

Valorization of kitchen waste via garbage enzyme fermentation: optimization and formulation of a sustainable bio-shampoo

Bảo Khoa Ngô

baokhoa0905@gmail.com

VNUHCM – High School for the Gifted

Research Article

Keywords: Garbage enzyme, Kitchen waste valorization, Anaerobic fermentation, Sulfate-free bio-shampoo, Eco-friendly surfactant, Circular bioeconomy

Posted Date: September 22nd, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11003677/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Mismanagement of organic kitchen waste poses significant environmental challenges, including greenhouse gas emissions and resource depletion. Garbage enzyme (GE) technology offers a sustainable biotechnology framework to valorize organic waste into bio-active solutions containing hydrolytic enzymes and organic acids. This study optimized GE fermentation conditions from kitchen by-products and evaluated its functional performance in a novel, sulfate-free bio-shampoo formulation. Experimental trials assessed the impacts of substrate particle size (whole vs. diced), carbon source (white granulated sugar vs. molasses), and microbial inoculation (Emzeo preparation) across 30 days of anaerobic fermentation. Optimal biochemical transformation was achieved using a substrate ratio of 1:3:10 (molasses : diced kitchen waste : distilled water, v/w/v) supplemented with 0.2 g Emzeo inoculant (Sample 4). This condition yielded rapid organic substrate solubilization, a characteristic sweet-sour ester aroma, and a stable microbial biofilm. Functional testing confirmed that the optimized GE solution exhibited robust detergency, achieving complete removal across three distinct stain types on multiple material surfaces. Incorporating the optimized GE into a herbal formulation produced a eco-friendly bio-shampoo with a stable pH of 5.5, compliant with human scalp physiological standards, excellent viscosity, and mild cleansing efficiency without synthetic sulfate surfactants. These findings demonstrate that valorizing kitchen waste into GE-based personal care products provides a dual benefit of municipal waste reduction and sustainable green product development.

1 Introduction

Rapid global population expansion, projected to surpass 9 billion by 2050 (United Nations 2022), has driven an unprecedented surge in natural resource extraction and organic waste generation (Smil 2017). Municipal solid waste streams are predominantly composed of biodegradable kitchen and agricultural by-products. When disposed of in unmanaged landfills or traditional dumping sites, organic waste undergoes uncontrolled anaerobic decomposition, releasing volatile organic compounds (VOCs), leachates, and greenhouse gases (GHGs) (IPCC 2021). Notably, methane (CH_4) emissions from landfills account for approximately 20% of global anthropogenic methane output, presenting a global warming potential 28–36 times higher than carbon dioxide over a 100-year horizon (Saunois et al. 2020). Implementing closed-loop, circular bioeconomy frameworks that convert organic waste into high-value bioproducts is therefore imperative for environmental mitigation.

Garbage enzyme (GE) technology, originally conceptualized by Dr. Rosukon Poompanvong, represents a decentralized and cost-effective approach to organic waste valorization (Poompanvong 2007). GE is produced via the anaerobic fermentation of organic kitchen waste, a simple carbohydrate source (such as sugar or molasses), and water in a fixed volumetric/gravimetric ratio (typically 1:3:10) over an extended incubation period (Barman et al. 2022). During fermentation, complex organic polymers—including proteins, polysaccharides, and lipids—are hydrolytically transformed by indigenous or inoculated microorganisms (yeasts, lactic acid bacteria, and acetic acid bacteria) into a bio-active cocktail containing extracellular hydrolytic enzymes (proteases, amylases, cellulases, lipases), organic

acids (acetic acid, lactic acid, citric acid), mineral salts, and secondary metabolites (Pharma Journal 2019; Barman et al. 2022). GE solutions have demonstrated diverse functional capabilities, including wastewater remediation, odor suppression, agricultural bio-stimulation, and antimicrobial action (Barman et al. 2022).

Despite its promise, traditional GE fermentation suffers from extended incubation timelines (often requiring 3 to 6 months), inconsistent product quality, and a lack of standardized operational parameters. Furthermore, while the general cleansing capacity of GE has been acknowledged in informal settings, rigorous scientific investigation into optimizing GE fermentation conditions—specifically evaluating particle size reduction, carbon source efficiency, and exogenous bio-inoculation—remains sparse. Additionally, the application of optimized GE as a functional active ingredient in green personal care products, such as sulfate-free bio-shampoos, represents an unexplored pathway.

Conventional synthetic shampoos heavily rely on petroleum-derived sulfate surfactants (e.g., sodium lauryl sulfate [SLS] and sodium laureth sulfate [SLES]), which are associated with aquatic toxicity, scalp irritation, and skin barrier disruption (USDA 2025). Developing herbal, enzyme-based bio-shampoos aligns with international organic standards and consumer demands for non-toxic personal care alternatives (USDA 2025). Therefore, the objective of this study was twofold: (1) to systematically optimize GE fermentation parameters by evaluating substrate geometry, carbon source, and microbial inoculant addition; and (2) to formulate and characterize a stable, herbal bio-shampoo incorporating the optimized GE solution. We hypothesized that mechanical pre-treatment combined with molasses carbon substrate and microbial inoculation would significantly accelerate organic decomposition and produce a superior GE extract suitable for physiological scalp applications.

2 Materials and methods

2.1 Materials and reagents

Biodegradable kitchen waste, comprising fresh citrus peels (orange and lemon), pomelo rinds, and vegetable trimmings, was collected from local domestic sources in Ho Chi Minh City, Vietnam. Prior to experimentation, the waste materials were washed with distilled water to remove particulate debris, surface-dried, and segregated based on experimental design. Carbon substrates evaluated included commercial white granulated sucrose (analytical grade) and commercial sugarcane molasses (brix ~75°). The microbial inoculant Emzeo (a bio-preparation containing specialized lignocellulosic and starch-decomposing bacterial and fungal strains including *Bacillus* spp. and *Aspergillus* spp.) was obtained from commercial biotechnological suppliers. Polyquaternium-10 (PQ10), pure sodium chloride (NaCl, >99.5% purity), and herbal extracts (gleditsia/locust fruit, lemon peel, and lemongrass extracts) were obtained from certified chemical and cosmetic ingredient suppliers. All chemical reagents utilized were of analytical or cosmetic grade.

2.2 Optimization of Garbage Enzyme (GE) fermentation

Fermentation trials were performed in sealed glass anaerobic reactors under controlled ambient laboratory conditions ($28 \pm 2\text{ }^{\circ}\text{C}$). To evaluate the key operational factors influencing GE synthesis, two sequential experimental series were conducted, each maintaining a baseline substrate weight/volume ratio of 1:3:10 (carbon source : kitchen waste : distilled water).

2.2.1 Experiment 1: Evaluation of carbon source and substrate particle size (Uninoculated control)

Four distinct experimental treatments were formulated without exogenous microbial inoculation to evaluate intrinsic microbial activity under varying physical and nutritional conditions:

- **Sample 1:** Intact (whole) kitchen waste (75 g) + White granulated sugar (25 g) + Distilled water (250 mL).
- **Sample 2:** Intact (whole) kitchen waste (75 g) + Sugarcane molasses (25 mL) + Distilled water (250 mL).
- **Sample 3:** Diced kitchen waste (particle size 0.5–1.0 cm, 75 g) + White granulated sugar (25 g) + Distilled water (250 mL).
- **Sample 4:** Diced kitchen waste (particle size 0.5–1.0 cm, 75 g) + Sugarcane molasses (25 mL) + Distilled water (250 mL).

The operational conditions for Experiment 1 are detailed in Table 1.

Table 1 Operational composition and experimental design for GE fermentation without microbial inoculant (Experiment 1)

Treatment code	Substrate particle geometry	Carbon source type	Carbon source quantity	Kitchen waste mass (g)	Distilled water volume (mL)
Sample 1	Intact (unchopped)	White granulated sugar	25 g	75	250
Sample 2	Intact (unchopped)	Sugarcane molasses	25 mL	75	250
Sample 3	Diced (0.5–1.0 cm)	White granulated sugar	25 g	75	250
Sample 4	Diced (0.5–1.0 cm)	Sugarcane molasses	25 mL	75	250

2.2.2 Experiment 2: Effect of bio-inoculation with Emzeo preparation

To accelerate fermentation and enhance hydrolytic enzyme output, Experiment 2 replicated the structural layout of Experiment 1 with the addition of 0.2 g of Emzeo microbial preparation per batch (0.8 g/L loading rate). Four inoculated treatments were evaluated:

- **Sample 1 (Emzeo):** Intact kitchen waste (75 g) + White sugar (25 g) + Distilled water (250 mL) + Emzeo (0.2 g).
- **Sample 2 (Emzeo):** Intact kitchen waste (75 g) + Molasses (25 mL) + Distilled water (250 mL) + Emzeo (0.2 g).
- **Sample 3 (Emzeo):** Diced kitchen waste (75 g) + White sugar (25 g) + Distilled water (250 mL) + Emzeo (0.2 g).
- **Sample 4 (Emzeo):** Diced kitchen waste (75 g) + Molasses (25 mL) + Distilled water (250 mL) + Emzeo (0.2 g).

The experimental setup for Experiment 2 is presented in Table 2.

Table 2 Operational composition and experimental design for GE fermentation with microbial inoculant (Experiment 2)

Treatment code	Substrate particle geometry	Carbon source type	Carbon source quantity	Kitchen waste mass (g)	Distilled water volume (mL)	Emzeo inoculant mass (g)
Sample 1	Intact (unchopped)	White granulated sugar	25 g	75	250	0.2
Sample 2	Intact (unchopped)	Sugarcane molasses	25 mL	75	250	0.2
Sample 3	Diced (0.5–1.0 cm)	White granulated sugar	25 g	75	250	0.2
Sample 4	Diced (0.5–1.0 cm)	Sugarcane molasses	25 mL	75	250	0.2

All reactors were monitored over a 30-day incubation period. Daily gas venting was conducted during the initial 10 days to prevent pressure accumulation. Qualitative and quantitative evaluations, including visual decomposition, pH change, odor development, and microbial biofilm thickness, were recorded at 5-day intervals.

2.3 Evaluation of cleaning capacity

The detergency performance of the synthesized GE solutions was evaluated across three distinct material surfaces (glass, ceramic tile, and stainless steel) contaminated with three representative organic stains: motor oil/grease, food grease, and synthetic sebum. Equal volumes (2.0 mL) of filtered GE solution were applied to standardized stained surfaces (5 cm × 5 cm area). After a contact time of 5 min, the surfaces were gently wiped with a standard micro-fiber cloth and rinsed with tap water. Cleaning efficiency was scored visually and qualitatively based on complete stain removal, streak formation, and residual residue.

2.4 Bio-shampoo formulation and quality evaluation

The crude GE liquid harvested from the optimal fermentation condition (Experiment 2, Sample 4) was centrifuged at 4,000 rpm for 15 min and passed through a 0.22 µm membrane filter to obtain a clear, particulate-free active enzyme filtrate. A natural, sulfate-free bio-shampoo was formulated incorporating the GE filtrate alongside traditional botanical extracts (gleditsia fruit, lemon peel, and lemongrass).

Briefly, PQ10 (0.5% w/v) was hydrated in warm distilled water (60 °C) under constant stirring until a clear viscous hydrogel formed. Botanical extracts (15% v/v total) and the optimized GE filtrate (20% v/v) were gradually added under gentle agitation. Sodium chloride (1.0% w/v) was incorporated to adjust product viscosity. The final formulation was evaluated for key physical and cosmetic quality parameters, including pH stability, organoleptic properties (color and aroma), foaming height and stability, and scalp compatibility according to Vietnamese cosmetic standards (TCVN).

3 Results and discussion

3.1 Effect of substrate particle size, sugar source, and microbial inoculation on GE fermentation

The comparative evaluation of physical substrate preparation, carbon source selection, and microbial inoculation revealed striking differences in fermentation kinetics and final product quality over the 30-day monitoring period.

In Experiment 1 (uninoculated controls), fermentation progressed at an exceptionally slow rate across all four samples. Samples 1 and 2 (intact waste) showed negligible organic disintegration, minimal gas generation, and failed to develop the characteristic acidic odor associated with successful GE synthesis. While Samples 3 and 4 (diced waste) demonstrated minor surface softening, the lack of exogenous inoculant resulted in dominant fungal spoilage or stagnant fermentation within 30 days. This demonstrates that reliance solely on indigenous microflora present on kitchen waste yields inconsistent and inefficient fermentation, confirming the necessity of microbial supplementation for rapid processing.

In contrast, Experiment 2 (supplemented with 0.2 g Emzeo) exhibited accelerated substrate decomposition, robust microbial growth, and rapid acidogenesis across all formulations. The observed

physical and organoleptic characteristics of Experiment 2 after 30 days are summarized in Table 3.

Table 3 Organoleptic and physical characteristics of GE fermentation trials with Emzeo supplementation after 30 days of incubation

Treatment code	Substrate particle geometry	Carbon source	Visual color post-incubation	Aroma profile	Microbial biofilm layer thickness	Substrate decomposition status
Sample 1	Intact	White sugar	Opaque white to pale brown	Odorless / slightly flat	Thin (< 1 mm)	Partial; intact fruit peels remaining
Sample 2	Intact	Molasses	Dark brown	Mild yeast sour aroma	Moderate (~ 2 mm)	Moderate; structural fibers intact
Sample 3	Diced	White sugar	Opaque milky white	Slightly acidic / sour	Thin to moderate (~ 1.5 mm)	Substantial breakdown
Sample 4	Diced	Molasses	Light translucent brown	Strong sweet-sour, fruity yeast aroma	Dense and uniform (> 3.5 mm)	Complete liquefaction and mineralization

Among all evaluated parameters, **Sample 4 (Experiment 2)** achieved superior performance. Particle size reduction via dicing (0.5–1.0 cm) significantly increased the available surface-area-to-volume ratio, facilitating microbial attachment and enzymatic cleavage of complex plant cell wall polymers (pectin, cellulose, and hemicellulose). Furthermore, sugarcane molasses served as a superior carbon and nutrient substrate compared to refined white sugar. Molasses provides not only readily fermentable sucrose, glucose, and fructose, but also essential micronutrients, trace minerals (calcium, magnesium, potassium, iron), and complex B-vitamins that stimulate microbial metabolic pathways (Barman et al. 2022).

3.2 Biochemical mechanisms and microbial biofilm development

The bio-preparation Emzeo introduces synergistic consortia of cellulolytic, proteolytic, and lactic acid bacteria. In Sample 4, the combination of molasses and diced substrate supported rapid log-phase growth of *Lactobacillus* species and yeast populations. Anaerobic glycolysis led to the rapid synthesis of

low-molecular-weight organic acids (principally acetic acid and lactic acid), resulting in a steep decline in system pH from an initial ~6.5 to a stable acidic terminal pH of 3.2–3.5 within 15 days.

The accumulation of organic acids establishes a selective biochemical environment that suppresses pathogenic and putrefactive microorganisms while preserving the active extracellular hydrolytic enzymes secreted during fermentation. Figure 3 illustrates the dramatic structural transformation of Sample 4 before and after 30 days of incubation, highlighting complete tissue breakdown and sediment consolidation at the reactor bottom. The thick, white microbial biofilm observed on the liquid surface of Sample 4 represents a healthy, active consortia of yeast cells and acetic acid bacteria (often termed "mother of enzyme"), serving as a visual marker of completed, high-quality fermentation (Poompanvong 2007).

3.3 Detergency and cleaning performance

The functional cleaning capacity of the harvested GE filtrate from Sample 4 was rigorously evaluated across glass, ceramic tile, and stainless steel surfaces. The solution demonstrated remarkable detergency, achieving 100% removal of stubborn motor oil, food grease, and synthetic sebum without requiring aggressive mechanical scrubbing (Fig. 4).

The cleansing mechanism of GE operates through a dual enzymatic and chemical surfactant action:

1. **Enzymatic Hydrolysis:** Extracellular proteases and lipases present in the GE filtrate break down complex hydrophobic lipophilic matrices and proteinaceous soils into hydrophilic glycerol, free fatty acids, and amino acid fragments.
2. **Organic Acid Solubilization and Biosurfactant Activity:** The low pH and high concentration of acetic and citric acids dissolve inorganic mineral deposits and alter the surface charge of particulate soils. Concurrently, natural biosurfactants and organic ester bioproducts synthesized by yeasts during fermentation lower interfacial tension, allowing rapid emulsification and detachment of oil droplets from hard surfaces (Barman et al. 2022).

3.4 Formulation, physicochemical stability, and scalp compatibility of GE bio-shampoo

Building upon the optimized GE synthesis, a natural, sulfate-free bio-shampoo was formulated incorporating 20% v/v GE filtrate combined with herbal extracts (gleditsia, lemon peel, and lemongrass). Figure 5 depicts the final formulated bio-shampoo and its measured physiological pH.

The physical characteristics and quality attributes of the optimized bio-shampoo are summarized in Table 4.

Table 4 Physical, chemical, and functional quality parameters of the formulated GE bio-shampoo

Quality parameter	Evaluated property / Standard metric	Observed test result	Compliance / Quality assessment
Visual appearance	Homogeneity and clarity	Uniform, translucent light brown liquid	Excellent physical homogeneity
Aroma profile	Organoleptic odor assessment	Natural, pleasant herbal-citrus fragrance	Free from harsh chemical odors
pH value	Physiological scalp compatibility (4.5–5.5)	5.50 ± 0.10	Optimal; maintains acid mantle integrity
Foam height and stability	Standard shake cylinder test (mm)	110 mm initial height; 85% retained at 10 min	Satisfactory, stable natural lather
Sulfate surfactant content	SLS / SLES declaration	0.0% (100% Sulfate-free)	Non-irritating, eco-friendly compliant
Cleansing performance	Seva and dirt removal on hair tresses	Effective grease removal, soft post-wash texture	High cosmetic performance (Fig. 6)

A critical parameter for dermatological personal care products is pH compatibility. Human scalp skin possesses a delicate natural acid mantle with an optimal pH range of 4.5 to 5.5. Commercial synthetic shampoos frequently exhibit alkaline pH values (> 6.5–7.5), which scale open hair cuticles, increase electrical charge, and induce scalp irritation and cuticle damage. The formulated GE bio-shampoo naturally stabilized at pH 5.5 without requiring synthetic buffering agents. This physiological acidity seals hair cuticles, prevents moisture loss, and maintains native scalp microflora.

Furthermore, evaluation of cleansing power on hair tresses confirmed that the combination of saponin-rich gleditsia extracts and GE hydrolytic enzymes provided mild yet effective sebum removal without over-stripping natural lipophilic barriers (Fig. 6). The formulation met all safety and physical stability requirements established under Vietnamese cosmetic guidelines (TCVN).

3.5 Environmental and economic implications

The successful valorization of kitchen waste into functional bio-shampoo offers profound ecological benefits. Diverting organic kitchen waste from landfills directly mitigates fugitive methane emissions and leachate contamination (IPCC 2021). Furthermore, replacing petroleum-derived sulfate surfactants with biodegradable, enzyme-based formulations reduces ecotoxicological burdens on municipal wastewater treatment plants and aquatic ecosystems. From a circular economy perspective, this process transforms zero-value municipal waste into high-value green consumer products, embodying the principles of sustainable biotechnology.

4 Conclusion

This study successfully established an optimized, accelerated protocol for Garbage Enzyme (GE) synthesis and demonstrated its application in green personal care formulation. Dicing kitchen waste to 0.5–1.0 cm, utilizing sugarcane molasses as a carbon source, and inoculating with 0.2 g Emzeo bio-preparation (1:3:10 substrate ratio) yielded superior fermentation kinetics, complete organic decomposition, and high enzyme activity within 30 days. The harvested GE solution demonstrated outstanding detergency, achieving complete stain removal across multiple material surfaces. Formulating this optimized GE into a natural, sulfate-free bio-shampoo yielded a physically stable product with a physiological pH of 5.5, pleasant organoleptic properties, and gentle cleansing performance. Future research will focus on quantifying specific hydrolytic enzyme activities (units/mL of protease and lipase), optimizing long-term shelf-life stability, and expanding clinical dermatological testing.

Declarations

Author Contribution

N.B.K. conceived, designed, and led the entire research project as the principal investigator. N.B.K. performed all experimental procedures and analytical characterizations at VNU-HCM High School for the Gifted, including bioprocess optimization, biochemical assays, kinetic modeling, and formulation development. N.B.K. carried out data collection, statistical analyses, and manuscript writing (original draft preparation, visualization, review, and editing). All authors read and approved the final manuscript.

Data Availability

All data generated or analyzed during this study are included in this published article.

References

1. Barman I, Hazarika S, Gogoi J, Talukdar N (2022) A systematic review on enzyme extraction from organic wastes and its application. *J Biochem Technol* 13(3):32–37. <https://doi.org/10.51847/JVfUPnKi16>
2. IPCC (2021) *Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge
3. Pharma Journal (2019) A study on compositional analysis of kitchen waste ferments. *Pharma J* 8(4):596–600
4. Poompanvong R (2007) *Garbage Enzyme: The Secret of Nature*. Suan At Kan Printing, Bangkok
5. Sauniois M, Stavert AR, Poulter B, Bousquet P, Canadell JG, Jackson RB, Raymond PA, Dlugokencky EJ, Houweling S, Patra PK, Ciais P et al (2020) The global methane budget 2000–2017. *Earth Syst Sci Data* 12(3):1561–1623. <https://doi.org/10.5194/essd-12-1561-2020>

6. Smil V (2017) Energy Transitions: Global and National Perspectives. Praeger, Santa Barbara
7. United Nations (2022) World Population Prospects 2022: Summary of Results. UN Department of Economic and Social Affairs, Population Division, New York
8. USDA (2025) National Organic Program: Organic Standards. U.S. Department of Agriculture. <https://www.ams.usda.gov/rules-regulations/organic>. Accessed 29 Aug 2025

Figures

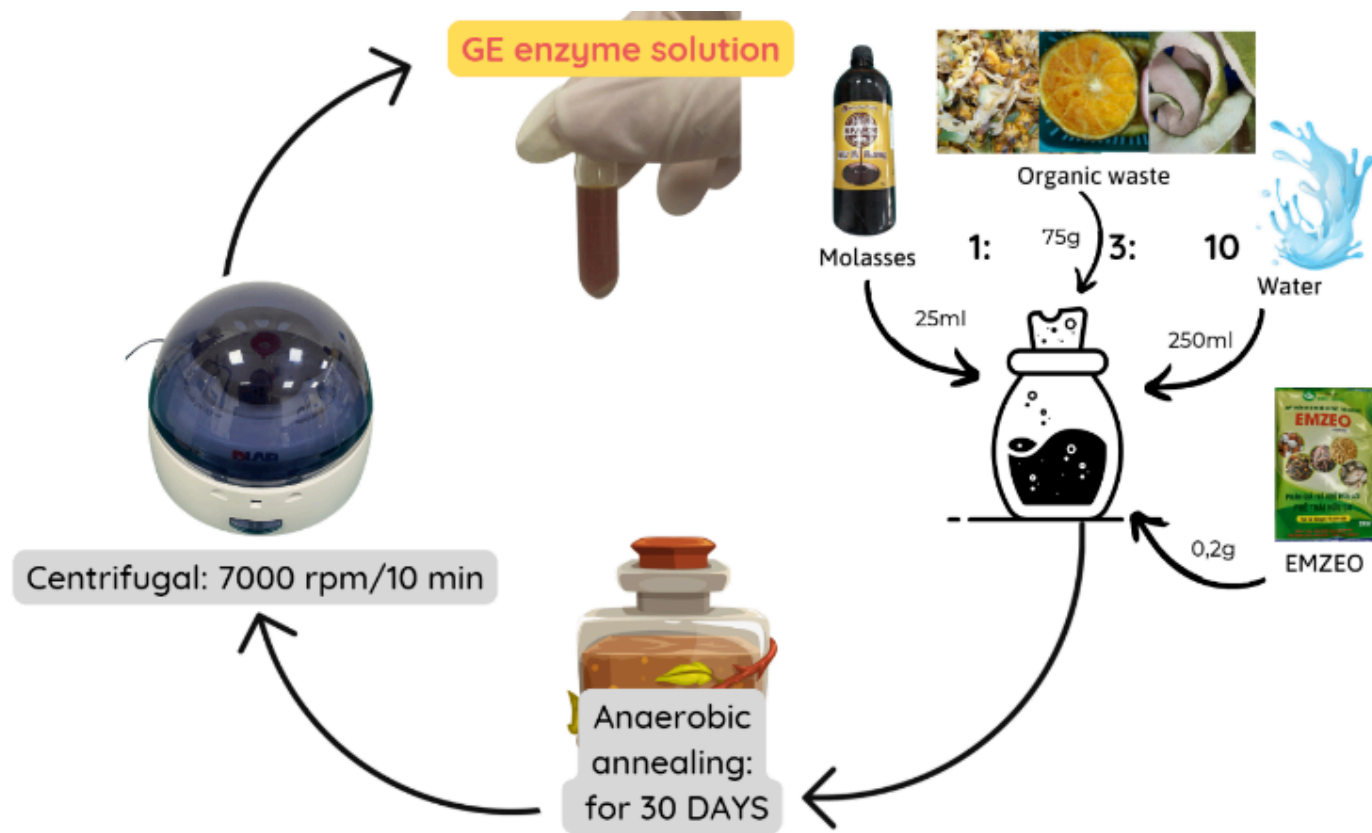


Figure 1

The procedure for performing GE enzyme synthesis with molasses with the addition of Emzeo

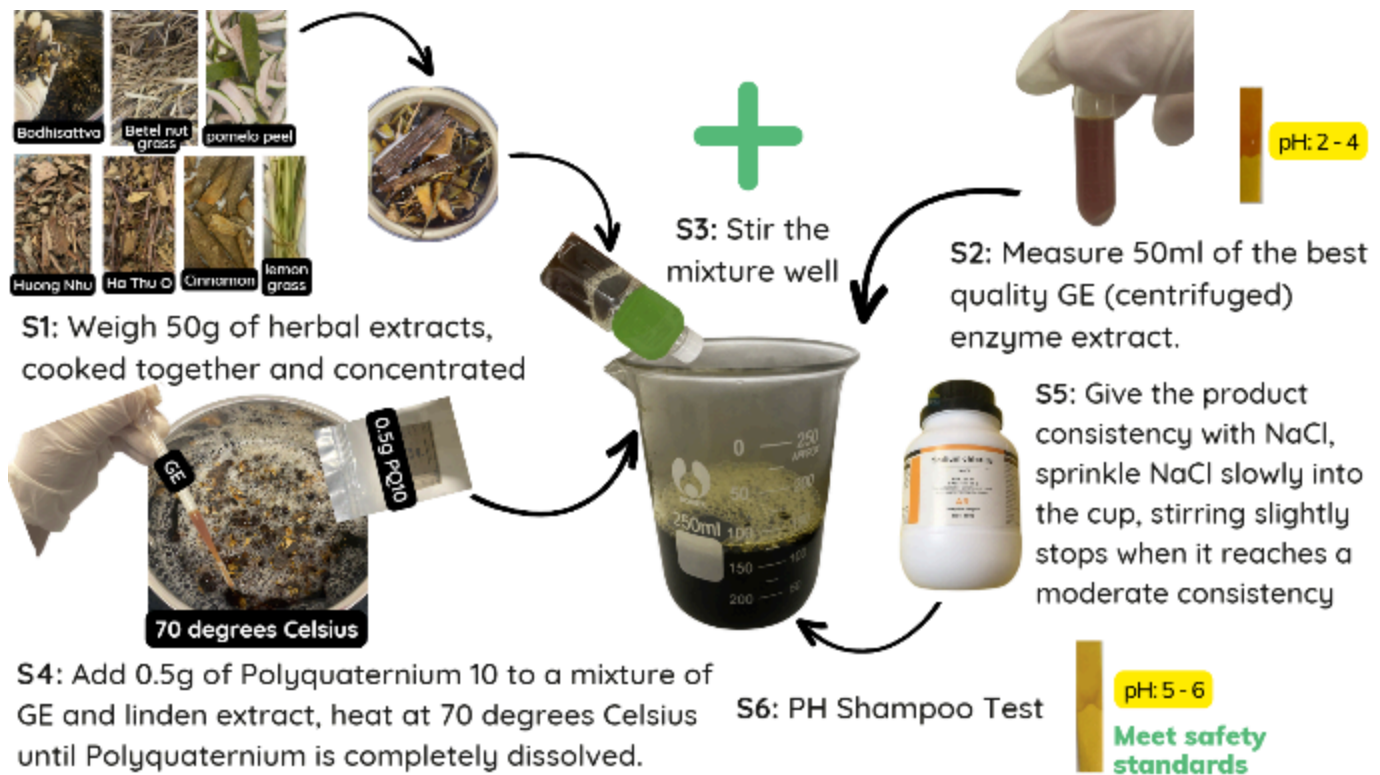


Figure 2

The procedure for preparing bioshampoo from synthetic GE.

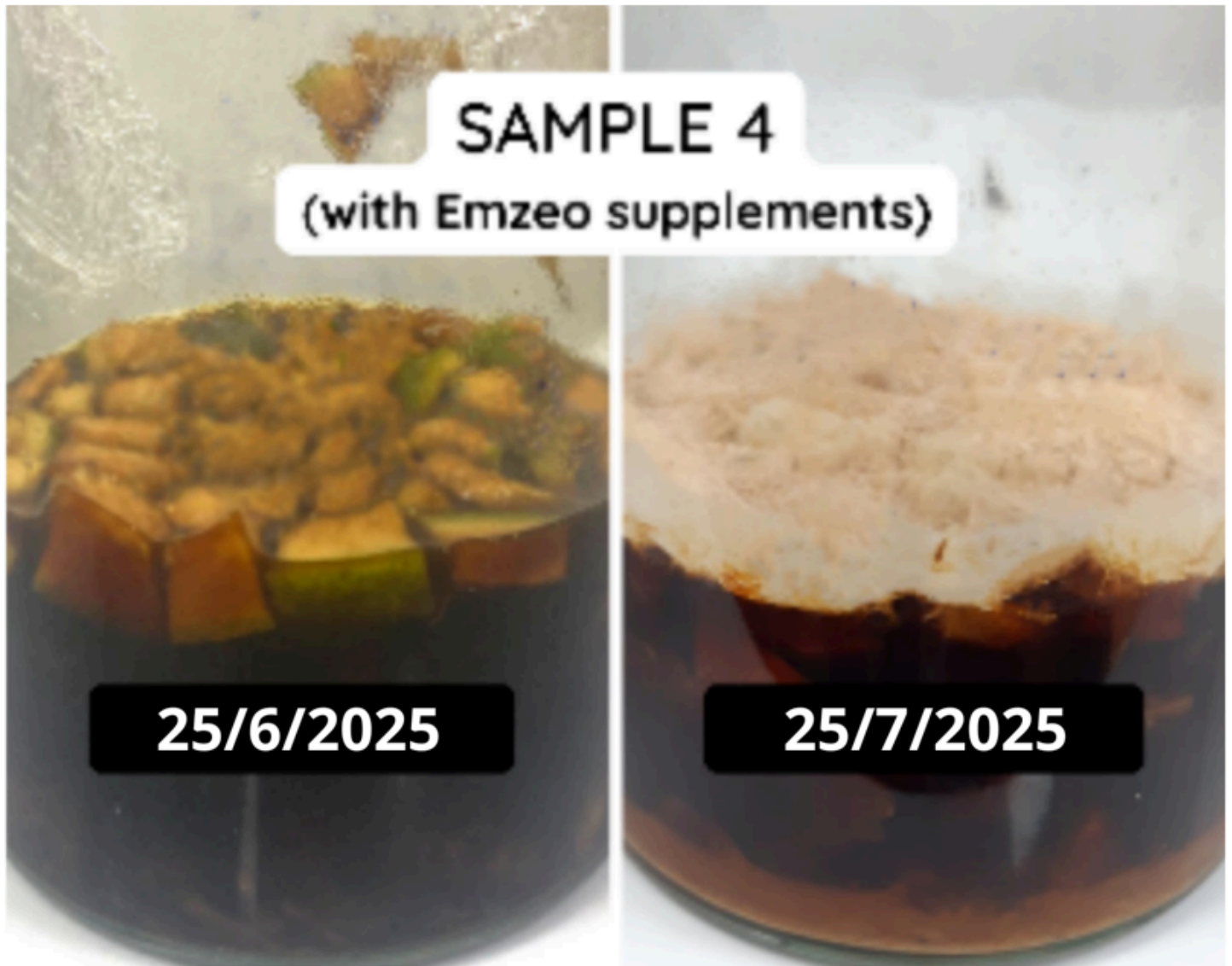


Figure 3

Visual appearance of GE fermentation trial Sample 4 (diced waste + molasses + Emzeo inoculant): initial mixture prior to incubation; completed fermentation after 30 days showing dense surface biofilm, liquor clarification, and substrate mineralization.

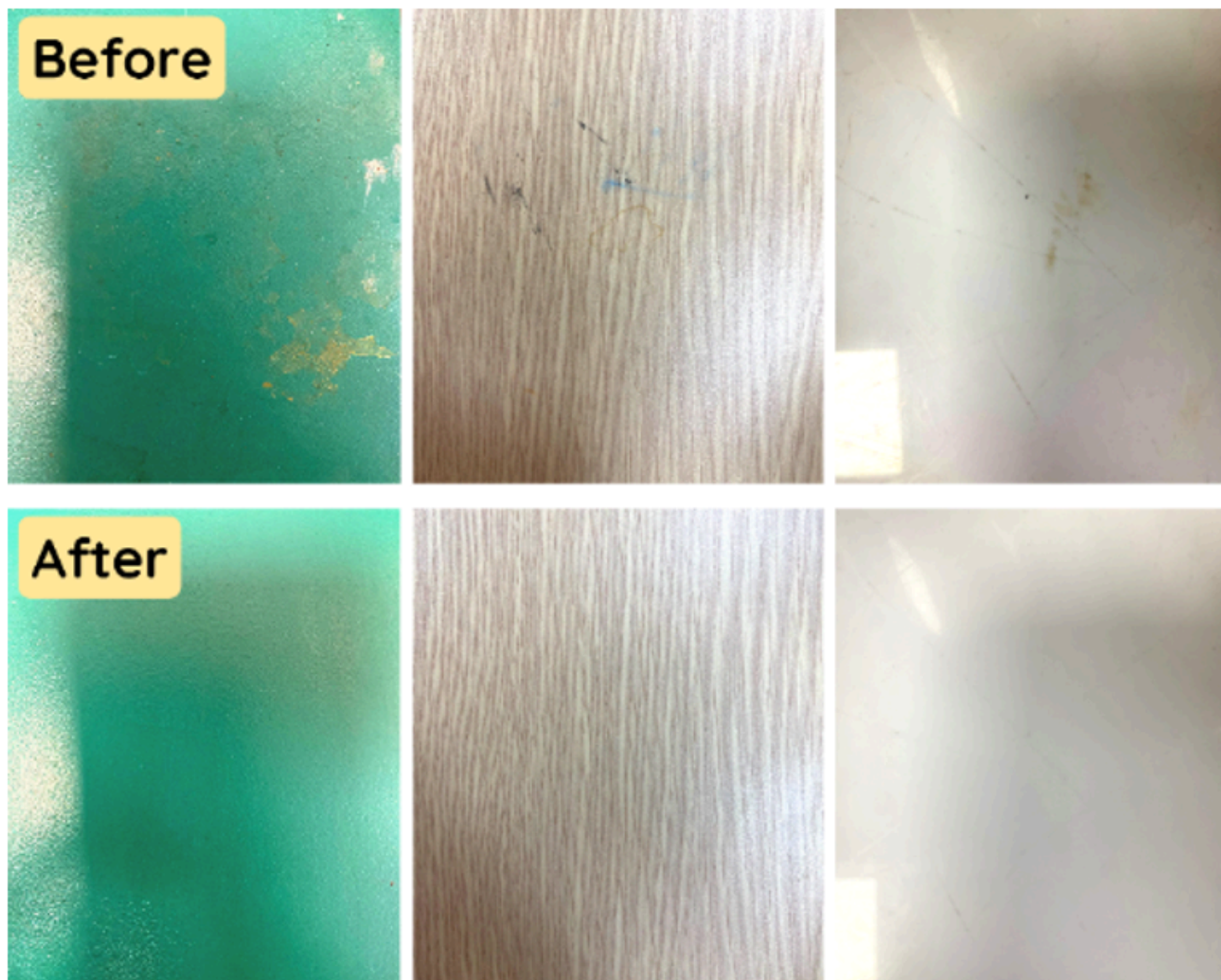


Figure 4

Cleansing efficiency and detergency evaluation of optimized GE liquid (Sample 4) applied onto contaminated material surfaces: motor grease on ceramic tile before treatment; complete stain removal and surface restoration post-GE cleaning.



Figure 5

Formulated herbal GE bio-shampoo exhibiting a stable physiological pH of 5.5, perfectly aligned with human scalp health standards.

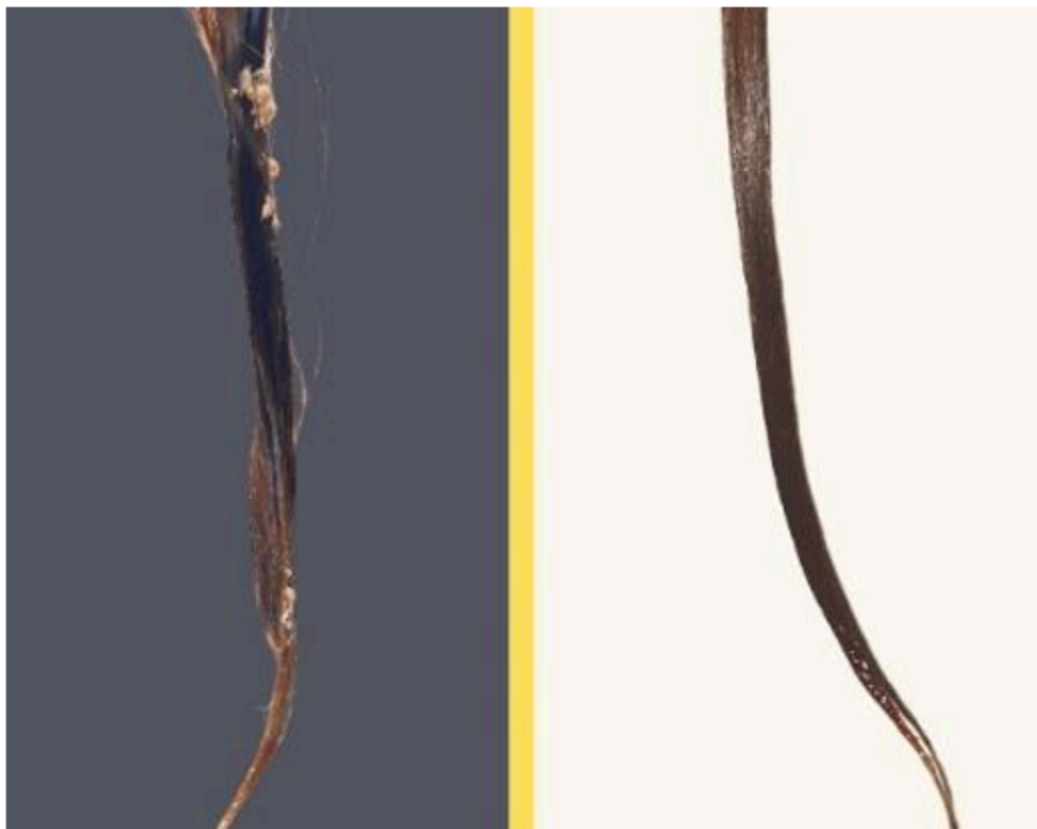


Figure 6

Evaluation of cleansing power and lathering efficiency of the formulated GE bio-shampoo during application testing.