An Integrated Bacteria-Microalgae-Biochar System for Microcystin Removal and Water Quality Improvement

Kritima Jitngam* and Jeeraporn Pekkoh
Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand
*Email: kritima_j@cmu.ac.th

Abstract

Cyanobacterial blooms caused by *Microcystis* produce microcystins that threaten aquatic ecosystems and water quality. Eco-friendly treatment using bacteria and microalgae, supported by biochar as a carrier material, has therefore attracted increasing interest. This study aimed to develop and evaluate a biochar-immobilized bacteria-microalgae system for microcystin reduction and water quality improvement. Three types of biochar i.e. macadamia, bamboo, and hemp were evaluated as support materials for immobilizing *Bacillus* sp. AK3 and the microalgae *Chlorella* sp. AARL G049 and *Tetradesmus* sp. AARL G090. The optimal conditions for cell immobilization were also investigated. Hemp biochar showed the best performance as a carrier, achieving a maximum immobilization of *Bacillus* sp. AK3 at 7.54 ± 3.43 × 10^7 CFU/cm². Among the tested microalgae, *Tetradesmus* sp. AARL G090 (13.6 ± 0.42 × 10^4 cells/cm²) demonstrated higher immobilization efficiency than *Chlorella* sp. AARL G049 (9.79 ± 0.73 × 10^4 cells/cm²). Surface modification of hemp biochar with Ca^{2+} did not enhance immobilization efficiency. In contrast, untreated biochar resulted in the highest immobilization of *Tetradesmus* sp. AARL G090 at 2.32 ± 1.82 × 10^7 cells/g. Further experiments on *Microcystis* removal and wastewater treatment are currently in progress.

Introduction

Eutrophication → **Harmful algal blooms (HABs)** increasingly threaten freshwater systems worldwide → The proliferation of ***Microcystis*** → **microcystin** production (Cyclic heptapeptide toxins) → threaten aquatic life and human health

Biochar offers **high surface area** and supports **microbial attachment** → **bacteria and microalgae** have demonstrated the ability to reduce nutrient levels in water → **microcystin** production (Cyclic heptapeptide toxins)

Objective

To deverlop and evaluate a biochar-immobilized bacteria-microalgae system for reduction of microcystin toxins and water quality improvement

Methodology

1 Study of Biochar-Immobilized *Bacillus* sp. AK3 and Microalgae *Chlorella* sp. AARL G049 and *Tetradesmus* sp. AARL G090

- **Selected biochar**

Biochar → Bamboo, Hemp, Macchadamia
Bacillus sp. AK3 → Total plate count
Chlorella sp. AARL G049 → Total cell count
Tetradesmus sp. AARL G090 → Total cell count

- **Comparison between Pre treatment and Untreat biochar**

Biochar / Microalga → Pre treatment biochar → washed with 6 mol/L HCl and coated with 1.5 mol/L $CaCl_2$ → Total cell count
Biochar / Microalga → Untreat biochar → Total cell count

2 Evaluation of *Microcystis* and Microcystin Removal and Nutrient reduction in polluted water

Microcystis AARL C028 + Synthetics wastewater → Control, Biochar, Immobillized biochar
- Inhibition of *Microcystis* AARL C028
- Water Quality Parameter
- Total bacteria count
- Microcystin

Immobillized biochar → *Bacillus* sp. AK3, *Tetradesmus* sp. AARL G090

Results

1 Study of Biochar-Immobilized *Bacillus* sp. AK3 and Microalgae *Chlorella* sp. AARL G049 and *Tetradesmus* sp. AARL G090

Study of Biochar-Immobilized *Bacillus* sp. AK3

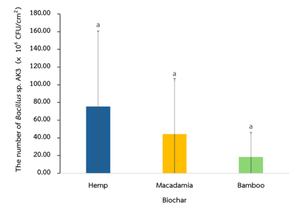


Figure 1 The number of *Bacillus* sp. AK3 attached to different biochar carriers derived from Hemp, Macadamia, and Bamboo

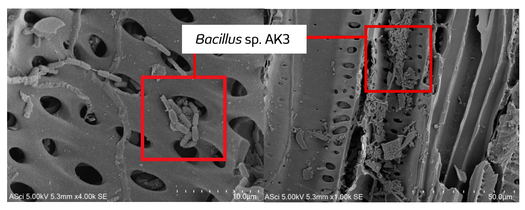


Figure 2 SEM of Hemp biochar immobiled *Bacillus* sp. AK3

Study of Biochar-Immobilized *Chlorella* sp. AARL G049 and *Tetradesmus* sp. AARL G090

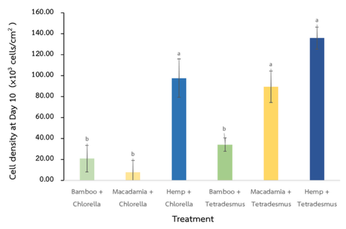


Figure 3 Cell density of Microalgae attached to different biochar carriers derived from Hemp, Macadamia, and Bamboo

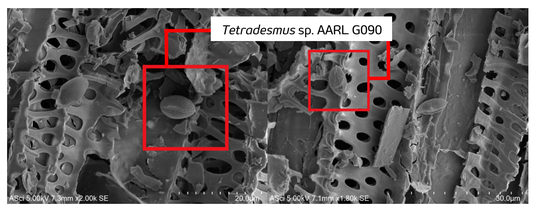


Figure 4 SEM of Hemp biochar immobiled *Tetradesmus* sp. AARL G090

2 Evaluation of *Microcystis* and Microcystin Removal and Nutrient reduction in polluted water

Inhibition of *Microcystis*

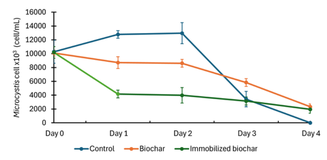


Figure 5 Cell density of *Microcystis* Day 0 - Day 4 across treatments

- Control: increased initially (Day 1–2), then dropped sharply (Day 3–4)
- Biochar: gradual decline (-77.1%)
- Immobilized biochar: much sharper early decline (already low by Day 1–2) (-80.9%)

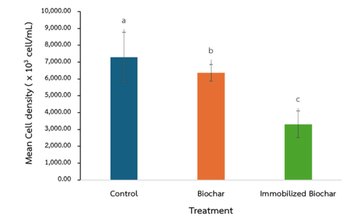


Figure 6 *Microcystis* cell density among different treatments.

Water Quality Parameter

- Nutrient

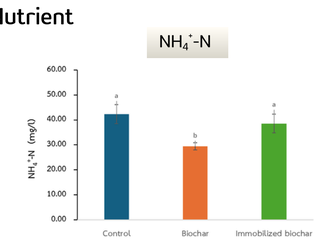


Figure 7 Ammonium-nitrogen concentration (mg/l)

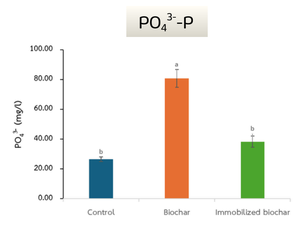


Figure 8 Phosphate concentration (mg/l)

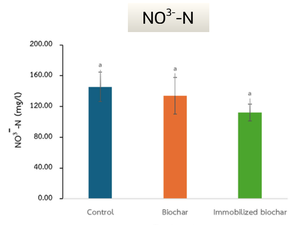


Figure 9 Nitrate-nitrogen concentration (mg/l)

Total bacteria count

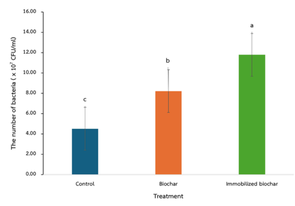


Figure 10 Total bacterial counts in different treatments .

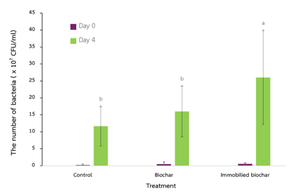


Figure 11 Comparison of total bacterial counts between Day 0 and Day 4 across different treatments.

Discussions

- Hemp-derived biochar is attributed to the inherent vascular structure of hemp, which provides continuous internal channels, high surface area, and exceptional porosity, significantly enhancing microbial adsorption and attachment.
- The immobilized biochar system markedly increased biological activity. Findings indicate that the biochar matrix serves as a superior scaffolding for bacterial colonization and proliferation. Furthermore, the substantial reduction of *Microcystis* in the immobilized treatments demonstrates a synergistic inhibitory effect exerted by both *Bacillus* sp. AK3 and *Tetradesmus* sp. AARL G090.
- The most effective nitrate removal was achieved by the immobilized biochar system, reflecting the efficient nitrate assimilation by *Bacillus* sp. AK3. Simultaneously, the biochar effectively reduced ammonium-nitrogen levels through physical sequestration and adsorption mechanisms within its complex porous architecture.

Conclusions

- Hemp-derived biochar was successfully developed as an effective carrier material for immobilizing *Bacillus* sp. AK3 and the microalga *Tetradesmus* sp. AARL G090
- The immobilized biochar system clearly enhanced biological activity, evidenced by early bacterial growth, *Microcystis* reduction, and the most effective nitrate removal
- The evaluation of microcystin removal efficiency is currently ongoing.

Acknowledgement

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References

- Arman, T., & Clarke, J. D. (2021). Microcystin Toxicokinetics, Molecular Toxicology, and Pathophysiology in Preclinical Rodent Models and Humans. Toxins, 13(8), 537.
- Ruangrit, K., Phinyo, K., Chailungka, S., Duangjan, K., Naree, A., Thasana, J., ... & Noirungsee, N. (2025). Enhanced nitrate removal in aquatic systems using biochar immobilized with algicidal *Bacillus* sp. AK3 and denitrifying *Alcaligenes* sp. M3: A synergistic approach. PloS one, 20(3), e0318416.
- Yang, R., Guo, X., Wu, H., Kang, W., Song, K., Li, Y., et al. (2022). Anisotropic hemp-stem-derived biochar supported phase change materials with efficient solar-thermal energy conversion and storage. Biochar, 4(1), 38.