbon remediation that integrates enzymatic catalysis with microbial augmentation.

3.3. Evaluation of Total Organic Carbon Removal in Hydrocarbon Pollutant Well Water

In aquatic environments, TOC is a critical parameter for quantifying organic carbon content and serves as a reliable analytical tool for optimizing treatment processes. In the present study, the treatment resulted in a pronounced reduction in TOC levels, with values decreasing to 12 and 9 ppm at station 1, and to 5 and 3 ppm at station 2, respectively.

These findings, summarized in Table 2 and illustrated in Figure 1, highlight the effectiveness of GE in reducing organic carbon concentrations within contaminated aquatic systems.

Table 2 Reduction of TOC for hydrocarbon pollutant well water stations 1 and 2 inoculated with a crude GE concentration (10 and 25 %), incubated for 24 h at room temperature

Concentration of GE (%)	Reduction of TOC of samples treated with crude GE incubated for 24 h at room temperature			
	Wastewater (station 1)		Wastewater (station 2)	
	ppm	Reduction rate %	ppm	Reduction rate %
Control	47		54	
10	12	74.4	5	90.7
25	9	80.8	3	94.4

GE reduces TOC through a synergistic set of mechanisms that operate simultaneously. Hydrolytic and oxidative enzymes directly cleave large, complex organic molecules into smaller, more biodegradable fragments, lowering the measurable TOC. At the same time, GE provides cofactors and nutrients that stimulate microbial communities, enhancing their metabolic activity and accelerating the biodegradation of these breakdown products. In addition, the enzymes catalyze redox reactions that promote oxidative conversion of recalcitrant organic matter into inorganic forms such as carbon dioxide and water. Together, these processes explain the sharp decline in TOC observed in the treated samples, highlighting GE's dual role as both a direct biocatalyst and an indirect microbial enhancer in wastewater remediation (29, 30).

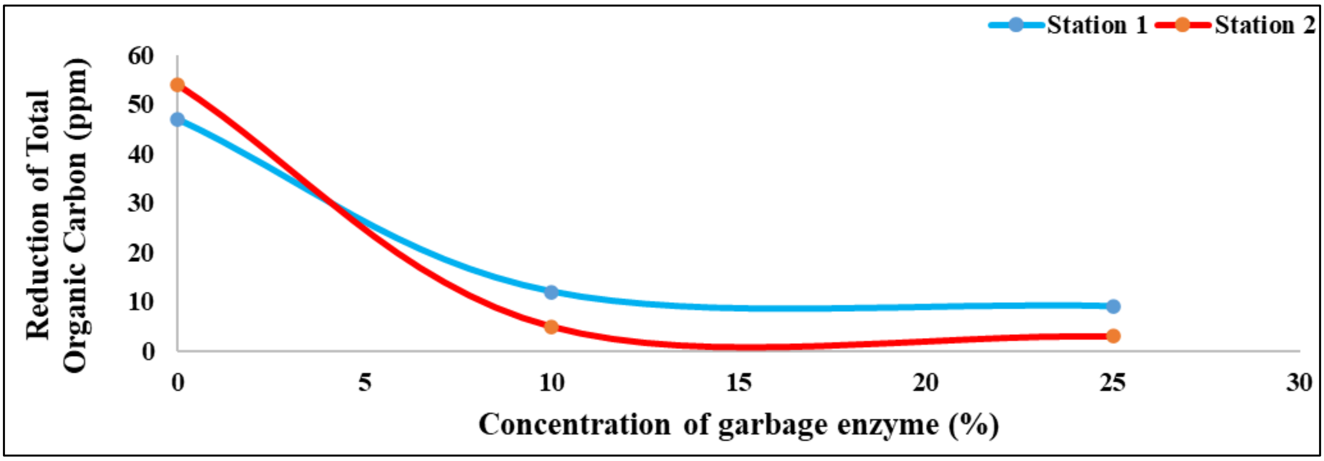

Figure 1 Crude GE (10 and 25 %) effectively reduces the TOC of hydrocarbon pollutant well water of stations 1 and 2, incubated for 24 h at room temperature

3.4. Determination of Chemical Oxygen Demand Decline in Hydrocarbon Pollutant Well Water

COD quantifies the amount of oxygen required to oxidize both soluble and insoluble organic matter, and is a critical parameter for evaluating water quality. In the presence of strong oxidizing agents, COD determination is widely used in wastewater treatment and process optimization (31). In the current study, HPWW samples collected from stations 1 and 2 in the Diyala region were subjected to biological treatment using crude GE at concentrations of 10% and 25%. The treatment resulted in a marked reduction in COD levels, with values decreasing to 38 and 27 ppm at station 1, and to 25 and 15 ppm at station 2, respectively. These findings are summarized in Table 3 and Figure 2.

Table 3 Reduction of the value of COD for hydrocarbon waste-contaminated water stations 1 and 2 inoculated with crude GE concentration (10 and 25 %), incubated for 24 h at room temperature

Concentration of GE (%)	Decrease in the COD value of samples treated with crude GE incubated for 24 h at room temperature			
	Polluted water (station 1)		Polluted water (station 2)	
	ppm	Reduction rate %	ppm	Reduction rate %
Control	143		163	
10	38	73.4	25	84.6
25	27	81.1	15	90.7

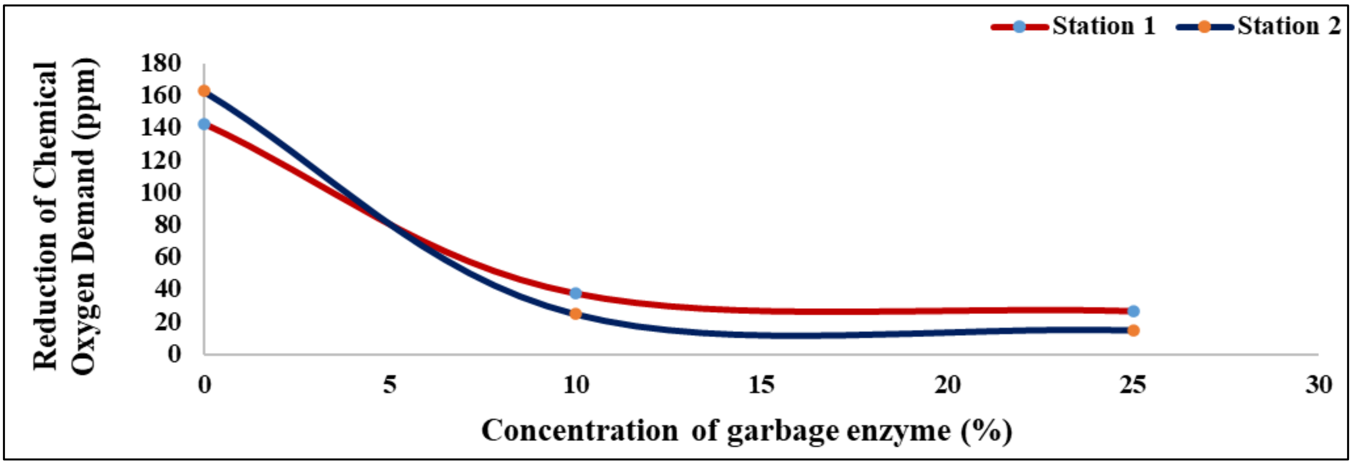

Figure 2 Demonstrates that after 24 h at room temperature, crude GE (10 and 25 %) effectively reduces the value of the COD of hydrocarbon waste-contaminated water stations 1 and 2

GE plays a decisive role in lowering the COD of HPWW, as reflected in the sharp reductions observed in both stations within just 24 hours. COD represents the oxygen required to oxidize organic matter, and its decline indicates that GE accelerates the breakdown of complex hydrocarbons and other organic pollutants into simpler compounds. This process reflects a combination of direct enzymatic degradation and enhanced microbial mineralization. Hydrolytic and oxidative enzymes cleave large molecules into smaller fragments, and cofactors and nutrients supplied by GE stimulate microbial communities to consume the resulting intermediates. In addition, enzymatic catalysis of redox reactions promotes the oxidative conversion of recalcitrant organic matter into inorganic carbon, further reducing oxygen demand (32).

The environmental significance of these findings lies in the rapid COD reduction achieved at relatively low GE concentrations, highlighting potential as a low-cost, eco-friendly biocatalyst for wastewater treatment. The higher efficiency observed at station 2 suggests that local water chemistry and microbial composition may favor enzymatic activity, underscoring the importance of environmental conditions in bioremediation success. Together, these mechanisms demonstrate that garbage enzymes can simultaneously act as direct catalysts and microbial enhancers, offering a sustainable alternative to conventional chemical treatments for hydrocarbon-contaminated water (28).

4. Conclusion

The results across all three experiments demonstrate the remarkable efficiency of garbage enzymes (GE) in treating hydrocarbon-polluted water. Within 24 hours of incubation, GE achieved near-complete (up to 99.6%) reduction of Total Organic Carbon (TOC) (up to 94.4%), and significant lowering of Chemical Oxygen Demand (COD) (up to 90.7%). These outcomes confirm that GE acts as a powerful biocatalyst, transforming complex organic pollutants into simpler, less harmful compounds.

Mechanistically, the observed improvements can be attributed to the synergistic action of enzymatic catalysis and microbial stimulation. Hydrolytic and oxidative enzymes within GE directly cleave hydrocarbons and organic matter, while cofactors and nutrients enhance microbial metabolism, accelerating mineralization into CO_2 and water. The reduction in TOC and COD highlights not only the breakdown of organic pollutants but also their conversion into inorganic forms, thereby lowering oxygen demand and improving water quality.

From an environmental perspective, these findings underscore the potential of GE as a low-cost, eco-friendly alternative to conventional chemical treatments in wastewater remediation. The consistently higher performance at Station 2 suggests that local water chemistry and microbial composition can influence enzymatic efficiency, pointing to the importance of site-specific conditions in bioremediation strategies. Overall, the integration of GE into wastewater treatment systems offers a sustainable pathway for managing hydrocarbon contamination while minimizing ecological impact.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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