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Biostimulation of *Chlorella vulgaris* with Indole-3-Acetic Acid and Epigallocatechin Gallate Enhances the Removal of Amoxicillin and Cephalexin from Water: Kinetics and Efficiency Evaluation

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ABSTRACT

This study investigated the ability of *Chlorella vulgaris* to remove amoxicillin and cephalexin from water and evaluated the effects of indole-3-acetic acid (IAA) as a biostimulant and the combined application of epigallocatechin gallate (EGCG) and sodium bicarbonate ($NaHCO_3$) as catalytic enhancers. Batch experiments were conducted using different initial concentrations of both antibiotics over a 13-day exposure period. The two antibiotics exhibited distinct removal behaviors. Amoxicillin showed a relatively linear reduction pattern and followed apparent first-order kinetics, characterized by a lower removal rate constant and a longer half-life. In contrast, cephalexin exhibited a non-linear, biphasic removal behavior, involving an initial slow adsorption phase followed by rapid biodegradation, and therefore did not fit well to a single first-order kinetic model. The presence of catalytic systems significantly enhanced antibiotic removal compared with non-catalytic treatments. IAA promoted algal activity and enzymatic pathways, whereas the EGCG + $NaHCO_3$ system achieved complete removal (100%) of both antibiotics under optimal conditions. The superior performance of EGCG + $NaHCO_3$ is attributed to synergistic redox reactions and buffering effects that create favorable pH and oxidative conditions for algal metabolism. Overall, the integration of catalytic enhancement with microalgal bioremediation represents a sustainable and environmentally friendly approach for the removal of pharmaceutical contaminants from wastewater.

1. INTRODUCTION

The omnipresence of pharmaceuticals in aquatic systems has been regarded as a global ecological concern [1]. Some of the most relevant are antibiotics and other pollutants, such as antibiotics, which remain one of the main contaminants due to their continual use in human and veterinary medicine, oxidation and partial metabolism in living organisms, and especially their resistance to noxious agents [2]. A proportion of these compounds is excreted unchanged, and they can enter wastewater treatment works with the potential to bypass conventional treatment with limited degradation [3]. As a result, further kinds of antibiotics such as Amoxicillin and Cephalexin are frequently detected in surface water, sediments, and even drinking water, posing potential hazards to the ecosystem and public health [4].

Antibiotics in water not only affect aquatic organisms, but can also contribute to the generation and spread of antibiotic-resistant bacteria and resistance genes, a major public health problem worldwide [5]. Therefore, it has caused an urgent need for efficient and sustainable removal methods of antibiotics from polluted water [6]. Carbon material as a promising structure has been extensively studied for the physicochemical methods such as adsorption, advanced oxidation, and membrane filtration; but it is generally expensive, gives rise to the development of secondary pollutants, and some of them may fail to achieve complete degradation. The attention has shifted to environmentally benign uptake systems, as (a) high cost, secondary pollutants, and incomplete degradation of chemical agents (b) impact EDTA treating wastes are the main factors against the growth of heavy metal removing plant-based industry [7].

Bioremediation by microalgae has emerged as a promising, eco-friendly method for eliminating pharmaceutical contaminants from aquatic environments. For example, microalgae have shown the ability to uptake and transform various kinds of contaminants through biomolecular routes [8, 9]. *Chlorella vulgaris* has been in advantage due to its fast growth, high thresholds to pharmaceutical contaminants, and in utilizing organic contaminants as nutrients. Because of photosynthesis, this microalgal also denitrifies and increases oxygen production and pH of the medium, which, in turn, may potentially facilitate oxidative degradation [10].

Algal bioremediation may emerge as an efficient

technology, whose efficacy, however, depends upon numerous environmental and physiological constraints like lowered enzymatic activity, nutrient availability, or stress responses in the form of pollutant exposure [11]. To overcome this shortfall, recent research has started employing catalytic or stimulatory agents with the aim of improving metabolic and oxidative capacity in the algae. Indole-3-acetic acid (IAA), a naturally occurring plant hormone, has been demonstrated to enhance algal cell proliferation, cell division, and enzymatic activities [12, 13]. Similarly, EGCG, a powerful green tea antioxidant, has also acted as a redox-active catalyst when coupled with sodium bicarbonate ($NaHCO_3$), thus favoring degradation of complex organic entities [14, 15].

IAA acts as a plant growth regulator, promoting the growth of microalgae, supporting photosynthesis, and stimulating metabolic processes to increase the uptake of pollutants. As opposed to IAA, EGCG may act as an electron donor or mediator in conjunction with sodium bicarbonate to facilitate electron transfer and enhance oxidative degradation pathways. Both mechanisms appear to be different; these observations indicate that IAA and EGCG + $NaHCO_3$ have unique effects on the removal of antibiotics.

The aim of this research is to study the kinetic behaviour of amoxicillin and cephalexin uptake by *Chlorella vulgaris* at different concentrations, determine the effects of IAA and EGCG + $NaHCO_3$ on the efficiency of removal of antibiotics, and assess the relative performance of IAA and EGCG + $NaHCO_3$ in terms of the rate of removal and the overall level of effectiveness.

2. MATERIALS AND METHODS

2.1 Preparation of algal culture

A pure culture of *Chlorella vulgaris* was obtained from the Department of Biology, College of Education for Pure Sciences, University of Baghdad. The culture was maintained in ch-13 medium under laboratory conditions at a temperature of 25 + 2°C with continuous aeration and illumination (2500 lux, 12: 12 h light/dark cycle) [16]. The algal cells were grown for 13 days until the exponential growth phase was reached, after which the biomass was harvested by centrifugation (4000 rpm for 10 minutes) and washed twice with distilled water [17].

2.2 Preparation of antibiotic solutions

Amoxicillin and Cephalexin stock solutions (100 mg/L) were prepared by dissolving the antibiotic powders in distilled water. Appropriate working concentrations of 5, 20, and 50 ppm for Amoxicillin and 5, 10, and 20ppm for Cephalexin were made using the stock solutions and stored at 4°C until use [18].

2.3 Experimental design

Batch experiments were carried out in 250 mL Erlenmeyer flasks containing 100 mL of antibiotic solution and 10 mL of *Chlorella vulgaris* suspension (OD ≈ 0.6 at 680 nm) [19]. Treatments were organized into two main groups, which contained either:

1) Control treatments - no catalyst; *Chlorella vulgaris* with antibiotics.

2) Catalytic treatments; investigating:

i) IAA - 5 mg/L

ii) EGCG and sodium bicarbonate ($NaHCO_3$) were applied simultaneously at concentrations of 10 mg/L each.

Control experiments were done with either antibiotic or catalyst, but without the addition of *Chlorella vulgaris*; this permitted the separation of the removal of pollutants by abiotically mediated processes and those removed by biotic means, i.e., through the growth of microalgae.

All experiments were performed in triplicate and were all incubated for 10 days under the same conditions of light and temperature. Samples were collected to be analyzed at 1, 4, 7, 10, and 13 days.

2.4 Analytical determination

The residual levels of Amoxicillin and Cephalexin were analyzed by High-Performance Liquid Chromatography (HPLC) using a UV detector set at 230 nm (SHIMADZU, Japan) at the Ministry of Industry and Minerals, the Industrial Research and Development Authority, Ibn Al-Bitar Research Center, Baghdad, Iraq. The mobile phase was composed of acetonitrile and phosphate buffer (60:40, v/v, pH 6.0) at a flow rate of 1 mL/min. The percentage of bioremediation efficiency was calculated using the following formula [20]:

$$\text{Removal efficiency (\%)} = (C_0 - C_t) / C_0 \times 100$$

where, C_0 is the initial antibiotic concentration, and C_t is the concentration at time.

2.5 Kinetic analysis

First-order kinetic modeling was applied to amoxicillin removal data. Cephalexin, however, exhibited a biphasic removal behavior characterized by an initial slow adsorption phase followed by rapid biodegradation and therefore did not fit well to a single first-order kinetic model. For datasets that adequately followed first-order kinetics, the apparent rate constant of antibiotic removal (k) was determined by evaluating the change in concentration with time according to the equation $\ln(C_t/C_0) = -kt$, where C_t is the concentration remaining at time t, and C_0 is the initial concentration. The half-life ($t_{1/2}$) was calculated using the equation $t_{1/2} = \ln(2)/k$. Kinetic parameters (k and $t_{1/2}$) were calculated based on experimental data obtained from control treatments performed in the absence of additional catalytic enhancement [21]. Accordingly, first-order kinetic modeling was applied only when the experimental data adequately supported this assumption.

2.6 Statistical analysis

Removal efficiency (%) was calculated based on the initial and residual antibiotic concentrations. All experiments were performed in triplicate, and results were shown as the mean ± standard error of a value (SE). Statistical significance among treatments and sampling days was obtained using one-way ANOVA, using a significance of $p \leq 0.05$ and SPSS Software/Desktop (version 26) [22].

3. RESULTS

3.1 Basic removal capacity of microalgae for the two antibiotics

Chlorella vulgaris demonstrated an inherent capacity to remove both amoxicillin and cephalexin from aqueous solutions in the absence of catalytic enhancers. Removal efficiency increased progressively with exposure time, indicating the combined contribution of biosorption during the early phase and biodegradation at later stages. Amoxicillin showed relatively higher initial removal, whereas cephalexin exhibited slower initial adsorption but achieved complete removal within a shorter overall time.

As exposure time increased, the bioremediation efficiency of *Chlorella vulgaris* for both antibiotics increased, as reflected by the values presented in Tables 1 and 2 (mean ± SE).

Table 1. Removal efficiency (%) of amoxicillin by *Chlorella vulgaris*

Day	5 ppm (%)	20 ppm (%)	50 ppm (%)
1	57.96 ± 2.10	77.13 ± 1.15	53.32 ± 2.33
4	72.68 ± 1.36	76.63 ± 1.17	85.10 ± 0.74
7	69.36 ± 1.53	75.83 ± 1.21	88.24 ± 0.59
10	100 ± 0.0	70.42 ± 1.48	73.34 ± 1.33
13	100 ± 0.0	100 ± 0.0	100 ± 0.0

*Significant differences at $p \leq 0.05$.

Table 2. Removal efficiency (%) of cephalexin by *Chlorella vulgaris*

Day	5 ppm (%)	10 ppm (%)	20 ppm (%)
1	1.62 ± 0.25	0.70 ± 0.50	0.35 ± 1.00
4	99.42 ± 0.01	99.56 ± 0.02	99.66 ± 0.03
7	98.96 ± 0.03	99.74 ± 0.01	100 ± 0.0
10	100 ± 0.0	100 ± 0.0	100 ± 0.0
13	100 ± 0.0	100 ± 0.0	100 ± 0.0

*Significant differences at $p \leq 0.05$.

Figure 1 shows that the removal of amoxicillin increased over time, reaching complete removal (100% removal) on day 13 regardless of concentration. The initial concentrations, which were higher, also had a slower removal rate in the beginning, meaning the removal of amoxicillin by microalgae was influenced by the concentration.

The two antibiotics exhibited distinct removal behaviors over the experimental period. Amoxicillin showed a relatively high initial adsorption efficiency on Day 1, ranging from 53.32% to 77.13% depending on the initial concentration (Table 1), indicating a rapid biosorption phase. In contrast, cephalexin exhibited a much lower initial adsorption on Day 1 (0.35–1.62%; Table 2). However, a marked increase in cephalexin removal efficiency was observed during the subsequent exposure period, with removal exceeding 99% by Day 4 for all tested concentrations (Figure 2). These results indicate differences in the temporal removal patterns of the two antibiotics rather than a direct comparison of their overall removal rates.

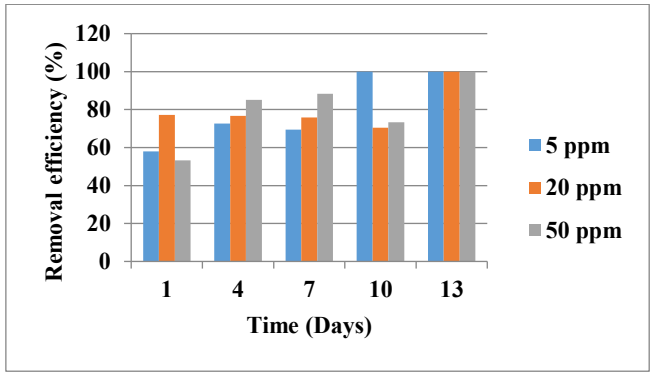

Figure 1. Time-dependent removal efficiency (%) of amoxicillin at different initial concentrations by *Chlorella vulgaris* during a 13-day exposure period

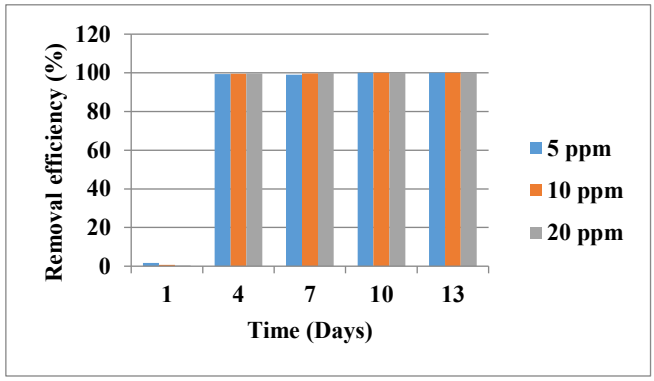

Figure 2. Removal efficiency (%) of cephalexin at different initial concentrations by *Chlorella vulgaris* as a function of exposure time

In the absence of catalysts, *Chlorella vulgaris* exhibited an increase in bioremediation capability over time (time = day, day = as the experiment progressed). Amoxicillin was essentially 100% degraded by 10 days at 5 ppm (low). At higher levels (20 and 50 ppm), amoxicillin degradation increased over time, but not as rapidly as at the lower concentration of 5 ppm. Cephalexin was completely degraded after 10 days of treatment with *Chlorella vulgaris* at all three concentrations tested, even without the use of catalysts (Tables 3 and 4).

Table 3. Removal efficiency (%) of amoxicillin under catalytic enhancement by IAA and EGCG + $NaHCO_3$ during 13-day exposure to *Chlorella vulgaris*

Day	5 ppm + IAA (%)	5 ppm + EGCG + $NaHCO_3$ (%)	20 ppm + IAA (%)	20 ppm + EGCG + $NaHCO_3$ (%)	50 ppm + IAA (%)	50 ppm + EGCG + $NaHCO_3$ (%)
1	61.98 ± 1.90	56.26 ± 2.19	40.02 ± 3.00	50.71 ± 2.47	41.60 ± 2.92	67.55 ± 1.62
4	88.72 ± 0.56	74.38 ± 1.28	85.33 ± 0.73	89.31 ± 0.54	89.25 ± 0.54	66.93 ± 1.65
7	49.30 ± 2.54	69.36 ± 1.53	98.65 ± 0.14	96.91 ± 0.31	92.78 ± 0.72	65.52 ± 1.72
10	100 ± 0.0	100 ± 0.0	76.02 ± 1.20	63.08 ± 1.85	76.37 ± 1.18	100 ± 0.0
13	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0

IAA: Indole-3-acetic acid; EGCG: Epigallocatechin gallate. *Significant differences at $p \leq 0.05$.

Table 4. Removal efficiency (%) of cephalexin under catalytic enhancement by IAA and EGCG + $NaHCO_3$ during 13-day exposure to *Chlorella vulgaris*

Day	5 ppm + IAA (%)	5 ppm + EGCG + $NaHCO_3$ (%)	10 ppm + IAA (%)	10 ppm + EGCG + $NaHCO_3$ (%)	20 ppm + IAA (%)	20 ppm + EGCG + $NaHCO_3$ (%)
1	1.98 ± 0.25	2.10 ± 0.25	1.05 ± 0.50	0.94 ± 0.50	0.50 ± 1.00	0.47 ± 1.00
4	99.40 ± 0.04	99.52 ± 0.02	99.65 ± 0.02	99.14 ± 0.04	99.54 ± 0.05	99.58 ± 0.04
7	98.68 ± 0.06	98.74 ± 0.06	99.37 ± 0.03	99.92 ± 0.00	99.97 ± 0.00	99.96 ± 0.00
10	99.94 ± 0.00	99.64 ± 0.02	99.93 ± 0.00	100 ± 0.0	100 ± 0.0	100 ± 0.0
13	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0

IAA: Indole-3-acetic acid; EGCG: Epigallocatechin gallate. *Significant differences at $p \leq 0.05$.

3.2 Effect of catalyst enhancer on removal kinetics

The addition of catalytic enhancers (IAA and EGCG + $NaHCO_3$) significantly altered the removal kinetics of both antibiotics. Compared with the control treatments, catalytic systems accelerated the degradation process and increased overall removal efficiency, particularly at higher initial concentrations. When IAA was added to the experimental groups treated with *Chlorella vulgaris*, the rate of removal of both antibiotics improved over time for both antibiotics treated with both *Chlorella vulgaris* alone and *Chlorella vulgaris* + IAA, with the 10-day (low) treatment levels of the experimental groups having the highest rates of removal for both antibiotics (Figures 3 and 4). Of all treatment combinations tested, the highest rates of degradation were observed in groups treated with EGCG + $NaHCO_3$ and both *Chlorella vulgaris* and IAA during the early treatment periods. These results indicate that multiple catalytic mechanisms may be working together to augment metabolic activity and increase the rate at which both antibiotics are degraded.

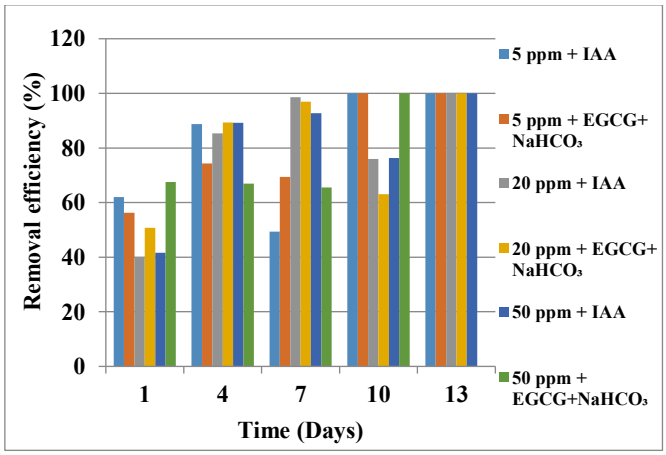

Figure 3. Effect of catalytic enhancement using IAA and EGCG + $NaHCO_3$ on the removal efficiency (%) of amoxicillin by *Chlorella vulgaris* over time
IAA: Indole-3-acetic acid; EGCG: Epigallocatechin gallate.

Trends in enhancement observed for cephalexin removal using bioremediation were similar between both catalytic systems. For both systems, the presence of EGCG + $NaHCO_3$ resulted in a consistently superior removal rate (for all concentrations of bioremediation). In fact, in most cases, nearly complete or complete removal occurred within ten days of treatment (Figures 3 and 4), supporting the effectiveness of catalyst(s) added to treatments.

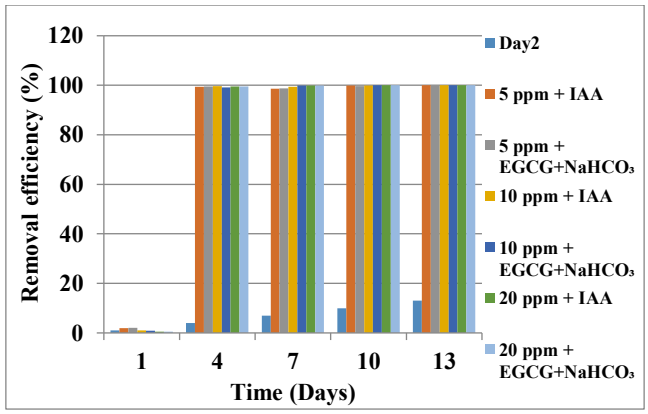

Figure 4. Catalytic enhancement of cephalexin removal efficiency (%) by *Chlorella vulgaris* using IAA and EGCG + $NaHCO_3$ during the 13-day treatment period
IAA: Indole-3-acetic acid; EGCG: Epigallocatechin gallate.

3.3 Quantitative analysis of removal kinetics

To provide a quantitative assessment of antibiotic removal behavior, a first-order kinetic model was applied to the experimental data presented in Tables 1 and 2 when appropriate. Due to the biphasic nature of cephalexin removal, characterized by an initial slow adsorption phase followed by rapid biodegradation, the overall dataset did not fit well to a single first-order kinetic model. Therefore, kinetic parameters (k and t½) were not reported for cephalexin (Table 5). In contrast, amoxicillin exhibited a lower rate constant and a longer half-life, indicating a fundamentally different removal kinetics over the experimental period.

Table 5. First-order kinetic parameters for antibiotic removal

Antibiotic	Removal Rate Constant, k (day^{-1})	Half-Life, $t_{1/2}$ (day)
Amoxicillin	0.177	3.91
Cephalexin	Not applicable (biphasic removal behavior)	

3.4 Statistical analysis

One-way ANOVA analysis revealed statistically significant differences ($p \leq 0.05$) among exposure times, treatment conditions, and initial antibiotic concentrations. The type of catalytic enhancer had a significant effect on removal efficiency, with EGCG + $NaHCO_3$ treatments showing the strongest enhancement. Low standard error values confirmed the reliability and reproducibility of the experimental data.

Therefore, *Chlorella vulgaris* provides a strong potential for use in the bioremediation of antibiotics, and the catalytic addition of either IAA or EGCG + $NaHCO_3$ results in

significantly increased efficiency when conducting such bioremediation under controlled laboratory conditions.

4. DISCUSSION

The differences between amoxicillin and cephalexin in terms of their removal from solution can be attributed to the chemical makeup of these compounds [23]. Amoxicillin has a phenolic functional group as well as a β-lactam structure that allows for strong binding to negatively charged microalgal cell walls through hydrogen bonds and electrostatic forces, promoting its attachment to the algae. On the other hand, cephalexin's amino group results in less strong binding to the algae because it does not have hydrogen bond-forming capabilities, but it allows cephalexin to pass through the algae more readily, leading to eventual degradation within the organism [24, 25]. Therefore, because cephalexin does not initially bind as well to microalgae, it is broken down more quickly [26, 27].

IAA increases the rate of growth at a rate greater than what is normally observed in a typical growth-stimulation assay. Kinetics show that IAA-treated systems exhibit an increased rate of removal or degradation during the mid-exposure period relative to the non-catalytic systems, indicating that IAA enhances the metabolic and enzymatic processes in treated systems. In addition, IAA stimulates photosynthesis and promotes the function of oxidoreductase enzymes, which can further increase the rate of biodegradation and the apparent kinetic constant of IAA systems relative to non-catalytic systems [2].

The synergistic effects of the two components work together to improve this combination's performance by providing both an antioxidant (EGCG) and pH buffering ($NaHCO_3$EGCG acts as an antioxidant due to its ability to transfer electrons during antioxidant oxidations and, therefore, to act as a Redox mediator. The addition of $NaHCO_3$ as a weakly alkaline buffering agent stabilizes the pH of the solution and aids in the formation of ROS, which further enhances the abiotic oxidative breakdown and the enzymatic biodegradation of the antibiotic through the addition of EGCG [28, 29].

When comparing different treatments, it was found that when the EGCG + $NaHCO_3$ was tested, it had superior removal capabilities over the IAA alone. It removed all of the contaminants within a shorter amount of time and had higher rates of effectiveness compared to just IAA. IAA also has some effect on increasing algal metabolism; however, it is not as effective for fast removal of antibiotics that are at very high levels as EGCG + $NaHCO_3$.

The results of the present study provide evidence that previous studies have reported that various approaches to improving the breakdown of pharmaceutical compounds by microalgae have provided proven success [30, 31]. Previous research has demonstrated that Chlorella species have demonstrated great promise in removing many types of antibiotics and other new contaminants from wastewater systems using a combination of metabolic degradation and adsorption [32-34]. Thus, these findings show the potential application of multiple catalytic enhancement technologies in combination with current microalgae bioremediation practices as a green, sustainable, and effective means for the removal of many types of resistant pharmaceutical contaminants from wastewater systems [35, 36].

First-order kinetic modeling revealed clear quantitative differences in the removal behavior of the two antibiotics. Although cephalexin reached complete removal faster than amoxicillin, its removal kinetics were non-linear and characterized by an initial lag phase followed by rapid degradation. This behavior indicates that cephalexin removal cannot be accurately described by a single first-order kinetic model. In contrast, amoxicillin showed a lower rate constant ($k = 0.177$ day^{-1}) and a longer half-life ($t_{1/2} = 3.91$ days), reflecting its slower overall removal despite its higher initial adsorption efficiency. This behavior suggests that amoxicillin removal was dominated by early-stage biosorption, whereas cephalexin removal was governed by rapid biodegradation during the later exposure period [37]. These kinetic parameters quantitatively confirm that the two antibiotics follow distinct removal dynamics rather than a simple faster–slower relationship [38].

5. CONCLUSIONS

This study found that both amoxicillin and cephalexin could be efficiently removed by *Chlorella vulgaris*, with increasing exposure time resulting in higher removal efficiencies through the combined action of biosorption and metabolic biodegradation. The kinetic behavior of cephalexin differed fundamentally from that of amoxicillin, showing a biphasic removal pattern rather than simple first-order decay.

The use of a catalytic enhancer (EGCG + $NaHCO_3$) significantly improved remediation performance, achieving complete removal of both amoxicillin and cephalexin under optimal conditions compared to systems without catalytic enhancement. This increased efficacy can be attributed to the synergistic catalytic and buffering effects that maintained favorable redox and pH conditions for algal activity. Overall, the integration of catalysis with microalgal bioremediation represents an environmentally sustainable approach for the treatment of antibiotic-contaminated wastewater.

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