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# I-PSAF 2025

# INTERNATIONAL POSTGRADUATE SEMINAR ON AGRICULTURE AND FORESTRY

**THEME : TECHNOLOGICAL INNOVATIONS IN SUSTAINABILITY**

**28-29 August 2025**
***Universiti Putra Malaysia Sarawak, Bintulu, Sarawak, Malaysia***

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# *e-Proceeding*

**Editors:**
**Anysia Hedy Ujat, Tan Toh Hii, Ameyra Aman-Zuki**

# INTERNATIONAL POSTGRADUATE SEMINAR ON AGRICULTURE AND FORESTRY 2025 (I-PSAF 2025)

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# FOREWORD

**PROF. DR. SHAHRUL RAZID SARBINI**
DIRECTOR
UNIVERSITI PUTRA MALAYSIA SARAWAK

Assalamualaikum warahmatullahi wabarakatuh and warm greetings to all,

It is with great pleasure that I welcome you to the International Postgraduate Seminar on Agriculture and Forestry (I-PSAF2025), organized by the Faculty of Agricultural and Forestry Sciences, Universiti Putra Malaysia Sarawak (UPMS). This year's seminar carries the theme "*Technological Innovations in Sustainability*", reflecting our shared commitment to harnessing science, technology, and innovation in building a greener and more resilient future.

Building upon the success of the past seminars, I-PSAF2025 continues to serve as a vital platform for postgraduate students and researchers from around the world to exchange knowledge, share discoveries, and engage in meaningful dialogue. The participation of international scholars highlights the growing recognition of UPMS as a hub of excellence in advancing sustainable solutions in agriculture and forestry, while also reinforcing our mission to foster global partnerships in research and education.

In today's rapidly changing world, sustainability can no longer be pursued in isolation. The integration of cutting-edge technologies, which range from digital agriculture and precision forestry to renewable energy and biotechnology, provides unprecedented opportunities to tackle the urgent challenges of climate change, biodiversity loss, and food security. By embracing innovation, we are not only improving efficiency and productivity but also ensuring that future generations inherit a healthier planet.

UPMS remains steadfast in promoting collaborative initiatives, including through the Konsortium Universiti Universitas Borneo (KUUB), which unites 16 universities across the Borneo archipelago. Together, we aim to strengthen research networks, foster interdisciplinary approaches, and drive technological solutions that support sustainable development in our region and beyond.

I would like to extend my deepest appreciation to the organizing committee for their dedication and hard work, as well as to the keynote speakers, presenters, and participants whose contributions make this seminar an enriching experience for all. I am confident that I-PSAF2025 will inspire new ideas, foster impactful collaborations, and pave the way for innovations that uphold sustainability as a guiding principle in agriculture and forestry.

Thank you, and I wish everyone a fruitful and meaningful seminar.

Wassalamualaikum warahmatullahi wabarakatuh.

# FOREWORD

**ASSOC. PROF. DR. NOORASMAH SAUPI**
DEAN, FACULTY OF AGRICULTURE AND FORESTRY SCIENCES
UNIVERSITI PUTRA MALAYSIA SARAWAK

Assalamualaikum warahmatullahi wabarakatuh and warm greetings,

It is my pleasure to welcome all participants and distinguished guests to the International Postgraduate Seminar on Agriculture and Forestry (I-PSAF2025), organized by Faculty of Agricultural and Forestry Sciences, Universiti Putra Malaysia Sarawak (UPMS). Now in its third edition, the seminar continues to serve as an important platform for postgraduate scholars and researchers to exchange knowledge, share discoveries, and strengthen collaborations.

The theme for this year, "*Technological Innovations in Sustainability*", emphasizes the critical role of innovation in addressing global challenges related to agriculture, forestry, and the environment. Through the application of technology, we are better equipped to advance sustainable practices and create long-term solutions that safeguard our planet.

As this seminar brings together participants from diverse backgrounds and institutions, it is my hope that I-PSAF2025 will not only foster academic discourse but also inspire enduring partnerships and collaborative initiatives. May the ideas and solutions shared here contribute meaningfully to the advancement of sustainable agriculture and forestry at both regional and global levels.

I extend my sincere appreciation to the organizing committee, keynote speakers, presenters, and participants for their valuable contributions. I am confident that I-PSAF2025 will be a meaningful and impactful seminar for all involved.

Thank you, and I wish everyone a fruitful and successful seminar.

# FOREWORD

**ASSOC. PROF. DR. LEONG SUI SIEN**
CHAIRMAN, IPSAF 2025
UNIVERSITI PUTRA MALAYSIA SARAWAK



It gives me great pleasure to welcome all distinguished speakers, participants, and guests to the International Postgraduate Seminar on Agriculture and Forestry: Technological Innovations in Sustainability (I-PSAF2025), held on 28–29 August 2025 at Universiti Putra Malaysia Sarawak, Bintulu, Sarawak, Malaysia. The I-PSAF 2025, organized by the Faculty of Agricultural and Forestry Sciences, UPMS serves as a vibrant platform for undergraduate students, postgraduate students, researchers, and industry experts to share their knowledge, exchange ideas, and present innovative solutions in advancing agriculture and forestry towards a more sustainable future.

The theme of I-PSAF 2025 emphasizes the critical role of technological innovations in addressing global challenges, particularly those related to food security, climate change, and environmental conservation. By bringing together diverse perspectives, we hope to inspire new collaborations and strengthen networks that will not only benefit research development but also contribute significantly to the well-being of communities and ecosystems.

On behalf of the organizing committee, I extend my sincere gratitude to all keynote speakers, presenters, and participants for their contributions and commitment. I would also like to sincerely thank all the committee members for their hard work, dedication, and support in ensuring the success of this event. My appreciation also goes to the UPM Sarawak management for their encouragement and support in making this seminar possible.

“Research is seeing what everybody else has seen and thinking what nobody else has thought.”

Thank you.

# FOREWORD

**DR. ANYSIA HEDY UJAT**
EDITOR IN CHIEF, IPSAF 2025
UNIVERSITI PUTRA MALAYSIA SARAWAK

It is my honour to present the e-Proceeding of the International Postgraduate Seminar on Agriculture and Forestry 2025 (I-PSAF 2025), organized by the Faculty of Agricultural and Forestry Sciences, Universiti Putra Malaysia Sarawak (UPMS). This year's theme, "*Technological Innovations in Sustainability*", reflects the need for innovative approaches that balance progress with environmental responsibility.

This seminar is especially significant as it is the first to be held under the name Universiti Putra Malaysia Sarawak (UPMS), following the official renaming of the former UPM Bintulu Campus. This milestone marks a new chapter for the university and affirms its role in advancing sustainability-focused research and education. The works included here highlight the contributions of postgraduate scholars whose research addresses critical issues in agriculture, forestry, and related disciplines.

The proceedings serve as a record of the knowledge shared during the seminar. Each paper represents not only the scientific effort of its author but also the wider commitment of our research community to provide solutions that support sustainable development.

I wish to extend sincere appreciation to the authors for their valuable contributions, to the reviewers for their constructive input, and to the organizing committee for their dedication in ensuring the success of this publication.

May this e-proceeding not only serve as a record of the seminar, but also as a reminder that our postgraduate scholars represent the next generation of researchers whose ideas, curiosity, and commitment will continue to shape the future of agriculture and forestry.

## Organizing Committee

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# Application of Biochar-Enriched Spent Coffee Compost and Actinobacteria Improves Maize Root Architecture

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## ABSTRACT

*This study examined the effects of compost from spent coffee grounds combined with agro-industrial byproducts like rice husk biochar, palm kernel shell biochar, and dried empty fruit bunches, with or without actinobacteria (Streptomyces sp.) on maize root development. Maize plants were subjected to ten treatments in a greenhouse trial, and their root length and surface area were quantified using WinRHIZO. Actinobacteria positively affected root morphology, with Treatment 2 (soil plus Streptomyces sp.) showing the greatest improvement. Meanwhile, compost only treatments showed limited improvement. In contrast, the combination of SCG based composts with actinobacteria, particularly Treatment 8, produced synergistic effects comparable to the microbial only control. Multivariate analysis (Wilks' Lambda=0.0979, F(18, 78)=9.52, p<0.001) confirmed that the treatments significantly influenced both root traits. These findings support the use of composted organic waste and beneficial microbes as a sustainable strategy to improve root architecture and nutrient uptake in maize*.

**Keywords:** spent coffee ground, biochar enriched compost, actinobacteria, maize root, sustainable soil amendment

## INTRODUCTION

Since 2020, global coffee consumption has surged, resulting in the generation of millions of tons of spent coffee ground (SCG) each year (Czekała et al., 2023). While SCG constitutes a significant organic waste, its direct application in agriculture is limited

due to the presence of phytotoxic compounds, such as caffeine (Pérez-Burillo et al., 2022). However, composting SCG with biochar presents a safe and sustainable option for reuse. Biochar offers multiple agricultural benefits, enhancing soil composition, boosting microbial activity, and promoting plant health (Murtaza et al., 2023). Its porous structure supports beneficial microbes like actinobacteria (Ren et al., 2020). Microbial communities play a crucial role in nutrient cycling, disease suppression, and root system enhancement.

Research conducted by Yan et al. (2022) highlights that the use of biochar significantly enhances microbial diversity, which contributes to improved soil resilience and ecological balance. The interaction between biochar and actinobacteria positively influences soil and root characteristics. Research has demonstrated that biochar-amended soils promote the proliferation of actinobacteria, thereby enhancing plant-microbe interactions within the rhizosphere (Yu et al., 2023).

When used in conjunction, biochar and actinobacteria work synergistically to improve maize root architecture and enhance soil aggregation (Silva et al., 2022; Boubekri et al., 2022). This study is designed to evaluate the effects of composted spent coffee grounds in conjunction with biochar, followed by the application of actinobacteria during the cultivation of sweet maize. The investigation focuses on key parameters, including root length and surface area, to determine how these integrated amendments can transform harmful waste into beneficial inputs for sustainable agricultural practices

## PROJECT APPROACHES

*Materials*

SCG was collected from R-Brand coffee shop in Bangi to create four compost formulations: H1 (SCG only), H2 (SCG with rice husk biochar), H3 (SCG, rice husk biochar, and dried empty fruit bunch), and H4 (SCG with palm kernel shell biochar). The composting process, based on Zainudin et al. (2022), lasted 10 weeks using aerated static piles and aimed for an initial moisture content of 60%. Each compost heap was about 120 cm tall and was turned weekly for aeration.

The composts were tested for their effects on maize root development with and without *Streptomyces* sp. strain PU-MM66, isolated from Sungai Menyala Forest Reserve (Adenan et al., 2024). For relevant treatments, 20 mL of a $10^8$ CFU/mL suspension in Tryptic Soy Broth was applied via soil drench during transplanting (Le et al., 2021; Chai et al., 2022). Cultivation was conducted in a greenhouse at Universiti Putra Malaysia using a completely randomized design with ten treatments. Maize was grown in 20 kg polybags of topsoil, organized as follows: T1 (soil only), T2 (soil + actinobacteria), T3 (soil + H1), T4 (soil + H1 + actinobacteria), T5 (soil + H2), T6 (soil + H2 + actinobacteria), T7 (soil + H3), T8 (soil + H3 + actinobacteria), T9 (soil + H4), and T10 (soil + H4 + actinobacteria), each replicated five times.

This study, based on Abdullah et al. (2022), applied compost in polybags for maize at a

1:5 to 1:10 compost-to-soil ratio (10–20% compost). All plants received twice 600 mL of water daily (Reeza et al., 2023), and NPK 15-15-15 fertilizer was applied per MARDI recommendations. Post-harvest (day 80), roots were extracted, cleaned, and weighed. Morphological characteristics, including total root length, surface area, and volume, were measured using WinRHIZO (Regent Instruments Inc., Quebec, Canada) and a scanner.

## FINDINGS

The longest root length was in Treatment 2, with soil supplemented with actinobacteria. Treatment 6, combining compost from SCG and rice husk biochar with actinobacteria, followed next. Treatment 8 included compost from SCG, rice husk biochar, and shredded empty fruit bunches with actinobacteria, while Treatment 10 utilized compost from SCG and palm kernel shell biochar, also enriched with actinobacteria (Figure 1).

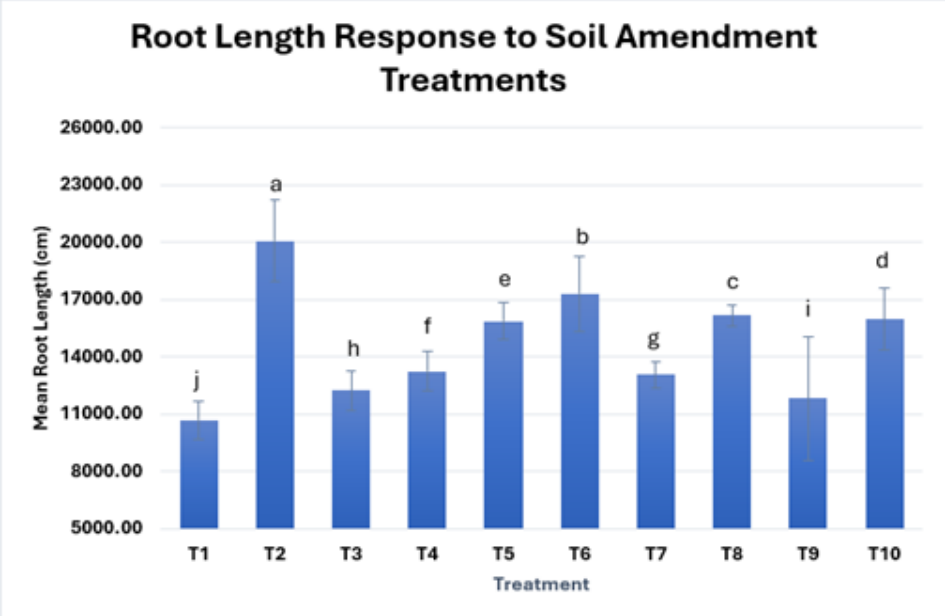


**Figure 1:** Mean root length of maize for each treatment. Error bars show standard deviation (n=5). Different letters indicate significant differences (Tukey's HSD, $p<0.05$). **T2** had the highest root length and was significantly better than all others, while **T1** had the lowest and differed significantly from all treatments

Based on the average maize root length for each treatment shown in Figure 1, a one-way ANOVA revealed a significant effect of treatment on root length ($F(9, 40)=15.86$, $p<0.001$), with mean values ranging from 10,686.55±1,014.72 cm in the control group to 20,088.08±2,141.71 cm in the best-performing treatment. Post-hoc Tukey's HSD test confirmed that all treatments were significantly different from one another ($p<0.05$). These results highlight the beneficial role of *Streptomyces* sp. based microbial inoculants and tailored composts in promoting root development in maize (Nozari et al., 2021).

As for the root surface area, one-way ANOVA showed a significant effect of soil

treatments on maize root surface area ($F(9, 40)=6.98$, $p<0.001$). The mean surface area ranged from 2,644.47±243.64 cm² in the control (T1) to 3,854.12±514.62 cm² in the best treatment (T2) (Figure 2).

Based on the mean root surface area of maize for each treatment shown in Figure 2, Tukey's HSD test revealed that treatment T2, which involved soil amended with *Streptomyces* sp., resulted in significantly greater root surface area compared to all other treatments. Additionally, treatments utilizing actinobacteria with enriched compost, specifically T4, T3, and T6 exhibited notable increases in root surface area compared to the control group. In contrast, T1 consistently yielded the lowest measurements. The findings align with Alori et al. (2019), emphasizing that combining microbial inoculants with compost enhances root surface area, which is vital for improving nutrient uptake in maize.

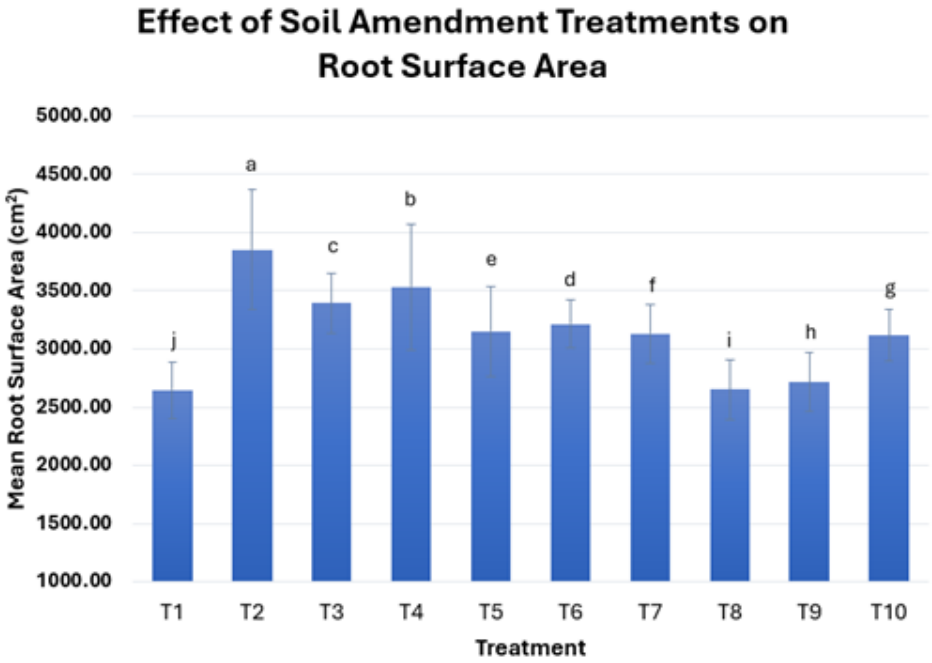


**Figure 2:** Mean root surface area of maize for each treatment. Error bars show standard deviation (n=5). Different letters indicate significant differences (Tukey's HSD, $p<0.05$). T2 had the highest surface area and was significantly better than all others, while T1 recorded the lowest and differed significantly from all treatments.

A multivariate analysis of variance (MANOVA) was conducted to examine the effect of compost treatments formulated with spent coffee grounds, various biochar, dried empty fruit bunches (EFB), and actinobacteria (*Streptomyces* sp.) on maize root morphology.

**Table 1**. Multivariate Analysis of Variance (MANOVA) Using Wilks' Lambda for Root Length and Surface Area

| Statistic | Value | Num DF | Den DF | F Value | p-value |
|---|---|---|---|---|---|
| **Wilks' Lambda** | 0.0979 | 18 | 78 | 9.52 | < 0.001 |

The analysis revealed a significant multivariate treatment effect, as indicated by Wilks' Lambda=0.0979, $F(18, 78)=9.52$, $p<0.001$. The low Wilks' Lambda value shows that root length and surface area variations were significantly influenced by compost and microbial amendments (Table 1).

This highlights that using SCG-based composts with rice husk biochar, palm kernel shell biochar, dried empty fruit bunches, and *Streptomyces* sp. enhances root development and nutrient absorption in maize. Improved root systems allow maize to access deeper soil layers, optimizing the use of nitrogen (N), phosphorus (P) and water, which are often limiting factors in crop production (Mishra et al., 2024).

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# Effect of Different Vaccination Programs on Growth Performance and Immune Status of Commercial Broiler Chickens

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## ABSTRACT

*This study investigated the effects of different infectious bronchitis virus (IBV) vaccination protocols on the immune response and growth performance of Arbor Acres broiler chickens. Four treatment groups were tested: an unvaccinated control, a single IBV strain (Mass 41 or 4-91), and a combined IBV strain group (Mass 41+4-91). Serological analysis revealed 100% seropositivity in most groups, with the highest average titre in the control group due to the post-infection immune response. RT-PCR and phylogenetic analysis confirmed the presence of QX-like IBV strains closely related to Chinese isolates, indicating regional adaptation. The combined vaccine group (T4) showed the best performance in terms of final body weight, bodyweight gain, and feed conversion efficiency. These findings suggest that combined vaccination provides broader protection and enhances production outcomes. This study highlights the importance of genotype-specific vaccination and ongoing surveillance to control IBV effectively and improve broiler health and productivity in endemic regions.*

**Keywords:** infectious bronchitis virus, broiler chickens, immune response, growth performance, vaccination protocol

## INTRODUCTION

Infectious bronchitis virus (IBV) is a highly contagious gamma coronavirus responsible for a significant loss of poultry production worldwide. It primarily infects the respiratory tracts of chickens, but nephropathogenic and reproductive strains are present, with progressively more virulent clinical presentations (Bhuiyan et al., 2021a). In broilers, IBV QX infection manifests as respiratory distress, growth retardation, and secondary bacterial infections. IBV QX control is challenging due to its antigenic variation and high prevalence (Barberis et al., 2018). Effective immunization efforts, therefore, remain vital in safeguarding both broiler and breeder flocks.

Vaccination is the mainstay for IBV control. However, due to the genetic variability of circulating field strains, a single-strain vaccine may not offer sufficient cross-protection. The strategic use of both killed and live vaccines in a prime-boost regimen overall offers a promising avenue against the effects of IBV QX in poultry (Shao et al., 2020). For broiler flocks, this translates into reduced early-life susceptibility and improved growth

performance (Chung et al., 2020). Despite ongoing difficulties with implementation and customisation, the mounting volume of evidence underscores the merit of this combined strategy as a foundation for sustainable control of IBV QX in modern poultry production (Leow et al., 2018).

## PROJECT APPROACHES

*Materials*

A total of 7,200 Arbor Acres broiler chickens were randomly assigned to four treatment groups, each consisting of five replicates. All birds received inactivated vaccinations prior to the administration of live vaccines on the first day of age. The four treatment groups were organized based on different vaccination protocols against infectious bronchitis virus (IBV). Treatment 1 (T1) served as the unvaccinated control group and did not receive any live vaccination on days 1 and 14. Treatment 2 (T2) broilers were vaccinated with a single IBV strain, Massachusetts (Mass 41). Treatment 3 (T3) broilers were vaccinated with another single IBV strain, Nobilis 4-91. Treatment 4 (T4) birds received a combination of both IBV strains Mass 41 and Nobilis 4-91 administered on days 1 and 14 of age.

The study was conducted over a 35-day period, during which data and biological samples were collected to evaluate both immunological response and growth performance. Immune response assessment was carried out using two diagnostic methods: enzyme-linked immunosorbent assay (ELISA), using a commercial IDEXX kit, and reverse transcription-polymerase chain reaction (RT-PCR), followed by gene sequencing to confirm the presence of IBV strains (Leow et al., 2018).

Growth performance was monitored throughout the study using standard broiler production parameters, including body weight (BW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) (Chung et al., 2020). These indicators were used to compare the efficacy of the different vaccination protocols in supporting optimal broiler health and productivity. This experimental setup aimed to determine whether single or combined live IBV vaccinations provide better protection without compromising growth performance in commercial broiler production systems.

## FINDINGS

Serological analysis using ELISA showed a high seroprevalence rate of 100% in T1, T2, and T4. The mean antibody titre was highest in T4 (4,506) and lowest in T1 (3,996), with the highest individual titre observed in T3 (14,007). The S/P ratio was highest in T4 (1.08±0.55) and lowest in T1 (0.96±0.77). RT-PCR confirmed IBV presence in 30.0% (12/40) of the samples. Among them, T1 had the highest challenge rates (60%), followed by T2 (20%) and T4 (10%). Molecular characterization via RT-PCR revealed amplification at 371 b. Phylogenetic analysis placed the samples isolate within the QX-like clade, clustering closely with Malaysian and Chinese variants. Although bootstrap support values were not provided in the report,

the genetic identity (98.7%) and query coverage (100%) confirm the robustness of this classification (Leow et al., 2018).

Growth performance data showed that all groups had similar trends from day 1 to 35. However, differences became more apparent from day 7 onward. T2 had significantly higher body weight than T1 and T3 at day 7 ($p<0.05$), suggesting improved early development. At day 28, T4 was significantly heavier than T1 and T3. By day 35, T4 had the highest average body weight (2049.45 g), significantly greater than T1 and T2 ($p<0.05$), indicating superior overall growth. Body weight gain (BWG) differed significantly between treatments in weeks 1 and 4. T2 had the highest BWG at week 1, while T4 had the highest at week 4. Feed intake (FI) varied significantly from weeks 1 to 3. T3 showed the highest intake at week 1, while T1 had significantly higher intake in weeks 2 and 3. Feed conversion ratio (FCR) differences were only significant in week 1, where T3 had a significantly poorer FCR (1.20) compared to T2 (1.08) and T4 (1.11).

## CONCLUSION

This study evaluated the impact of different IBV vaccination programs on the immune response and growth performance of Arbor Acres broiler chickens. Four treatment groups were tested, including single and combined live IBV vaccines (Mass 41 and Nobilis 4-91), alongside an unvaccinated control. Results demonstrated that combined vaccination (T4) produced superior outcomes, including the highest final body weight and the lowest infection rate. Serological and molecular analyses confirmed high seroprevalence and the circulation of QX-like IBV strains in Sabah. RT-PCR and phylogenetic analyses identified genetic similarities between local isolates and Chinese QX variants, indicating potential regional adaptation. Significant differences were observed in body weight gain, feed intake, and feed conversion ratio across treatments, particularly favouring T4 in the mid-to-late stages. This trial highlights the value of using combined IBV vaccines to enhance broiler immunity and productivity while emphasizing the need for continuous monitoring of evolving local IBV strains.

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# Gas Chromatography-Mass Spectrometry (GC-MS) Validated Microbial Inoculation: Saving Wild Agarwood Through Sustainable Biotechnological Cultivation

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## ABSTRACT

*This study develops an innovative fungal biotechnology to accelerate resin formation in Aquilaria species for sustainable agarwood production. Three microbial consortia, including F1 (Trichoderma sp. and Aspergillus sp.), F2 (Fusarium sp. and Penicillium sp.), and F3 (Trichoderma sp., Lasiodiplodia sp. and Curvularia sp.), were inoculated into Aquilaria species using a bottle drip method, an efficient and low-cost technique. Gas Chromatography-Mass Spectrometry (GC-MS) analysis revealed that F3 produced the superior resin yield, with exclusive aromatic compounds (e.g., guaia-1(10),11-diene and cryptomeridiol) during the dry seasons. This biotechnological approach using optimised fungal consortia and inoculation methods can enhance agarwood production sustainably, reducing reliance on wild harvesting. This study method aligns with green technology and SDG 15 by promoting eco-friendly cultivation, minimising environmental impact, and meeting global demand without endangering natural populations. The success of F3 inoculants demonstrates the potential of fungal biotechnology as a scalable solution for sustainable agarwood industries.*

**Keywords:** agarwood, microbial inoculants, GC-MS, sustainable biotechnology, green innovation

## INTRODUCTION

The global demand for agarwood, a highly valuable resin in the trees *Aquilaria*, *Gonystylus* and *Gyrinops* spp. has led to severe overharvesting of wild trees in natural forests, threatening their survival. With a premium grade of agarwood that can reach up to USD 100,000 per kilogram (Justin et al., 2020), sustainable alternatives are necessitated. Wild agarwood formation occurs naturally over decades, making wild harvesting both ecologically damaging and economically unsustainable (Chhipa & Kaushik, 2017). Hence, this study presents an innovative biotechnological approach by developing optimised fungal consortia to accelerate the resin formation in cultivated *Aquilaria* species.

Three microbial consortia (F1: *Trichoderma* sp. and *Aspergillus* sp., F2: *Fusarium* sp.

and *Penicillium* sp., F3: *Trichoderma* sp., *Lasiodiplodia* sp. and *Curvularia* sp.) were injected into *Aquilaria* trees by using the bottle drip method (Tang, 2012; Justin et al., 2020). GC-MS analysis served as a validation tool for the inoculated agarwood quality by investigating: (1) determining the key aromatic compounds (sesquiterpenes and chromones), (2) comparing resin quality across consortia, and (3) assessing seasonal variations in metabolic profiles. This research bridges the gaps in forestry biotechnology by replacing destructive wild harvesting with controlled cultivation and establishing scientific protocols for quality assessment in artificially induced agarwood. This study aligns with the United Nations Sustainable Development Goal 15 (Life on Land) by promoting biodiversity conservation while supporting economic development through sustainable forest management (United Nations, 2025). The integration of microbial biotechnology with advanced analytical validations brings significant benefits in balancing ecological preservation while meeting market demands for this valuable natural resource.

## PROJECT APPROACHES

*Materials*

This study uses a biotechnological approach to enhance agarwood formation by inoculating the *Aquilaria* species with three optimised fungal consortia (F1: *Trichoderma* sp. and *Aspergillus* sp., F2: *Fusarium* sp. and *Penicillium* sp., F3: *Trichoderma* sp., *Lasiodiplodia* sp. and *Curvularia* sp.) using the bottle drip method, a cost-efficient inoculating technique (Tang, 2012; Justin et al., 2020). The selection of fungal strains was based on their synergistic effects and their known roles in resin induction. GS-MS was used to identify the biochemical compounds (e.g., sesquiterpenes and chromones) present in the resin formed. It is also to compare consortia performance and assess the seasonal variations in the metabolic profiles. The study also aligned with SDG 15 by implementing sustainable practices in minimising environmental impacts. This approach combines biotechnology and analytical chemistry to provide a scalable, eco-friendly solution for agarwood cultivation.

## FINDINGS

GC-MS analysis revealed that the fungal consortium F3 (*Trichoderma* sp., *Lasiodiplodia* sp., and *Curvularia* sp.) demonstrated the best resin quality, with its chromatographic profiles showing a 28% higher sesquiterpene content compared to controls ($p < 0.05$). The inoculated F3 agarwood also exhibited unique aromatic compounds, including guaia-1(10),11-diene (0.45%) and cryptomeridiol (0.93%). These compounds are also previously linked to premium-grade agarwood oil (Ahmed and Kulkarni, 2017), validating the consortium's ability to induce commercially valuable resin profiles within months rather than decades. Three key innovations emerged: (1) Consortium-Specific Synergy, F3 outperformed the other inoculants by enhancing resin/terpenoid pathways, yielding major aromatic compounds (sesquiterpenes, chromone, and fatty acid), while avoiding antimicrobial interference seen in less effective inoculants such as F2 (*Fusarium* sp. and *Penicillium* sp.)

which underperformed due to interference by *Penicillium's* antimicrobial activity. (2) Seasonal variation, with dry season inoculations yielding 35–42% higher peak intensities and 89% abundance of key chromones, suggesting commercial quality. (3) Quality benchmarking, as F3 resin matched 78% of chemical compounds found in wild *Aquilaria* (NIST library) and introduced a novel compound, crytomeridiol, previously unreported in agarwood, which was identified as a potential quality indicator.

The study validates that fungal consortia, particularly F3, outperform physical wounding in agarwood induction, with seasonal timing significantly impacting resin quality. As F3 can replicate premium "wild-type" aromas within months, this method offers a scalable and conservation-friendly agarwood production. Ultimately, this highlights the crucial role of microbial biotechnology in sustainable agroforestry, which balances ecological conservation with economic viability.

## CONCLUSION

This study presents GC-MS as an essential tool for optimising sustainable agarwood production. The F3 microbial consortium has been validated through comprehensive metabolic profiling and offers a commercially viable solution that could reduce the resin induction period from decades to months while replicating the chemical constituents of premium wild agarwood. By optimising dry-season inoculation and the bottle drip inoculation methods, we can further enhance resin yield and consistency. These findings establish a sustainable biotechnology platform that aligns with both ecological conservation needs (SDG 15) and industry demands. Future research should explore field-scale applications and the genetic mechanisms underlying the observed fungal-plant interactions. In this regard, analytical chemistry can bridge conservation and commercial forestry objectives.

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# Assessment of Proximate Composition and Antioxidant Properties of *Chlorella vulgaris* In Comparison to Conventional Feed Ingredients

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## ABSTRACT

*Chlorella vulgaris is increasingly recognized as a functional alternative to conventional feed in ruminant diets. This study evaluated the proximate composition and antioxidant capacity of C. vulgaris compared to soybean meal and black soldier fly. Proximate analysis was conducted to determine the nutritional profile, while antioxidant activity was assessed through 2,2-diphenyl-1-picrylhydrazyl assay (DPPH), ferric reducing ability of plasma assay (FRAP), and total polyphenol content assays. C. vulgaris yields the highest value of DM (93.10 g), and ash (8.40 g), yet exhibited lower crude fat content (0.85g). Antioxidant properties, including 2,2-diphenyl-1-picrylhydrazyl assay (DPPH), ferric reducing ability of plasma assay (FRAP), and total polyphenol content assays, were also recorded as the highest in Chlorella vulgaris by 0.15 mg AAE/ml of FRAP, 81.86% of DPPH, and 291.23 mg GAE/g of TPC. Therefore, C. vulgaris possesses strong nutritional composition and antioxidant potential as a viable partial substitute for conventional feed in ruminant feeding strategies.*

**Keywords:** proximate analysis, antioxidant analysis, *Chlorella vulgaris*, soybean meal, black soldier fly

## INTRODUCTION

Sustainable intensification of animal production requires nutritionally balanced diet with protein sources being critical to livestock growth and productivity. Soybean meal (SBM), the predominant plant-based protein, is valued for its favourable amino acid composition and low anti-nutritional factors (ANFs) (Schwarz et al., 2021). However, its sustainability is increasingly challenged by market volatility and ecological concerns such as deforestation and greenhouse gas emissions. These challenges highlight the

urgent need for alternative protein sources that can support animal performance while reducing environmental burdens.

Insects and microalgae have emerged as promising candidates for sustainable livestock nutrition. The Black Soldier Fly (BSF; *Hermetia illucens*) efficiently converts organic waste into protein-rich biomass (Campbell et al., 2020) and exhibits a low carbon and water footprint, underscoring its ecological potential. Similarly, *Chlorella vulgaris* (CV), a unicellular green alga, is notable for its high protein content (50–60%) and bioactive compounds, including peptides, polyunsaturated fatty acids (PUFAs), and pigments (Abdelnour et al., 2019; Saadaoui et al., 2021) that may enhance animal health and productivity.

Despite the recognized benefits, comparative assessments of BSF, CV, and SBM, particularly in terms of nutrient bioavailability and antioxidant potential, remain sparse within the context of animal dietary supplementation. Therefore, this study aimed to assess the nutritional composition and antioxidant potential of *Chlorella vulgaris* and BSF in relation to SBM, which may provide insights into the utilization of these materials as a supporting strategy for sustainable protein alternatives in the animals' diets.

## PROJECT APPROACHES

*Materials*

Commercial powder of *Hermetia illucens* (black soldier fly; BSF, T1) and soybean meal (SBM, T2) were obtained from the local producer, while *Chlorella vulgaris* (CV, T3) was obtained from an e-commerce supplier (Alibaba.com). The samples were ground by using a hammer mill (1mm screen) and stored in vacuum-sealed bags at -20°C until further analysis. The samples were further analysed for their proximate composition, and antioxidant properties. All processing methods were performed in triplicate for each treatment using a completely randomized design.

All nutritional parameters of dry matter, ash, crude protein (CP), and crude fat (CF) were measured according to the Association of Official Analytical Chemists (AOAC, 1990). Antioxidant activities of the samples were assessed using three assays: (1) 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, (2) ferric reducing antioxidant power (FRAP), and total polyphenol content (TPC), following the methods described by Singleton et. al. (1999), with slight modification.

Data were analysed by analysis of variance (ANOVA) within the General Linear Model (GLM) procedure of SAS software (Statistical Analysis System software version 9.4; SAS Institute Inc., Cary, NC, USA). Significant differences between means ($p<0.05$) were compared using Duncan's multiple range test.

## FINDINGS

*Chlorella vulgaris* is widely recognized for its valuable nutritional constituents, particularly

high crude protein content, complemented by a diverse array of bioactive compounds (Kholif & Olafadehan, 2021). This study revealed that *Chlorella vulgaris* yields a notably high dry matter (DM) content of 93.10 g and ash content of 8.40 g, while its crude fat content remains comparatively low at 0.85 g, relative to soybean meal and black soldier fly. Interestingly, the crude protein content from the three respective materials was not significantly different from each other ($p>0.05$), suggesting that *Chlorella vulgaris* may serve as a viable alternative protein source in ruminants' diets.

Furthermore, *Chlorella vulgaris* exhibited superior antioxidant properties, as evidenced by the highest recorded value across several assays: 0.15 mg AAE/ml of FRAP, 81.86% of DPPH, and 291.23 mg GAE/g of TPC compared to soybean meal and black soldier fly. This pronounced antioxidant activity, attributed to the abundance of phytochemicals inherent in microalgae (Madeira et al., 2017), positioned *Chlorella vulgaris* as a highly valuable feed additive. The nutritional and bioactive profile underscores its potential application not only as a protein-rich dietary supplement in ruminant nutrition but also as a functional ingredient with ancillary roles in animal health and biofuel production. Consequently, *Chlorella vulgaris* emerges as a perfect candidate for integration into sustainable feed strategies.

## CONCLUSION

This study highlights the potential of *Chlorella vulgaris* as a sustainable source of protein and bioactive compounds. The findings suggest that incorporating *Chlorella vulgaris* may improve the protein content and antioxidant properties of feed components. Moving forward, future studies should delve deeper into the animal application and subsequently investigate the scalability and feasibility of *Chlorella vulgaris* utilization to bridge the gap in real-world applications. Ultimately, this study contributes to the growing body of research supporting the incorporation of microalgae as a substitute for conventional protein sources, thus paving the way for more sustainable food systems and solutions.

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# Evaluation of Hydrothermally Synthesized Fe-Doped $BiVO_4$ for Photocatalytic Activity under Low-Intensity UV Irradiation

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## ABSTRACT

*This study demonstrates the potential of Fe-doped $BiVO_4$ as an efficient photocatalyst for the degradation of tetracycline (TC). The Fe-doped $BiVO_4$ was synthesized using the hydrothermal method, resulting in a monoclinic heterostructure with a crystallite size ranging from 27.37 to 29.10 nm, and an optical band gap of 2.69-2.87 eV. Photocatalytic tests revealed that 3 wt% Fe-doping exhibited the highest degradation efficiency, removing 73.37% of TC in 120 minutes under a low-power (13W) UV lamp. The enhanced photocatalytic performance was attributed to the improved electron-hole separation and optimized Fe doping concentration, which facilitated more efficient light absorption and charge carrier dynamics. These findings contribute to global efforts in mitigating waterborne antibiotic and microbial pollution, particularly in regions where access to high-energy treatment technologies is limited.*

**Keywords**: hydrothermal, photocatalysis, antibacterial, Fe-doping, water treatment

## INTRODUCTION

Water sources are contaminated by chemicals and microbes from diverse origins, disrupting ecosystems and limiting water. Significant contributors to this crisis include household chemicals, industrial effluents, agricultural runoff, and a range of emerging contaminants (ECs). ECs such as pesticides, personal care products, endocrine disruptors, and antibiotics are commonly present in wastewater, groundwater, and surface waters at concentrations higher than anticipated, posing ongoing risks to ecosystems and human health (Li et al., 2024). Among these ECs, the antibiotic tetracycline (TC) is particularly concerning. Widely used in medical, veterinary, and agricultural sectors for its affordability and broad-spectrum antibacterial properties, TC contamination in water bodies introduces several risks, including toxicity, endocrine disruption, and the development of antibiotic-resistant bacteria.

Bismuth vanadate ($BiVO_4$) is a promising semiconductor photocatalyst for solar-driven applications, valued for its eco-friendly synthesis, minimal secondary pollution, and ability to degrade pollutants via reactive oxygen species (ROS) generation. However,

$BiVO_4$'s photocatalytic activity is limited by rapid electron-hole recombination and relatively poor adsorption efficiency (Kamble et al., 2023). To address these challenges, modifications such as ion doping, noble metal incorporation, and heterojunction formation have been investigated to enhance $BiVO_4$'s performance. Specifically, doping with transition metals has shown potential for improving photocatalytic efficiency by reducing recombination rates and expanding light absorption (Kalidasan et al., 2024; Sultana et al., 2023). Doping semiconductors with metal and non-metal ions has proven effective in reducing the recombination rate of photoinduced charge carriers (Zheng et al., 2024). Previous study has found that the substitution of a fraction of $Bi^{3+}$ ions with $Fe^{3+}$ ions represents an effective strategy to augment the photoexcitation mechanisms and the adsorption characteristics of $BiVO_4$, culminating in a significant enhancement in the selective partial oxidation of methane (Afriyie & Zhang, 2024). By examining these properties, this work aims to optimize photocatalytic efficiency for TC degradation under low-power UV light, addressing crucial challenges in sustainable environmental applications.

## PROJECT APPROACHES

### *Materials*

Bismuth (III) nitrate pentahydrate ($Bi(NO_3)_3 \cdot 5H_2O$) and ammonium metavanadate ($NH_4VO_3$) were obtained from Chemiz (M) Sdn. Bhd, while iron (II) sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$), with 99% purity, was purchased from Mallinckrodt Baker, Inc (USA). Tetracycline hydrochloride (TC) was obtained from Wako Chemicals, Japan, and the reagents concentrated nitric acid ($HNO_3$) and sodium hydroxide (NaOH) were from Sigma-Aldrich. All reagents were of analytical grade and used without further purification.

### *Preparation of $BiVO_4$ and Fe-Doped $BiVO_4$*

$BiVO_4$ and Fe-doped $BiVO_4$ were synthesized hydrothermally using a Teflon-lined autoclave as shown in Figure 1 as previously reported (Hemavibool et al., 2022).

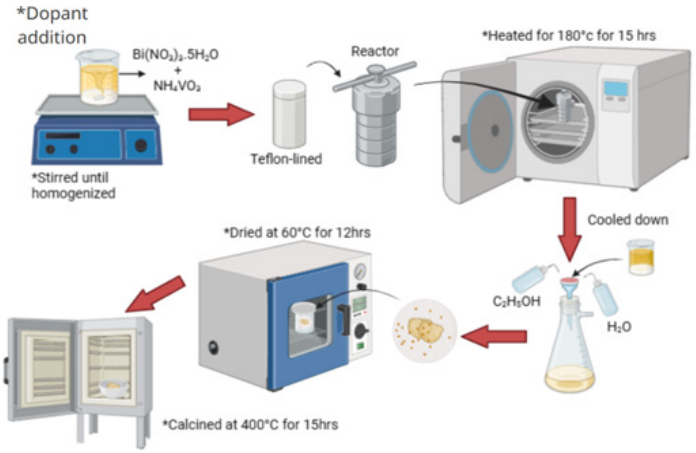


**Figure 1:** Flowchart for synthesis of bare and Fe-doped $BiVO_4$.

*Characterization*

XRD measurements were carried out using a Shimadzu XRD-6000 diffractometer. Scanning was conducted in the 2θ range of 10–80° with a step size of 0.02° and a scanning speed of 1°/min. Crystallite sizes were calculated using the Scherrer equation (Eq. 1), focusing on shifts in diffraction patterns to observe any phase changes due to Fe incorporation.

$$D = k\lambda/\beta \cos \theta \quad \text{(Eq. 1)}$$

where, D is the average crystallite thickness perpendicular to the crystal plane direction, k=0.89 is the Scherrer constant of the diffraction peak, λ=1.54056 nm is the X-ray wavelength of Cu-Kα radiation, θ is the diffraction angle, and β is the half peak width.

*Photocatalytic Testing*

The photocatalytic activity was evaluated using TC degradation under low-intensity UV light irradiation. A 13W UV aquarium lamp was positioned 10 cm above the reaction vessel to maintain consistent light exposure. Prior to illumination, the catalyst was suspended in the TC solution and stirred in the dark for 30 minutes to establish adsorption-desorption equilibrium, verified by preliminary trials. For degradation measurements, 10 mL aliquots were sampled every 15 minutes, centrifuged, and analyzed at 357 nm. The TC degradation efficiency, based on concentration changes, was calculated using a pseudo-first-order (PFO) kinetic model to compare degradation rates across samples.

## FINDINGS

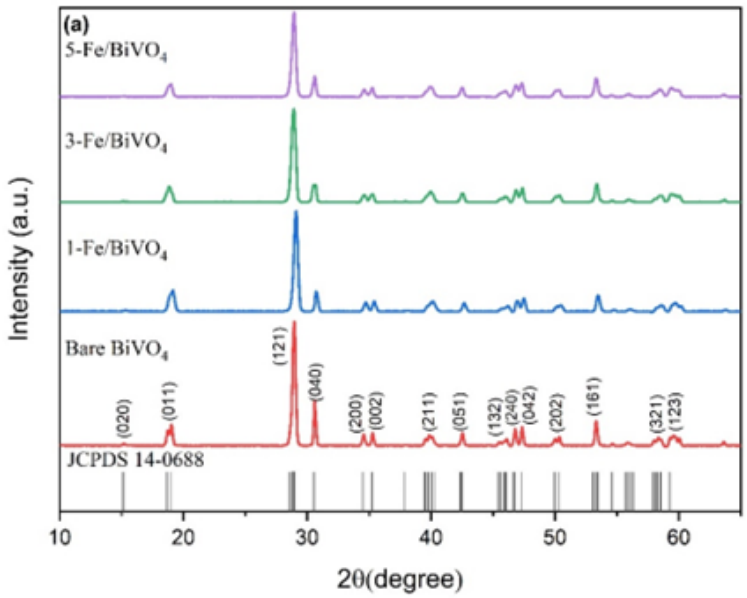


**Figure 2:** XRD patterns of $BiVO_4$ with various wt% of Fe dopant.

The X-ray diffraction (XRD) patterns of bare and Fe-doped $BiVO_4$ samples were obtained to confirm the crystal structure, phase purity, and any structural modifications induced by Fe doping as shown in Figure 2.The XRD patterns of the bare $BiVO_4$ samples display characteristic peaks at 2θ values of 18.9° (011), 28.9° (121), 30.6° (040), 34.6° (200), and others, which correspond to the monoclinic scheelite structure of $BiVO_4$ (JCPDS No. 14-0688). This confirms that the synthesized BiVO4 has the desired monoclinic crystal phase (Mammeri et al., 2024). Notably, no additional peaks are observed after Fe doping, indicating that Fe incorporation did not introduce secondary phases or impurities and that the material maintains its phase purity and structural stability (Silambarasan et al., 2015). This stability is advantageous for photocatalytic applications, as it suggests that Fe doping enhances the photocatalyst's properties without compromising its structural integrity.

*Photodegradation test*

The photocatalytic degradation of TC under low-power UV irradiation was investigated using bare and Fe-doped $BiVO_4$ photocatalysts, as shown in Figure 3. The degradation performance was monitored by the concentration ratio ($C/C_0$), where $C_0$ represents the initial concentration of TC, and C is the concentration at different time intervals. Figure 3(a) demonstrates the variation of TC concentration over time under both dark and light conditions. In the dark phase (0–30 min), no significant reduction in TC concentration was observed for any of the photocatalysts, indicating minimal adsorption of TC onto the photocatalyst surfaces. This behavior confirms that the degradation mechanism is predominantly photocatalytic and not due to adsorption. Upon exposure to light, the degradation of TC is significantly enhanced, particularly for Fe-doped $BiVO_4$ samples.

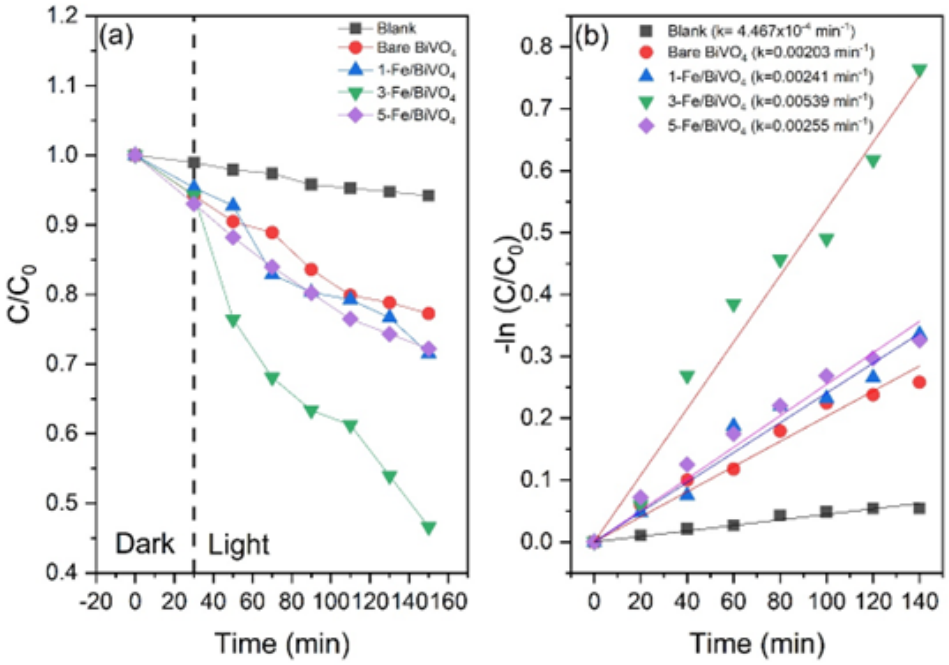


**Figure 3: (a)** Degradation profiles showing TC concentration ($C/C_0$) versus time for different photocatalysts under dark and UV irradiation. **(b)** Kinetic plots based on PFO model fitting with corresponding rate constants ($-\ln(C/C_0)$ vs. time).

Among the tested photocatalysts, 3 wt% Fe-doped $BiVO_4$ exhibited the highest TC degradation efficiency, achieving 73.37% within 120 minutes. In contrast, bare $BiVO_4$ and other Fe-doped samples (1 wt% and 5 wt%) showed relatively moderate photocatalytic activities. Figure 3(b) presents the kinetic plots of $-\ln(C/C_0)$ versus time for TC degradation. The highest rate constant was observed for 3 wt% Fe-doped $BiVO_4$, which was approximately 2.65 times higher than that of bare $BiVO_4$, demonstrating enhanced photocatalytic efficiency due to improved charge separation and surface reactivity.

The photocatalytic degradation of TC under low-power UV irradiation was investigated using bare and Fe-doped $BiVO_4$ photocatalysts, as shown in Figure 3. The degradation performance was monitored by the concentration ratio ($C/C_0$), where $C_0$ represents the initial concentration of TC, and C is the concentration at different time intervals. Figure 3(a) demonstrates the variation of TC concentration over time under both dark and light conditions. In the dark phase (0–30 min), no significant reduction in TC concentration was observed for any of the photocatalysts, indicating minimal adsorption of TC onto the photocatalyst surfaces. This behavior confirms that the degradation mechanism is predominantly photocatalytic and not due to adsorption. Upon exposure to light, the degradation of TC is significantly enhanced, particularly for Fe-doped $BiVO_4$ samples. Among the tested photocatalysts, 3 wt% Fe-doped $BiVO_4$ exhibited the highest TC degradation efficiency, achieving 73.37% within 120 minutes. In contrast, bare $BiVO_4$ and other Fe-doped samples (1 wt% and 5 wt%) showed relatively moderate photocatalytic activities. Figure 3(b) presents the kinetic plots of $-\ln(C/C_0)$ versus time for TC degradation. The highest rate constant was observed for 3 wt% Fe-doped $BiVO_4$, which was approximately 2.65 times higher than that of bare $BiVO_4$, demonstrating enhanced photocatalytic efficiency due to improved charge separation and surface reactivity.

## CONCLUSION

In conclusion, this study successfully demonstrates the potential of Fe-doped $BiVO_4$ as an effective photocatalyst for the degradation of TC. The synthesis of bare and Fe-doped $BiVO_4$ using the hydrothermal method resulted in a monoclinic-tetragonal heterostructure with crystallite size of 27.37–29.10 nm. Photocatalytic tests showed that the 3% Fe-doped $BiVO_4$ had the highest degradation efficiency for TC, achieving a removal rate of 73.37% under low-power (13 W) UV lamp in 120 minutes. The increased photoactivity was attributed to improved electron-hole separation and an optimized Fe doping concentration, highlighting the catalyst's potential for environmental remediation applications.

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# Preparation of Food-Derived Biochar for Adsorption of Methylene Blue

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## ABSTRACT

*This work details the synthesis of biochar (BC) via controlled carbonization of food-derived waste collected from offshore platforms and vessels, developed as a sustainable adsorbent for methylene blue (MB) removal from aqueous systems. The biomass underwent thermal treatment at 300°C, 500°C, and 700°C for two hours to induce structural transformation, enhance porosity, and activate surface functionalities. Comprehensive physicochemical characterization using FTIR, TGA, SEM, and XRD confirmed the formation of thermally stable, porous carbon structures with surface features conducive to adsorption. Batch experiments conducted under varying operational conditions demonstrated high MB uptake, with adsorption kinetics best described by the pseudo-second-order model, indicative of chemisorption-driven interactions. The findings highlight the practical potential of valorising offshore-generated organic waste into functional carbon materials, offering a low-cost, circular approach to wastewater remediation and advancing sustainable practices within offshore waste management systems.*

**Keywords:** adsorption, biochar, carbonization, methylene blue, wastewater treatment

## INTRODUCTION

Water contamination by synthetic dyes represents a persistent environmental threat, particularly in nations experiencing rapid industrialization in the textile, leather, and paper sectors. Among these dyes, methylene blue (MB) is extensively utilized due to its intense coloration, structural stability, and broad-spectrum applications, including in medical, aquacultural, and food-related industries (Khan et al., 2022; Oladoye et al., 2022). However, MB's high resistance to biodegradation, strong light absorbance (~664 nm), and chemical stability enable it to persist in aquatic ecosystems which brings serious health complications.

Adsorption is widely favoured for its operational simplicity, cost-effectiveness, and high efficiency (Satyam & Patra, 2024). The removal of MB through adsorption typically involves multiple interactions such as electrostatic attraction, hydrogen bonding,

π-π stacking, and pore-filling that depend on the physicochemical properties of the adsorbent (Samadi Kazemi & Sobhani, 2023; Somsiripan & Sangwichien, 2023). Biochar (BC) derived from such food waste is a carbon-rich, porous material with high surface reactivity, making it a promising candidate for dye removal from wastewater (Mian & Liu, 2018). This research aims to prepare and characterize biochar (BC) derived from food waste, evaluate its adsorption efficiency for the removal of methylene blue (MB) under different operational parameters, and further examine the adsorption behavior through kinetic and isotherm modeling.

## PROJECT APPROACHES

### *Materials*

BC was synthesized via a carbonization method using food-derived waste collected from offshore platforms and vessels. Once dried, the material was manually crushed and sieved to obtain a uniform particle size suitable for thermal treatment. For the carbonization process, precisely 10.00g of the prepared biomass was placed into a ceramic crucible. The crucible was then inserted into a muffle furnace and subjected to controlled thermal treatment at three distinct temperatures: 300°C, 500°C, and 700°C. The heating was carried out at a constant rate of 10°C per minute until the target temperature was reached. The preparation process of food-derived biochar is illustrated in Figure 1.

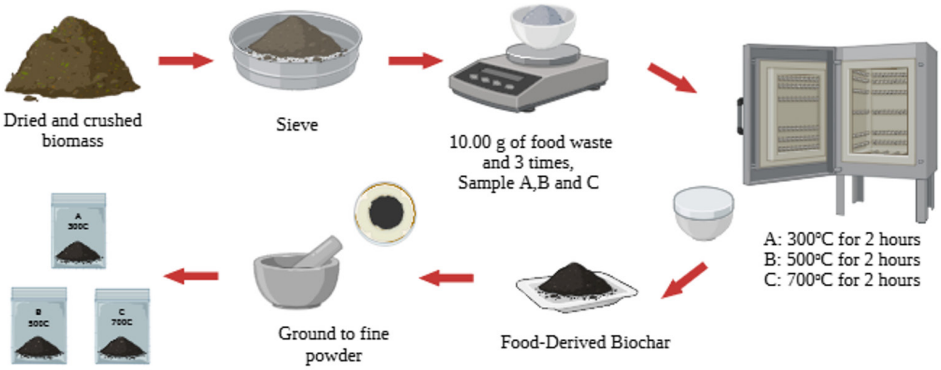


**Figure 1:** Schematic of the preparation of food-derived BC

### *Adsorption Test*

The prepared BC powder was added to 100 mL of a 10 mg/L MB solution. During each experimental run, 1 mL aliquots were withdrawn from the dye solution at predetermined time intervals and centrifuged at 4000 rpm for 10 minutes to remove suspended BC particles prior to absorbance measurement. The residual concentration of MB was determined using a calibration curve generated by measuring the absorbance of standard MB solutions at 664 nm. The adsorption capacity (Qe) of the BC adsorbent was subsequently calculated using the corresponding equation (Wang et al., 2024):

$$Qe = \frac{(C_0 - C_t)}{m} \times V$$

The percentage removal efficiency was calculated using the following equation (Ulfa et al., 2022):

$$\%\ removal = \frac{(C_o - C_t)}{m} \times 100$$

Where;
$C_o$= initial concentration of MB (mg/L),
$C_t$= concentration at a specific time (mg/L),
$V$= volume of the dye solution (L),
$m$= mass of the adsorbent (mg).

## FINDINGS

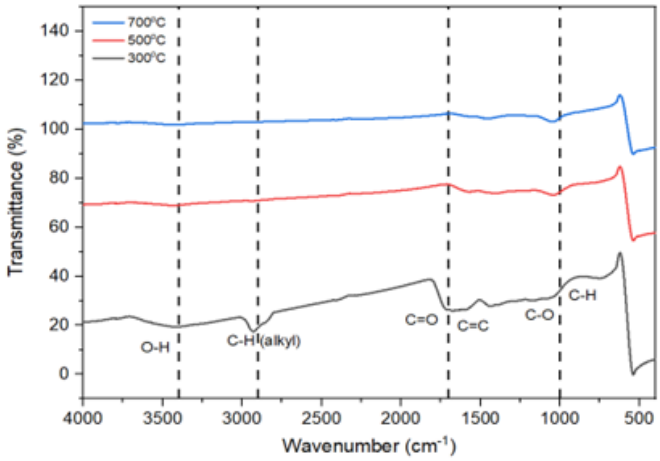


**Figure 2:** FTIR analysis of BC prepared at different carbonization temperatures

As shown in Figure 2, the Fourier Transform Infrared (FTIR) spectra of food-derived BC prepared at 300°C, 500°C, and 700°C show distinct functional groups. At 300°C, a broad band near 3400 $cm^{-1}$ corresponds to O–H stretching from hydroxyl groups and adsorbed water. Peaks at ~2920 $cm^{-1}$ are due to C–H stretching in alkyl groups, while a sharp band at ~1700 $cm^{-1}$ indicates C=O stretching of carbonyl groups. The peak near 1600 $cm^{-1}$ is assigned to C=C stretching in aromatic rings. A band at ~1050 $cm^{-1}$ corresponds to C–H vibrations, and another at ~1000 $cm^{-1}$ is attributed to C–O stretching. Increasing the carbonization temperature to 500°C and 700°C results in a reduced intensity of oxygen-containing functional group peaks (O–H, C=O, C–O), reflecting the removal of volatile matter and the formation of more condensed aromatic structures, which is beneficial for MB adsorption.

## CONCLUSION

This study demonstrates the successful preparation and application of BC derived from offshore food waste as an efficient adsorbent for the removal of MB from aqueous solutions. The carbonization process enhanced the BC's porosity, surface chemistry, and structural stability, enabling effective adsorption behaviour dominated by chemisorption interactions. Characterization analyses confirmed the presence of

key functional groups and a thermally stable, porous structure that facilitated strong dye surface binding under varying operational conditions. The findings highlight the potential of converting offshore food waste into a high-performance, value-added material for environmental remediation. This approach contributes to sustainable wastewater treatment by offering a low-cost, waste-derived adsorbent with reliable adsorption kinetics. Moreover, it aligns with circular economy goals by integrating waste valorisation with pollution control, offering a promising platform for future advancements in dye-contaminated water treatment technologies.

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# Photocatalytic Degradation of Tetracycline: Engineering a Magnetic $MnFe_2O_4$/Biochar Composite for Efficient and Sustainable Antibiotic Removal

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## ABSTRACT

*This study presents the synthesis and evaluation of a magnetic manganese ferrite/biochar ($MnFe_2O_4$BC) composite for the photocatalytic degradation of tetracycline (TC) under low-intensity UV irradiation. $MnFe_2O_4$ nanoparticles were prepared via co-precipitation and hydrothermal methods and integrated with duckweed-derived BC (Lemna minor), selected for its high surface area and renewable origin. The composite exhibited improved photocatalytic performance compared to bare $MnFe_2O_4$, within 120 minutes under UV light (λ = 365 nm, 15 W). This enhancement is attributed to synergistic effects between the $MnFe_2O_4$ and BC, including better charge separation and increased adsorption capacity. The material's magnetic properties enabled facile recovery, and recyclability tests confirmed its operational stability over multiple cycles. Antibacterial testing revealed selective inhibition against Escherichia coli, with no activity against Staphylococcus aureus, suggesting the composite's additional potential for microbial control in water treatment. By employing a sustainable biomass precursor and a low-energy synthesis strategy, this study contributes to the development of cost-effective and environmentally friendly photocatalysts for antibiotic removal in wastewater treatment systems.*

**Keywords:** manganese ferrite, biochar, tetracycline degradation, photocatalysis, wastewater treatment

## INTRODUCTION

Tetracycline (TC), a broad-spectrum antibiotic widely used in human medicine, veterinary practices, and aquaculture, is often detected in surface water and wastewater due to its high stability and resistance to biodegradation (Kovalakova et al., 2020). Its persistence in aquatic environments disrupts microbial ecosystems and accelerates the spread of antibiotic resistance, making its removal an urgent environmental priority (Mirizadeh et al., 2024) Conventional treatment methods such as adsorption, membrane filtration, and advanced oxidation frequently face drawbacks including low efficiency, high cost, and risks of secondary pollution.

Photocatalysis has gained attention as a sustainable and energy-efficient approach for degrading organic contaminants, including pharmaceuticals, under mild conditions. Among photocatalysts, $MnFe_2O_4$ is notable for its magnetic recoverability and narrow band gap, enabling activity under UV light. However, practical use is hindered by rapid charge carrier recombination and limited surface area (Wang et al., 2022). To overcome these limitations, $MnFe_2O_4$ can be coupled with biochar (BC), a porous carbonaceous material derived from biomass, known for its strong adsorption capacity and environmental compatibility.

In this study, duckweed (*Lemna minor*), a fast-growing aquatic plant, was selected as a renewable and low-cost precursor for BC. Previous work reported superior performance of $MnFe_2O_4$/BC in levofloxacin degradation (Meng et al., 2023), while Zhang et al. (2022) showed that combining sol-gel synthesis with sawdust-derived BC enhanced $MnFe_2O_4$ dispersion and pore structure. Building on these findings, this research aims to synthesize $MnFe_2O_4$/BC composites using co-precipitation and hydrothermal methods and to evaluate their performance in TC degradation under low-intensity (13W) UV light.

The composite is expected to enhance photocatalytic activity through improved light harvesting, adsorption, and charge separation, while enabling simple magnetic recovery and reuse. This work contributes to sustainable water treatment by valorizing biomass waste into functional materials, advancing practical antibiotic remediation in real wastewater systems.

## PROJECT APPROACHES

*Materials*

The preparation of $MnFe_2O_4$ and $MnFe_2O_4$/BC is illustrated in Figure 1. In brief, manganese sulfate monohydrate ($MnSO_4 \cdot H_2O$) and ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) were dissolved in 100 mL of deionized water at a molar ratio of 1:2 and stirred for 60 minutes. For the $MnFe_2O_4$/BC, 4.0 g of BC was added to the metal salt solution and stirred for an additional 30 minutes to ensure uniform dispersion. The pH of the mixture was then adjusted using 1 M NaOH. For the hydrothermal synthesis, the mixture was preheated at 70°C for 60 minutes, transferred to a Teflon-lined autoclave, and heated at 180°C for 15 hours. In contrast, for the co-precipitation method, the mixture was maintained at 70°C under continuous stirring until a dark brown precipitate formed, followed by natural cooling to room temperature. The resulting precipitates were collected by vacuum filtration, washed with deionized water and ethanol, and dried at 60°C for 24 hours. Finally, the dried powders were ground and calcined at 500°C for 3 hours to enhance crystallinity and thermal stability.

The photocatalytic activity was evaluated using TC degradation under low-intensity UV light irradiation. A 13 W UV aquarium lamp was positioned 10 cm above the reaction vessel to maintain consistent light exposure. Prior to illumination, the photocatalyst was suspended in the TC solution and stirred in the dark for 30 minutes to establish adsorption-desorption equilibrium, verified by preliminary trials. For degradation

measurements, 10 mL aliquots were sampled every 15 minutes, centrifuged, and analyzed at 357 nm. The TC degradation efficiency, based on concentration changes, was calculated using a pseudo-first-order kinetic model to compare degradation rates across samples.

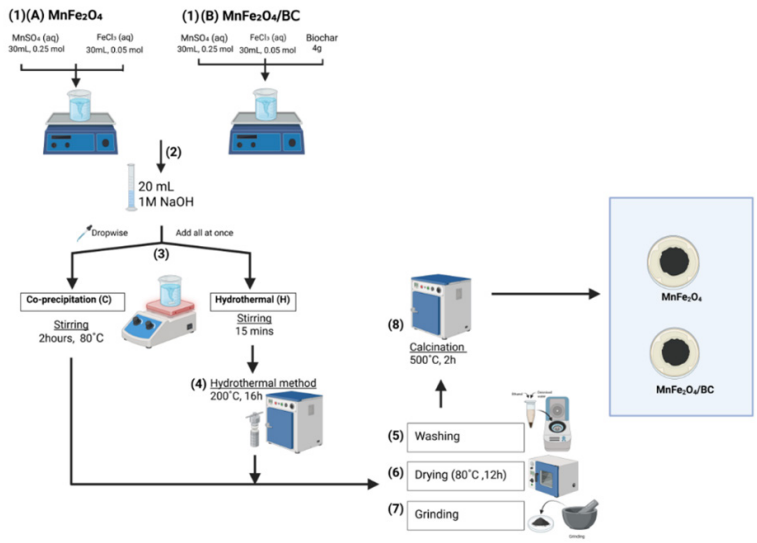


**Figure 1:** Schematic flowchart illustrating the synthesis process of bare $MnFe_2O_4$, and the $MnFe_2O_4$/BC via co-precipitation and hydrothermal method.

## FINDINGS

The FT-IR spectra of BC, $MnFe_2O_4$, and their $MnFe_2O_4$/BC composites synthesized via both hydrothermal and coprecipitation methods show distinct vibrational changes, confirming successful integration of the metal oxide into the carbon matrix as shown in Figure 2. A broad O–H stretching band (3400–3200 $cm^{-1}$) is strong in BC, weaker in $MnFe_2O_4$, and reduced in the composite, indicating interaction between hydroxyl groups and $MnFe_2O_4$. The C=O band (1600–1500 $cm^{-1}$) shifts and weakens in the composite, implying structural changes or bonding. C–O stretching (1100–1000 $cm^{-1}$) decreases, possibly due to metal–O–C bond formation (Lai et al., 2019). Strong Fe–O and Mn–O peaks (<800 $cm^{-1}$) appear in $MnFe_2O_4$, but are reduced in the composite, further supporting interaction between the BC and metal oxide.

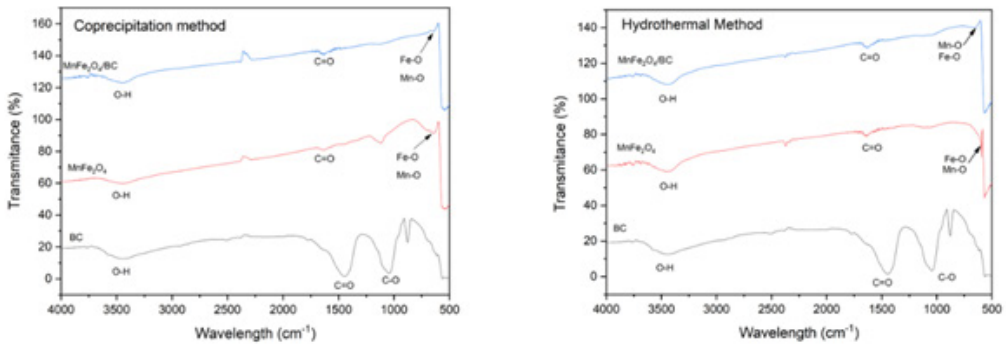


**Figure 2:** FTIR spectra of BC, $MnFe_2O_4$, and $MnFe_2O_4$/BC via hydrothermal and co-precipitation methods.

The antibacterial activity of $MnFe_2O_4$/BC synthesized via co-precipitation (Sample A) and hydrothermal (Sample B) methods was evaluated against *E. coli* and *S. aureus* using the agar well diffusion method. As shown in Table 1, Sample A exhibited a zone of inhibition (ZOI) measuring 1.33 ± 0.20 cm against *E. coli*, while Sample B showed a ZOI of 1.13 ± 0.15 cm. Both composites showed no inhibitory effect on *S. aureus*. Standard antibiotics, including streptomycin and chloramphenicol, served as positive controls and demonstrated significantly larger inhibition zones. Ethanol was used as the negative control and showed no antibacterial activity. These results indicate that the $MnFe_2O_4$/BC possess selective antibacterial activity, particularly against Gram-negative bacteria. This selectivity may be attributed to differences in cell wall structure and permeability (Huy et al., 2017). The observed antibacterial effects support the potential use of these composites in targeted microbial control within wastewater treatment applications

**Table 1:** Assay of Antibacterial Activity of $MnFe_2O_4$/BC

| Sample / ZOI (cm) | *E. coli* | *S. aureus* |
|---|---|---|
| **$MnFe_2O_4$/BC (Sample A)** | 1.33 ± 0.20 | 0.00 ± 0.00 |
| **$MnFe_2O_4$/BC (Sample B)** | 1.13 ± 0.15 | 0.00 ± 0.00 |
| **Streptomycin** | 2.14 ± 0.09 | 3.19 ± 0.21 |
| **Chloroamphenicol** | 3.08 ± 0.13 | 2.18 ± 0.22 |
| **Ethanol** | 0.00 ± 0.00 | 0.00 ± 0.00 |

## CONCLUSION

In conclusion, this study successfully demonstrated the synthesis of $MnFe_2O_4$and $MnFe_2O_4$/BC composites using duckweed-derived BC as a sustainable support material. The composite photocatalyst showed enhanced performance in degrading tetracycline under low-intensity UV light, highlighting its potential as an effective and environmentally friendly solution for wastewater treatment. The incorporation of BC not only improved the photocatalytic efficiency but also contributed to material sustainability and ease of magnetic recovery. Overall, the findings offer valuable insights into the development of green photocatalysts for addressing emerging contaminants in water, paving the way for future advancements in sustainable environmental remediation technologies.

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# One-Pot Hydrothermal Synthesis of $BiVO_4$/g-$C_3N_4$ Composite for Efficient Photocatalytic Degradation of Tetracycline

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## ABSTRACT

*This study presents a one-pot hydrothermal synthesis approach for the fabrication of $BiVO_4$/g-$C_3N_4$ composite photocatalysts, designed for the efficient degradation of tetracycline (TC) in aqueous environments. $BiVO_4$ was synthesized by reacting $Bi(NO_3)_3 \cdot 5H_2O$ and $NH_4VO_3$ in acidic and basic media, respectively, followed by hydrothermal treatment at 180°C for 15 hours and subsequent calcination at 500°C. Separately, g-$C_3N_4$ was obtained from melamine under identical hydrothermal and thermal conditions. For the composite preparation, melamine powder was incorporated into the BiVO4 precursor solution prior to hydrothermal treatment, facilitating in-situ formation of a $BiVO_4$/g-$C_3N_4$ heterojunction. The resulting photocatalysts were washed, dried, and thermally treated to ensure high crystallinity and structural stability. The $BiVO_4$/g-$C_3N_4$ composite synthesized via this green, scalable method exhibits promising potential for photocatalytic degradation of TC under visible light, highlighting its relevance in addressing pharmaceutical contaminants in wastewater.*

**Keywords:** bismuth vanadate, graphitic nitride, hydrothermal, tetracycline, wastewater treatment

## INTRODUCTION

The widespread use of tetracycline (TC) in medicine, veterinary care, and agriculture is due to its broad-spectrum antibacterial activity and affordability (Aruljothi et al., 2023). However, its extensive use has led to its widespread presence in the environment, including surface water, groundwater, and soil, posing potential risks to ecosystems and human health. Traditional wastewater treatment methods are often insufficient to completely remove TC, necessitating the development of more effective and sustainable degradation technologies (Behera et al., 2024). Photodegradation, an advanced oxidation process (AOPs), has emerged as a promising technology for the removal of organic pollutants, including antibiotics, from water (Thangavelu et al., 2023). This process utilizes semiconductor photocatalysts to accelerate the degradation of pollutants under light irradiation. Among various photocatalytic materials, bismuth vanadate ($BiVO_4$) and graphitic carbon nitride (g-$C_3N_4$) have gained considerable

attention due to their unique optical and electronic properties (Kang et al., 2021).

The strategic coupling of $BiVO_4$ and g-$C_3N_4$ has garnered increasing attention due to their complementary band structures and visible-light-driven photocatalytic potential. Recent work has illuminated the nuanced mechanisms underpinning charge separation at their interface, with some studies confirming a type-II heterojunction configuration, while others have demonstrated a Z-scheme mechanism, particularly when electron mediators such as Au nanoparticles or fullerene derivatives are employed. Notably, direct contact between $BiVO_4$ and g-$C_3N_4$ favours a type-II heterojunction, but introducing mediators transforms the system into a Z-scheme configuration with improved charge recombination suppression and enhanced pollutant degradation efficiency, particularly for caffeine (CAF) under visible light. This duality highlights the importance of interface engineering and mediator choice in tailoring photocatalytic mechanisms.

## PROJECT APPROACHES

*Materials*

The $BiVO_4$/g-$C_3N_4$ composite was synthesized via a hydrothermal method as shown in Figure 1. Initially, 3.3954 g of $Bi(NO_3)_3 \cdot 5H_2O$ was dissolved in 30 mL of 1.5M $HNO_3$, while 0.8189 g of $NH_4VO_3$ was dissolved in 30 mL of 1.5M NaOH. The two solutions were then gradually mixed, resulting in the formation of an orange precipitate. Subsequently, 20 g of melamine powder was introduced into the mixture, followed by stirring for 30 minutes. The prepared mixture was then transferred to a Teflon-lined reactor and subjected to hydrothermal treatment at 180°C for 15 hours. The obtained precipitate was collected, thoroughly washed with deionized water and ethanol, and dried at 60°C for 12 hours. Finally, the dried product underwent calcination at 500°C for 12 hours as shown in Figure 1.

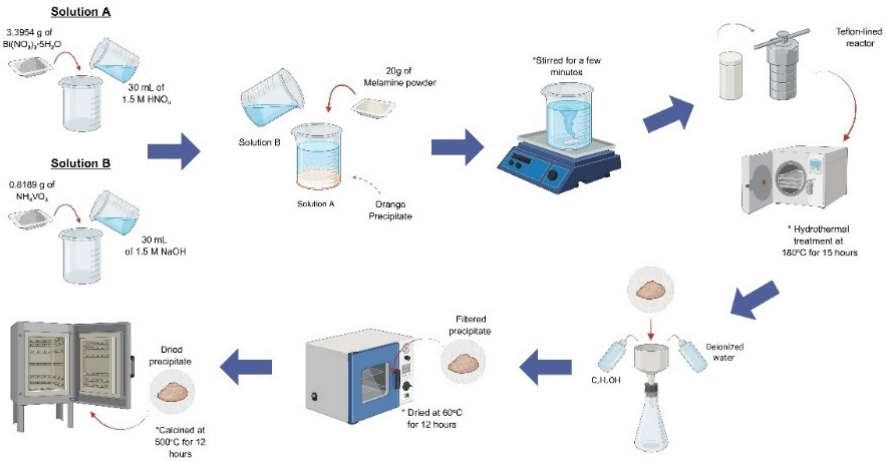


**Figure 1.** Flowchart of the $BiVO_4$/g-$C_3N_4$ synthesis.

The photocatalytic performance of $BiVO_4$, g-$C_3N_4$ and $BiVO_4$/g-$C_3N_4$ was probed by examining the degradation of the TC under vertical low intensity UV irradiation (13W). The photocatalytic reactions were performed in a handmade wooden chamber.

Typically, the photocatalytic test was performed in a dark condition for the first 30 minutes to take into account of the adsorption process. The photodegradation test were conducted in a conical flask wrapped with aluminum foil containing 100 mL aqueous solutions of TC. About 10 mg sample of the catalyst was used. After photo illumination, 2 ml aliquots were collected, and the concentration of TC were determined using UV-Vis spectrometry. The maximum absorbance wavelengths (λmax) used for TC will be 354 nm.

## FINDINGS

As shown in Figure 2, the FTIR spectra of $BiVO_4$, g-$C_3N_4$ and $BiVO_4$/g-$C_3N_4$ composite reveal the key functional groups present as shown in Figure 4. In the spectrum of g-$C_3N_4$ , characteristic absorption bands are observed around 1200–1650 $cm^{-1}$, corresponding to the stretching vibrations of C–N and C=N bonds, which are typical of the aromatic heptazine units that form the framework of carbon nitride. A broad absorption band near 3000–3500 $cm^{-1}$ is attributed to N–H and O–H stretching vibrations, indicating the presence of surface amine groups and adsorbed water molecules. The $BiVO_4$spectrum exhibits a strong absorption peak around 800–900 $cm^{-1}$, corresponding to the V–O stretching vibration, which is a signature of the $BiVO_4$ lattice structure. In the $BiVO_4$/g-$C_3N_4$ composite spectrum, the characteristic peaks of both C–N/C=N and V–O bonds are clearly present, along with the broad N–H and O–H bands. The coexistence of these features confirms the successful formation of the $BiVO_4$/g-$C_3N_4$ composite and indicates that the structural integrity of both components was preserved during synthesis, which is crucial for maintaining the desired photocatalytic properties.

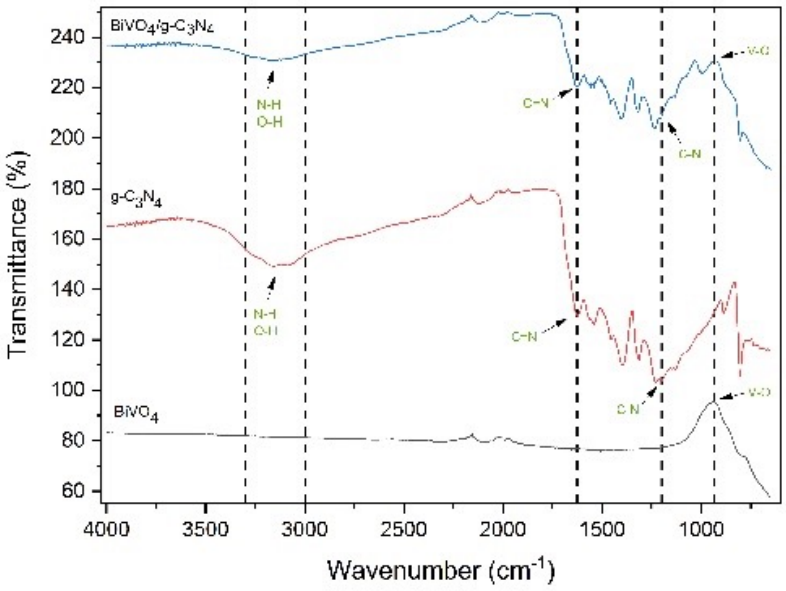


**Figure 2**. FTIR Analysis Of $BiVO_4$, g-$C_3N_4$ and $BiVO_4$/g-$C_3N_4$

## CONCLUSION

In summary, this work successfully demonstrates the fabrication and photocatalytic

potential of a $BiVO_4$/g-$C_3N_4$ composite for the degradation of TC in aqueous environments. The heterojunction structure facilitated improved light absorption and charge separation, leading to superior performance under UV light compared to individual components. The synergy between $BiVO_4$ and g-$C_3N_4$ not only enhanced photocatalytic activity but also underscored the benefits of combining semiconductors with complementary electronic properties for environmental applications. These findings contribute to the growing field of green photocatalyst development and offer promising directions for creating efficient, sustainable technologies to address emerging water pollutants.

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# The Bidayuh Ethnic's Fermented Foods: A Review as Potential Sources of Probiotics

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## ABSTRACT

*This study reviews over eight Bidayuh fermented foods (e.g., tipuyak, budu, rebung, belacan, tuak, tapai, sawipahit, daun ubi) as probiotic sources. Microbiological analysis shows that plant-based ferments (e.g., tempoyak) are dominated by Lactobacillus, while animal-based ones (e.g., budu) feature halophilic Tetragenococcus. Most Lactic acid bacteria (LAB) isolates exhibit strain-specific probiotic traits: acid/bile tolerance, bacteriocin production, cyanide detoxification, immune modulation, and cholesterol reduction. Safety concerns include high histamine in fish products and cyanogens in rebung will be discussed. Mitigation involves histamine-degrading strains, optimized fermentation, starter quality, and salt control. Although some strains meet FAO/WHO criteria, traditional knowledge loss and insufficient probiotic validation require urgent policy for biocultural protection. More clinical validation and strain documentation are critically needed.*

**Keywords:** Bidayuh Ethnic food, Traditional Fermentation, Probiotic

## INTRODUCTION

Fermented foods provide essential nutrition, preservation, and probiotic benefits globally. While commercial products like yogurt dominate research, indigenous Southeast Asian ferments remain critically understudied. Sarawak hosts over 30 ethnic groups, yet Bidayuh fermentation—practiced by 8% of Sarawak's population—receives only 15% of Malaysian fermented food research. This neglect persists despite Bidayuh foods (e.g., Budu, Kasam Babi, Tempoyak) containing unique lactic acid bacteria (LAB) with tropical adaptations like thermal tolerance and antimicrobial properties (Tharmabalan et al., 2025). We review eight Bidayuh ferments against FAO/

WHO probiotic criteria, addressing: (1) Traditional process of preparation of Bidayuh fermented food, (2) LAB diversity in Bidayuh fermented food, and (3) Mechanism of action of probiotic. By bridging ethnobiology and probiotic science, this study validates Bidayuh ferments as sustainable sources of tropical-adapted probiotics while combating antimicrobial resistance—a key WHO priority.

## BIDAYUH FERMENTED FOOD

*Budu (Fermented Fish Sauce)*

Budu is the traditional sauce of Malaysia, made with the anchovies mixed with one-third salt and followed by fermentation for six months in close containers. *Tetragenococcuswas* the most prevalent genus, making up to 98.62% of the total. However, the presence of microbiota depends on the time of fermentation. Other genera, including *Staphylococcus*, *Acinetobacter*, *Halanaerobium*, and *Bacillus* were also isolated (Tharmabalan et al., 2025).

*Pekasam (Fermented Fish/Rice)*

Fermentation of fish is the significant process of preserving fish for a longer period. Pekasam is the traditional fermented fish of Malaysia, prepared in two steps; in the first step, the fish is washed properly to avoid fishy odors. Fermentation of fish is an important way of preserving it. After washing, the fish is kept in tightly sealed containers with a sufficient amount of salt at low temperature for 4 days. After the completion of the first fermentation stage, the fish will undergo an additional fermentation process for a couple of weeks, applying ground toasted rice grains, brown sugar, water, and natural acidulants, like dried tamarind slices that assist acidification during fermentation for 14–21 days (John et al., 2025). Lactiplantibacillus plantarum, *Bacillus cereus*, *Bacillus* sp., *Lactobacillus plantarum*, *Pediococcuspentosaceus*, *Leuconostoc*, *Lactobacillus* spp., were LAB isolated from pekasam (Ida Muryany et al., 2017; Alysha et al., 2024).

*Belacan (Shrimp Paste)*

Belacan is a globally known fermented food in Malaysia. Belacan, frequently referred to as shrimp paste, is primarily made up of small shrimp and salt that undergo fermentation and are transformed into paste or blocks for business sale. The process of forming belacan involves complete shrimps of Acetesspecies with both head and shells washed and cleaned thoroughly. After that, they are combined with 4–10%(w/w) salt. This is followed by overnight fermentation by lactic acid bacteria at 30 to 32 days followed by drying in the sun for several hours to minimize the bacterial count and evaporate water content (Tharmabalan et al., 2025).

*Tipuyak (Fermented Durian)*

Tipuyak or Tempoyak is a fermented fruit paste made from durian fruit by adding salt. It is a cultural food and has been enjoyed in enormous quantities across Southeast Asia.

Tempoyak has a diverse microflora, but most of it is mainly composed of *Lactobacillus* species. Tempoyak is made by crushing durian flesh, adding salt toit, and letting the homogeneous mixture sit at room temperature in a covered container for 4–7 days (Anggadhania et al., 2023).

*Tuak (Palm wine)*

Tuak is produced from the sap of various trees, including palm tree (*Arenga pinnata*), rice, and coconut sap (Gunam et al., 2022). The palm tree is tapped to get palm sap, it is then mixed with a natural starter that comes from Raru a coconut fiber. After fermentation for 12–24 hourstuak is produced, which is further distilled until a high-quality wine is collected in bottles and traded at a commercial level (Koay et al., 2022).

*Tapai (Rice Wine)*

Rice wine commonly known as Tapai in Malaysia, is primarily made from cereal grains of rice or wheat in some countries like China. The microbiota of rice wine is composed of two main phyla (*Proteobacteria* and *Firmicutes*) and ten genera (*Kosakonia*, *Weissella*, *Enterobacter*, *Lactococcus*, *Pseudomonas*, *Bacillus*, *Chromobacterium*, *Paludibacterium*, *Enterococcus*, and *Gluconobacter*) (Koay et al., 2024).

*Sawi Pahit*

A famous cultural fermented vegetable food from the Bidayuh people in Sarawak (Malaysia Borneo) is Sawi Pahit. It is made from white mustard (*Brassica rapa* subsp. *pekinensis*), which is the species of the Brassicaceae family. The process of forming of Sawi Pahit is as follows: leaves are assembled, washed, wilted, cut into smaller pieces, and mixed with 5% salt (NaCl). This combination is stored in an airtight jar at 23-25 °C for 4–7 days. *L. plantarum* was isolated from it as a potential probiotic. It has antimicrobial properties against pathogens (Olawunmi et al., 2023).

*Daun ubi (Cassava leaves)*

Daun ubi is a traditional fermented leafy food made by native inhabitants of Sarawak, Malaysia, specifically the Dayak (Bidayuh group). The process of forming Daun ubi consists of the following steps: collection of leaves, washing, cutting it in small pieces, and mixing them with 2.5 or 4.5% of salt (John et al., 2025). Bamigbade et al. (2023) reported *Lactiplantibacillus plantarum* and *Limosilactobacillus fermentum* in cassava leaves. These probiotics have several benefits, including antimicrobial and acid tolerance.

## CORE MECHANISMS OF ACTION OF PROBIOTIC

- Competitive Exclusion: Adhesion to gut lining, blocking pathogens (e.g., Pekasam, Sawi Pahit) (Rusli et al., 2023).
- Antimicrobial Production: Bacteriocins, organic acids killing pathogens (e.g., Budu, Belacan, Pekasam) Rossi et al. (2021).

- Detoxification: Enzymatic degradation of toxins like histamine (Budu) (Kanjan and Sakpetch, 2020) or cyanide (Rebung, Daun Ubi) (Uprarawanna et al., 2025).
- Bioactive Metabolite Production: Antioxidants, immunomodulators, antihypertensive/ antidiabetic compounds (e.g., Tipuyak, Tuak, Tapai) (Hussin et al., 2022).
- Gut Barrier Enhancement: Supporting intestinal integrity (e.g., Belacan LAB) (Hamid et al., 2020).

## CONCLUSION

This study reviews traditional Bidayuh fermented foods (e.g., budu, tempoyak, pekasam, sawipahit), which are significant reservoirs of diverse lactic acid bacteria (LAB) and yeasts with strain-specific probiotic potential. LAB profiles are distinct, with plant-based ferments dominated by Lactobacillus and animal-based (e.g., budu) by halophilic *Tetragenococcus*. Key isolates demonstrate essential probiotic traits: acid/ bile tolerance, antimicrobial production (bacteriocins), pathogen exclusion, immune modulation, cholesterol reduction, and detoxification (e.g., cyanide in rebung, histamine in fish products). While some strains meet FAO/WHO criteria, safety concerns (histamine, cyanogens) exist but are mitigable via fermentation control and specific strains. Urgent action is needed for clinical validation, strain documentation, biocultural heritage protection, and policies supporting traditional knowledge preservation to harness these functional foods fully.

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# Carbonization of KOH-Activated Duckweed-Derived Biochar for Methylene Blue Removal

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## ABSTRACT

*This study examines the influence of carbonization temperature on the yield of potassium hydroxide (KOH)-activated biochar derived from duckweed (Lemna minor). Duckweed, a rapidly growing aquatic plant with high carbon content, was carbonized at 500°C and 700°C to produce biochar, followed by KOH activation to enhance its surface properties. KOH activation significantly improved porosity, surface functionality, and adsorption potential, making the material suitable for dye removal applications. The integration of duckweed as a renewable precursor with chemical activation offers a sustainable pathway for producing high-performance adsorbents for environmental remediation.*

**Keywords:** Duckweed-derived biochar, carbonization, methylene blue, adsorption

## INTRODUCTION

Biochar (BC), a carbon-rich material produced through the thermochemical conversion of biomass, has shown promise as an adsorbent; however, its pristine form typically lacks sufficient surface reactivity for effective dye removal (C. Yang et al., 2021; Zhang et al., 2022). To enhance its adsorption efficiency, chemical activation using potassium hydroxide (KOH) was employed, a method recognized for its ability to significantly improve the structural and surface characteristics of BC. During activation, KOH penetrates the carbon matrix and promotes the development of a well-connected porous network through redox and dehydration reactions that release volatile compounds and etch the carbon surface. This process generates a high specific surface area, abundant micropores, and enriched surface functionalities such as hydroxyl and carboxyl groups, which collectively enhance dye uptake through electrostatic interactions and π–π stacking (Liu et al., 2024; Wu et al., 2024).

Duckweed (*Lemna minor*), a fast-growing aquatic plant with high carbon content, was selected as the biomass precursor due to its widespread availability and renewable nature (Jeffrey et al., 2024; G.-L. Yang, 2022). It was converted into BC via carbonization, followed by KOH activation to produce a highly porous adsorbent. The novelty of

this work lies in the strategic integration of duckweed, an underutilized yet rapidly proliferating aquatic biomass, with a straightforward KOH activation process to produce a high-performance BC for dye adsorption. While numerous studies have explored BC derived from terrestrial biomass sources such as wood (Di Domenico et al., 2024), agricultural waste (Alam et al., 2020), and coconut shells (Nisa et al., 2023), few have investigated the potential of aquatic plants like duckweed, which offers advantages in terms of biomass yield, short harvesting cycles, and non-competition with food crops. This study contributes to a better understanding of the thermal behaviour of aquatic biomass precursors under chemical activation conditions and provides baseline data for optimizing biochar synthesis for various environmental applications.

## PROJECT APPROACH

*Materials*

The natural duckweed (*Lemna minor*) was collected from a pond in Bintulu. After collection, the duckweed was washed with tap water to remove solid impurities and then sun-dried for 72 h. Following this, the sample was further oven-dried at 80°C for 24 h to ensure complete moisture removal. The dried duckweed was milled and sieved to 2 mm and then stored in a sealed container for subsequent experiments. Figure 1 depicts the illustration for the preparation of the duckweed.

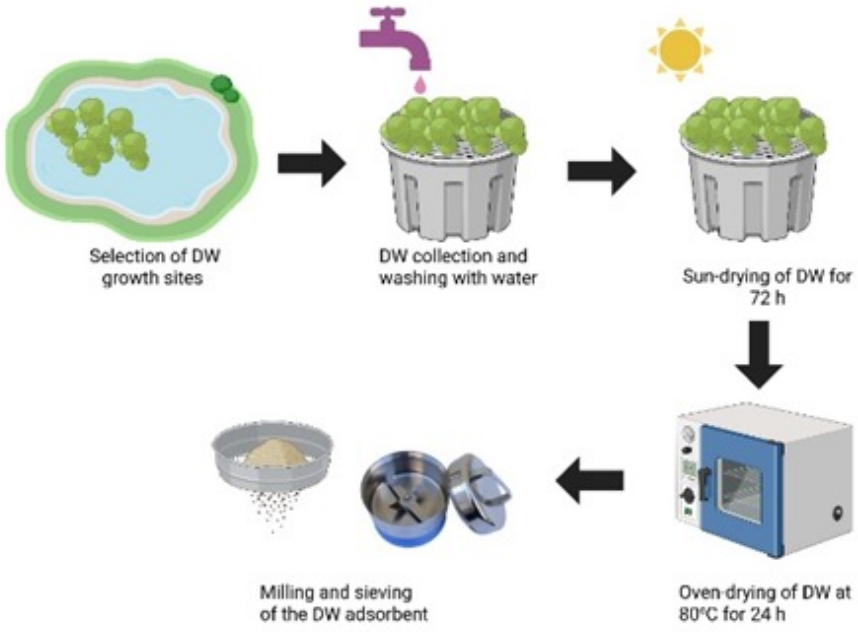


**Figure 1:** Schematic illustration of the preparation process of duckweed.

Once dried, the duckweed was ground into a fine powder using a mortar and pestle. Separately, potassium hydroxide (KOH) pellets were weighed and mixed with the duckweed powder in a 3:1 weight ratio. The mixture was homogenized thoroughly and then transferred to a crucible for carbonization at 500°C and 700°C for 2 hours. Upon completion, the product was naturally cooled to room temperature, then washed repeatedly with deionized water and 0.5 M HCl to remove residual KOH until the pH of

the filtrate was neutral. The resulting material was dried again at 60°C for 12 hours to obtain the final KOH-activated duckweed-derived BC as depicted in Figure 2.

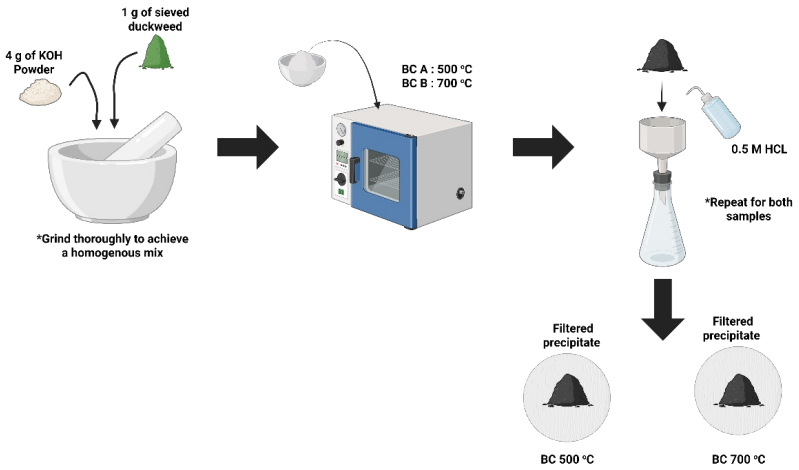


**Figure 2:** Schematic illustration of the preparation process of KOH activated BC.

## FINDINGS

The mass yield of BC following KOH activation and hydrothermal carbonization was evaluated to assess the efficiency of the conversion process. Starting with 1.0 gram of dried duckweed biomass, the final mass of KOH-activated BC obtained after carbonization at 500°C was approximately 0.31 grams, indicating a yield of 31.0%.

**Table 1:** Mass Yield of KOH-Activated Duckweed BC

| Carbonization Temperature (°C) | Initial Mass of Biomass (g) | Final Mass of Biomass (g) | Mass Yield (%) |
|---|---|---|---|
| **500** | 1.00 | 0.31 | 31.0 |
| **700** | 1.00 | 0.23 | 23.0 |

When the carbonization temperature was increased to 700°C, the final mass was further reduced to approximately 0.23 grams, resulting in a lower yield of 23.0% as shown in Table 1. This progressive decrease in yield with increasing temperature is attributed to the enhanced volatilization of organic compounds and the more complete breakdown of thermally stable biomass components at higher temperatures (Wang et al., 2022). The decline in residual mass reflects the intensified removal of non-carbon elements and structural rearrangements facilitated by KOH activation, which improves the textural properties of the BC. These observations align with trends reported in similar studies, affirming that elevated carbonization temperatures, although yielding less solid residue, result in a more functionally efficient adsorbent material (Řimnáčová et al., 2024).

## CONCLUSION

This study demonstrated that carbonization temperature plays a critical role in determining the yield of KOH-activated biochar derived from *Lemna minor*. Increasing the carbonization temperature from 500°C to 700°C resulted in a reduction in mass

yield from 31.0% to 23.0%, primarily due to enhanced volatilization and decomposition of biomass constituents at higher temperatures. While elevated temperatures reduce solid residue, they promote the development of a more porous structure and enriched surface functionalities, improving the material's adsorption potential. The integration of an abundant aquatic biomass such as duckweed with an efficient KOH activation process offers a sustainable and low-cost approach for producing high-performance adsorbents suitable for environmental remediation, particularly in dye removal applications. Future work will focus on detailed characterization of textural properties and adsorption performance to further optimize synthesis conditions for practical deployment.

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# Ground Sago Bark Ash as a Soil Amendment: Mitigating Major Nutrient Leaching in Fertilized Tropical Peatlands

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## ABSTRACT

*Despite the extensive use of chemical fertilizers to improve fertility, tropical peatlands remain inherently low in productivity. Ground sago bark ash (GSBA), an alkaline by-product of the sago industry, in conjunction with urea, Egyptian Rock Phosphates (ERP), and Muriate of Potash (MOP), can be used to mitigate losses of nitrogen (N), phosphorus (P), and potassium (K) in tropical peat soil. A leaching study was carried out for 30 days to evaluate the effects of GSBA on nutrient retention in tropical peat soil. The study followed a completely randomized design with three replications, using 1 kg of soil and treatments placed in the polypropylene container. Treatments consisted of soil, urea, ERP and MOP and ground sago bark ash rates, which varied by 60%, 80%, and 100%. At the end of the 30 days leaching study, GSBA effectively improved P and K availability in tropical peat soil compared with peat soil fertilized with conventional fertilizers alone. The incorporation of GSBA with conventional fertilizers helps to retain major nutrients in tropical peat soil. Treatments (T5, T6, and T8) showed the best results compared with conventional fertilizers, highlighting GSBA as a practical and sustainable soil amendment. Further field studies are in progress to validate its effectiveness under real soil and crop interaction conditions.*

**Keywords:** nutrient retention, leaching, ground sago bark ash (GSBA), tropical peat soil

## INTRODUCTION

Tropical peat soils in Malaysia have high organic matter, waterlogged conditions, and low pH (Pakir et al., 2025). These conditions contribute to poor nutrient retention, particularly for mobile nutrients such as nitrogen (N), phosphorus (P), and

potassium (K). In Sarawak, peatlands are commonly used for agriculture but often experience high nutrient losses due to leaching exacerbated by high rainfall and weak soil buffering capacity (Page et al., 2011). Furthermore, the high acidity and presence of toxic elements such as Al3+ and Fe2+ hinder root development and nutrient uptake (Paramisparam et al., 2021). Conventional fertilization practices using urea, Egyptian Rock Phosphate (ERP), and muriate of potash (MOP) often lead to nutrient leaching and environmental risks in tropical peat soils. Recent studies have explored strategies to mitigate these issues, including the use of clinoptilolite zeolite and forest litter compost. According to Krishnan et al. (2021), these amendments can improve potassium retention and soil properties. Sustainable and cost-effective soil management strategies are urgently needed in these fragile ecosystems.

Ground sago bark ash (GSBA) could be incorporated with commercial fertilizers to improve the productivity of peat soil. GSBA is an alkaline (Demeyer et al., 2001), mineral-rich by-product generated as a waste during sago starch flour processing. According to Johan et al. (2021), sago bark ash is qualified to be a liming material because of Ca and Mg carbonates. Empirical evidence shows that GSBA co-applied with charcoal raises soil pH, enhances soil CEC, and improves the availability of K, P, Ca, and other base cations in acidic soils (Hamidi et al., 2021). For instance, Paramisparam et al. (2021) demonstrated that blending sago bark ash with charcoal increased soil-exchangeable K, reduced exchangeable acidity, and mitigated Al and Fe toxicity (Paramisparam et al., 2021). However, the potential of GSBA to reduce nutrient leaching in fertilized tropical peatlands is not widely explored. This study evaluates the combined use of GCBA with standard fertilizers in mitigating nutrient leaching from fertilization in peat soil. The work seeks to advance theoretical understanding, offer a replicable methodological framework, and propose a practical, circular economy approach aligned with SDG 2 (Zero Hunger), SDG 12 (Responsible Consumption and Production), and SDG 13 (Climate Action).

## PROJECT APPROACHES

*Materials*

A soil leaching study was carried out for 30 days to determine the potential of GSBA as a soil amendment to mitigate N, P, and K leaching from urea, ERP, MOP in tropical peat soil. Peat samples were collected from Kg Sau, Mukah, Sarawak, Malaysia, at a 0–25 cm depth, air-dried, and sieved (2 mm) for initial physicochemical characterization. GSBA was applied at rates of 60%, 80%, and 100% of the standard chemical fertilizer recommendation (based on a planting density of 66,666 plants $ha^{-1}$) in combination with the fertilizers in a 30-day leaching experiment. The study followed a completely randomized design with three replications, using 1 kg of soil placed in the polypropylene container. Treatments consisting of soil, urea, ERP and MOP and ground sago bark ash rates, which varied by 60%, 80%, and 100% are summarized in Table 1. The container was perforated and lined with filter paper to collect leachates. Treatments were moistened to 60% field capacity, surface-applied with fertilizer (except the control), and leached at 5-day intervals using distilled water volumes equivalent to the average monthly rainfall derived from 10-year data.

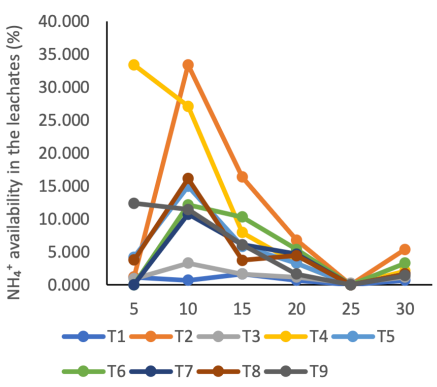
**Table 1:** Amounts of muriate of potash (MOP), Egyptian Rock Phosphate (ERP), urea, and ground sago bark ash

| Treatment | Soil (kg) | Ground Sago Bark Ash (g) | Urea (g) | ERP (g) | MOP (g) |
|---|---|---|---|---|---|
| **TI** | 1 | - | - | - | - |
| **T2** | 1 | - | 7 | 7 | 4 |
| **T3** | 1 | 50 | - | - | - |
| **T4** | 1 | 50 | 5.6 | 5.6 | 3.2 |
| **T5** | 1 | 50 | 4.2 | 4.2 | 2.4 |
| **T6** | 1 | 40 | 5.6 | 5.6 | 3.2 |
| **T7** | 1 | 40 | 4.2 | 4.2 | 2.4 |
| **T8** | 1 | 30 | 5.6 | 5.6 | 3.2 |
| **T9** | 1 | 30 | 4.2 | 4.2 | 2.4 |

Leachates were analyzed for pH, exchangeable ammonium, available nitrate, available P and exchangeable K. At the end of the leaching study, soils were tested for pH, total carbon, organic matter, total N, exchangeable acidity, cation exchange capacity, total K, exchangeable K, total P and available P by using standard procedures. Statistical analysis was conducted via ANOVA (SAS 9.4) with means separated by Tukey's HSD at $p \leq 0.05$. This approach aimed to quantify the efficacy of GSBA in enhancing nutrient retention, minimizing leaching losses, and improving the nutrient status of tropical peat soils thus offering a sustainable alternative to conventional fertilizer management for peatland agriculture.

## FINDINGS

The incorporation of GSBA demonstrated significant interactions with conventional fertilizers. Among all treatments, ammonium and nitrate leaching were more pronounced in T2 due to the application of urea, ERP and MOP (Figure 1–4). Despite no chemical fertilizers being added in T3 (Figure 1–4), higher ammonium and nitrate leaching could be due to the higher inherent content of those forms of nitrogen in GSBA.

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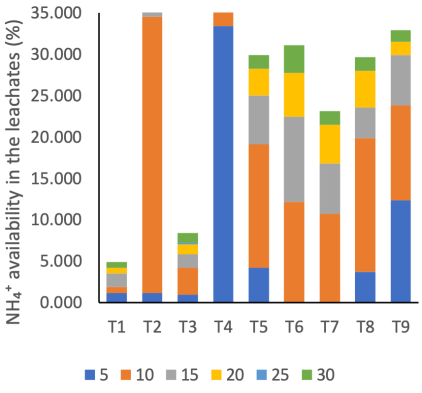


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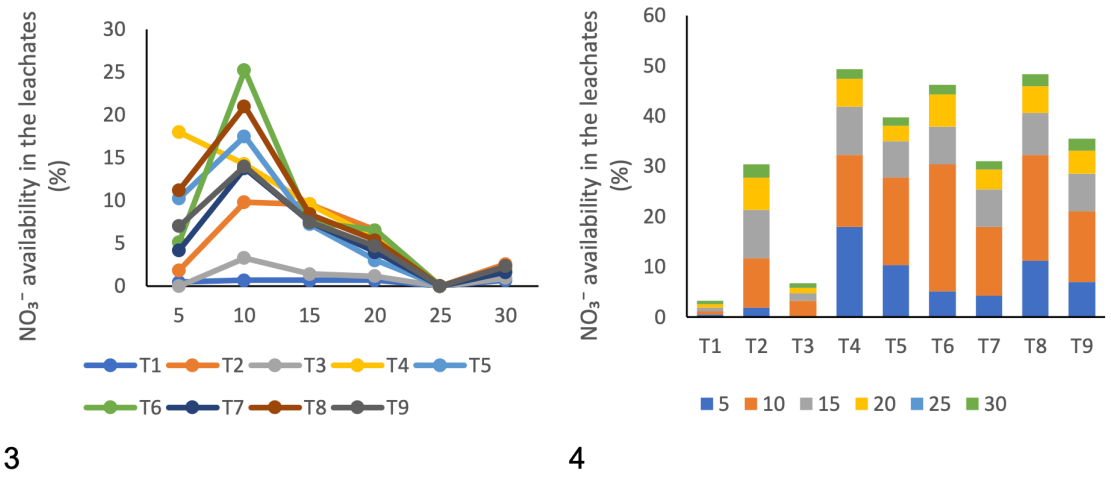
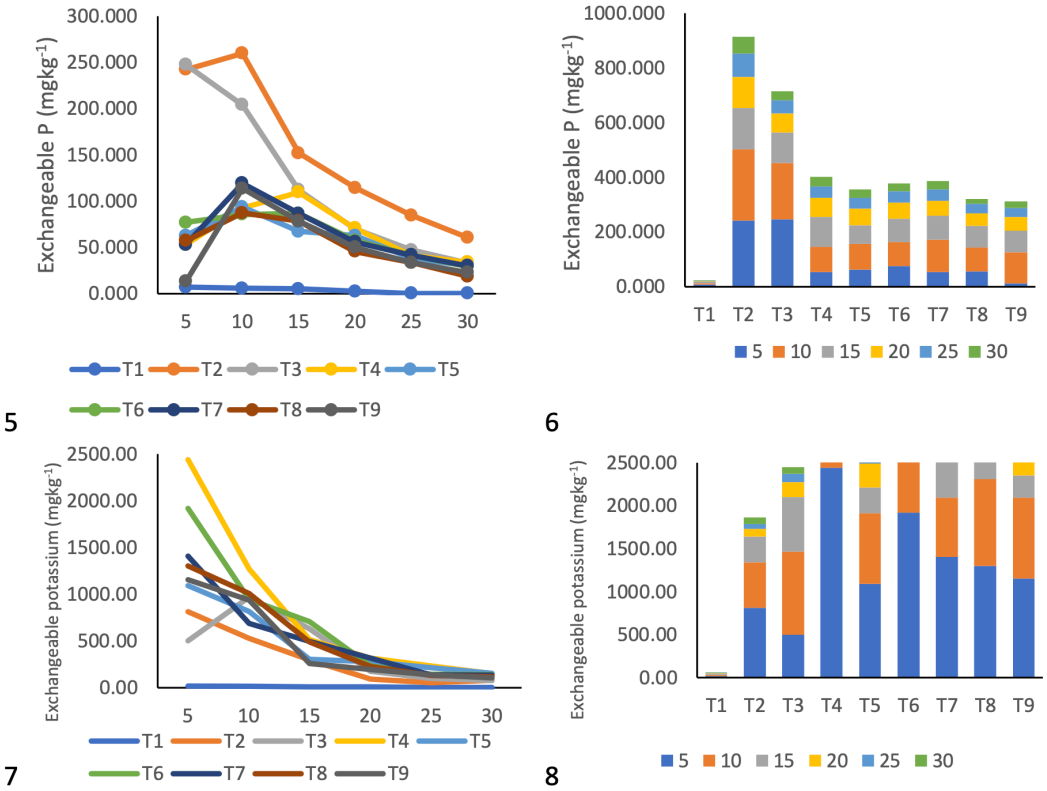
**Figure 1:** Ammonium availability in the leachate over thirty days of leaching.
**Figure 2.** Cumulative concentration of ammonium availability in leachate over thirty days of leaching




3 4

**Figure 3:** Nitrate availability in the leachate over thirty days of leaching.
**Figure 4:** Cumulative concentration of nitrate availability in leachate over thirty days of leaching

Although GSBA increased K and P leaching (Figure 5-8) relative to T1, GSBA simultaneously contributed to higher soil nutrient reserves, indicating a dual role in nutrient mobility and availability. The increase in K leaching was expected, as GSBA is naturally rich in K.

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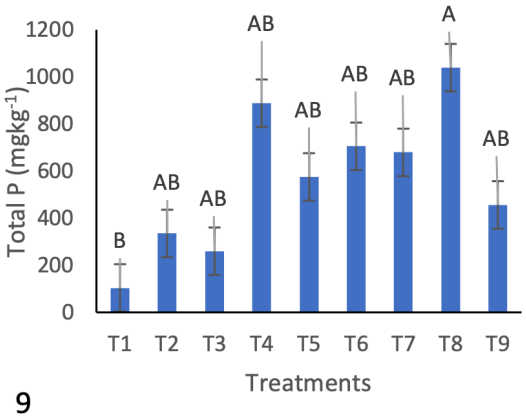
7 8

**Figure 5:** Phosphorus availability in leachate over thirty days of leaching.
**Figure 6:** Cumulative concentration of P in leachate over thirty days of leaching.
**Figure 7:** Exchangeable potassium in the leachates over thirty days of leaching
**Figure 8:** Cumulative concentration of exchangeable potassium in leachate over thirty days of leaching

Nevertheless, GSBA treatments improved soil total P and K concentrations (Figures 9 and 10) compared with T2 (full fertilization), demonstrating its potential as a supplementary nutrient source. These results suggest that while GSBA can enhance nutrient pools, careful management is required to minimize leaching losses and ensure greater synchronization between nutrient release and crop uptake. Overall, the study confirms the potential of GSBA to improve peat soil fertility by enriching the soil with essential nutrients such as P and K. Treatments incorporating GSBA consistently demonstrated higher total nutrient concentrations compared to commercial fertilization, highlighting its capacity to partially substitute chemical fertilizers while recycling local agro-industrial waste.

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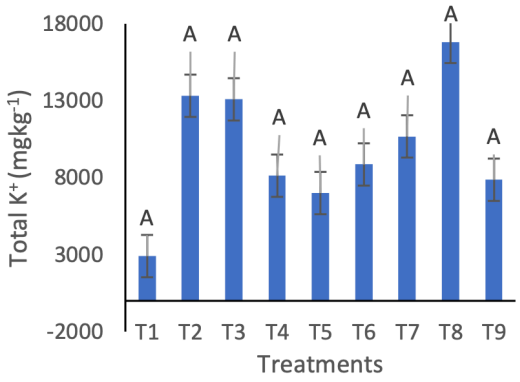


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**Figure 9:** Effect of treatments on total phosphorus at 30 days of leaching.
**Figure 10:** Effect of treatments on total potassium at 30 days of leaching

## CONCLUSION

Ground sago bark ash effectively improved P and K availability in tropical peat soil compared with peat soil fertilized with conventional fertilizers alone. The incorporation of GSBA with conventional fertilizers helps to retain major nutrients in tropical peat soil with T5, T6, and T8 showed the best results compared with conventional fertilizer, highlighting GSBA as a practical and sustainable soil amendment. Field studies are in progress to validate its effectiveness under real soil and crop interaction conditions.

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# Morphological and Molecular Characterization of Fungal Pathogens Infecting Trout in Pakistan

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## ABSTRACT

*Fungal pathogens pose significant threats to aquaculture particularly through diseases such as saprolegniasis and aspergillosis, which severely impact trout production in Pakistan. This study aimed to perform morphological identification of fungi infesting Oncorhynchus mykiss in the Khyber Pakhtunkhwa region of Pakistan and to conduct molecular characterization and phylogenetic analysis of the identified fungal species,targeting the ITS and 16S regions. fungal isolates were obtained from infected trout tissues, cultured on selective media, and characterized based on colony morphology and microscopic features. DNA was extracted, and ITS1–ITS2 regions were amplified for sequencing and phylogenetic analysis. Morphological characterization revealed distinct colony structures and conidial morphologies, such as chlamydospore formation in Paecilomyces variotii. Molecular analysis showed high sequence similarity (>99%) with reference strains in GenBank. Phylogenetic analysis confirmed species-level clustering and provided insights into evolutionary relationships among the isolates. These findings contribute to fungal taxonomy in aquaculture and provide baseline data to strengthen disease management strategies, biosecurity, and sustainability in trout farming.Molecular analysis of the ITS1–ITS2 regions confirmed these morphological identifications, showing high sequence similarity (>99%) with GenBank reference strains. Phylogenetic analysis further verified species-level clustering with strong bootstrap support, clarifying evolutionary relationships among the isolates. Together, these findings provide the first detailed account of fungal pathogens infecting farmed trout in northern Pakistan and establish a baseline for taxonomy, disease surveillance, and biosecurity measures in aquaculture.*

**Keywords:** Aquaculture, fungal pathogens, saprolegniasis, morphological identification, molecular characterization, phylogenetic analysis

## INTRODUCTION

Aquaculture is a vital component of global food security but faces major threats from fungal diseases, especially in *Oncorhynchus mykiss* trout farming in Pakistan. Pathogens like *Alternaria*, *Aspergillus*, *Trichoderma*, *Penicillium*, and *Paecilomyces* cause significant economic losses by infecting fish and increasing mortality (Ebrahimi et al., 2022). These fungi exploit environmental conditions, spreading via water. Although traditional morphological methods aid in identification, they have limitations. Therefore, combining morphology with molecular techniques such as ITS sequencing and SCAR marker development enables accurate and rapid identification (Magray et al., 2019). In addition to molecular techniques, morphological characterization remains a cornerstone in fungal taxonomy and identification. Morphological features such as colony morphology, hyphal characteristics, and spore morphology provide valuable diagnostic information that complements molecular data (Seifert et al., 2007). This study investigates fungal pathogens infecting trout through an integrated taxonomic and molecular approach to improve aquaculture biosecurity.

## PROJECT APPROACHES

Approximately 200 infected trout samples were collected from fish farms in Shino hatchery, Kunhar River (Naran), and Swat (Khyber Pakhtunkhwa, Pakistan). Samples were cultured on Sabouraud Dextrose Agar (SDA) at 28–30°C for 6–7 days. Isolates of *Aspergillus niger*, *Aspergillus flavus*, *Penicillium chrysogenum*, and *Paecilomyces variotii* were preserved in glycerol at -80°C.

Genomic DNA was extracted using the CTAB method. The ITS1 and ITS2 regions were amplified using PCR and sequenced with a 3130 Genetic Analyzer (Applied Biosystems). BioEdit version 7.2.6 was used for sequence alignment, and MEGA version 11.0.13 was used for phylogenetic tree construction using the maximum likelihood method.

ISSR-PCR with 20 primers from Macrogen (Seoul, Korea) was used to detect polymorphisms. SCAR (Sequence Characterized Amplified Region) markers were developed by sequencing the ISSR-PCR products, aligning sequences to identify unique bands, and designing primers specific to these regions using Primer3 software. The specificity of these primers was tested against all isolates, ensuring their diagnostic utility. SCAR primers were designed from ISSR fragments and synthesized by Macrogen. PCR amplification was performed using the designed SCAR primers under the same conditions as the ISSR analysis. Specificity tests for SCAR primer pairs were conducted against the fungal strains with accession numbers PQ036835.1–PQ036841.1. Sensitivity assays involved PCR with varied quantities of genomic DNA and were replicated at least twice.

## FINDINGS

Morphological and microscopic analysis revealed species-specific structures:

*Alternaria alternata* had dark conidiophores and large conidia with short beaks; *Aspergillus niger* showed radial conidia and smooth conidiophores; *A. flavus* displayed branching hyphae and spherical conidia; *Trichoderma citrinoviride* secreted yellow pigment and had a unique branching conidiophore; *Penicillium chrysogenum* exhibited classic brush-like conidiophores; *Paecilomyces variotii* showed chlamydospores and long chains of conidia.

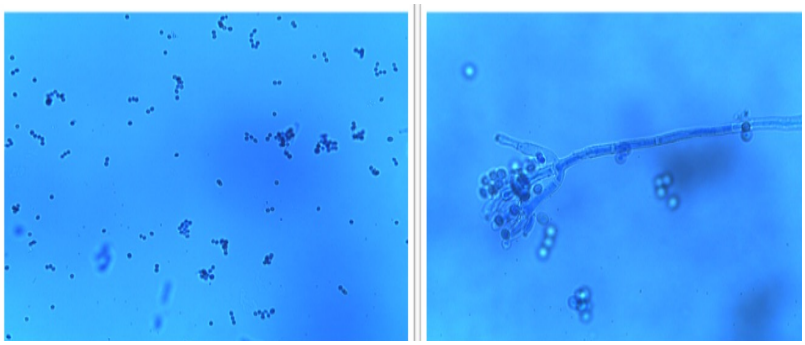

**Figure 1**: Morphological and Molecular Characterization of *Penicillium chrysogenum.*

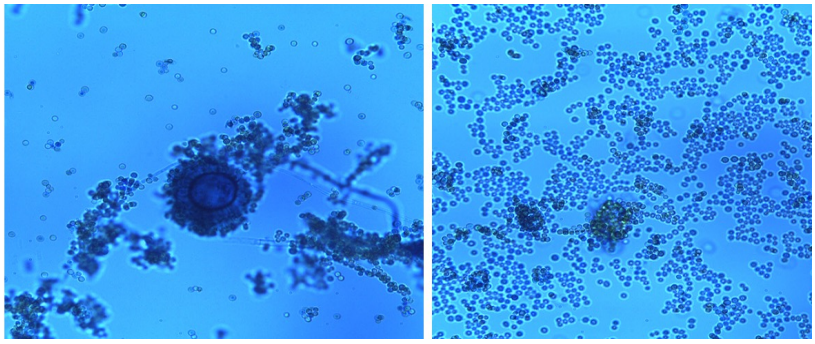

**Figure 2**: Morphological and Molecular Characterization of *Aspergillus flvus.*

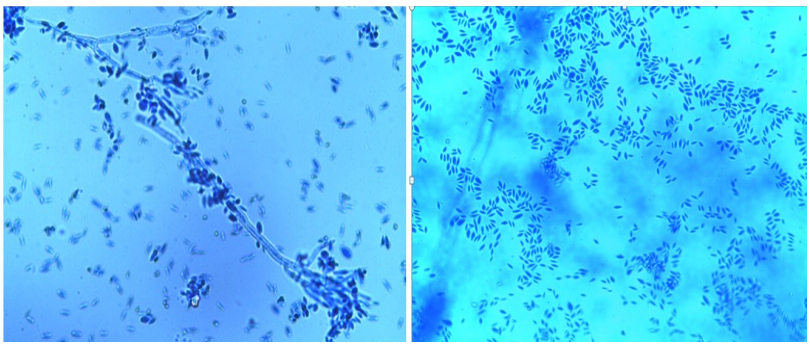

**Figure 3**: Morphological and Molecular Characterization of *Paecilomyces variotii.*

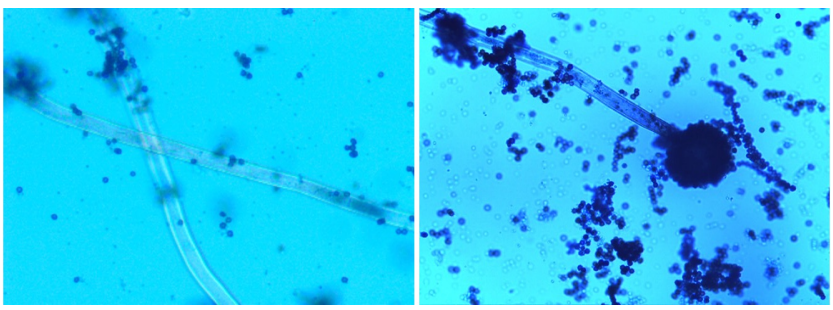

**Figure 4:** Morphological and Molecular Characterization of *Aspergillus niger*

Molecular analysis showed high ITS sequence similarity (>99%) and low genetic distances (0.01–0.05). Phylogenetic trees had strong bootstrap support (>80%), confirming species clusters. The *Alternaria* isolates clustered with *A. brassicicola, A. flavus* and *A. niger* matched Chinese and Indian isolates.

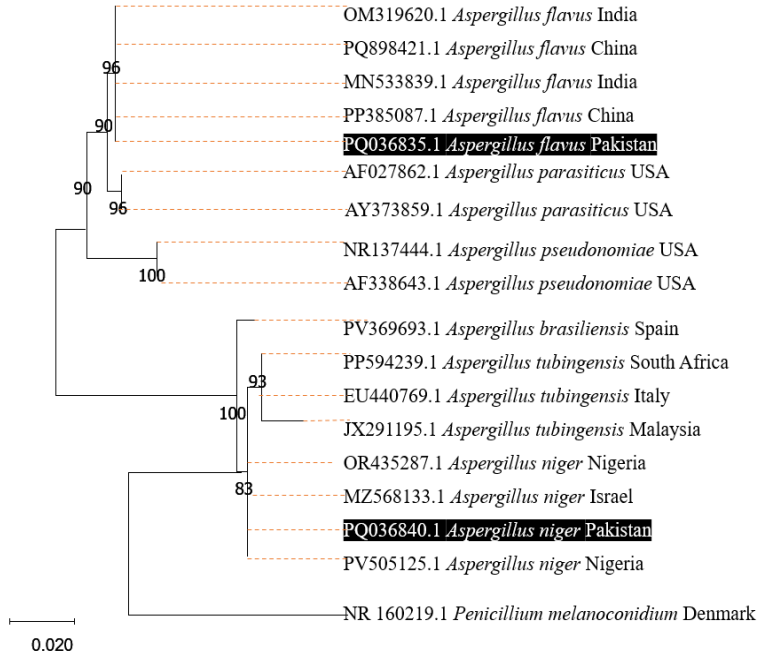


**Figure 5**: Phylogenetic tree illustrating the inferred evolutionary relationships among various species of the genus *Aspergillus*, along with one outgroup species from the genus *Penicillium* (NR160219) *Penicillium melanoconidium* from Denmark).

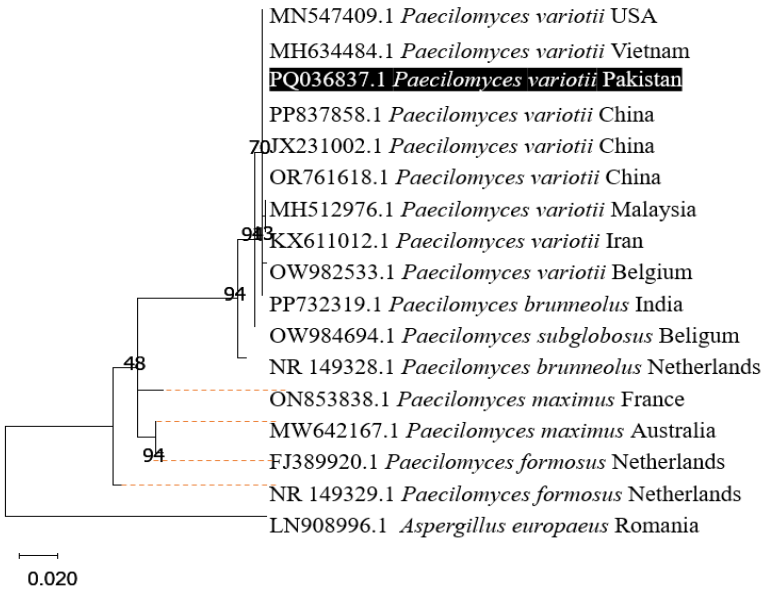


**Figure 6**: The Maximum Likelihood technique, using 1000 bootstrap iterations of the incomplete (ITS) sequence, was used to create the phylogenetic tree of *Paecilomyces variotii*.

**Table 1**. Haplotype and Nucleotide diversity of the ITS sequences of fungal species.

| Organism name | Accession number(s) | Total sequences | Hd | Kt | PiT |
|---|---|---|---|---|---|
| *Alternaria flavus* | PQ036835.1 | 7 | 0.90476 | 1.85714 | 0.00337 |
| *Paecilomyces variotii* | PQ036837.1 | 9 | 0.41667 | 0.66667 | 0.00124 |
| *Penicillium chrysogenum* | PQ036839.1 | 7 | 0.66667 | 0.76190 | 0.00129 |
| *Aspergilla niger* | PQ036840.1 | 7 | 0.71429 | 1.42857 | 0.00265 |

## CONCLUSION

This study comprehensively identifies fungal pathogens infecting trout in Pakistan using morphological and molecular methods. Species-specific traits were confirmed by high ITS sequence identity and phylogenetic clustering. SCAR markers proved to be a rapid, reliable tool for accurate fungal identification. These findings offer critical insights into trout disease diagnostics and enhance aquaculture biosecurity.

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# Biodegradation of Polyethylene (PE) by Thermophilic Bacteria Isolated from Paku Hot Spring, Sarawak

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## ABSTRACT

*Plastic pollution, particularly from polyethylene (PE), remains a significant environmental concern due to its high resistance to natural degradation. This study investigated the biodegradation potential of thermophilic bacteria isolated from Paku Hot Spring, Sarawak. The objectives were to isolate and screen thermophilic bacteria capable of degrading low-density polyethylene (LDPE), to characterize the selected isolates using the Bacterial Adhesion to Hydrocarbons (BATH) test, clear zone assay, GTG5-PCR fingerprinting, and 16S rRNA gene sequencing, and to evaluate their biodegradation efficiency based on pH variation, bacterial growth (OD600), and PE weight loss for a 30-day incubation period. Two potential PE-degrading bacteria, SPK(W)M1(1) and SPK(SD)P1(2) demonstrated notable PE-degrading activity. Over the 30-day biodegradation assay, pH levels decreased from the initial pH 7.0 to a range of 6.32–6.66. Weight loss analysis indicated that SPK(W)M1(1) achieved the greatest PE degradation at 2.52% (OD600: 0.06 ± 0.02), followed by SPK(SD)P1(2) at 2.03% (OD600: 0.08 ± 0.01). These findings highlight the potential of thermophilic bacteria from geothermal environments as sustainable and eco-friendly agents for plastic waste biodegradation, supporting their further exploration for large-scale environmental applications in Malaysia.*

**Keywords:** Biodegradation, Polyethylene, Thermophilic Bacteria

## INTRODUCTION

Plastic pollution, especially from polyethylene (PE), constitutes one of the most enduring and widespread environmental challenges of the age of humanity. Polyethylene, a synthetic polymer extensively employed in packaging and consumer products, demonstrates significant resistance to natural breakdown, leading to its persistent accumulation in terrestrial and aquatic ecosystems. Traditional waste disposal methods,

such as landfilling and burning, frequently pose considerable environmental hazards, including greenhouse gas emissions and leachate pollution. Conversely, microbial biodegradation has arisen as a sustainable and eco-friendly alternative. Thermophilic bacteria, particularly those residing in natural hot springs, are noteworthy for their enzymatic versatility and ability to decompose complex polymers at high temperatures. Notwithstanding this promise, the biodegradation capacities of thermophiles from Malaysian hot springs are yet little investigated. This research seeks to extract and characterise thermophilic bacteria from Paku Hot Spring, Sarawak, and evaluate their efficacy in decomposing polyethylene.

*Objectives*

1. To isolate and screen thermophilic bacteria from Paku Hot Spring with potential for LDPE biodegradation.
2. To characterize the selected isolates using the Bacterial Adhesion to Hydrocarbons (BATH) test, clear zone assay, $GTG_5$-PCR fingerprinting, and 16S rRNA gene sequencing.
3. To evaluate the biodegradation efficiency of selected thermophilic isolates based on changes in pH, bacterial growth (OD600), and LDPE weight loss over 30 days.

## PROJECT APPROACH

This project was conducted to investigate the potential of thermophilic bacteria from Paku Hot Spring, Sarawak, to biodegrade polyethylene (PE), a common and persistent plastic pollutant. The project began with in situ collection of water and sediment samples from various zones of the hot spring. Samples were then transported under controlled conditions to the laboratory, where 96 thermophilic bacterial isolates were obtained using Carbon Free Synthetic Medium (CFSM) agar incubated at elevated temperatures (55°C) (Balasubramanian et al., 2010). Preliminary screening of isolates for biodegradation potential involved the Bacterial Adhesion to Hydrocarbons (BATH) test to assess cell surface hydrophobicity, a key trait for plastic adhesion and colonization (Devi, 2019). Enzymatic degradation capability was further evaluated using clear zone assays on PE-emulsified agar plates (Soud, 2019). Isolates that exhibited positive results were subjected to GTG5-PCR fingerprinting to assess genetic diversity and select unique strains for further study (Kathleen et al., 2014). Promising isolates were identified using 16S rRNA gene sequencing to determine taxonomic identity. For biodegradation evaluation, sterilized PE films were incubated with the selected strains in CFSM at thermophilic conditions (55°C) for 30 days. During incubation, pH changes, optical density (OD600) as an indicator of bacterial growth, and percentage weight loss of PE were monitored. These parameters were used to determine the isolates' efficiency in degrading polyethylene under controlled laboratory conditions (Toh et al., 2023).

## FINDINGS

A total of 96 thermophilic bacterial isolates were successfully acquired from Paku Hot Spring. The preliminary assessment utilising the Bacterial Adhesion to Hydrocarbons

(BATH) test indicated that 11 isolates (Figure 1) demonstrated significant hydrophobicity, suggesting their capacity to attach to hydrophobic substrates like polyethylene (PE). Clear zone assays (Figure 2) further validated the existence of enzymatic activity linked to PE breakdown in various isolates. Two strains, SPK(W)M1(1) and SPK(SD) P1(2), were chosen for further analysis because of their reliable performance in both the BATH test and clear zone assay, together with distinct genetic profiles verified by $GTG_5$-PCR fingerprinting (Figure 4)

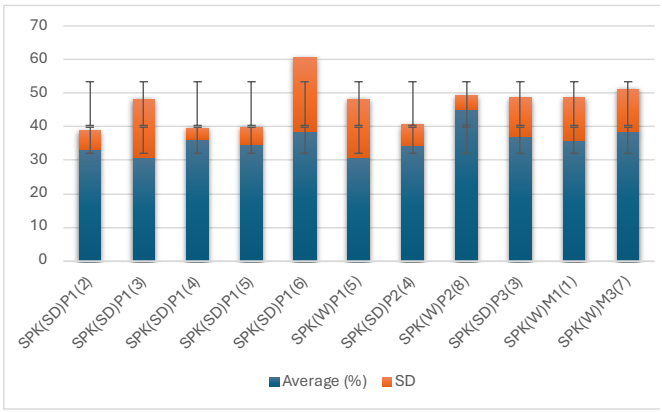


**Figure 1:** 11 Isolates with highest BATH Test percentage

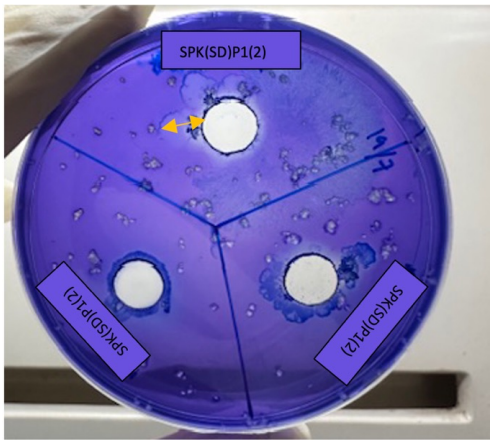


**Figure 2:** The clear zone was conducted by streaking 11 sample of thermophilic bacteria on PE agar.
Yellow arrow: present of clear zone

*Screening Selection Processing*

The selection of isolates for further biodegradation assay was analyzed using Heatmap (Figure 3). The heatmap was generated using open access heatmap software available at (Morpheus:https://software.broadinstitute.org/morpheus) accessed on 23 July 2025. Isolates with a 'YES' result in both tests were highlighted and prioritized for subsequent degradation studies, resulting in the selection of 7 candidate strains

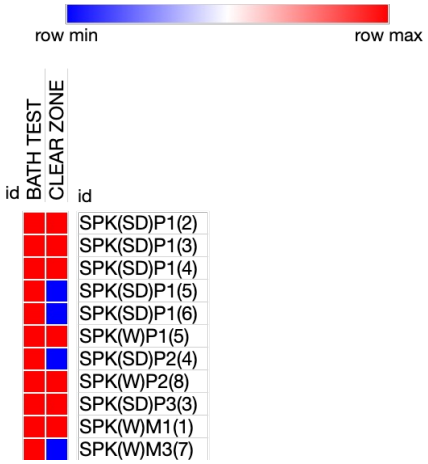


**Figure 3***:* Heatmap illustrating the distribution of Thermophilic bacteria from screening process. Columns denote screening result of BATH test and Clear Zone. The color gradient represents the presence of the screening. Red: Yes Blue: No

*$GTG_5$ Fingerprinting*

Figure 4 illustrates the dendrogram of thermophiles isolates obtained from Paku Hot Spring. The three thermophiles' isolates were grouped into two cluster.


**Figure 4**. Dendrogram showing thermophiles distribution isolated from Paku hot spring.

*16s rRNA*

Identification using 16S rRNA gene sequencing was conducted to enable accurate taxonomic identification.

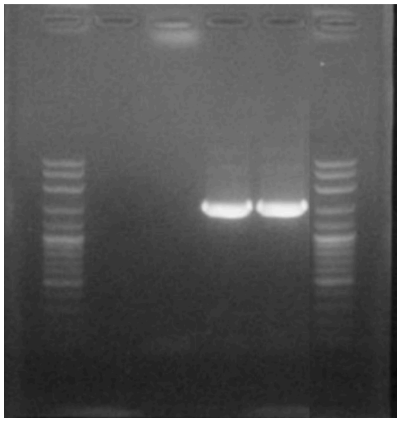

**Figure 5**. 100 bp ladder, negative, SPK(SD)P1(3), SPK(SD)P1(2), SPK(W)M1(1), 100 bp ladder.

However, only three isolates SPK(SD)P1(2), SPK(SD)P1(3) and SPK(W)M1(1) produced distinct bands suitable for analysis in the GTG5 fingerprinting (Figure 4) while only two isolates exhibit strong, amplifiable bands in the 16s rRNA PCR (Figure 5). Subsequent sequencing and BLAST anlysis confirmed the identify of two isolates SPK(SD)P1(2) and SPK(W)M1(1), with both assigned to genus *Anoxybacillus* sp. UARK-01 (97.65%). Genus *Anoxybacillus* sp. UARK-01, is a thermophilic species previously associated with environmental resilience and hydrocarbon degradation. Despite their identical identification as *Anoxybacillus* sp. UARK-01 by 16S rRNA gene analysis, these two isolates were found to cluster on different branches in the $GTG_5$ fingerprinting dendrogram (Figure 4), suggesting intraspecific genomic diversity. despite the $GTG_5$ result showing a distinct result.

A 30-day biodegradation study demonstrated active polyethylene breakdown by both bacteria. The pH (Table 1) of the culture medium reduced from the initial neutral value (pH 7.0) to a range of 6.32 to 6.66, indicating active microbial metabolism. Optical density (Table 2), measurements indicated significant bacterial proliferation, with SPK(SD) P1(2) attaining an OD600 of 0.08±0.01, and SPK(W)M1(1) achieving 0.06±0.02. The weight loss (Table 3) study of the PE films further substantiated biodegradation, with

SPK(W)M1(1) exhibiting the maximum degradation at 2.52%, succeeded by SPK(SD) P1(2) at 2.03%.

**Table 1**. Mean value of the pH recorded after 30 days of LDPE biodegradation process using selected two thermophiles isolated from Paku hot spring water, Sarawak.

| Thermophilic Isolates | pH measurement | |
|---|---|---|
| | Before treatment | After Treatment |
| ***Anoxybacillus* sp. UARK-01 –(SPK(W)M1(1))** | 7 | 6.5 ± 0.08 |
| ***Anoxybacillus* sp. UARK-01 (SPK(SD)P1(2))** | 7 | 6.5 ± 0.06 |
| **Control** | 7 | 7.0 |

**Table 2**. Mean value of the bacteria growth (OD600) recorded after 30 days of LDPE biodegradation process using selected two thermophiles isolated from Paku hot spring water, Sarawak.

| Thermophilic Isolates | Bacterial growth (OD600) |
|---|---|
| **Anoxybacillus sp. UARK-01 –(SPK(W)M1(1))** | 0.06 ± 0.02 |
| **Anoxybacillus sp. UARK-01 (SPK(SD)P1(2))** | 0.08 ± 0.02 |
| **Control** | 0 |

**Table 3**. Mean value of the weight loss (g) for the LDPE plastic sheet recorded after 30 days of LDPE biodegradation process using two thermophiles isolated from Paku hot spring water, Sarawak.

| Thermophilic Isolates | Weight loss of the LDPE film (g) | | Weight reduction (%) |
|---|---|---|---|
| | Before treatment | After Treatment | |
| ***Anoxybacillus* sp. UARK-01 –(SPK(W)M1(1))** | 0.5 | 0.45 ± 0.05 | 10 |
| ***Anoxybacillus* sp. UARK-01 (SPK(SD)P1(2))** | 0.5 | 0.42 ± 0.00 | 2 |
| **Control** | 0.5 | 0.5 | 0 |

Despite the modest percentage of weight loss, the results are deemed encouraging due to the brief incubation period and the intrinsic resistance of PE to degradation. The findings indicate that thermophilic bacteria from Paku Hot Spring can initiate the degradation of polyethylene at elevated temperature settings. The research endorses the prospective utilisation of extremophilic bacteria in formulating biotechnological strategies for plastic waste management, especially in tropical areas such as Malaysia.

## CONCLUSION

This study effectively revealed the capacity of thermophilic bacteria sourced from Paku Hot Spring, Sarawak, to commence the biodegradation of polyethylene (PE), a very resistant and pervasive plastic contaminant. Utilising microbial isolation, phenotypic and molecular characterisation, along with a 30-day degradation experiment, two promising strains designated as *Anoxybacillus* UARK-01 species demonstrated a reduction in polyethylene weight exceeding 2%, accompanied by definitive evidence of metabolic activity and growth. The degradation rates, however small, are noteworthy considering the brief incubation duration, single-strain methodology, and the intrinsic recalcitrance of polyethylene. These findings underscore the unexploited potential of

thermophilic bacteria from geothermal environments as sustainable agents for plastic waste reduction. The findings of this research provide a significant basis for future investigations aimed at improving degradation efficiency via microbial consortia, enzymatic optimisation, and extended trials, thereby aiding the advancement of sustainable biotechnological solutions for plastic pollution in Malaysia and beyond.

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# Effect of Anti-Browning Treatments on Quality Retention of Fresh-Cut Terap (*Artocarpus odoratissimus*) During Cold Storage

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## ABSTRACT

*Flesh browning is a major concern in fresh-cut Artocarpus odoratissimus, affecting its visual appeal, sensory quality, and market value. Despite its popularity in Sarawak, the fruit remains underutilized due to its short shelf life and limited postharvest handling research. This study evaluated the effectiveness of five anti-browning treatments on enzymatic browning and phytochemical stability in vacuum-packed fresh-cut samples stored at 7°C for 35 days. Results indicated that Treatment 3 (2% ascorbic acid + 1% citric acid + 1% calcium chloride), can prolong the shelf life of the A. odoratissimus flesh up to a month. It significantly reduced browning activity (0.34±0.02), total colour changes (ΔE) (24.35±1.02), microbial count and retained ascorbic acid (2.36±0.34 µg $g^{-}1$). It also suppressed phenylalanine ammonia-lyase (PAL) (0.14±0.02 UE $g^{-1}$ $min^{-1}$) and peroxidase POD (0.21±0.01 UE $g^{-1}$ $min^{-1}$) activities, after 4 weeks of storage. These findings support the potential of fresh-cut A. odoratissimus for commercial development.*

**Keywords:** anti-browning, indigenous fruit, shelf life, storage, terap

## INTRODUCTION

*Artocarpus odoratissimus*, like many tropical fruits, tends to brown quickly once they are opened due to enzymatic reactions. When the fruit is cut and exposed to air, phenolic compounds from the vacuoles interact with polyphenol oxidase (PPO). This leads to the formation of brown pigments that affect its appearance and quality (Wang et al., 2023; Zhu et al., 2023). With demand rising sharply during its peak season, it is important to improve both pre-harvest and post-harvest practices to keep the fruit available and of good quality, even during the off-season. Local sellers often market the fruits because the fruits deteriorate quickly. These losses happen mainly because of challenges in packaging, primarily due to the fruit's size and latex.

Preventing browning in food processing has become an important research area (Ioannou and Ghoul, 2013) since it can reduce costs losses in the fresh produce industry. In food preservation, effective anti-browning agents can inhibit polyphenol

oxidase enzymes (PPOs), preventing browning reactions from occurring. These anti-browning agents improve food appearance, extend shelf life, and reduce food waste. A study by Colas-Meda et al. (2016) showed that ascorbic acid, citric acid, and calcium chloride are effective as anti-browning agents by slowing down the browning appearance and maintaining the overall quality of fruits and vegetables.

This study aimed to explore the effects of vacuum-sealed anti-browning treatments on the color, enzymatic browning, and microbial load of fresh-cut *A. odoratissimus* under cold storage conditions. This study investigated the efficacy of various anti-browning treatments and packaging methods to enhance shelf life and maintain the postharvest quality of *A. odoratissimus*.

## RESEARCH APPROACHES

The fruits were harvested from an orchard located at Bekenu Sarawak, Malaysia. Defect-free fruits were brought to the laboratory without delay. Any dirt adhering to the surfaces of the fruit samples was scrubbed off before sample processing. The *A. odoratissimus* fruits were dissected and the pulp was removed from the core, and the seeds were retained. Only firm and blemish-free flesh portions were soaked in 5 anti-browning treatments, which were T1 (control distilled water), T2 (NatureSeal®), T3 (2% ascorbic acid, 1% citric acid, 1% calcium chloride), T4 (2% calcium ascorbate with 1% calcium chloride), T5 (2% sodium ascorbate with 1% calcium chloride). The samples were vacuum packaged and stored at 7°C. The treatments were arranged in a CRD and observed over 35 days. Data on the CIE lab parameter were collected on a weekly basis, and the total colour changes were computed using the method proposed by Nambi et al. (2017). Brandelli and Lopes (2005) methods were used to evaluate the degree of browning. Enzymatic activities on PAL were assessed based on Peixoto et al. (1999) for PAL, Cano et al. (1997) for PPO, and Rossi et al. (1997) for POD. The microbial quality was assessed following ISO methodologies. All experiments were performed in triplicates. The data was analyzed using SAS 9.4 software. Means were compared using single-factor analysis of variance (ANOVA) with post hoc Tukey's test ($p<0.05$) which was used to compare the mean values of A. odoratissimus flesh for the physicochemical parameters, degree of browning, enzymatic activities, and phytochemical contents.

## FINDINGS

Findings showed that the rapid increase in total colour changes was observed in treatment 1 from 21.42±0.49 to 27.52±1.08 treat with distilled water compared to the T3 with slowed increased from 9.97±0.12 to 24.35±1.02 and T2 increased from 11.32±0.46 to 25.27±0.88 (positive control) where the flesh remains fresh along the storage period. This was associated with the degree of browning that lower degree of browning was found in the fruits treated with NatureSeal® in T2 value at 0.44±0.01 and T3 0.35±0.01 (ascorbic acid, citric acid and calcium chloride, while higher degree of browning was recorded in T1 value 48.25±0.03 (distilled water), T4 at 0.51±0.02 (calcium ascorbate, calcium chloride)

and T5 at 0.48±0.02 (sodium ascorbate, calcium chloride) after 35 days storage. Previous studies by Laksana et al. (2024) found that ascorbic acid is an effective inhibitor of enzymatic browning because it can reduce quinones. However, once the ascorbic acid is fully oxidized, quinones may accumulate and trigger the browning process.

Meanwhile, the enzyme activities for PAL, PPO, and POD show similar results. T3 has lower enzyme activity due to the combination of citric acid and ascorbic acid, which inhibit browning during storage. This indicates that ascorbic acid regulates PPO activity by converting o-quinones back to their phenolic substrates while it oxidizes into dehydroascorbic acid (Moon et al., 2020; Techavuthiporn and Boonyaritthonghai, 2016). In contrast, citric acid has a dual inhibitory effect. It lowers the pH below the optimal level for PPO activity and chelates copper at the active site of PPO (Moon et al., 2020).

The growth of psychrotrophic microbes (PM) and lactic acid bacteria (LAB) was effectively reduced during low-temperature storage at 7±1°C for up to three weeks. A slight increase was noted in weeks four and five, especially in Treatment 4 (T4), where the count rose from 0.00±0.00 to 15.63±0.48 for psychrotrophic microbes (PM) and from 0.00±0.00 log CFU/g to 3.27±1.25 log CFU/g for lactic acid bacteria (LAB). This defines that it is essential to analyze the microbial load on fresh cut fruit for safety consumption.

This study further examined correlations using a person correlation matrix. The results showed a strong positive correlation between TPC and PPO activity (r=0.94). This means that as the concentration of phenolic compounds increases, PPO activity also rises. This increase may lead to more enzymatic browning and contribute to the decline in quality of fresh-cut fruits.

## CONCLUSION

Terap fruits treated with ascorbic acid, citric acid, and calcium chloride effectively reduce browning and maintain phenolic activities during storage. This process prolongs the shelf life of the fruits and reduces postharvest losses while promoting fresh-cut terap. The result is improved external quality and a longer shelf life without decay, which reduces postharvest losses and supports the potential commercialization of fresh-cut terap. Improvements in post-harvest technology are crucial for producing fresh-cut fruit, opening new opportunities for terap in the market.

## ACKNOWLEDGEMENT

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# R4Next-Gen Fertilizer: Nitrogen-Rich Hydrogels from Industrial Byproducts

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## ABSTRACT

*A promising pathway toward sustainable agriculture involves transforming nitrogen rich industrial waste into hydrogel-based slow-release fertilizers (SRFs). In Malaysia, this type of waste is often classified as scheduled waste, requiring costly and environmentally demanding disposal methods. By embedding the waste into biodegradable hydrogels, nutrients can be released gradually in response to soil moisture, which may improve nutrient use efficiency while enhancing soil water retention. This dual-function approach not only reduces the environmental footprint of industrial operations but also supports more resilient and productive farming systems. Drawing from existing research, hydrogels have shown potential to reduce nutrient leaching, support healthy plant growth, and align with circular economy and environmental sustainability goals. This review explores the potential of hydrogel-based SRFs as a dual-purpose strategy: converting nitrogen-rich waste into value-added agricultural inputs while advancing circular economy. The approach offers a scalable, environmentally responsible pathway for improving both industrial and agricultural sustainability in Malaysia.*

**Keywords:** slow-release fertilizer, nitrogen-rich industrial waste, waste valorisation, environmental stewardship

## INTRODUCTION

In Malaysia, some industrial operations generate large volumes of nitrogen-rich waste as a byproduct. This byproduct is classified as scheduled waste under national environmental regulations, which requires strict handling and disposal to prevent ecological harm (DOE Malaysia, 2005; Maharajh et al., 2021). To dispose this, the traditional disposal methods like incineration and chemical neutralization are not only costly but also environmentally taxing and contributing to emissions (Capanoglu et al., 2022). Meanwhile, Malaysia's agriculture sector remains heavily reliant on conventional nitrogen fertilizers to maintain crop yields. These fertilizers, however, are vulnerable to nutrient losses through leaching and volatilization. Hence these inefficiencies pose risks to groundwater quality and long-term soil health (Galloway et al., 2003; Fan et al., 2020). This intersection of industrial waste challenges and agricultural inefficiencies

presents an opportunity for innovation. Hydrogels are biodegradable materials known for their excellent water retention and controlled nutrient release, offer a promising solution (Rizwan et al., 2021; Chamorro et al., 2024). When this hydrogel is used as nutrient carriers, they can reduce fertilizer losses, improve soil moisture, and support more sustainable farming practices. This review explores the potential of hydrogel-based slow-release fertilizers (SRFs) as a dual-purpose strategy: converting nitrogen-rich waste into value-added agricultural inputs while advancing circular economy and ESG goals. The approach offers a scalable, environmentally responsible pathway for improving both industrial and agricultural sustainability in Malaysia.

## PROJECT APPROACHES

With increasing pressure to manage industrial waste responsibly and promote sustainable agriculture, this project proposed a practical and forward-thinking solution: converting nitrogen-rich by-products into environmentally friendly, slow-release fertilizers. Instead of discarding these nutrient-rich materials, the study reimagines them as valuable inputs for farming. By embedding the waste into biodegradable hydrogels, the nutrients are transformed into a responsive delivery system that will be released gradually releasing gradually.

The development process focuses on creating a hydrogel matrix that not only retains water but also regulates nutrient availability. Biopolymers are first dissolved in distilled water and enhanced with natural additives to improve elasticity and structural integrity (Chamorro et al., 2024). Nitrogen-rich waste is then incorporated under controlled laboratory conditions to ensure even dispersion without compromising the hydrogel's properties. Once formulated, the final mixture is cast into molds and dried at room temperature to form a stable, solid fertilizer. Its physicochemical properties and effect on plant growth will be performed following protocols by Ostrand et al. (2020) and Rizwan et al. (2021).

## FINDINGS

Based on insights from current literature, it suggests that hydrogel-based fertilizers incorporating nitrogen-rich waste offer several advantages over conventional fertilizer systems. One of the most notable benefits is their ability to retain water effectively, which is especially valuable in tropical agricultural settings where moisture stress and irregular rainfall are common challenges. By holding water within the soil matrix, hydrogels help reduce irrigation frequency and improve plant resilience under dry conditions (Ostrand et al., 2020; Rizwan et al., 2021). In addition to water retention, these hydrogels provide a controlled nutrient release mechanism that aligns more closely with plant uptake patterns. This slow and steady release minimizes nutrient losses due to leaching and volatilization issues that often compromise the efficiency of traditional fertilizers.

As a result, hydrogel-based fertilizers can enhance nutrient use efficiency while reducing the risk of environmental pollution (Melaj et al., 2019). Studies also indicate

that hydrogel formulations contribute positively to early plant development. Consistent moisture and balanced nutrient availability promote better germination rates, stronger root systems, and improved overall plant vigor (Hou et al., 2022). These agronomic benefits make hydrogels a promising alternative for regions facing water scarcity and nutrient management challenges. However, literature also cautions against excessive incorporation of nitrogen-rich waste, which may lead to salt stress or phytotoxic effects. Achieving the right balance between waste content and polymer composition is essential to ensure plant safety and soil health. Overall, hydrogel-based fertilizers show strong potential to support sustainable agriculture by improving productivity and reducing environmental impact.

## CONCLUSION

Overall, this review highlights the promising role of hydrogel-based slow-release fertilizers in addressing two major challenges, i.e., managing nitrogen-rich industrial waste and improving agricultural efficiency. By repurposing waste into a valuable input, this approach not only reduces the environmental burden of disposal but also enhances nutrient delivery and water retention in soil. It aligns well with circular economic principles and supports broader sustainability goals in both industry and agriculture. The dual benefits of environmental stewardship and improved crop performance make hydrogel-based fertilizers a compelling alternative to conventional practices.

## ACKNOWLEDGEMENT

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# Optimizing Substrate and Additive Formulations in Seedballs to Enhance Germination

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## ABSTRACT

*Seedballs offer a low-cost, efficient method for large-scale ecological restoration, but optimizing their composition is crucial for maximizing germination success. This study evaluated the germination performance of Mucuna bracteata seedballs using different substrate mixtures and polymer additives under laboratory and field conditions. Substrates included combinations of red soil, clay, sand, compost, cocopeat, and peatmoss. Among them, a 2:1 mixture of red soil and peatmoss (T4) produced the highest germination rate in the laboratory (83.67%). However, performance declined under open-field conditions, with 63.33% germination in vegetated areas and 52.87% in bare land. Incorporations of 0.5%–1% natural polymer (NP) additives recorded significantly higher germination rates (86.67% to 91.11%). These findings highlight the importance of strategic substrate and additive selection to optimize seedball effectiveness across diverse environments, ultimately contributing to more successful reforestation and ecosystem restoration initiatives.*

**Keywords:** restoration, germination, water retention, substrates, seedball

## INTRODUCTION

Seedballs offer an affordable and innovative way to grow plants in areas with limited vegetation, such as deforested lands, deserts, grasslands, and abandoned agricultural or horticultural fields. This method has become an important tool in natural farming and global conservation efforts (Kannan et al., 2021). It is especially useful for replanting large dry areas in a cost-effective manner (Tamilarasan et al., 2021; Atkinson, 2003). Successful large-scale forest restoration requires methods that are sustainable, low-cost, and involve local communities (Tamilarasan et al., 2021; Holl, 2017; Shoo et al., 2016). Seedball technology supports forest ecosystem conservation, especially in areas where traditional planting methods are difficult to apply. Seedballs can be spread efficiently over rough or hard-to-reach terrain using drones, making

them a practical and scalable solution (Kannan et al., 2021).

Restoring vegetation on degraded lands has become a critical focus in environmental research, as it plays a vital role in combating biodiversity loss and land degradation (Kannan et al., 2021). For large-scale forest restoration to be successful, the planting media must be both effective and environmentally sustainable (Shoo et al., 2016; Holl, 2017). Since different seed types have specific requirements for optimal growth, this study aims to evaluate the effects of various substrate compositions and additives on the germination rates of selected seed.

## PROJECT APPROACHES

*Mucuna bracteata* seedballs were prepared using different substrates and additive compositions to evaluate their germination performance under laboratory and field conditions. Four substrate mixtures were tested: red soil with sand (T1), cocopeat (T2), compost (T3), and peatmoss (T4). Field trials were conducted in two locations, where vegetated areas and bare land to compare germination under different environmental conditions. Additionally, seedballs were mixed with varying concentrations of NP additive (0.5%, 1.0%, 2.0%, and 4.0%) to observe its effect on germination. Germination percentages were recorded, and statistical analysis was used to determine significant differences among treatments.

## FINDINGS

Figure 1 showed that seedballs formulated with T4 (2 parts red soil + 1 part peatmoss) achieved the highest germination percentage, significantly outperforming other treatments. The germination rate for T4 exceeded 80%, which was notably higher than T1 (2 parts red soil + 1 part sand), T2 (2 parts red soil + 1 part cocopeat), and T3 (2 parts red soil + 1 part compost) under controlled condition. All three treatments exhibited comparable and significantly lower germination rates, with no significant difference among them. These results show that peatmoss helps keep moisture and creates a better environment to grow under controlled conditions (Schmilewski, 2018).

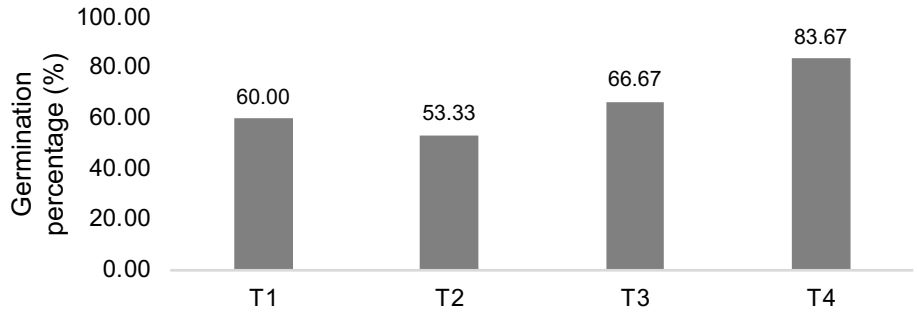


**Figure 1**: Germination performance of Mucuna bracteata seedballs under laboratory conditions using various substrate compositions; T1-2Red soil + 1Sand, T2-2Red soil + 1Cocopeat, T3-2Red soil + 1Compost, and T4- 2Red soil + 1Peatmoss

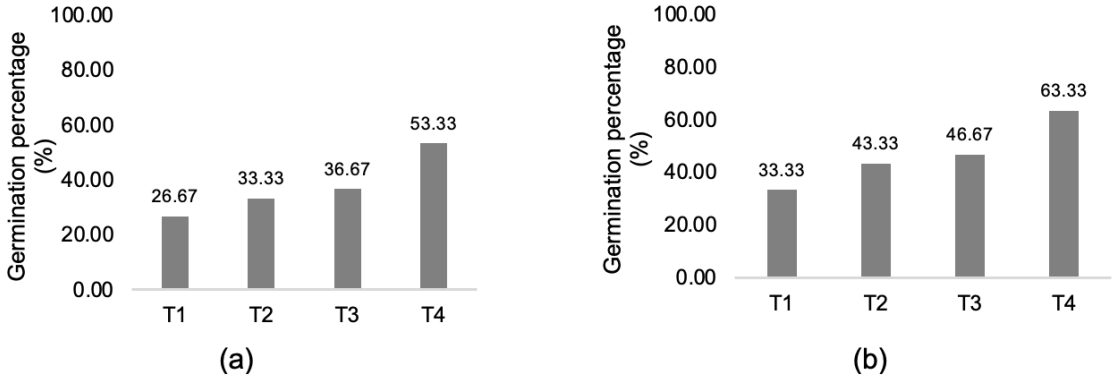


**Figure 2**: Germination performance of Mucuna bracteata seedballs under field conditions (a) vegetated area and (b) bare land using various compositions; T1-2Red soil + 1Sand, T2-2Red soil + 1Cocopeat, T3-2Red soil + 1Compost, and T4- 2Red soil + 1Peatmoss

In the vegetated area (Figure 2a), T4 (2 parts red soil + 1 part peatmoss) recorded the highest germination rate, which was significantly higher than T1, T2, and T3. Similarly, in the bare land condition (Figure 2b), T4 also achieved the highest germination percentage compared to the other treatments. However, the germination percentage was comparatively lower than under controlled conditions. Therefore, seedballs should be supplemented with water-retaining materials to enhance their water-holding capacity and support germination under open-field conditions.

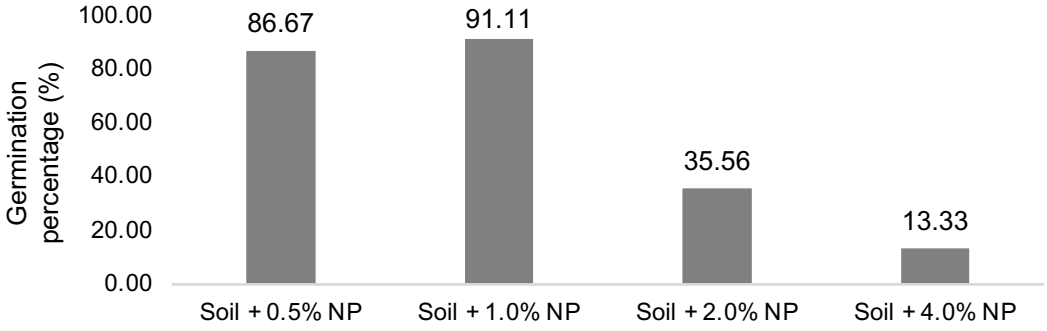


**Figure 3**: Germination performance of *Mucuna bracteata* seedballs under various additive compositions; T1-0.5% NP, T2-1.0% NP, T3-2.0% NP and T4-4.0% NP.

Addition of NP showed to improve the water holding capacity in seedball (Figure 3). The germination percentage of *Mucuna bracteata* seedballs changed depending on the concentration of NP added to the soil. T1 (0.5% NP) and T2 (1.0% NP) had the highest germination rates, both above 85%, However, T3 (2.0% NP) and T4 (4.0% NP) showed much lower germination percentages. This may be because of when NPs absorb water, they form a gel-like matrix around seeds. In high concentrations, this gel acts as a physical barrier, restricting oxygen diffusion to the seed which can substantially delay or inhibit germination (MacDonald et al., 2020).

## CONCLUSION

*Mucuna bracteata* seedballs grow best with peatmoss and low NP levels. Peatmoss helped seeds grow better in both lab and field tests. Low concentrations of NP

(0.5% and 1.0%) enhanced germination by increasing the water-holding capacity of the growth medium. These results can help improve seedball planting for land restoration.

## ACKNOWLEDGEMENT

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# Chilling Injury in *Artocarpus* Fruits: Physiological disorders, Quality deterioration and Mitigation Strategies

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## ABSTRACT

*Chilling injury (CI) presents a major challenge in the postharvest handling of Artocarpus fruits such as jackfruit (Artocarpus heterophyllus), cempedak (Artocarpus integer), terap (Artocarpus odoratissimus), breadnut (Artocarpus camansi), and sukun (Artocarpus altilis). These tropical species are highly susceptible to low-temperature stress, often resulting in physiological disorders including surface pitting, peel discoloration, uneven ripening, and increased susceptibility to pathogens. This review consolidates current knowledge on the mechanisms of CI, associated physiological disorders, and recent advances in mitigation strategies. Approaches such as heat preconditioning, modified atmosphere packaging, and the application of natural compound, such as salicylic acid and antioxidants that have shown potential in enhancing chilling tolerance in Artocarpus fruits. Understanding and mitigating CI in Artocarpus fruits is crucial for enhancing postharvest quality, extending shelf life, and reducing economic losses in tropical fruit supply chains.*

**Keywords:** chilling injury, fruit physiology, shelf life, storage, *Artocarpus*

## INTRODUCTION

*Artocarpus* is a genus within the Moraceae family, comprising over 50 species found in tropical and subtropical regions across Asia. It is indigenous to the Western Ghats in India, Malaysia, and is also present in Central and Eastern Africa as well as Southeast Asia (Krupa et al., 2020). Nevertheless, *Artocarpus* species have a limited shelf life and often exhibit off-flavours, a mushy texture, and surface browning, resulting in significant post-harvest losses (Ismail et al., 2023).

These climacteric fruits are highly sensitive to low-temperature storage, often suffering irreversible physiological disorders when stored below 10–13 °C above freezing point known as chilling injury (CI) (Patel et al., 2016; Yuan et al., 2024). Common symptoms of CI include surface browning, flesh discoloration, water-soaked lesions, and increased susceptibility to microbial infection (Kaur et al., 2024). Such injuries not only compromise fruit quality and consumer acceptability but also shorten shelf life and result in substantial economic losses.

This paper reviews recent findings on chilling injury in *Artocarpus* fruits, focusing on physiological responses, quality deterioration during storage, and postharvest strategies to reduce CI. Understanding these factors is key to improving temperature management and extending shelf life for local and export markets.

## RESEARCH APPROACHES

### *Quality deterioration*

Chilling injury in *Artocarpus* fruits leads to significant deterioration in quality, influencing aspects like appearance, texture, and taste (Patel et al., 2016). Symptoms of chilling injury encompass a dull green hue on the skin and spines, the presence of water-soaked spots, and browning of the skin, flesh, and seed. Additionally, there is a translucency observed in the seed's internal tissue, along with irregular brown coalesced pitted lesions on the seed surface and browning that extends from the seed's edge into its internal tissue. Other effects include unfavorable changes in flavor, increased water loss, and a heightened vulnerability to secondary infections (Mohammed and Wickham, 2011).

(a) (b) (c) (d)

**Figure 1:** Chilling injury symptoms in (a) jackfruit, (b) breadnut, (c) cempedak and (d) terap after prolong storage

### *Physiological disorders*

Exposure to low temperatures induces oxidative stress that leads to lipid peroxidation, damaging cell membranes and increasing their permeability, which results in electrolyte leakage and loss of compartmentalization (Wongs-Aree et al., 2024) (Figure 2). In some experiments, researchers have used the rate of electrolyte leakage to indicate chilling injury symptoms (Zainal et al., 2024).

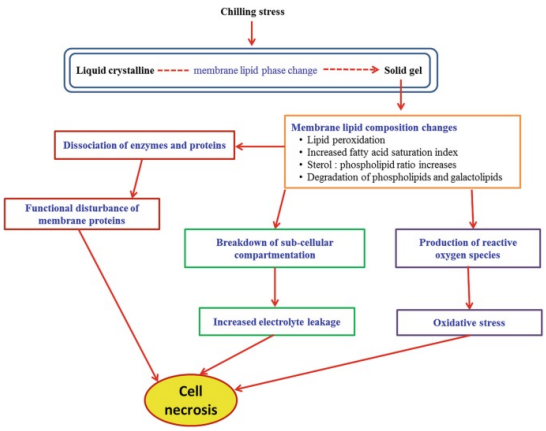


**Figure 2:** Physiological effects of cold storage that leads to CI (Zainal et al., 2024)

## FINDINGS

Physical methods such as hot water dips, intermittent warming, and modified atmosphere packaging help stabilize membranes and limit oxidative stress during cold storage (Wongs-Aree et al., 2024). Chemical treatments including citric acid, calcium chloride, methyl jasmonate, and antioxidant coatings (e.g., chitosan) boost defense responses and reduce ROS accumulation (Kaur et al., 2024; Patel et al., 2016). The result is improved external quality and a longer shelf life without decay, which reduces postharvest losses and supports the potential commercialization of ready-to-eat products, opening new opportunities in the market.

**Table 1**. Physical treatments in alleviating chilling injury

| Treatments | Storage | Effects | Reference(s) |
|---|---|---|---|
| Hot water | 50°C for 10–15 min | Reduces surface browning, delays softening | Kaur et al. (2024) |
| Low-temperature conditioning | 15°C for 24–48 h | Enhances chilling tolerance, reduces pitting | Wongs-Aree et al. (2024) |
| Modified atmosphere packaging | 5% $O_2$ + 10% $CO_2$ at 13°C | Maintains firmness, reduces respiration rate | Patel et al. (2016) |
| Pre-storage heat | 40–45°C for 2–4 h | Enhances membrane integrity, improves storage life | Kaura et al. (2024) |

**Table 2**. Chemical treatments in alleviating chilling injury

| Treatments | Storage | Effects | Reference(s) |
|---|---|---|---|
| Salicylic acid dip | 1–2 mM for 10 min | Enhances antioxidant activity, delays CI symptoms | Tareen et al. (2012) |
| Chitosan coating | 1.5% chitosan, dipping 2 min | Reduces microbial growth, maintains firmness | Basumatary et al. (2021) |
| Citric acid dip | 1% citric acid, 5 min dip | Controls enzymatic browning, maintains sensory quality | Javan et al. (2015) |
| Methyl jasmonate | 1–10 µM vapor exposure for 6–12 h | Activates stress defence pathways, improves cold tolerance | Aluko et al. (2021) |

## ACKNOWLEDGEMENT

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# Utilizing Liquid Ammonium Sulphate (LAS) for Enhanced Composting of Palm Oil Mill By-Products in a Circular Economy Framework

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## ABSTRACT

*Palm oil mill by-products such as decanter cake, boiler ash, and palm oil mill effluent (POME) sludge present environmental challenges due to slow decomposition and nutrient imbalances. Liquid ammonium sulphate (LAS), an aqueous solution of ammonium sulphate classified as scheduled waste when discarded, can potentially be repurposed to enhance composting. This study evaluates the integration of LAS into composting of palm oil by-products to accelerate decomposition, improve nutrient recovery, and produce high-quality organic fertiliser. Five compost treatments were constructed and monitored, with temperature as a key indicator of microbial activity. Compost piles without LAS maintained 45–55 °C, confirming active composting. Good-quality compost is typically characterised by a stable temperature near ambient, neutral pH, reduced C/N ratio (<20), dark crumbly texture, and absence of foul odour. By converting industrial by-products into agricultural inputs, this research promotes a circular economy approach, supporting sustainable waste management in Malaysia's agriculture and heavy industry.*

**Keywords:** agricultural wastes, compost, liquid ammonium sulphate, nitrogen, fertiliser, circular economy

## INTRODUCTION

Malaysia is the second largest global producer of crude palm oil (CPO), with over 450 palm oil mills processing more than 123 million tonnes of fresh fruit bunches annually (MPOB, 2025). Large volumes of by-products such as empty fruit bunches, decanter cake, boiler ash, and palm oil mill effluent (POME) sludge are often left unmanaged, leading to biomass accumulation and environmental pollution.

Composting these by-products can produce organic fertilisers, enhance plant growth

and soil health, and reduce reliance on chemical fertilisers. However, the process typically takes 8–12 weeks and often results in low-quality compost due to imbalanced carbon-to-nitrogen (C/N) ratios (Supriatna et al., 2022).

Meanwhile, industrial wastewater treatment plant that produces liquid ammonium sulphate (LAS) as its by-product, incur high disposal cost as scheduled waste to licensed premises regulated by the Department of Environment (DOE) Malaysia. Despite its potential as a composting additive rich in nitrogen (N) and sulphur (S), and as a pH adjuster, its application in agriculture remains limited.

This study investigates the potential of LAS in balancing the C/N ratio that accelerates the composting process, subsequently improving compost quality, crop growth, and yield. This addresses gaps in integrating waste repurposing strategies towards circular and sustainable cross-sector waste management solutions between the agricultural and heavy industry sectors in Malaysia.

## PROJECT APPROACHES

This study employed a structured experimental design to assess the effectiveness of liquid ammonium sulphate (LAS) in enhancing composting of palm oil mill (POM) by-products. Five compost piles were prepared using a 60:40 ratio of decanter cake to boiler ash, supplemented with 7% alkaline sludge and 1–7% chicken manure as nitrogen sources. By weight ratio, Pile 1 consisted of decanter cake:boiler ash:alkaline sludge (55:38:7); Pile 2 included decanter cake:boiler ash:alkaline sludge:chicken manure (56:36:7:1); Pile 3 was composed of decanter cake:boiler ash:liquid ammonium sulphate (60:38:2); and Pile 4 comprised decanter cake:boiler ash:liquid ammonium sulphate:chicken manure (60:31:2:7). Pile 5, which served as the negative control, contained decanter cake:boiler ash (68:32). LAS, a nitrogen- and sulphur-rich additive, was applied at 2% via spraying in two treatment groups, while one negative control and two positive controls were maintained without LAS. Composting was conducted over 12 weeks with biweekly pile turning to ensure aeration and uniform decomposition. Temperature was recorded regularly as an indicator of microbial activity, while maturity was evaluated based on standard compost quality parameters: stable temperature close to ambient, neutral pH, C/N ratio below 20, dark crumbly texture, and absence of foul odour. This approach provides insight into optimising nutrient recovery and sustainable waste management.

## FINDINGS

Preliminary observations show that all compost piles maintained active thermophilic phases, with temperatures ranging from 45–55°C, confirming microbial activity. LAS-treated piles are anticipated to sustain higher peak temperatures for longer durations compared to controls, reflecting enhanced microbial metabolism and accelerated decomposition. The addition of chicken manure is also expected to further stimulate heat generation and improve nutrient balance in the composting process.

By the end of the 12-week period, it is expected that LAS-amended treatments will yield compost with improved maturity indicators, including a reduced C/N ratio (<20), stable temperature near ambient, neutral pH, dark crumbly structure, and absence of foul odour. These improvements suggest that LAS can serve as an effective additive to enhance nutrient recovery and shorten composting time. Ultimately, the study is expected to demonstrate that integrating LAS into composting offers a circular economy solution for sustainable waste management in Malaysia's palm oil industry.

## CONCLUSION

Integration of LAS generated from industrial wastewater treatment plants into the composting of POM by-products offers a promising waste-to-resource strategy to enhance composting efficiency, produce high-quality organic fertilisers, and reduce environmental impacts from both the agricultural and heavy industry sectors.

Through comprehensive scientific data and analysis, this study will enable agencies such as the Department of Environment (DOE) and the Department of Agriculture (DOA) to evaluate the potential for approving the safe and effective use of LAS in agricultural practices, thereby providing a strong evidence base to guide future policy and regulatory decisions.

The approach aligns with circular economy principles by transforming industrial by-products into valuable agricultural inputs, creating a practical link between sustainable waste management and sustainable agriculture practices in Malaysia and beyond.

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