

BIOSYNTHESIS OF SILVER NANOPARTICLES USING THE CYANOBACTERIUM SYNECHOCYSTIS SALINA

Characterization by FTIR and SEM and Application in Industrial Wastewater Treatment

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Abstract: The contamination of water bodies with heavy metal and nutrient loads from industrial effluent is a growing environmental and public-health concern and conventional remediation methods are often costly, energy intensive or reliant on hazardous chemicals. Biologically synthesized silver nanoparticles (AgNPs) possess large surface area-to-volume ratio and strong affinity for metal ions, making them low cost and eco-friendly. In this study, AgNPs were biosynthesized by employing a culture-derived extract of saline cyanobacterium, *Synechocystis salina*, which was isolated and molecularly identified prior to nanoparticle synthesis. The reduction of Ag⁺ ions were confirmed by the visual change in the color from a pale yellow-green to a dark brown, which is consistent with the surface plasmon resonance. The nanoparticles were characterized by Fourier-transform infrared spectroscopy (FTIR) which identified surface bound amine, amide, carboxyl, alkane, ketone and alkyl-amine functional groups responsible for capping and stabilization, and Scanning Electron Microscopy (SEM) which revealed an irregular, aggregated nanostructured surface morphology. Then the AgNP-enriched microalgal suspension was applied to real industrial wastewater collected from a local effluent discharge site. During the four days of treatment, nitrate concentration decreased from 1.102 to 0.89 mg/L (19.3% reduction), phosphorus from 1.939 to 0.756 mg/L (61.0% reduction) and ammonia from 1.143 to 0.130 mg/L (88.6% reduction), cadmium concentration decreased from 3.5 to 3.1 mg/kg, measured by ICP-OES/ICP-MS. The results suggest the potential of *S. salina*-derived AgNPs to reduce the nutrient and heavy-metal loads in real industrial effluent and support the further development of microalgal nanoparticle systems as a sustainable wastewater-treatment strategy.

Keywords: Silver nanoparticles; Biologically synthesized; *Synechocystis salina*; FTIR; SEM; Industrial wastewater; Heavy-metal loads

I. INTRODUCTION

One of the major contributors to heavy-metal pollution of surface waters has been the industrial effluents, which has been one of the most serious environmental problems in the last decade. These contaminants are non-biodegradable and bioaccumulate up the food chain and thus represent a persistent risk to ecosystems and human health. Traditional treatment technologies (chemical precipitation, ion exchange, and membrane filtration) are often limited by high operating costs, incomplete removal and the generation of secondary waste, which motivates the search for cheaper, greener alternatives.

Nanotechnology-based remediation has become a promising complementary tool for conventional remediation methods. Silver nanoparticles (AgNPs) are especially attractive because they have a high surface area to volume ratio and tunable surface chemistry, allowing for efficient adsorption of metal ions through chemical bonding, ion exchange and electrostatic interaction, while their well-documented antibacterial activity offers an additional disinfection benefit in water-treatment applications. However, the physicochemical synthetic routes for AgNPs usually involve the use of toxic reducing and capping agents. This has raised the interest in biological or “green” synthesis of AgNPs using bacteria, fungi and microalgae as reducing and stabilizing agents.

Cyanobacteria such as *Synechocystis salina* are promising platforms for nanoparticle biosynthesis because they grow rapidly, produce large amounts of biomass, and tolerate heavy metals. This tolerance also makes *S. salina* a good candidate for bioremediation applications. This study aimed to (i) standardize the culture conditions for *Synechocystis salina*, (ii) confirm *Synechocystis salina* identity by sequencing, (iii) produce AgNPs using an extract from *Synechocystis salina*, (iv) characterize the AgNPs by FTIR and SEM, and (v) evaluate how effectively the AgNP–*Synechocystis salina* system reduces nutrient and heavy-metal loads in industrial wastewater.

II. MATERIALS AND METHODS

2.1 Microalgal Culture and Growth Conditions

A strain of *Synechocystis salina* was selected because it divided rapidly and produced abundant biomass. Cultures were grown in 200 mL containers at 24 ± 1 °C under a light intensity of 700–800 lux, with a photoperiod of 16 h light and 8 h dark. Salinity was maintained between 13% and 15%. This culture regime was maintained for a total of four months (Table 1).

Table 1. Culture parameters used for growth of *Synechocystis salina*.

Parameter	Value
Working volume	200 mL
Temperature	24 ± 1 °C
Light intensity	700–800 lux
Photoperiod	16:8 h (light/dark)
Culture duration	4 months
Salinity	13–15%

Growth was monitored microscopically at 40× and 45× magnification. Biomass progression was followed using cell counts on a Neubauer hemocytometer. Optical density was measured at 620 nm and 700 nm. Chlorophyll content was determined spectrophotometrically at 405 nm and 450 nm, after methanol extraction of cell pellets.

2.2 Molecular Identification

Genomic DNA was extracted from the biomass using the CTAB method described by Doyle and Doyle in 1987. The 16S rRNA gene was amplified using PCR. The PCR conditions included a denaturation at 94 °C for 3 minutes. This was followed by 40 cycles of 94 °C for 30 seconds, 55 °C for 30 seconds, and 72 °C for 1 minute, with a final extension step at 72 °C for 7 minutes. The resulting amplicons were checked by agarose gel electrophoresis, and the bands of interest were purified from the gel and sequenced. Assembled contigs were compared against sequences in the NCBI database using BLAST, and phylogenetic analysis was performed on the matching sequences to construct a phylogenetic tree.

2.3 Biosynthesis of Silver Nanoparticles

Cell-free microalgal extract (50 mL) was mixed with 50 mL of prepared 1 mM AgNO₃ solution. The mixture was stirred vigorously for 30 minutes, at room temperature. After that it was left to stand for 2 hours. A brown product formed. This product was collected by centrifugation at 6,000 rpm for 20 minutes. The collected material was dried overnight at room temperature. It was then stored in an airtight container for analysis. This procedure followed the method described by Azizi et al. (2013).

2.4 Nanoparticle Characterization

FTIR spectra were recorded from 4000 to 400 cm⁻¹ to identify the groups present on the nanoparticle surface. These groups are responsible for the reduction process and capping of the nanoparticles. The surface morphology was studied using scanning electron microscopy or SEM. The instrument operated at an accelerating voltage of 15 kV. Images were taken at magnifications ranging from ×500 up, to ×7,000.

III. APPLICATION IN INDUSTRIAL WASTEWATER TREATMENT

Industrial wastewater was collected from a river receiving industrial discharge. A 25 mL aliquot of the AgNP-enriched *S. salina* culture was added to 25 mL of wastewater, with an untreated control (no culture) run in parallel. Temperature, salinity (measured with a hand-held refractometer), and pH were recorded at the start of treatment. Nitrate (brucine–sulfuric acid method, 410 nm), phosphorus (ascorbic acid method, 880 nm), and ammonia (phenol–hypochlorite method) were measured before adding the culture and again after four days of treatment. Each measurement was performed in triplicate, and results are reported as mean ± standard deviation. Cadmium concentration was measured before and after treatment using ICP-OES and ICP-MS following acid digestion of the samples, according to EPA Method 3005A.

IV. RESULTS AND DISCUSSION

4.1 Microalgal Growth

Cell density increased steadily over the culture period, rising from 0.718×10^7 cells/mL on day 0 to 4.863×10^7 cells/mL by day, 8.231×10^7 cells/mL by day 43, and reaching a peak of 8.562×10^7 cells/mL by day 50, after which growth plateaued and the biomass was harvested. Optical density at 620 nm and 700 nm and chlorophyll content at 405 nm and 450 nm increased in parallel with cell density, confirming that the culture was actively growing and photosynthetically active throughout this period.

4.2 Molecular Identification

BLAST analysis of the amplified 16S gene sequence was performed against the NCBI nucleotide database. The results showed a number of high-confidence matches. All top hits had 100% query coverage and an E-value of 0.0. This means the full length of the query sequence matched reference sequences with a level of confidence that's statistically indistinguishable from perfect alignment (Table 2).

The three ranking matches were *Synechocystis salina* strain BO22, *Synechocystis* sp. LEGE 07073 and *Synechocystis salina* strain LEGE 06155. Each of these returned 100.00% sequence identity, the highest possible match in BLAST, confirming unambiguously that the isolate belongs to the species *Synechocystis salina*.

The next group of matches included *Synechocystis* sp. PCC 7339 and PCC 7338, both showing 99.55% sequence identity, while a broader set of related *Synechocystis* strains and chromosome sequences matched at 99.28% identity. This indicates the isolate is closely related to the *Synechocystis* genus but differs slightly from the well-known PCC 6803 reference lineage.

Table 2. Complete list of top-ranking BLAST hits for the amplified 16S rRNA gene sequence, ranked by percent identity. All hits returned 100% query coverage and an E-value of 0.0.

Description	Query Cover	E value	Per. Identity	Accession
<i>Synechocystis salina</i> strain BO22 16S rRNA gene, partial sequence	100%	0.0	100.00%	ON944122.1
<i>Synechocystis</i> sp. LEGE 07073 16S rRNA gene, partial sequence	100%	0.0	100.00%	HM217083.1
<i>Synechocystis salina</i> LEGE 06155 16S rRNA gene, partial sequence	100%	0.0	100.00%	HQ832911.1
<i>Synechocystis</i> sp. PCC 7339 chromosome, complete genome	100%	0.0	99.55%	CP063659.1
<i>Synechocystis</i> sp. PCC 7338 chromosome, complete genome	100%	0.0	99.55%	CP054306.1
<i>Synechocystis</i> sp. CACIAM 05 chromosome	100%	0.0	99.28%	CP019225.1
<i>Synechocystis</i> sp. YACCYB507 16S rRNA gene, partial sequence	100%	0.0	99.28%	MH683774.1
<i>Synechocystis</i> sp. YACCYB361 16S rRNA gene, partial sequence	100%	0.0	99.28%	MH683753.1
<i>Synechocystis</i> sp. YACCYB325 16S rRNA gene, partial sequence	100%	0.0	99.28%	MH683746.1
<i>Synechocystis</i> sp. YACCYB315 16S rRNA gene, partial sequence	100%	0.0	99.28%	MH683741.1
<i>Synechocystis</i> sp. YACCYB308 16S rRNA gene, partial sequence	100%	0.0	99.28%	MH683737.1

Synechocystis sp. YACCYB307 16S rRNA gene, partial sequence	100%	0.0	99.28%	MH683736.1
Synechocystis sp. IPPAS B-1465 chromosome, complete genome	100%	0.0	99.28%	CP028094.1
Synechocystis sp. KSU-AQIQ-1 partial 16S rRNA gene	100%	0.0	99.28%	LN997853.1
Synechocystis sp. PCC 6803 substr. GT-G chromosome, complete genome	100%	0.0	99.28%	CP012832.1
Synechocystis sp. KSU-WH-2 16S rRNA gene, partial sequence	100%	0.0	99.28%	KT807477.1
Synechocystis sp. PCC 6803 substr. GT-T chromosome, complete genome	100%	0.0	99.28%	CP094998.1
Synechocystis sp. PCC 6803 chromosome, complete genome	100%	0.0	99.28%	CP073017.1
Synechocystis sp. PCC 6803, complete genome	100%	0.0	99.28%	CP003265.1
Aphanocapsa cf. rivularis UAM 390 16S rRNA gene, partial sequence	100%	0.0	99.28%	JQ070058.1
Synechocystis sp. PCC 6803 substr. PCC-P DNA, complete genome	100%	0.0	99.28%	AP012278.1
Synechocystis sp. PCC 6803 substr. PCC-N DNA, complete genome	100%	0.0	99.28%	AP012277.1

Table 2 shows the set of top-ranking matches found by the search. The matches are sorted by percent identity. Each entry includes query coverage, E-value, percent identity, and the NCBI accession number. Every match listed had 100% query coverage and an E-value of 0.0, meaning that the entire length of the amplicon matched perfectly to each reference sequence, with no significant chance that this similarity occurred by chance. This is the benchmark for reliable BLAST-based species identification.

The alignment-score distribution for the 113 BLAST hits is shown in Table 3. Every matching sequence received a score in the highest confidence range (alignment score ≥ 200) which is marked in red. These sequences aligned across the full length of the query from position 1 to about 1,100 base pairs. This pattern—scores across nearly the full length—is typical of a clean high-quality sequencing read. It also suggests that many close relatives of the sample are already in the reference database. This gives confidence in the identification beyond the percent identity values reported in Table 2.

Table 3. Distribution of alignment scores for the top 113 BLAST hits against 100 subject sequences, color-coded by confidence bin. Every hit fell in the highest-confidence (≥ 200) category.

Alignment Score Category	Color Code	Number of Hits (of 113)	Percentage
≥ 200 (highest confidence)	Red	113	100%
80–200	Magenta	0	0%
50–80	Green	0	0%

40–50	Blue	0	0%
< 40	Black	0	0%

Phylogenetic reconstruction of the amplified sequence along with the BLAST-matched reference sequences (Table 4) placed the isolate in a weakly supported clade (bootstrap value 55) together with its two closest, 100% sequence-identity relatives, *Synechocystis salina* and its closest relatives, HM217083.1 (*Synechocystis* sp. LEGE 07073) and HQ832911.1 (*Synechocystis salina* LEGE 06155). This isolate-containing clade is joined, with moderate bootstrap support (64), by a separate and well-supported (bootstrap value 95) sister pair — *Synechocystis* sp. PCC 7339 and PCC 7338 — which share 99.55% sequence identity with the isolate. This combined five-taxon clade is nested within a larger, well-supported cluster (bootstrap value 86) that additionally includes the YACCYB-group strains, IPPAS B-1465, KSU-WH-2, and KSU-AQIQ-1 (99.28% identity). This entire cluster is clearly separated, with strong bootstrap support (100), from a distinct clade formed by additional loci of the PCC 6803 substrains, CACIAM 05, and duplicate loci of PCC 7339/PCC 7338 — likely representing paralogous 16S rRNA gene copies rather than a distinct taxon. Together, the BLAST hit table, the alignment-score distribution, and the phylogenetic tree provide three independents, converging lines of molecular evidence that the cultured isolate is *Synechocystis salina*, though its immediate sister relationship with LEGE 07073 and LEGE 06155 (bootstrap 55) should be interpreted with some caution given the modest bootstrap support at that node.

Table 4. Summary of phylogenetic clade groupings and bootstrap support values for the isolate and its closest BLAST-matched reference sequences, based on 16S rRNA gene sequence comparison.

Clade / Node	Bootstrap Support	Representative Taxa	Relationship to Isolate
Innermost clade	55	<i>S. salina</i> (isolate); <i>Synechocystis</i> sp. LEGE 07073 (HM217083.1); <i>S. salina</i> LEGE 06155 (HQ832911.1)	Direct sister taxa — 100.00% identity; weak bootstrap support
Sister pair (PCC 7339/7338)	95	<i>Synechocystis</i> sp. PCC 7339 (CP063659.1); PCC 7338 (CP054306.1)	Well-supported sister pair — 99.55% identity; unites with isolate clade at bootstrap 64
Broader supported cluster	86	+ YACCYB-group strains (MH683736–MH683774), IPPAS B-1465, KSU-WH-2, KSU-AQIQ-1	Related lineage — 99.28% identity
Separate clade (duplicate loci)	100	PCC 6803 substr. GT-G/GT-T/PCC-P/PCC-N, CACIAM 05, additional PCC 7339/7338 loci	Deeply branching, molecularly distinct lineage

4.3 Formation and Characterization of AgNPs

When AgNO_3 was added to the extract, a visible colour change occurred, shifting from yellow-green to dark brown. This change is consistent with excitation of surface plasmon resonance in the formed AgNPs, indicating that bioreduction of Ag^+ to Ag^0 was successful.

FTIR analysis of the dried nanoparticles revealed absorption bands at 3418.46, 2920.11, 2850.97, 1444.96, 1368.93, 1260.09, 1032.03, 574.94, and 526.71 cm^{-1} , with additional bands at 1618.85 and 1537.93 cm^{-1} (Figure 1). The broad band at 3418 cm^{-1} is assigned to O–H/N–H stretching from hydroxyl groups and protein amines that coat the particle surface. The bands at 2920 and 2850 cm^{-1} correspond to asymmetric and symmetric C–H stretching of aliphatic $-\text{CH}_2-$ groups, which likely come from lipid or fatty-acid chains in the extract. The bands at 1618.85 cm^{-1} and 1537.93 cm^{-1} correspond to the amide I vibration (C=O stretch of the peptide backbone) and the amide II vibration (N–H bend/C–N stretch) respectively. Together these bands give evidence that proteins from the algal extract are attached to the nanoparticle surface. The bands at 1444.96 and 1368.93 cm^{-1} arise from C–H bending and carboxylate (COO^-) stretching, which are consistent with amino-acid side chains or organic acids that bind to the particle surface. The bands at 1260.09 and 1032.03 cm^{-1} correspond to C–N stretching and C–O–C stretching of polysaccharide backbones. The low-wavenumber bands at 574.94 and 526.71 cm^{-1} lie in a region normally associated with metal-ligand (Ag–O/Ag–N) vibrations. These bands provide the direct spectral evidence that biomolecules are bound to the silver surface.

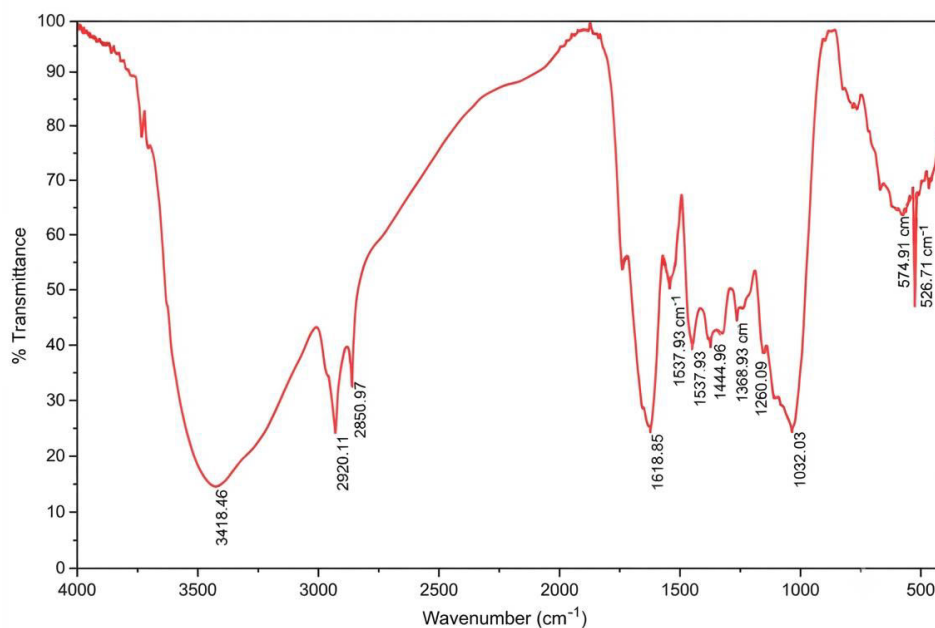


Figure 1. FTIR spectrum of the biosynthesized silver nanoparticles, recorded over 4000–400 cm^{-1} .

This spectral profile does not reflect a single functional group but rather a mix of signals — amide I, amide II, carboxylate, and C–O–C bands all appearing together — pointing to a protein–polysaccharide composition. This makes sense because the biomolecules in the extract serve a dual role: they act as reducing agents, donating electrons that convert Ag^+ ions into metallic silver (Ag^0), while simultaneously serving as capping and stabilizing agents that coat the newly formed particles, limiting further growth and aggregation in solution.

SEM imaging was performed at magnifications from $\times 500$ to $\times 7000$ at 15 kV, providing a progressively clearer view of the nanoparticle surface morphology. At $\times 500$ the surface appeared as a single uneven, fused area rather than as separate, distinct particles — an appearance common for dried biosynthesized materials, where the surrounding organic material made up of biomolecules holds particles together in clusters during sample preparation. At higher magnification ($\times 1,500$), grainy regions and short rod-like shapes began to appear within this matrix, suggesting that fine particles are trapped inside the capping layer. By $\times 7000$ the image revealed a porous network of small, irregular grains resembling clusters of nanoparticles held together by the organic coating identified earlier through FTIR analysis.

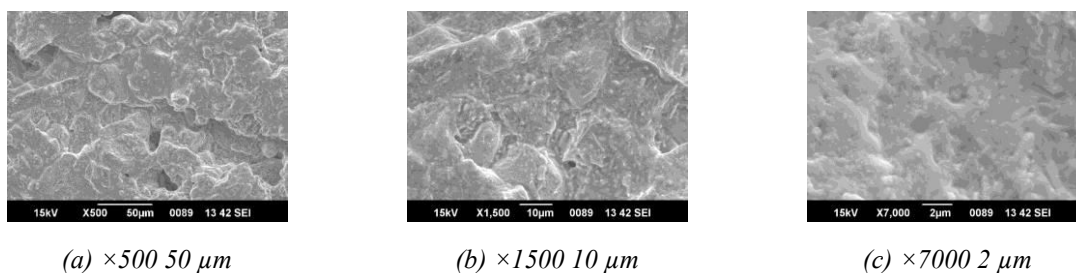


Figure 2. SEM micrographs of the biosynthesized silver nanoparticles at (a) $\times 500$ (b) $\times 1500$ and (c) $\times 7000$ magnification (15 kV), showing an irregular, aggregated, biomolecule-capped surface morphology.

The progressive magnification series shows nanoparticles aggregated within an organic matrix rather than as well-isolated, individual particles. This is an expected result for biologically capped AgNPs, since the same biomolecules that produce the amide and carboxylate bands seen in FTIR are also likely responsible for the clustering that occurs as the sample dries. Consequently, the SEM images alone cannot give a precise size distribution for individual particles; complementary methods such as transmission electron microscopy (TEM) or dynamic light scattering (DLS) would be needed for that purpose. The SEM data do confirm, however, that the surface has a nanostructured, nonuniform, biomolecule-embedded morphology consistent with biologically synthesized nanoparticles, though they do not establish precise particle size or shape.

4.4 Efficacy in Industrial Wastewater Treatment

Treatment of the wastewater sample with the AgNP-enriched *S. salina* culture led to measurable reductions in all three nutrient parameters tested over four days (Table 5): nitrate decreased by 19.3 %, phosphorus by 61.0 %, and ammonia by 88.6 % relative to baseline. Cadmium concentration decreased modestly, from 3.5 mg L⁻¹ to 3.1 mg L⁻¹, over the same period (Table 6).

Table 5. Nutrient parameters in industrial wastewater before and after 4 days of treatment with AgNP-enriched *Synechocystis salina* culture.

Parameter	Before treatment (mg/L)	After 4 days (mg/L)	Reduction (%)
Nitrate	1.102	0.890	19.3
Phosphorus	1.939	0.756	61.0
Ammonia	1.143	0.130	88.6

Table 6. Cadmium concentration in wastewater before and after treatment.

Sample	Method	Cadmium (mg/L)
Untreated wastewater (IWW)	ICP-MS	3.5 (LOQ = 0.05)
Treated wastewater (IWW-NP)	ICP-OES	3.1 (LOQ = 0.05)

The pronounced drop in ammonia and phosphorus is consistent with microalgal uptake of nitrogen and phosphorus for growth and metabolism. The smaller decrease in nitrate suggests that nitrate uptake or conversion occurred more slowly under the conditions tested. The decrease in cadmium is consistent with metal binding to the AgNP–microalgae surface, likely through the chemical bonding, ion-exchange, and electrostatic mechanisms identified as active on the nanoparticle surface by FTIR. Use of the culture also lowered the acidity of the wastewater, which may help maintain a more environmentally favourable pH when the water is released downstream. These results are consistent with studies reporting that nanocomposite-microalgae treatments outperform microalgae alone at removing NH₄⁺, PO₄³⁻, NO₃⁻, and SO₄²⁻ from wastewater, supporting the idea that *S. salina*-derived AgNPs are a practical, low-cost tool for treating industrial wastewater.

V. CONCLUSION

Silver nanoparticles were successfully synthesized using an extract from the cyanobacterium *Synechocystis salina*, whose identity was confirmed at the molecular level by 16S rRNA gene sequencing. Conversion of Ag⁺ ions was indicated by a colour change and confirmed by FTIR, which revealed amine, amide, carboxyl, and ketone groups capping and stabilising the silver nanoparticles. SEM showed a nanostructured surface consistent with a biological synthesis. When this silver nanoparticle-enriched *Synechocystis salina* culture was applied to industrial wastewater, it lowered ammonia by 88.6 %, phosphorus by 61.0 %, and nitrate by 19.3 %, and also reduced cadmium concentration. These findings demonstrate that microalgae can be used to produce silver nanoparticles in an eco-friendly, low-cost way that also cleans wastewater. Further research is needed to optimize the treatment conditions, evaluate the method against other types of contaminants and effluents, and assess whether this approach can be scaled up economically for field use.

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