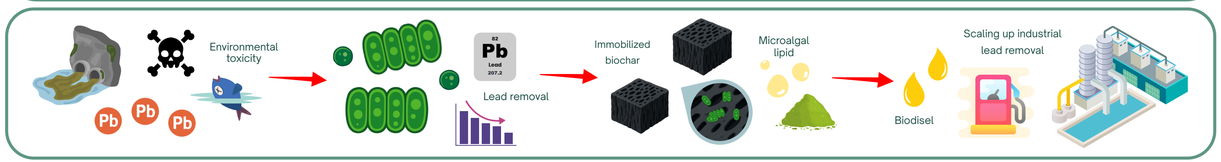
Application of Biochar-Immobilized Microalgae for Lead Removal from Wastewater

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Abstract

Bioremediation of heavy metals using microalgae has received increasing attention due to its eco-friendly nature, high efficiency, and the potential conversion of biomass into high-value products. This study aimed to compare two microalgal species, *Tetradesmus obliquus* AARL G090 and *Chlorella* sp. AARL G049, in terms of growth performance, biochemical composition, and lead adsorption efficiency. In addition, the application of microalgae immobilized on biochar for lead removal was investigated. The results showed that lead concentrations of 3, 30, and 300 mg/L significantly affected the growth of both species. At low concentrations, the specific growth rates were comparable to those of the control group. However, growth rates decreased at higher concentrations. Regarding lead removal efficiency, *T. obliquus* AARL G090 and *Chlorella* sp. AARL G049 achieved maximum removal efficiencies of 89.73% and 90.34%, regarding pigments and high-value substances at 30 mg/L of lead. *T. obliquus* AARL G090 showed a maximum increase in chlorophyll a of 43.3%, while *Chlorella* sp. AARL G049 increased by 31.8%. In terms of high-value compounds, the lipids are currently under investigation to determine their suitability as a biofuel feedstock. When testing immobilized biochar with *T. obliquus* AARL G090, the results show that algae immobilized on biochar can reduce lead levels by up to 98.65%, free cell algae by up to 99.12%, and biochar by up to 98.76%, respectively. Therefore, the use of algae in combination with biochar for lead removal can be further developed into an industrial scale.

Graphical abstract



Introduction

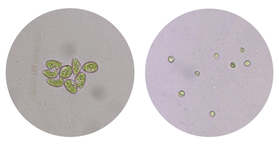
Heavy metal contamination is a critical environmental issue due to its non-biodegradable nature and its ability to bioaccumulate in the food chain, posing severe risks to human health. While conventional treatments like chemical precipitation and ion exchange are widely used, they face significant limitations, including high energy consumption, high costs, and the generation of toxic secondary sludge. Consequently, microalgae-based bioremediation here is a great alternative for sustainable and eco-friendly remediation.

Objectives

- To compare microalgal growth, biochemical composition, and lead adsorption capacity of selected microalgae.
- To assess the effect of lead exposure on microalgal growth and the production of high-value metabolites.
- To evaluate the efficiency of lead removal from aqueous system using microalgae immobilized biochar.

Materials and methods

1 Selected microalgae



- *Tetradesmus obliquus* AARL G090
- *Chlorella* sp. AARL G049



conc. 0, 3, 30, and 300 mg/L of Pb

Day 14

parameters

- Growth rate
- Biomass
- Lipid profile*
- Chlorophyll a
- Lead removal



immobilized on biochar

Day 0-6

parameters

Growth rate microalgae on biochar and supernatant*

2 Microalgae immobilized biochar



Tetradesmus obliquus AARL G090



conc. 0, 30, 100, and 200 mg/L of Pb

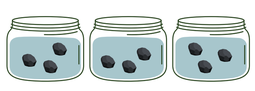
Day 8

parameters

- Growth rate*
- Biomass*
- Lipid profile*
- Chlorophyll a*
- Lead removal
- ROS*



immobilized on biochar



conc. 0, 30, 100, and 200 mg/L of Pb

Day 8

parameters

biochar
- Growth rate*
- Lipid profile*
- Lead removal
- SEM

supernatant
- Growth rate*

note: (*) = Data currently being processed

Discussions

- **Impact of Lead on Growth & Morphology:** Low to moderate lead concentrations (3–30 mg/L) did not significantly affect algal growth. However, at 300 mg/L, growth was severely inhibited, leading to cellular bleaching and death. This is attributed to the production of reactive oxygen species (ROS) and enzymatic inhibition (Dao & Beardall, 2016).
- **Adsorption Efficiency:** Free algae performed well at low concentrations, but efficiency declined sharply at higher levels. In contrast, immobilized algae on biochar exhibited significantly higher stability and consistent performance. This trend suggests that while extreme toxicity may lead to cell mortality in free systems, the biochar matrix provides a protective shield, maintaining removal efficiency (Waqas *et al*., 2023).
- **Potential for High-Value Metabolites (Ongoing Study):** Lead exposure is expected to trigger lipid accumulation as a metabolic response to oxidative stress, potentially enhancing the overall lipid yield for biofuel conversion. This biochemical shift is currently under investigation to evaluate the post-treatment biomass value.
- **Microscopic Confirmation:** SEM analysis on day 8 confirmed successful colonization and a high density of algal cells within the biochar pores. This confirms that the algae-biochar combination remains viable and effective for industrial-scale lead removal.

Conclusions

Lead concentration acts as a critical factor influencing the growth and biochemical profiles of both strains. While *Chlorella* sp. AARL G049 demonstrates high resistance to lead toxicity. *Tetradesmus obliquus* AARL G090 is more effective for lead removal at lower concentrations. Furthermore, the use of algae in combination with biochar for lead removal has the potential for industrial-scale adaptation.

References

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Results

1 Selected microalgae

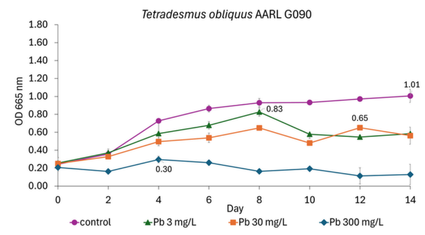


Figure 1: Absorbance of *Tetradesmus obliquus* AARL G090 at 665 nm in culture media containing lead at concentrations of 0, 3, 30, and 300 mg/L.

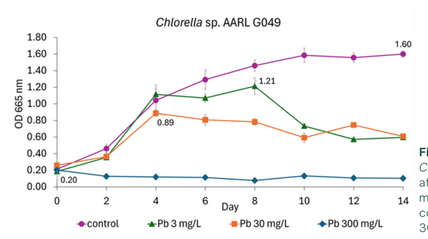


Figure 2: Absorbance of *Chlorella* sp. AARL G049 at 665 nm in culture media containing lead at concentrations of 0, 3, 30, and 300 mg/L.

OD 665 nm

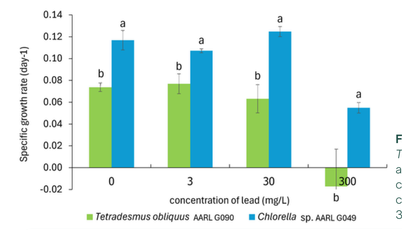


Figure 3: Specific growth rate of *Tetradesmus obliquus* AARL G090 and *Chlorella* sp. AARL G049 in culture media containing lead at concentrations of 0, 3, 30, and 300 mg/L. ($p<0.05$)

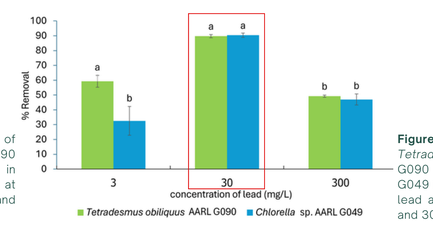


Figure 4: % lead removal of *Tetradesmus obliquus* AARL G090 and *Chlorella* sp. AARL G049 in culture media containing lead at concentrations of 3, 30, and 300 mg/L. ($p<0.05$)

Specific growth rate | **% Pb Removal**

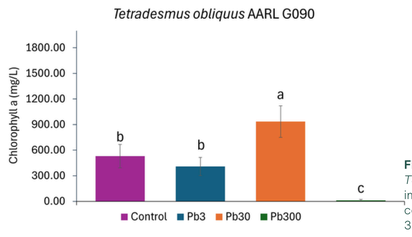


Figure 5: Chlorophyll a content of *Tetradesmus obliquus* AARL G090 in culture media containing lead at concentrations of 0, 3, 30, and 300 mg/L. ($p<0.05$)

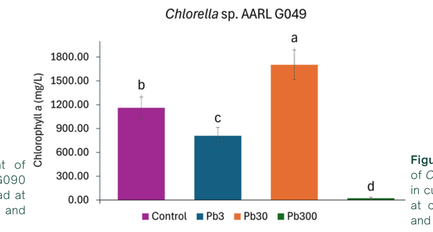


Figure 6: Chlorophyll a content of *Chlorella* sp. AARL G049 in culture media containing lead at concentrations of 0, 3, 30, and 300 mg/L. ($p<0.05$)

Chlorophyll a

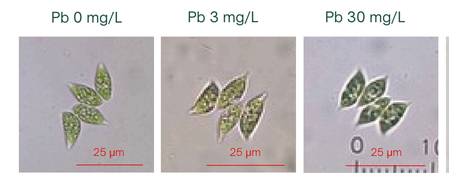


Figure 7: Microscopic morphology of *Tetradesmus obliquus* AARL G090 exposed to varying concentrations of lead in the culture medium.

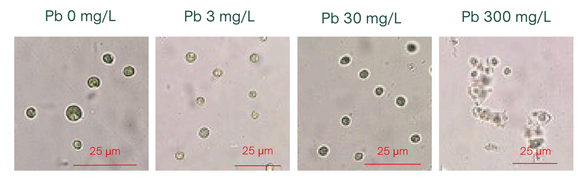


Figure 8: Microscopic morphology of *Chlorella* sp. AARL G049 exposed to varying concentrations of lead in the culture medium.

***Tetradesmus obliquus* AARL G090** | ***Chlorella* sp. AARL G049**

2 Microalgae immobilized biochar

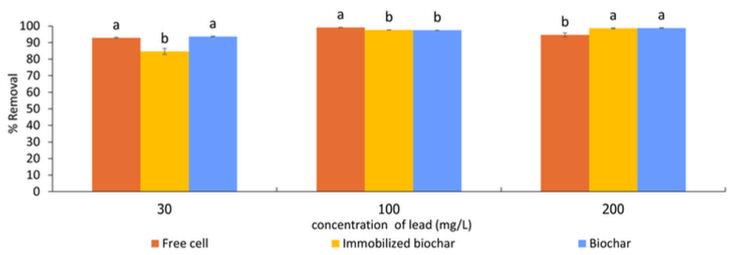


Figure 9: % lead removal of *Tetradesmus obliquus* AARL G090, immobilized biochar, and biochar in culture media containing lead at concentrations of 30, 100, and 200 mg/L. ($p<0.05$)

% Pb Removal

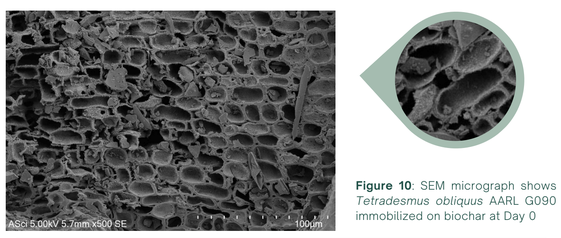


Figure 10: SEM micrograph shows *Tetradesmus obliquus* AARL G090 immobilized on biochar at Day 0

SEM Control Day 0

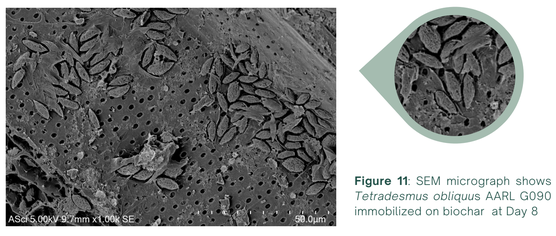


Figure 11: SEM micrograph shows *Tetradesmus obliquus* AARL G090 immobilized on biochar at Day 8

SEM Control Day 8

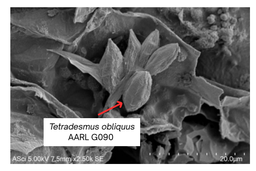


Figure 12: SEM micrograph shows the morphology and attachment of *Tetradesmus obliquus* AARL G090 cells on biochar surface at a lead concentration of 30 mg/L.

SEM Pb 30

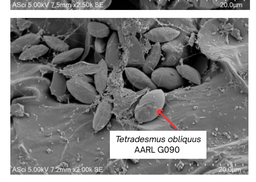


Figure 13: SEM micrograph shows the morphology and attachment of *Tetradesmus obliquus* AARL G090 cells on biochar surface at a lead concentration of 100 mg/L.

SEM Pb 100

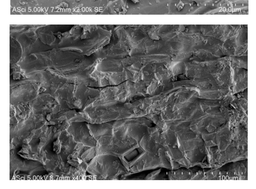


Figure 14: SEM micrograph shows the morphology and attachment of Tetradesmus obliquus AARL G090 cells on biochar surface at a lead concentration of 200 mg/L.

SEM Pb 200

High-Value Metabolites (Ongoing Study)

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