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Cultivation of *Chlorella Vulgaris (C. vulgaris)* for Tetracycline Degradation

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Abstract
Tetracycline (TC), a commonly used antibiotic, is frequently detected in aquatic environments and poses a serious ecological threat. This study aimed to evaluate the ability of *Chlorella vulgaris (C.vulgaris)* to degrade TC under varying conditions of pH, TC concentration, and exposure time. Cultivation was carried out in BG-11 medium, and a Box-Behnken Design (BBD) was applied to determine optimal degradation parameters. Tetracycline degradation was quantified using high-performance liquid chromatography (HPLC), while chlorophyll a, chlorophyll b, and carotenoid contents were analyzed using a spectrophotometer. Results showed that *C. vulgaris* effectively degraded TC, particularly at pH 5.5 with low initial TC concentrations and longer exposure times. Pigment analysis revealed that exposure to TC altered algal metabolism, characterized by reduced pigment levels at higher concentrations and shorter exposure periods. This study confirms the potential use of *C. vulgaris* in pharmaceutical wastewater treatment through an eco-friendly, biological approach.
Keywords: *Chlorella vulgaris*; tetracycline; bioremediation; HPLC; pigment analysis

Introduction

Tetracycline (TC) is a widely used antibiotic for treating human and animal infections. However, improper disposal and extensive application in agriculture have led to its frequent detection in aquatic ecosystems, raising environmental and health concerns (Amangelsin et al. 2023). The persistence of TC in water bodies contributes to the development of antibiotic-resistant bacteria, potentially causing harm to aquatic organisms (Anbarani et al., 2023). Therefore, the removal of TC from wastewater is an urgent global priority. Physical and chemical methods, such as membrane filtration, photodegradation, and adsorption, have been applied for TC removal; however, they are often limited by high operational costs, energy demands, and the risk of producing toxic by-products. In contrast, biological treatment using microalgae offers an eco-friendly and cost-effective alternative. Microalgae have the ability to absorb, accumulate, and biodegrade contaminants, including antibiotics, while simultaneously producing valuable biomass.

Among microalgae, *Chlorella vulgaris* has demonstrated significant potential in bioremediation due to its rapid growth, tolerance to stress, and capabilities for degrading pollutants. This study aims to evaluate the ability of *C. vulgaris* to degrade tetracycline under various conditions, including pH, antibiotic concentration, and exposure time. The pigment content of *C. vulgaris* was also assessed to examine physiological changes in response to tetracycline exposure.

Materials and methods

Chlorella vulgaris was obtained from the Algae Culture Collection from Agri Season Sdn Bhd. The microalga was cultivated in BG-11 medium under continuous illumination with white fluorescent light at an intensity of 3000–5000 lux and a photoperiod of continuous light supply. The culture was maintained at room temperature (25–28°C) and continuously aerated using an aquarium air pump to enhance growth and mixing. Prior to the experimental treatment, the algal culture was grown for 10 days to reach the exponential phase.

The experimental design employed a Box-Behnken Design (BBD) using Design-Expert 13 software. Three independent variables were selected: pH (5.5, 7.0, 8.5), tetracycline concentration (5, 15, 25 mg/L), and exposure time (24, 72, 120 hours). A total of 14 experimental runs were conducted. After each exposure period, samples were filtered through a 0.45 μm membrane to remove unwanted particles.

Residual tetracycline concentration was measured using high-performance liquid chromatography (HPLC) equipped with a C18 column. The mobile phase consisted of acetonitrile and 0.01 M oxalic acid (70:30 v/v) at a flow rate of 1 mL/min with detection at 355 nm. A calibration curve was prepared using standard tetracycline solutions to quantify degradation. Pigment analysis was performed by centrifuging 5 mL of culture at 5500 rpm for 10 minutes. The pellet was extracted with 5 mL of 80% acetone and incubated at 4°C in the dark for an hour. The absorbance was measured at 664 nm, 644 nm, and 470 nm using a UV-Vis spectrophotometer. Chlorophyll a, chlorophyll b, and carotenoid content were calculated using the Lichtenthaler and Wellburn equations (1983).

Results and discussion

The degradation of tetracycline (TC) by *Chlorella vulgaris* under different environmental conditions was successfully evaluated using a Box-Behnken Design (BBD). The experimental responses demonstrated that *C. vulgaris* exhibited substantial ability to reduce tetracycline levels across a range of pH, concentration, and exposure times. The results indicated that tetracycline degradation increased with longer exposure times and lower initial concentrations, particularly at a slightly acidic pH (5.5), which is in agreement with previous findings that acidic environments enhance tetracycline biodegradation (Anbarani et al., 2023).

C. vulgaris showed its highest removal efficiency at 120 hours of exposure with 5 mg/L tetracycline at pH 5.5. At higher concentrations (25 mg/L), the removal rate decreased, possibly due to the inhibitory effect of TC on algal growth and metabolism (Amangelsin et al. 2023). The pigment contents of *C. vulgaris*, including chlorophyll a, chlorophyll b, and carotenoids, were measured as indicators of metabolic response under TC stress.

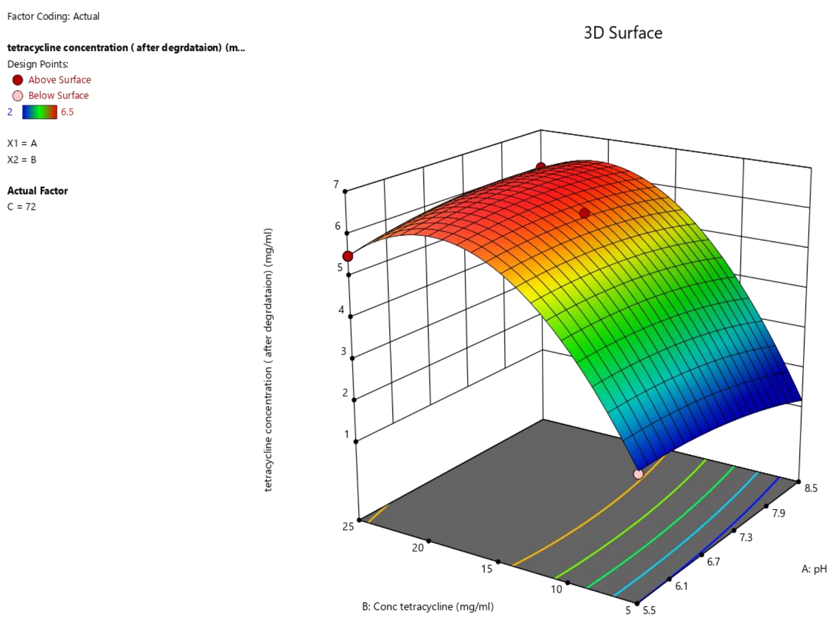

Figure 1 Degradation efficiency of tetracycline by *Chlorella vulgaris* under different treatment conditions.

To assess the influence of key environmental and operational factors on tetracycline degradation by *Chlorella vulgaris*, an Analysis of Variance (ANOVA) was performed using Design Expert 13. The selected quadratic model was found to be statistically significant ($p < 0.05$), indicating that it adequately describes the experimental data. The significant factors included tetracycline concentration (B), temperature (A), and light intensity (C), while the interaction between temperature and light intensity (AC) also showed notable effects on degradation efficiency.

Tetracycline concentration (B) had the highest sum of squares and F-value among all tested variables, indicating that it exerted the most substantial influence on the degradation process. At higher concentrations, degradation efficiency decreased, likely due to inhibition of algal metabolism, as previously observed in studies by Mirizadeh et al. (2024). Light intensity (C) and temperature (A) also exhibited significant effects, aligning with findings that these factors impact microalgal photosynthetic activity and enzymatic functions essential for pollutant removal (Li et al., 2022).

The significance of the AC interaction term reveals that optimal degradation is not only dependent on individual factors but also on their combined effects. Specifically, suitable light intensity can enhance enzymatic reactions at favorable temperatures, as similarly noted in studies involving *Desmodesmus* species under varying environmental conditions (Xu et al., 2024).

Table 1: ANOVA table for quadratic model

Source	Sum of Squares	Mean Square	F-value	p-value	Significance
Model	25.86	2.87	123.15	0.0081	Significant
A – pH	0.0653	0.0653	2.8	0.2362	
B-Conc. tetracycline	11.21	11.21	480.29	0.0021	
C – hours	0.1333	0.13333	5.71	0.1393	
AB	0.1905	0.1905	8.16	0.1038	
AC	0.16	0.16	6.86	0.1201	
BC	0.2305	0.2305	9.88	0.0881	
A²	0.1375	0.1375	5.89	0.1359	
B²	8.6	8.6	368.76	0.0027	
C²	0.1756	0.0175	0.7519	0.4773	
Residual	0.0467	0.0233			
Lack of Fit	0.0267	0.0267	1.33	0.4544	Not Significant
Pure Error	0.02	0.02			
Cor Total	25.911				

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Model Equation (in terms of coded factors):

$$\text{Tetracycline concentration (after degradation)} = -4.76620 - 1.24074A + 0.65444B - 0.26544C - 0.00222A - 0.002778AC - 0.000764BC - 0.103704A^2$$

This quadratic model indicates a nonlinear relationship between the tested variables and the residual TC concentration. The negative coefficient for A^2 suggests diminishing returns at extreme temperature values, supporting the concept of an optimal temperature range for algal degradation activity. The observed trends are consistent with those reported by Tang et al. (2022), who found that high tetracycline levels reduce photosynthetic efficiency in *C. pyrenoidosa*, and further confirm the roles of light and temperature in regulating microalgal degradation potential.

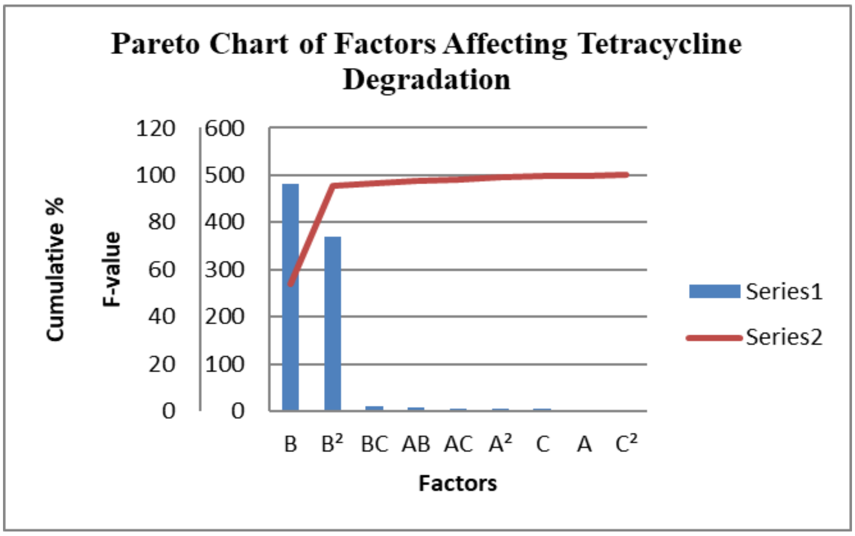

Figure 2 Pareto Chart for factors affecting tetracycline degradation.

Figure 2 presents the Pareto chart used to evaluate the relative impact of factors on tetracycline degradation. The analysis was based on experimental data involving three independent variables: pH (A), tetracycline concentration (B), and exposure time (C). The chart visually ranks the significance of these variables and their interactions based on the magnitude of their effects. From the chart, tetracycline concentration (B) and its squared term (B^2) exert the greatest influence on degradation. This suggests that the amount of tetracycline present plays a dominant role in determining the rate and extent of degradation. This aligns with findings by Mojica and Aga (2011), who reported that initial antibiotic concentration strongly determines degradation pathways and persistence in aquatic systems, particularly under microalgal treatment conditions (Xiao et al., 2023).

Interaction terms, such as BC (concentration × time) and AB (concentration × pH), also showed a notable influence. This implies that the effects of tetracycline concentration are modulated by both the duration of exposure and the surrounding pH. In other words, the degradation outcome is not solely driven by concentration, but also by the duration of exposure of *Chlorella vulgaris* to the antibiotic and the acidity or alkalinity of the medium. This finding is consistent with comprehensive reviews on tetracycline fate, which report that degradation mechanisms often depend on interactive environmental factors such as temperature, pH, and pollutant concentration (Ahmad, Zhu, & Sun, 2021). These interactions influence not only degradation rates but also the formation of transformation products and the dynamics of microbial communities.

In contrast, individual factors such as pH (A), exposure time (C), and their squared terms (A^2, C^2) contributed minimally in this study, suggesting that their isolated impact was limited within the tested experimental range. While these variables remain mechanistically relevant in biological systems, their effects were overshadowed by the dominant role of tetracycline concentration (Li et al., 2022). In summary, the Pareto analysis underscores tetracycline concentration as the most critical factor influencing degradation — both as a main effect and through interaction with time and pH. These findings suggest that environmental remediation strategies should prioritize managing initial antibiotic concentrations, while still considering synergistic effects of exposure duration and pH to enhance overall degradation efficiency.

Conclusion

This study demonstrated the potential of *Chlorella vulgaris* in degrading tetracycline (TC) under varying environmental conditions. Using a Box-Behnken Design, the effects of pH, tetracycline concentration, and exposure time on degradation efficiency were successfully modelled. Among the tested variables, tetracycline concentration emerged as the most influential factor, both independently and through interactions with time and pH. Optimal degradation was achieved at low tetracycline concentrations, acidic pH (5.5), and longer exposure periods (up to 120 hours). Pigment analysis further confirmed that TC exposure affects the metabolic activity of *C. vulgaris*, as evidenced by reduced chlorophyll and carotenoid content, especially under high TC stress. However, recovery of pigment levels over time indicated the microalga's ability to adapt to antibiotic-induced stress. The findings suggest that *C. vulgaris* is a promising candidate for eco-friendly bioremediation of pharmaceutical pollutants in wastewater, with practical implications for developing sustainable antibiotic removal strategies.

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References

Ahmad, F., Zhu, D., & Sun, J. (2021). Environmental fate of tetracycline antibiotics: Degradation pathway mechanisms, challenges, and perspectives. *Environmental Sciences Europe, 33*(1), 64.

Amangelsin, Y., Semenova, Y., Dadar, M., Aljofan, M., & Bjørklund, G. (2023). The impact of tetracycline pollution on the aquatic environment and removal strategies. *Antibiotics, 12*(3), 440.

Anbarani, M. Z., Ramavandi, B., & Bonyadi, Z. (2023). Modification of *C. vulgaris* carbon with Fe_3O_4 nanoparticles for Tc elimination from aqueous media. *Heliyon, 9*(3), e14356.

Li, S., Show, P. L., Ngo, H. H., & Ho, S. H. (2022). Algae-mediated antibiotic wastewater treatment: A critical review. *Environmental Science & Ecotechnology, 9,* 100145.

Mirizadeh, M., Hosseini, S. M., Mohebbi, A., & Gholami, M. (2024). Effects of tetracycline on growth, pigment content, and oxidative stress in *Chlorella vulgaris*: Implications for wastewater bioremediation. *Science of the Total Environment, 905,* 167857.

Mojica, K. D. A., & Aga, D. S. (2011). Antibiotics pollution in aquatic environments: A review of occurrence and biodegradation. *Chemosphere, 82*(4), 455–465.

Tang, J., Yang, J., Zhao, S., Wang, X., & Xie, Z. (2022). Toxic effects of tetracycline and its removal by the freshwater microalga *Chlorella pyrenoidosa*. *Agronomy, 12*(10), 2497.

Xiao, Y., Hu, C., Gao, R., Chen, X., & Wang, Z. (2023). Antibiotic degradation and removal mechanisms by microalgae: Recent advances and future perspectives. *Journal of Hazardous Materials, 445,* 130512.

Xu, X., Yang, Z., Liu, Y., Liu, C., Liang, J., Zhang, M., & Zhang, Z. (2024). Influence of antibiotics on the growth and photosynthesis of microalgae: A review. *Biology, 13*(11), 901.