

1 **Using bio-based CaCO₃ functionalized sediment to simultaneously remove**
2 **algae and COD through adsorption and sedimentation in water source**
3 **reservoirs**

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Abstract

In-situ turbidity enhancement can suppress algal growth in reservoirs but often exacerbates chemical oxygen demand (COD) accumulation due to incomplete organic removal. This study presents a biologically synthesized bio-CaCO₃-modified sediment, engineered via *Bacillus*-induced carbonate precipitation, to simultaneously control algae and reduce COD. The material forms 15–30 nm core–shell clusters with enriched –OH/–COOH groups and mesopores (~19.76 nm), confirmed by SEM, XRD, FTIR, and BET (+1.02 m² g⁻¹). Adsorption tests against *Microcystis aeruginosa*, *Chlorella*, and *Limnithrix* showed Langmuir-type monolayer binding ($R^2 > 0.97$) and pseudo-second-order kinetics. XDLVO theory and DFT analysis revealed strong EPS–Bio-CaCO₃ interactions ($\Delta E_{AB} = 31.28$ mJ m⁻²; $\Delta E_{ads} = -1.07$ eV). Optimal conditions (7.5 wt% CaCO₃, 56% residual Ca²⁺, 85 min) achieved 93.8% Chl-a removal, 88.6% COD reduction, and 87.5% turbidity control ($R^2 = 0.98$), with minimal Ca²⁺ leaching. By integrating chemisorption, interfacial adhesion, and pore confinement, this material provides a stable, eco-friendly strategy for dual pollutant control and *in-situ* sediment remediation.

Keywords: Micro-nano CaCO₃; Sediment modification; Response surface; Extracellular polymeric substances (EPS); XDLVO.

I Introduction

Algal blooms in source water reservoirs pose significant environmental and public health challenges by releasing odorous compounds (Su et al., 2015), organic pollutants, and algal toxins (Wnorowski, 1992). These impacts disrupt aquatic ecosystems, degrade water quality, increase treatment costs, and compromise the reliability of drinking water provision (Huisman et al., 2018; Jan et al., 2023). Sediment resuspension (SR) has emerged as a viable *in-situ* strategy for algal bloom control, primarily by attenuating underwater light and promoting particle-induced sedimentation (Fang et al., 2024). When implemented under well-oxygenated conditions, SR can function not merely as a physical suppression measure but also as a biogeochemical process that promotes nutrient retention—stabilizing phosphorus through Fe-P co-precipitation and limiting the mobilization of redox-sensitive metals (Su et al., 2025)—rather than triggering nutrient release. This dual role addresses concerns over internal loading and underscores SR’s potential compatibility with drinking-water reservoir management. Nevertheless, turbulence inherent to SR can mobilize organic matter, increasing chemical oxygen demand (COD). Although SR introduces no external reagents and is therefore inherently suited to potable water applications (Ewis et al., 2022; Srinivasan, 2011), its overall performance is strongly governed by sediment geochemistry and structure—highlighting the necessity for targeted sediment optimisation.

In reservoir environments, the adsorption efficiency between suspended sediment particles and algae is generally low due to electrostatic repulsion. Modifying clay particles has been explored as a strategy to enhance their adsorption capacity (Barçante et al., 2020; Bergaya and Lagaly, 2013; Farrokhpay and Bradshaw, 2012; Obaje et al., 2013). Key modification techniques include altering the zeta potential (Li et al., 2015), adjusting suspension viscosity, and increasing the number of exchangeable anions and cations, all of which influence the adsorption flocculation-sedimentation process of clay particles. Common cationic modifiers, such as polyaluminum compounds, quaternary ammonium salts (Cao et al., 2006), polyacrylamide (Yu

et al., 2017), and aluminum chloride/aluminum sulfate (Liu et al., 2016) primarily function by modifying surface functional groups (Cao and Yu, 2003). Beyond inorganic clay modifications, organic compounds such as chitosan (Li et al., 2023; Yin et al., 2021) and starch (Cui et al., 2023; Shi et al., 2016) have been investigated for improving adsorption performance. However, their practical application is often hindered by poor stability in aqueous environments, high self-aggregation tendencies, and elevated costs (Pan et al., 2019). Consequently, recent research has focused on developing low-cost, stable, and biodegradable biological modification methods (Sanghi et al., 2006), which offer a more sustainable and environmentally friendly solution for algal control.

Bacillus sp. BF-VB2 modifies kaolin within a pH range of 4.0–10.0, forming a clay-aggregated flocculant capable of treating high-turbidity wastewater (Bisht and Lal, 2019). Compared with chemical flocculants, bio-modified clay-based flocculants have garnered increasing attention due to their biodegradability and high adsorption efficiency. N, N, N-trimethylglycine-grafted cellulose nanocrystals (CNC), in combination with kaolin, form a clay-aggregated flocculant that effectively removes freshwater *Chlorella vulgaris* and marine *Nannochloropsis oculata* (Blockx et al., 2021). Similarly, chitosan, a biopolymer derived from shrimp and crab shells, binds with clay and soil particles to facilitate pollutant sedimentation (Zou et al., 2006). The surface of sediment particles can be modified by algae-derived polysaccharides, which adsorb multivalent metal cations, bind polar organic molecules, and gradually form soil aggregates through coordination complexes and organic-inorganic interactions (Arduino et al., 1989).

Calcium (Ca) in clay plays a crucial role in soil aggregation, adhering to clay and silicate minerals while forming aggregates with carbonates or Ca hydrates that fill pore spaces (Safar and Whalen, 2023). Building on this, bio-CaCO₃ is most effectively deployed as MICP-derived amorphous/micro-nano “seeds” bearing residual EPS, rather than relying on live-cell formation in the field; once introduced into resuspended sediments, these seeds act as persistent nucleation substrates that continue to grow and transform within the sediment matrix under

80 ambient pore-water chemistry, thereby delivering the practical benefits of biogenic crystal-
81 lization without maintaining microbial activity. In MICP, microorganisms such as *Bacillus* or
82 *Sporosarcina* mediate carbonate formation via urease or carbonic-anhydrase pathways, and
83 EPS serves as a nucleation template that tunes polymorph selection (e.g., calcite, vaterite)
84 and interfacial reactivity (Zhuang et al., 2018). Biogenically synthesized CaCO_3 commonly
85 exhibits hierarchical textures (e.g., dense core-grainy shell, raft-like or needle-like assem-
86 blies) (Chekroun et al., 2004; Pérez and García, 2020), widely interpreted as signatures of
87 EPS-regulated nonclassical crystallization with surfaces enriched in $-\text{COOH}/-\text{OH}$ that promote
88 electrostatic attraction, multidentate Ca-bridging, and steric entrapment with algal cells and
89 organic macromolecules (Kim et al., 2017; Seifan and Berenjian, 2019). Similarly, alkalinity-
90 regulated *Thauera* can enhance carbonate availability, with CO_2 reacting with Ca^{2+} to yield
91 micro-/nano- CaCO_3 , facilitating clay aggregation (Zhao et al., 2024). Thus, the main advantage
92 of bio- CaCO_3 over chemically synthesized CaCO_3 lies in EPS-mediated organic-inorganic
93 coupling that endows seeds with adaptive polymorph evolution and highly functionalized
94 surfaces, while the in-sediment (“seeded”) secondary growth clarifies how benefits are realized
95 operationally; nevertheless, the roles of nanoparticle concentration, pore architecture, and
96 specific surface area—particularly under dynamic resuspension—remain underexplored.

97 In light of these considerations, we propose that sediment modification via micro-nano CaCO_3
98 represents a feasible strategy to enhance clay aggregation and adsorption capacity, thereby
99 simultaneously inhibiting algal growth and improving COD removal in source water. In this
100 study, micro-nano CaCO_3 -modified sediments were synthesized using *Bacillus* bacteria, which
101 are known for their high urease activity and calcium carbonate precipitation ability. The physic-
102 ochemical properties, algal inhibition efficiency, and COD reduction performance of the modi-
103 fied sediments were systematically investigated. The underlying mechanisms were elucidated
104 through XDLVO theory and complementary modeling approaches. Furthermore, optimal oper-
105 ational parameters—including micro-nano CaCO_3 dosage and residual free Ca^{2+} dosage—were
106 identified to maximize treatment efficacy. This technology not only addresses the organic pol-

lutant surge linked to sediment resuspension in algal control but also advances in situ ecological restoration by providing a chemical-free, material-efficient solution for water body management.

2 Materials and methods

2.1 Sampling information

The sediment was collected from the 0-10 cm water depth sample of the reservoir river channel of the Lianghui Reservoir in Zhejiang Province China (39°54'39.33"N, 116°24'48.18"E) (Table S1). The sediment was sieved through a 100-mesh sieve and refrigerated at 4°C. Major components were SiO₂ (68.72%) and Al₂O₃ (19.56%) (mass ratio 3.5:1), with minor Fe₂O₃ (4.19%) (Table S2). The activated sludge was collected from the aeration tank of the Fourth Wastewater Treatment Plant in Xi'an. *Chlorella* was isolated from the Shiquan Reservoir in Shanxi Province (China) and cultured in Aquatic Medium No. 6 (AM-6) at 25 °C under an illumination intensity of 2000 lux, with a 12 h:12 h light-dark photoperiod. *Microcystis aeruginosa* and *Limnothrix* sp. were obtained from the Freshwater Algae Culture Collection in the Institute of Hydrobiology, Chinese Academy of Science and were cultured using BG11 culture medium.

2.2 Preparation of bio-CaCO₃-modified sediment

Eight liters of *Bacillus* culture extract, containing residual extracellular polymeric substances (EPS) and metabolic products, were introduced into a 12 L jacketed reactor as the organic template for seed formation. The pH was maintained at 8–10 by automated titration with 5 mol L⁻¹ NaOH via an injection pump. Aeration was applied for 1.5 h, and dissolved oxygen (DO) was controlled at 2–5 mg L⁻¹. After 24 h, the supernatant was collected and analyzed for CO₃²⁻ and HCO₃⁻ concentrations to determine the Ca²⁺ dosage required for 2%, 5%, 8%, 10%, and 14% (w/w) micro-/nano-scale bio-CaCO₃ seeds. The residual free Ca²⁺ concentration was adjusted to

0%, 50%, 100%, or 150% of the measured carbonate alkalinity. Calcium chloride solution was then introduced dropwise under continuous stirring to induce nucleation within the EPS matrix. This procedure produced amorphous/micro-nanocrystalline CaCO_3 seeds enriched with structural defects and EPS coatings, which were used directly in subsequent sediment–water experiments to promote secondary mineral growth and contaminant immobilization.

Modified sediment was prepared by mixing 150 mL of a 25 g L^{-1} clay suspension with bio- CaCO_3 -modified *Bacillus* extract at a 3:1 volume ratio (clay:extract) in a 500 mL beaker. The suspension was stirred at 200 rpm for 30 min to promote surface binding and carbonate precipitation. After 12 h of static aging, the mixture was centrifuged at 4000 rpm for 10 min. The resulting pellet was washed twice with deionized water and oven-dried at 50°C for 24 h. The dried solid was designated as bio- CaCO_3 -modified sediment and used in SEM, XRD, BET, and adsorption analyses.

2.3 Adsorption Kinetics and Isotherm Modeling

Adsorption behavior of algae onto bio- CaCO_3 -modified sediment was assessed through kinetic and isotherm experiments using three representative species (*Chlorella vulgaris*, *Microcystis aeruginosa*, and *Limnothrix* sp.). In each experiment, 100 mL of algal suspension was mixed with 50 mL of modified sediment and agitated at 120 rpm and 25°C . For kinetics, fixed initial Chl-a concentrations (*Chlorella* = $1145 \mu\text{g L}^{-1}$, *M. aeruginosa* = $3273 \mu\text{g L}^{-1}$, *Limnothrix* = $980 \mu\text{g L}^{-1}$) were used, and samples were collected at specific time intervals to fit pseudo-first-order and pseudo-second-order models (Eqs. S1–S5).

Equilibrium studies employed algal suspensions with species-specific Chl-a gradients: *Chlorella vulgaris* ($23.5\text{--}424.9 \mu\text{g L}^{-1}$), *Microcystis aeruginosa* ($91.7\text{--}1046.5 \mu\text{g L}^{-1}$), and *Limnothrix* sp. ($20.0\text{--}91.1 \mu\text{g L}^{-1}$). Each 100 mL of algal solution was mixed with 50 mL of bio- CaCO_3 -modified sediment (prepared under optimal conditions), and incubated at 25°C with shaking at 120 rpm for 4 h to ensure adsorption equilibrium. Following centrifugation (1500 g, 20 min), the supernatants were analyzed for residual Chl-a concentration. Adsorption data were fitted

to Langmuir and Freundlich isotherm models (Eqs. S6–S9), with calculated parameters summarized in Table S6. All experiments were conducted in triplicate to ensure reproducibility.

2.4 Interfacial Interaction Analysis (XDLVO and DFT)

Zeta potential of the bio-CaCO₃-modified sediment and algal biomass was measured using a Zetasizer Pro Blue (Malvern, UK). Static contact angles were measured on dried compressed pellets using ultrapure water, formamide, and diiodomethane (DAS3, KRÜSS, Germany). Each measurement was performed in quintuplicate. Surface energy components (Lifshitz–van der Waals, electron donor, and electron acceptor) were calculated using the extended Young’s equation (ADAM, 1957; White, 1977) (Eqs. S10–S12), and the detailed calculation of the total energy is described in the appendix (Table S7, Table S8).

Density functional theory (DFT) calculations were performed using the periodic plane-wave approach implemented in VASP with the GGA-PBE functional and DFT-D3 dispersion correction, which has been shown to reliably capture both hydrogen bonding and van der Waals interactions at organic–mineral interfaces (Zhao et al., 2018). SiO₂ (001) and calcite (104) surfaces were constructed according to established crystallographic terminations (Kulkarni et al., 2012), with CaCO₃ nanoclusters representing Ca²⁺ bridging domains, a validated strategy for modeling divalent cation-mediated adhesion (Cruz et al., 2012). Representative monosaccharide fragments from algal EPS, such as glucuronic acid, were selected as model compounds due to their prevalence in EPS and their ability to retain the key –COOH and –OH functionalities responsible for interfacial coordination, while offering computational tractability (Zhu et al., 2019). Adsorption energies (ΔE) were calculated from total energy differences, providing molecular-scale insight into EPS–mineral adhesion mechanisms. Computational details and model structures are provided in Supporting Information.

2.5 Determination of Chl-a and COD in water samples

After a 15-min reaction, 10 mL supernatants were collected for analysis. Chl-a and COD were measured according to Chinese standard methods HJ 897-2017 and HJ 828-2017, respectively. All tests were performed in triplicate, and results are reported as mean values. Removal efficiency was calculated as Eq. 1:

$$\text{Removal efficiency(\%)} = \frac{100 (C_i - C_f)}{C_i} \quad (1)$$

where C_i and C_f are the initial and final concentrations ($\mu\text{g L}^{-1}$), respectively. This equation was applied to both Chl-a and COD measurements to evaluate algal inhibition and organic matter removal performance.

2.6 Response surface methodology for process optimization

The effects of three operational variables—micro-nano CaCO_3 mass fraction, residual free Ca^{2+} dosage, and contact time—on Chl-a and COD removal were evaluated using response surface methodology (RSM). A Box-Behnken design (BBD) was implemented via Design Expert 8.0 (Stat-Ease Inc., USA) at a 95% confidence level (Table S4). A quadratic polynomial regression model was used to predict removal efficiency. Detailed model equations and coefficient definitions are provided in Supplementary Eq. (S16).

2.7 Microscopic characterization and structural analysis

The morphological characteristics of the sediment were captured by scanning electron microscopy (SEM) (FEI Quanta 600 FEG, USA), and the surface functional groups were reflected by infrared spectroscopy FTIR (Nicolet™ iS™5, Thermo Scientific, USA).

2.8 Materials and data processing

All the chemical reagents needed for the experiment, such as sodium hydroxide, sodium carbonate, calcium chloride, glucose, and urea, were purchased from Sigma-Aldrich (MO, USA) and confirmed as analytical grade. All experimental data were processed and visualized using Origin 2021 and ggplot2 in R (v4.0).

3 Results and discussion

3.1 Removal Efficiency Modulated by Multivariable Conditions

The removal efficiency of Chl-a and COD by Bio-CaCO₃-modified sediment exhibited distinct nonlinear dependencies on CaCO₃ dosage, residual Ca²⁺ dosage, and reaction time (Fig. 1A–1B). For Chl-a, maximum removal (>92%) was achieved at CaCO₃ dosages >10% and reaction times >60 min, with residual Ca²⁺ showing negligible influence. The response surface revealed a steep gradient along the CaCO₃ axis and a plateau at high dosages and prolonged reaction times, indicating that Chl-a removal was predominantly controlled by sorption site availability and surface-mediated flocculation rather than dissolved Ca²⁺.

This behavior aligns with established mechanisms of Ca-based algal aggregation, where electrostatic interactions and bridging with extracellular polymeric substances (EPS) enhance cell destabilization (Gao et al., 2025; Wu et al., 2024). The minimal impact of residual Ca²⁺ suggests that surface-bound CaCO₃, rather than free ions, drives flocculation, likely through charge neutralization and interparticle bridging (Fig. S1A). The observed plateau implies a saturation effect, where additional CaCO₃ or extended contact time no longer improves efficiency—a critical consideration for optimizing operational parameters in practical applications.

The response surface analysis revealed distinct removal patterns for COD compared to Chl-a (Fig. 1B). Optimal COD removal efficiency (~88%) was achieved at intermediate conditions: 6–

8% CaCO_3 dosage, 50%-60% residual Ca^{2+} , and 60 min reaction time. This system displayed unique sensitivity to ionic strength, following a parabolic response curve where removal efficiency initially improved with increasing Ca^{2+} concentration but declined sharply beyond 80%. This reversal suggests a transition from beneficial complexation at moderate ionic strength to inhibitory effects at higher dosage, potentially due to competitive binding and oversaturation of active sites (Sengco, 2001; Zhang et al., 2009). These findings highlight the dual role of calcium ions in COD removal - while CaCO_3 provides the structural framework for adsorption, the dissolved Ca^{2+} dosage critically regulates removal efficiency through modulation of molecular interactions.

To further dissect the role of each individual factor, a single-variable statistical analysis was performed (Fig. 1C). For Chl-a, increasing CaCO_3 dosage from 2% to 14% resulted in a significant elevation in median removal efficiency from 76.4% to 91.5% ($p < 0.01$), with a concomitant narrowing of interquartile ranges (Fig. S1B). A similar trend was observed with reaction time, where removal peaked at 60 min and plateaued thereafter. In contrast, variations in residual Ca^{2+} dosage had negligible impact on Chl-a removal, reinforcing the conclusion that algal aggregation is predominantly driven by solid-phase mineral properties.

In contrast, COD removal exhibited more complex behavior, with residual Ca^{2+} emerging as the dominant control factor. The system showed a clear optimum at 50% Ca^{2+} , with median removal efficiency increasing from 63.2% to 81.6% before declining at higher concentrations ($p < 0.05$). The wider interquartile ranges observed throughout the COD tests, particularly at elevated Ca^{2+} levels, reflect the system's sensitivity to ionic environment fluctuations and suggest multiple competing interaction mechanisms. This fundamental difference in response patterns between Chl-a and COD removal underscores the distinct physicochemical processes governing particulate versus dissolved pollutant sequestration, with important implications for system optimization in complex water treatment scenarios.

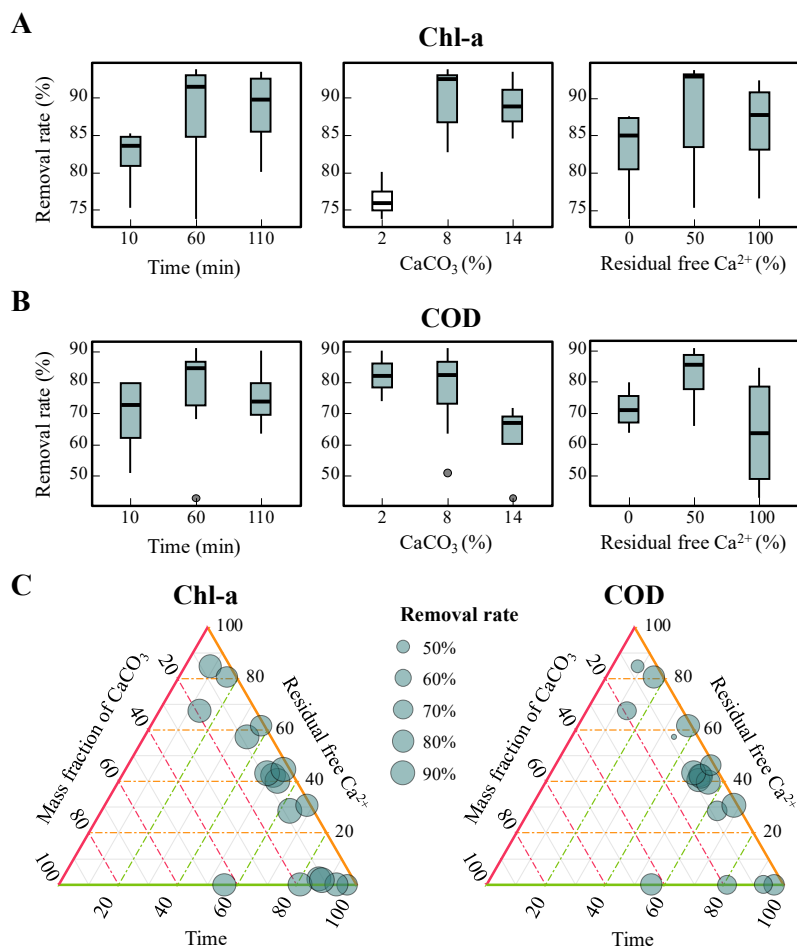


Fig. 1: Bio- CaCO_3 -modified sediment removes Chl-a under the combined influence of CaCO_3 mass fraction, residual free Ca^{2+} dosage, and reaction time (A); and removes COD under the same factor combination (B). Chl-a removal rate is influenced by individual parameters including reaction time (10, 60, 110 min), CaCO_3 dosage (2%, 8%, 14%), and residual free Ca^{2+} (0%, 50%, 100%) (C); COD removal rate responds to the same experimental conditions (D).

3.2 Functional-Group Coordination and Interfacial Energetics

Validate Adsorption Kinetics

Adsorption of algae onto micro-nano CaCO_3 -modified sediment conforms to monolayer chemisorption, as indicated by the higher coefficients of determination (R^2) for both the Langmuir isotherm and the pseudo-second-order (PSO) kinetic model (Fig. S2). The Langmuir model outperformed the Freundlich model (Fig. 2A, Table S6), while the PSO model exhibited R^2 values ranging from 0.971 to 0.997 (Fig. 2B), significantly exceeding those of the pseudo-

first-order (PFO) model (0.533–0.910) (Liu et al., 2020; Zhang et al., 2020). The separation factor ($R_L < 1$) confirms the thermodynamic feasibility of the process (Table S6). The Langmuir affinity constant (K_L) revealed an order of *Chlorella* (0.011) > *Microcystis aeruginosa* (0.003) $\approx$ *Limnothrix* (0.003), reflecting the relative binding affinities between algal surface functional groups and active sites on the modified sludge (Liu et al., 2020).

At equilibrium, the maximum adsorption capacities (Q_m) of the modified sediment reached 1113 $\mu\text{g g}^{-1}$ for *Microcystis aeruginosa*, 617 $\mu\text{g g}^{-1}$ for *Chlorella*, and 2699 $\mu\text{g g}^{-1}$ for *Limnothrix*, corresponding to differences in initial algal concentration. Experimental equilibrium adsorption capacities (q_e) ranged from 378 to 634 $\mu\text{g g}^{-1}$ and were consistent with calculated values (396.8–662.9 $\mu\text{g g}^{-1}$) (Andersen et al., 1991; Safar and Whalen, 2023). Initial adsorption rates followed the order: *Microcystis aeruginosa* > *Limnothrix* > *Chlorella*, which is governed by the initial concentration gradient (Fig. S3). A higher initial concentration (C_0) provides a greater mass transfer driving force, accelerating algal cell diffusion to the adsorbent surface and enhancing the initial adsorption rate (Caliskan et al., 2011). According to the PSO model, the instantaneous adsorption rate is proportional to $\frac{dq_t}{dt} \propto k_2 q_e^2$; at early stages ($q_t \approx 0$), higher (C_0) values correspond to increased (q_e), promoting faster initial adsorption (Tran, 2023).

The enhanced adsorption performance of *Limnothrix* is attributed to its elevated extracellular polymeric substance (EPS) secretion (Han et al., 2024), which is rich in polysaccharides and anionic functional groups such as carboxyl and sulfate (Fig. S4). These groups facilitate calcium-mediated coordination (e.g., Ca–O bonding), reinforcing interfacial adhesion (Rao et al., 2012). Additionally, the filamentous morphology of *Limnothrix* increases the effective contact area and provides entanglement sites, enhancing spatial compatibility with the porous structure of the modified sludge and contributing to its significantly higher Q_m following modification (Young, 2006).

Notably, the variation in Chl-a removal efficiency among the three algal species reflects differences in cellular morphology, EPS production, and initial concentration gradients. Fil-

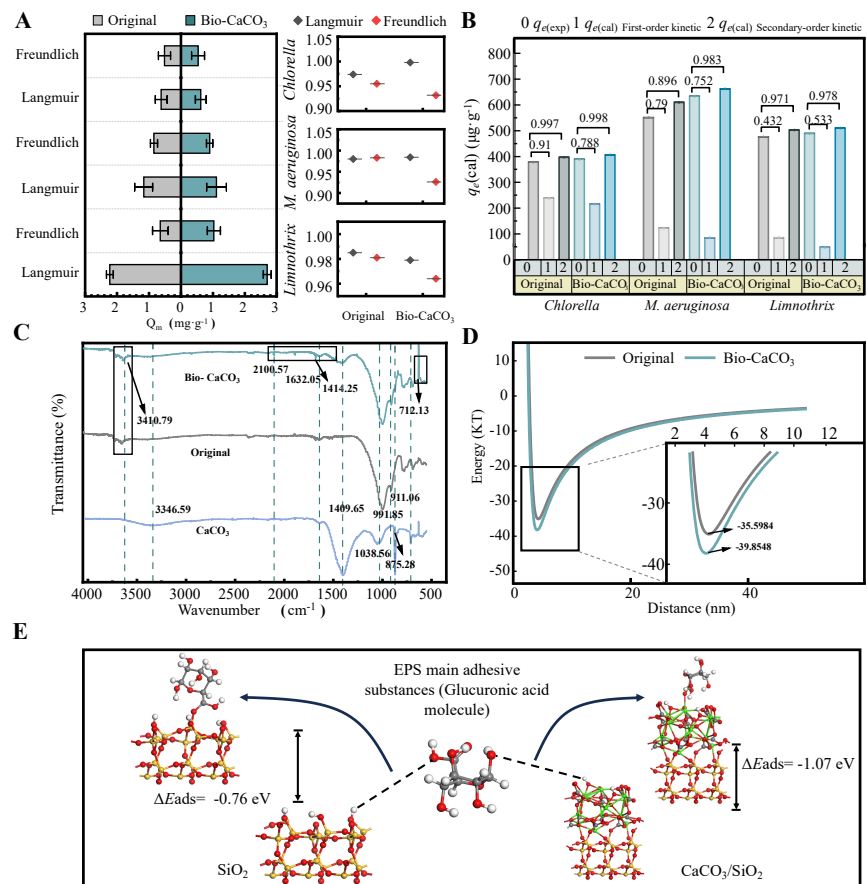


Fig. 2: The adsorption isotherms of *Chlorella*, *Microcystis aeruginosa*, and *Limnithrix* were better described by the Langmuir model than by the Freundlich model, as indicated by higher R^2 values, suggesting monolayer adsorption onto homogeneous surfaces. The equilibrium adsorption capacities (q_e , mg g⁻¹) were substantially enhanced following Bio-CaCO₃ modification, particularly for *Chlorella* (A). Adsorption kinetics were more consistent with the pseudo-second-order model, indicating that chemisorption was the rate-limiting mechanism. The modified sediment exhibited markedly improved adsorption rates and capacities across all algal species (B). FTIR absorbance spectra (a.u.) of CaCO₃, original sediment, and Bio-CaCO₃ composites plotted against wavenumber (cm⁻¹) demonstrate enhanced signals corresponding to carbonate groups (ν_3 : ~1400 cm⁻¹, ν_2 : ~870 cm⁻¹) and organic functionalities (C=O, -OH), confirming successful biomineral integration (C). Extended DLVO (XDLVO) interaction energy profiles (ΔG_{AB} , mJ m⁻²) plotted against separation distance (nm) reveal that Bio-CaCO₃ surfaces exhibit deeper primary energy minima and lower energy barriers compared to unmodified sediment, indicating stronger attractive interactions (D). Density functional theory (DFT) simulations of glucuronic acid, a representative adhesive saccharide in algal EPS, interacting with sediment surfaces. The adsorption energy (ΔE_{ads}) on pristine SiO₂ is -0.76 eV, whereas the energy decreases to -1.07 eV on CaCO₃-modified SiO₂, indicating stronger interfacial binding after mineral modification (E).

amentous *Limnithrix* sp. exhibited higher removal rates due to its tendency to form dense flocs via self-entanglement and Ca²⁺-mediated EPS bridging, thereby enhancing sedimentation and incorporation into bio-CaCO₃ aggregates (Han et al., 2024). In contrast, *Microcystis*

281 *aeruginosa* forms buoyant colonies stabilized by gas vesicles, rendering it less susceptible to
282 gravitational settling or bridging flocculation (Verspagen et al., 2006). Additionally, kinetic
283 experiments employed fixed, high initial Chl-a concentrations, whereas equilibrium assays
284 used species-specific gradients (Mackay et al., 2016), further contributing to variability in ad-
285 sorption behavior. While these differences do not undermine the demonstrated effectiveness
286 of the modified sediment, they underscore the interplay of multiple removal mechanisms—
287 adsorption, bridging, and sedimentation—whose relative contributions vary across algal taxa.
288 Future work will incorporate direct biomass quantification, and EPS characterization to more
289 accurately resolve the dominant removal pathways (Deng et al., 2019; Lai et al., 2018).

290 Complementary spectroscopic and theoretical analyses collectively elucidate the enhanced
291 algae-sediment interaction mechanisms induced by bio-CaCO₃ modification. FTIR spectra
292 of the modified sediment revealed carbonate vibrational bands at 1414, 875, and 712 cm⁻¹
293 (aragonite/vaterite phases)(Andersen et al., 1991; Nilsen et al., 2004; Zou et al., 2019), together
294 with hydroxyl peaks at 3612 and 3400 cm⁻¹ (Al-OH, adsorbed -OH) and amide/carboxyl absorp-
295 tions at ~1630 cm⁻¹ (Fig. 2C), confirming the retention of EPS-derived organic moieties and
296 indicating organic-inorganic coupling between residual EPS and the mineral phase (Alexander
297 et al., 2018; Andersen et al., 1991; Sand et al., 2011). DFT simulations using glucuronic acid as
298 a model EPS ligand showed that, on silanol-rich silica, carboxyl oxygen atoms formed strong
299 hydrogen bonds (1.62–1.78 Å) with surface Si-OH groups, contracting the C-O-Si bond length
300 from 1.39 Å to 1.33 Å and reducing the O-C-O bond angle from ~166° to ~138°, consistent
301 with reorientation of surface hydroxyls to accommodate the saccharide (Fig. S11A-D). The
302 adsorption energy was $\Delta E_{ads} = -0.76$ eV, reflecting moderately strong but reversible binding
303 (Yu et al., 2011; Yu et al., 2024). On Ca²⁺-modified surfaces, a dual-binding mode emerged,
304 where Ca²⁺ bridged carboxylate oxygens of glucuronic acid and oxygen atoms from surface
305 silanols (Ca-O: 1.92–2.08 Å) (Fig. S11E-H), increasing stability ($\Delta E_{ads} = -1.07$ eV, ~40% stronger)
306 (Fig. 2E) (Desmond et al., 2017). The corresponding dynamic structural evolution can be
307 visualized in the GIF animations for C-Si-O (Fig. S9) and Ca-Si-O (Fig. S10), which clearly

show the persistence of Ca–O coordination and the more rigid adsorption geometry on Ca²⁺-modified surfaces. Time-resolved trajectories indicated slower relaxation and persistent Ca–O coordination, supporting a multi-point anchoring mechanism that restricts conformational mobility and reduces desorption under shear. At the interfacial scale, XDLVO analysis showed deepening of the primary minimum from -36.8 kT to -40.8 kT after bio-CaCO₃ modification (Fig. 2D) (Wu et al., 2020), indicating stronger attractive forces and greater colloidal stability (Table S8). The consistency between molecular-level DFT results and interface-level XDLVO findings provides strong evidence that Ca²⁺-mediated hydrogen bonding and cation bridging dominate the enhanced EPS–mineral adhesion, with organic–inorganic coupling, molecular coordination, and interfacial energy optimization acting synergistically.

3.3 Structural refinement of sediment induced by Bio-CaCO₃ integration

The addition of micro-nano CaCO₃ results in substantial changes in the sediment pore structure. The mesopore proportion increases to 73.5%, and the BET-specific surface area rises from 10.08 to 11.71 m² g⁻¹ (Fig. 3A), improving structural openness and enlarging the interaction interface with algal cells (Bennett et al., 2012). This structural refinement promotes multi-site adsorption of organic matter within and between clay mineral particles (Kleber et al., 2015). While the micropore volume remains stable, the total pore volume expands from 10.55 to 12.44 cm³ g⁻¹, and the average pore diameter decreases from 20.17 nm to 18.39 nm, reflecting a finer and more complex pore network (Fig. 3B).

The unmodified sediment exhibited an angular, lamellar morphology with loosely aggregated clay minerals. In contrast, the bio-CaCO₃-modified sediment displayed a uniform coating of spherical particles (15–30 nm), which at higher magnification (200 k×) revealed dense-core and rough-shell features composed of ~20 nm nodular subunits ($D_1 = 19.76\text{ nm}$) (Fig. 3D–E). These nanospheres were embedded within the clay matrix, forming grape-like aggregates and a hi-

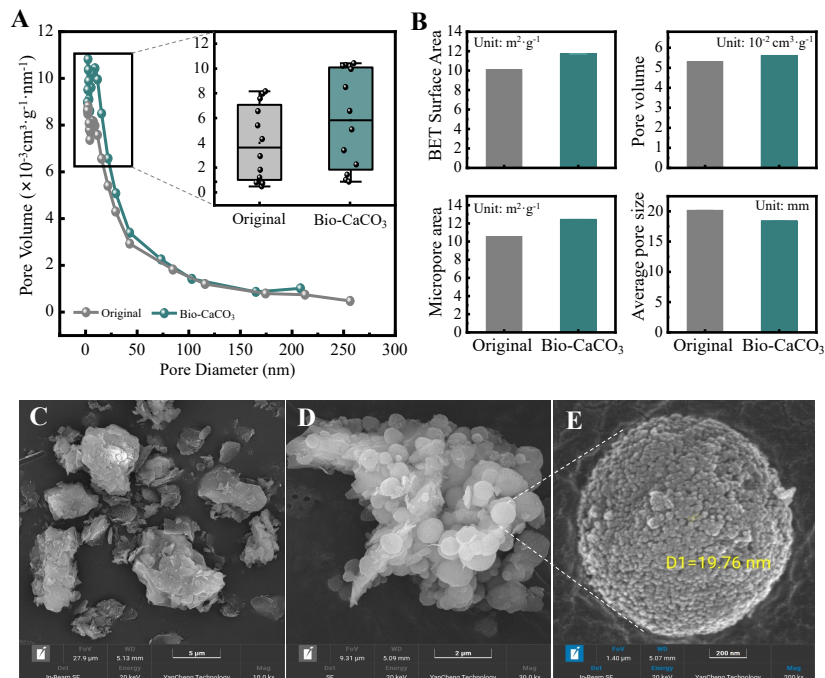


Fig. 3: Particle size distribution curves before and after modification. The modified sample shows a significant shift towards finer particles, as indicated by the red-marked region and arrow. Insets show the statistical distribution of D10, D50, and D90 values ($n = 5$) (A). Comparison of specific surface area and porosity between original and Bio-CaCO₃-modified samples (B). SEM image of raw sediment particles, showing irregular, compact, and angular morphology (C). SEM image of Bio-CaCO₃-modified particles, forming aggregated spherical structures with increased porosity (D). High-resolution SEM of a single spherical Bio-CaCO₃ particle ($D_1 \approx 19.76 \text{ nm}$), showing a nanoscale porous surface, favorable for enhanced adsorption and surface interaction (E).

erarchical pore framework (Fig. S8). XRD analysis further confirmed that bio-modification induced *in-situ* CaCO₃ formation, with pronounced calcite reflections at $2\theta \approx 29.4^\circ$ (104), 35.9° (110), 39.4° (113), and 47.5° (018) (PDF#05-0586), accompanied by a broad amorphous hump spanning $\sim 20\text{--}35^\circ 2\theta$ (Fig. S5). The coexistence of well-defined calcite peaks and an amorphous background is indicative of partial crystallization from an amorphous calcium carbonate (ACC) precursor, a transformation pathway commonly associated with EPS-mediated nonclassical nucleation (Rodríguez-Navarro et al., 2016; Rodríguez-Navarro et al., 2016). The absence of calcite peaks in the unmodified sediment excludes the possibility of inheritance from the raw material, confirming that crystalline domains were generated during the bio-modification process. Moreover, the relatively low peak intensities and broadened full width at half maximum (FWHM) suggest nanocrystalline dimensions and lattice disorder, consistent with the presence of defect-

rich seeds capable of continued polymorphic transformation within the sediment microenvironment.

The combination of nanospherical morphology and crystalline phase transformation suggests the successful formation of bio-synthesized micro/nano-scale CaCO_3 on the sediment surface. This hierarchical pore structure facilitates electron transfer and the directional aggregation of amino acids and small proteins (Wang, 2016), regulates the microbial membrane microenvironment, and promotes the self-organization of CaCO_3 into a mesopore-dominated architecture. The morphological evolution aligns with EPS-mediated nucleation and self-assembly mechanisms reported for biogenic minerals, underscoring the synergistic role of microbial interfaces in directing pore-scale organization and improving the capacity for algal and COD removal (Nilsen et al., 2004; Zou et al., 2019).

Notably, the formation of organized CaCO_3 nanostructures is accompanied by the development of moderately hydroxylated surfaces, as revealed by FTIR and supported by DFT analysis (Section 3.2). These surfaces provide reactive Ca–OH sites capable of multidentate complexation with EPS functional groups, reinforcing the interfacial affinity established by the hierarchical morphology. This structural–chemical consistency validates the dual role of bio- CaCO_3 in promoting both surface reactivity and architectural stabilization, thereby supporting the observed enhancements in EPS retention and overall removal performance (Bowers et al., 2015).

3.4 Predictive Modeling of Dual Pollutant Removal Using Box–Behnken Design

F value or p value is often used to evaluate the significance of each influencing factor in the regression model (Zou et al., 2006). The model equation was evaluated by analysis of variance (ANOVA) (Table S9). Only when the test condition of $p < 0.05$ was the model and the influencing factor considered significant (Table 1).

Table 1: ANOVA analysis for Chl-a and COD removal regression. (* p values > 0.05 were considered to be not statistically significant.)

Model term	Coefficient	F-value	P-value	Coefficient	F-value	P-value
Response	Chl-a			COD		
prob	< 0.0001			<0.0001		
R ²	0.9939			0.9827		
Pred. R ²	0.9187			0.8054		
CV (%)	0.95			3.64		
Intercept	93.30	126.91	< 0.0001	87.52	44.10	< 0.0001
A	6.27	466.53	< 0.0001	-10.00	105.76	< 0.0001
B	1.63	31.31	0.0008	-3.87	15.83	0.0053
C	3.15	118.03	< 0.0001	3.17	10.63	0.0139
AB*	-0.08	0.00946	0.9251	-9.01	42.99	0.0003
AC	1.04	6.35	0.0398	-1.16	0.71	0.4285
BC*	1.26	2.35	0.1689	10.39	57.10	0.0001
A ²	-7.40	341.29	< 0.0001	-5.45	16.52	0.0048
B ²	-3.86	92.99	< 0.0001	-14.68	120.02	< 0.0001
C ²	-2.55	40.58	0.0004	-5.19	15.01	0.0061

The coefficient of variation (CV%) of the model reflects the relative dispersion of the data. The lower the value, the smaller the dispersion of the data set. In this study, the maximum value of the coefficient of variation (CV%) of COD removal efficiency was 7.31% (far below the 15% threshold), confirming the reliability of the experimental data (Yetilmezsoy et al., 2009).

The response-surface analysis indicates two fundamentally different interaction regimes. For Chl-a, only the AC term ($\text{CaCO}_3 \times \text{time}$) is significant ($F = 6.35$, $p = 0.040$), pointing to a time-dependent generation of chemisorption sites. During the first hour, moderate dosages (4–6 wt

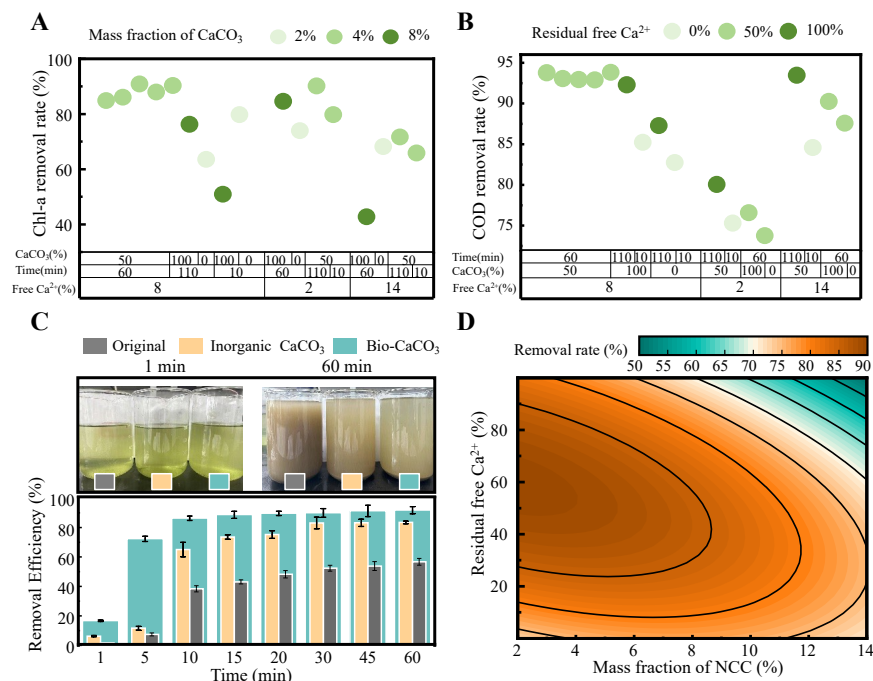


Fig. 4: Bio- CaCO_3 -modified sediment achieves optimal Chl-a removal efficiency under the combined effects of CaCO_3 dosage, residual free Ca^{2+} dosage, and reaction time, with CaCO_3 dosage identified as the dominant factor (A). COD removal efficiency under the same conditions is primarily influenced by residual free Ca^{2+} concentration (B). Turbidity reduction during the resuspension–settling process reflects the sediment’s flocculation-enhancing effect (C). Interactive effects of CaCO_3 dosage and residual free Ca^{2+} on COD removal efficiency are shown in a response surface plot, indicating a non-linear synergistic relationship (D).

%) (Fig. 4A) recrystallise into calcite/vaterite nanospheres whose freshly exposed Ca–OH groups form inner-sphere complexes with uronic and sulfonic residues in algal EPS ($\Delta E_{\text{ads}} \approx -1.07$ eV) (Li and Stenstrom, 2014; Okoth et al., 2008). When these sites approach saturation (~93 % removal) the uptake curve flattens, and neither higher dosage nor extended contact adds benefit—hence the non-significance of BC ($\text{Ca}^{2+} \times \text{time}$). This behaviour agrees with reports that particulate algal destabilisation is governed by solid-phase Ca sites rather than bulk Ca^{2+} concentration (Keiluweit and Kleber, 2009).

Notably, the response surface analysis (Fig. 4D) reveals steep gradients and pronounced curvature, indicating a strong nonlinear synergy between CaCO_3 dosage and residual Ca^{2+} in enhancing COD removal. Under optimal conditions ($\approx 6\text{--}8$ wt% CaCO_3 , residual $\text{Ca}^{2+} \leq 60\%$), divalent Ca^{2+} serves as an electrostatic bridge between carboxylate and phenolic groups in dissolved or

organics and silanol (Si–O) or aluminol (Al–OH) groups on mineral surfaces (Keiluweit and Kleber, 2009; Najafi et al., 2021). Simultaneously, CaCO₃-induced mesoporosity increases the accessible binding surface area, while hydrolysis-derived bicarbonate promotes the accumulation of hydrophilic α-helical proteins in algal EPS, enhancing microalgae–sediment adhesion (Adamczyk et al., 1992; Safar and Whalen, 2023). Moderate free Ca²⁺ levels (≈ 8–10%) also facilitate coordination with algal carboxyl groups, promoting aggregation and further stabilizing flocs (Cao et al., 2025).

In contrast, when residual Ca²⁺ exceeds ≈ 80%, the system enters an inhibitory regime. Excessive Ca²⁺ compresses the electric double layer, reverses surface charge, and displaces weakly bound fulvate, thereby suppressing COD adsorption (Najafi et al., 2021). This is further supported by the positive coefficient of the Ca²⁺ × time (BC) interaction term in the quadratic model, reflecting that prolonged contact intensifies Ca²⁺ accumulation and exacerbates adsorption decline—consistent with observations from carbonate-rich riverine systems (Safar and Whalen, 2023). Elevated Ca²⁺ also electrostatically shields Si–O and Al–OH groups, weakening their hydrogen-bonding capacity with organics (Greenland and Quirk, 1962; Sand et al., 2011). These results underscore the need for precise regulation of CaCO₃ dosage, Ca²⁺ levels, and reaction time to sustain the favorable coordination regime while avoiding overloading effects that impair treatment efficiency.

According to the specific parameters (Table 1), the regression model is statistically significant at the 95% confidence level. The regression equations of the predicted response surface quadratic model are shown in Eq. (11) and Eq. (12):

$$Y_1(\%) = 61.206 + 4.132A + 0.173B + 0.145C + 0.00345AC - 0.205A^2 - 0.0015B^2 - 0.001C^2 \quad (11)$$

$$Y_2(\%) = 67.676 + 2.487A + 0.5B + 0.136C - 0.030AB + 0.0042BC - 0.151A^2 - 0.0058B^2 - 0.0021C^2 \quad (12)$$

The dosage of micro-nano CaCO₃ was 7.5%, the residual free Ca²⁺ was 56%, the contact time was 85.35 min, and the maximum removal rates of Chl-a and COD were 93.83% and 88.64%,

respectively (Table S10).

Biologically modified sediment exhibits markedly enhanced stability during the resuspension process, achieving over 90% reduction in suspended particulate levels within 60 minutes (Fig. 4C). After 30 minutes, the measured value drops to 48.6 NTU, significantly lower than those observed for inorganic-modified (81.3 NTU) and unmodified sediments (231 NTU). This improved performance under both short- and long-term conditions indicates stronger resistance to hydrodynamic disturbance and more effective maintenance of water clarity (Fig. S7). These findings support the use of micro-nano CaCO_3 -based biological modification as a low-disturbance and efficient strategy for algal control and aquatic environmental enhancement.

3.5 Synergistic Effects of Surface Potential and Mn Redox Cycling

The surface electrical properties are pivotal for modulating algal removal efficiency (Yu et al., 1995). Bio- CaCO_3 modification led to a Zeta potential shift from -13.2 mV to -9.65 mV, attenuating electrostatic repulsion between negatively charged algal cells and sediment particles. Simultaneously, the extended DLVO interaction energy (ΔG_{AB}) increased from 26.373 to 31.282 mJ m^{-2} , indicating enhanced exposure of electron-donating groups and stronger interfacial interactions.

The improved performance of the bio- CaCO_3 -modified sediment can be attributed to several interconnected mechanisms that operate consistently across molecular, interfacial, and geochemical scales. First, the modification process enriches the sediment surface with hydroxylated sites and Ca^{2+} -mediated bridging domains (Woods Jr, 2004), increasing the density of active coordination points for algal EPS and mineral particles. Second, extended XDLVO analysis revealed a deeper secondary minimum (-40.8 kT compared to -36.8 kT for unmodified sediment), which favors reversible adsorption and temporary retention, while the high primary barrier (~ 1900 kT) inhibits irreversible fouling (Adamczyk et al., 1992). Third, the enhanced EPS-

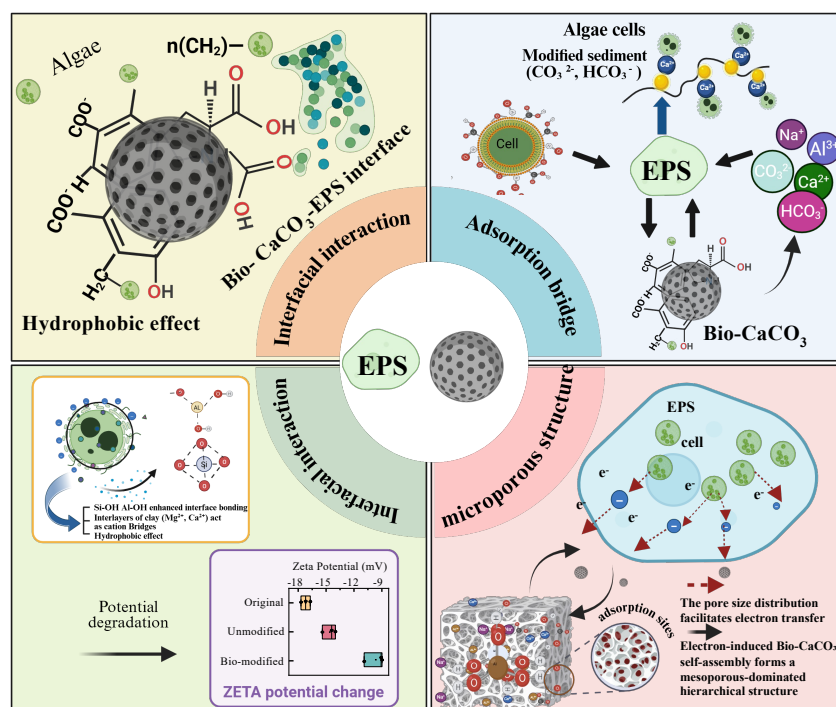


Fig. 5: Schematic illustration of the interaction mechanisms between Bio- CaCO_3 -modified sediment and algal extracellular polymeric substances (EPS).

surface interaction—particularly with the bound EPS fraction—was associated with a 15.3% reduction in contact angle, indicating increased surface hydrophobicity that further promotes controlled cell attachment. Together, these effects yield an interface that combines strong yet reversible binding, preventing both inefficient capture and long-term blockage.

Additionally, manganese (Mn) fractionation was employed as a redox-sensitive geochemical tracer to evaluate the interfacial behavior of the bio- CaCO_3 -modified sediment (Su et al., 2025). Under aerobic sediment resuspension, where dissolved oxygen exceeded 5 mg L^{-1} , the dispersion of bio- CaCO_3 seeds within the sediment matrix provided persistent nucleation sites for continuous *in-situ* crystal growth, while maintaining an oxidative microenvironment that is known to suppress internal nutrient release through Fe-P co-precipitation. This environment also likely promoted the activity of indigenous Mn-oxidizing bacteria, facilitating the oxidation of soluble Mn^{2+} to higher-valent particulate Mn species (e.g., $\text{Mn}^{3+}/\text{Mn}^{4+}$ oxides) (Tebo et al., 2005; Zhou and Fu, 2020). These oxidized Mn forms, though not the primary removal pathway,

can form coordination complexes or co-precipitate with CaCO_3 and algal EPS, contributing to aggregate formation and interfacial stabilization (Pulsawat et al., 2003). Correspondingly, a notable shift from exchangeable and reducible Mn to more stable oxidized fractions was observed, indicating enhanced mineral–organic complexation and reduced ion mobility. Given that Mn speciation responds sensitively to redox and binding environment changes, this behavior serves as an indirect indicator of adsorption strength and interface robustness. These coupled processes—surface potential modification by Ca^{2+} -mediated organic–inorganic coupling and Mn redox cycling under well-oxygenated SR—collectively support the dual removal of Chl-a and COD via adsorption-driven pathways without stimulating nutrient release.

4 Conclusion

Bio- CaCO_3 functionalisation of reservoir sediment enables efficient, *in-situ* control of algal biomass and dissolved organics during resuspension, achieving up to 92% Chl-a and 88% COD removal at the optimal condition (7.5 wt% CaCO_3 , 56% residual Ca^{2+} , 85 min). The bio-mineral coating forms 15–30 nm CaCO_3 nanospheres, increasing BET surface area ($10.1 \rightarrow 11.7 \text{ m}^2 \text{ g}^{-1}$) and generating ~18 nm mesopores, serving as crystallisation seeds that drive and sustain amorphous-to-crystalline transformation within the sediment matrix. This process preserves organic–inorganic coupling with residual EPS, while Ca^{2+} release modulates surface potential ($-13.2 \rightarrow -9.7 \text{ mV}$) and deepens the secondary XDLVO minimum, enhancing reversible EPS-mediated adhesion. Together with the redox-responsive sediment microenvironment, these structural and chemical attributes create a self-regenerating interface capable of maintaining high removal performance under dynamic reservoir conditions, offering a practical and environmentally benign strategy for long-term water quality protection.

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References

- ADAM, N.K., 1957. Use of the term “young’s equation” for contact angles. *Nature* 180, 809–810. <https://doi.org/10.1038/180809a0>
- Adamczyk, Z., Siwek, B., Zembala, M., 1992. Reversible and irreversible adsorption of particles on homogeneous surfaces. *Colloids and Surfaces* 62, 119–130. [https://doi.org/10.1016/0166-6622\(92\)80043-2](https://doi.org/10.1016/0166-6622(92)80043-2)
- Alexander, J.A., Ahmad Zaini, M.A., Surajudeen, A., Aliyu, E.-N.U., Omeiza, A.U., 2018. Surface modification of low-cost bentonite adsorbents—a review. *Particulate Science and Technology* 37, 538–549. <https://doi.org/10.1080/02726351.2018.1438548>
- Andersen, F.A., Brecevic, L., Beuter, G., Dell’Amico, D.B., Calderazzo, F., Bjerrum, N.J., Underhill, A.E., 1991. Infrared spectra of amorphous and crystalline calcium carbonate. *Acta Chem. Scand* 45, 1018–1024.
- Arduino, E., Barberis, E., Boero, V., 1989. Iron oxides and particle aggregation in b horizons of some italian soils. *Geoderma* 45, 319–329. [https://doi.org/10.1016/0016-7061\(89\)90014-1](https://doi.org/10.1016/0016-7061(89)90014-1)
- Barçante, B., Nascimento, N.O., Silva, T.F.G., Reis, L.A., Giani, A., 2020. Cyanobacteria dynamics and phytoplankton species richness as a measure of waterbody recovery: Response to phosphorus removal treatment in a tropical eutrophic reservoir. *Ecological Indicators* 117,

106702. <https://doi.org/10.1016/j.ecolind.2020.106702>

Bennett, R.H., Hulbert, M.H., Curry, K.J., Curry, A., Douglas, J., 2012. Organic matter sequestered in potential energy fields predicted by 3-d clay microstructure model. *Marine Geology* 315–318, 108–114. <https://doi.org/10.1016/j.margeo.2012.04.009>

Bergaya, F., Lagaly, G., 2013. General introduction, in: *Handbook of Clay Science*. Elsevier, pp. 1–19. <https://doi.org/10.1016/b978-0-08-098258-8.00001-8>

Bisht, V., Lal, B., 2019. Exploration of performance kinetics and mechanism of action of a potential novel bioflocculant BF-VB2 on clay and dye wastewater flocculation. *Frontiers in Microbiology* 10. <https://doi.org/10.3389/fmicb.2019.01288>

Blockx, J., Verfaillie, A., Deschaume, O., Bartic, C., Muylaert, K., Thielemans, W., 2021. Glycine betaine grafted nanocellulose as an effective and bio-based cationic nanocellulose flocculant for wastewater treatment and microalgal harvesting. *Nanoscale Advances* 3, 4133–4144. <https://doi.org/10.1039/d1na00102g>

Bowers, G.M., Argersinger, H.E., Reddy, U.V., Johnson, T.A., Arey, B., Bowden, M., Kirkpatrick, R.J., 2015. Integrated molecular and microscopic scale insight into morphology and ion dynamics in Ca²⁺-mediated natural organic matter floccs. *The Journal of Physical Chemistry C* 119, 17773–17783. <https://doi.org/10.1021/acs.jpcc.5b05509>

Caliskan, N., Kul, A.R., Alkan, S., Sogut, E.G., Alacabey, İ., 2011. Adsorption of zinc(II) on diatomite and manganese-oxide-modified diatomite: A kinetic and equilibrium study. *Journal of Hazardous Materials* 193, 27–36. <https://doi.org/10.1016/j.jhazmat.2011.06.058>

Cao, D.-Q., Song, Y.-X., Wu, Y.-F., Yihuo, G., Jin, J.-Y., 2025. Calcium ion mixing modes govern membrane fouling mitigation during membrane-based recovery of extracellular polymeric substances. *Membranes* 15, 169. <https://doi.org/10.3390/membranes15060169>

Cao, X., Song, X., Yu, Z., Wang, K., 2006. [Mechanisms of removing red tide organisms by organo-clays]. *Huan Jing Ke Xue* 27, 1522–1530.

Cao, X., Yu, Z., 2003. [Extinguishment of harmful algae by organo-clay]. *Ying Yong Sheng Tai Xue Bao = The Journal of Applied Ecology* 14, 1169–1172.

- Chekroun, K.B., Rodriguez-Navarro, C., Gonzalez-Munoz, M.T., Arias, J.M., Cultrone, G., Rodriguez-Gallego, M., 2004. Precipitation and growth morphology of calcium carbonate induced by myxococcus xanthus: Implications for recognition of bacterial carbonates. *Journal of Sedimentary Research* 74, 868–876. <https://doi.org/10.1306/050504740868>
- Cruz, L.F., Cobine, P.A., De La Fuente, L., 2012. Calcium increases xylella fastidiosa surface attachment, biofilm formation, and twitching motility. *Applied and Environmental Microbiology* 78, 1321–1331. <https://doi.org/10.1128/aem.06501-11>
- Cui, J., Niu, X., Zhang, D., Ma, J., Zhu, X., Zheng, X., Lin, Z., Fu, M., 2023. The novel chitosan-amphoteric starch dual flocculants for enhanced removal of microcystis aeruginosa and algal organic matter. *Carbohydrate Polymers* 304, 120474. <https://doi.org/10.1016/j.carbpol.2022.120474>
- Deng, X., Qi, M., Ren, R., Liu, J., Sun, X., Xie, P., Chen, J., 2019. The relationships between odors and environmental factors at bloom and non-bloom area in lake taihu, china. *Chemosphere* 218, 569–576. <https://doi.org/10.1016/j.chemosphere.2018.11.121>
- Desmond, J.L., Juhl, K., Hassenkam, T., Stipp, S.L.S., Walsh, T.R., Rodger, P.M., 2017. Organic-silica interactions in saline: Elucidating the structural influence of calcium in low-salinity enhanced oil recovery. *Scientific Reports* 7. <https://doi.org/10.1038/s41598-017-10327-9>
- Ewis, D., Ba-Abbad, M.M., Benamor, A., El-Naas, M.H., 2022. Adsorption of organic water pollutants by clays and clay minerals composites: A comprehensive review. *Applied Clay Science* 229, 106686. <https://doi.org/10.1016/j.clay.2022.106686>
- Fang, J., Li, Y., Su, M., Cao, T., Sun, X., Ai, Y., Qin, J., Yu, J., Yang, M., 2024. Mitigating harmful cyanobacterial blooms in drinking water reservoirs through in-situ sediment resuspension. *Water Research* 267, 122509. <https://doi.org/10.1016/j.watres.2024.122509>
- Farrokhpay, S., Bradshaw, D., 2012. Effect of clay minerals on froth stability in mineral flotation. A review, XXVI. IMPC, New Delhi, India, Paper 313.
- Gao, X., Zhang, H., Zhang, X., Zhang, C., Mao, C., Shan, S., Wei, F., Mortimer, M., Fang, J., 2025. Interactions between extracellular polymeric substances and engineered nanoparticles in

aquatic systems and their environmental effects: A comprehensive review. *Environmental Science: Nano* 12, 2177–2192. <https://doi.org/10.1039/d5en00073d>

Greenland, D.J., Quirk, J.P., 1962. Adsorption of 1-n-alkyl pyridinium bromides by montmorillonite, in: *Clays and Clay Minerals*. Elsevier, pp. 484–499. <https://doi.org/10.1016/b978-1-4831-9842-2.50039-0>

Han, S.-F., Jin, W., Qu, F., Hanelt, D., Abomohra, A., 2024. Integrated municipal wastewater treatment and lipid accumulation by a self-flocculating/floating microalga *limnotherix* sp. *Biore-source Technology* 394, 130165. <https://doi.org/10.1016/j.biortech.2023.130165>

Huisman, J., Codd, G.A., Paerl, H.W., Ibelings, B.W., Verspagen, J.M.H., Visser, P.M., 2018. Cyanobacterial blooms. *Nature Reviews Microbiology* 16, 471–483. <https://doi.org/10.1038/s41579-018-0040-1>

Jan, S., Mishra, A.K., Bhat, M.A., Bhat, M.A., Jan, A.T., 2023. Pollutants in aquatic system: A frontier perspective of emerging threat and strategies to solve the crisis for safe drinking water. *Environmental Science and Pollution Research* 30, 113242–113279. <https://doi.org/10.1007/s11356-023-30302-4>

Keiluweit, M., Kleber, M., 2009. Molecular-level interactions in soils and sediments: The role of aromatic π -systems. *Environmental Science & Technology* 43, 3421–3429. <https://doi.org/10.1021/es8033044>

Kim, H., Jo, B.Y., Kim, H.S., 2017. Effect of different concentrations and ratios of ammonium, nitrate, and phosphate on growth of the blue-green alga (cyanobacterium) *microcystis aeruginosa* isolated from the nakdong river, korea. *ALGAE* 32, 275–284. <https://doi.org/10.4490/algae.2017.32.10.23>

Kleber, M., Eusterhues, K., Keiluweit, M., Mikutta, C., Mikutta, R., Nico, P.S., 2015. Mineral–organic associations: Formation, properties, and relevance in soil environments, in: *Advances in Agronomy*. Elsevier, pp. 1–140. <https://doi.org/10.1016/bs.agron.2014.10.005>

Kulkarni, A.D., Truhlar, D.G., Goverapet Srinivasan, S., Duin, A.C.T. van, Norman, P., Schwartzen-truber, T.E., 2012. Oxygen interactions with silica surfaces: Coupled cluster and density func-

tional investigation and the development of a new ReaxFF potential. The Journal of Physical Chemistry C 117, 258–269. <https://doi.org/10.1021/jp3086649>

Lai, H., Fang, H., Huang, L., He, G., Reible, D., 2018. A review on sediment bioflocculation: Dynamics, influencing factors and modeling. Science of The Total Environment 642, 1184–1200. <https://doi.org/10.1016/j.scitotenv.2018.06.101>

Li, B., Stenstrom, M.K., 2014. Research advances and challenges in one-dimensional modeling of secondary settling tanks – a critical review. Water Research 65, 40–63. <https://doi.org/10.1016/j.watres.2014.07.007>

Li, H., Yu, Z., Cao, X., Song, X., 2023. Chitosan modification and its synergism with clay to mitigate harmful algal blooms. Environmental Technology & Innovation 29, 103028. <https://doi.org/10.1016/j.eti.2023.103028>

Li, L., Zhang, H., Pan, G., 2015. Influence of zeta potential on the flocculation of cyanobacteria cells using chitosan modified soil. Journal of Environmental Sciences 28, 47–53. <https://doi.org/10.1016/j.jes.2014.04.017>

Liu, L., Liu, X., Wang, D., Lin, H., Huang, L., 2020. Removal and reduction of Cr(VI) in simulated wastewater using magnetic biochar prepared by co-pyrolysis of nano-zero-valent iron and sewage sludge. Journal of Cleaner Production 257, 120562. <https://doi.org/10.1016/j.jclepro.2020.120562>

Liu, Y., Cao, X., Yu, Z., Song, X., Qiu, L., 2016. Controlling harmful algae blooms using aluminum-modified clay. Marine Pollution Bulletin 103, 211–219. <https://doi.org/10.1016/j.marpolbul.2015.12.017>

Mackay, D., Celsie, A.K.D., Arnot, J.A., Powell, D.E., 2016. Processes influencing chemical biomagnification and trophic magnification factors in aquatic ecosystems: Implications for chemical hazard and risk assessment. Chemosphere 154, 99–108. <https://doi.org/10.1016/j.chemosphere.2016.03.048>

Najafi, H., Farajfaed, S., Zolgharnian, S., Mosavi Mirak, S.H., Asasian-Kolur, N., Sharifian, S., 2021. A comprehensive study on modified-pillared clays as an adsorbent in wastewater treatment

processes. *Process Safety and Environmental Protection* 147, 8–36. <https://doi.org/10.1016/j.psep.2020.09.028>

Nilsen, O., Fjellvåg, H., Kjekshus, A., 2004. Growth of calcium carbonate by the atomic layer chemical vapour deposition technique. *Thin Solid Films* 450, 240–247. <https://doi.org/10.1016/j.tsf.2003.10.152>

Obaje, S., Omada, J., Dambatta, U., 2013. Clays and their industrial applications: Synoptic review. *International Journal of Science and Technology* 3, 264–270.

Okoth, G., Centikaya, S., Brüggemann, J., Thöming, J., 2008. On hydrodynamic optimisation of multi-channel counter-flow lamella settlers and separation efficiency of cohesive particles. *Chemical Engineering and Processing: Process Intensification* 47, 90–100. <https://doi.org/10.1016/j.cep.2007.08.003>

Pan, S.-Y., Haddad, A.Z., Gadgil, A.J., 2019. Toward greener and more sustainable manufacture of bauxite-derived adsorbents for water defluoridation. *ACS Sustainable Chemistry & Engineering* 7, 18323–18331. <https://doi.org/10.1021/acssuschemeng.9b03649>

Pérez, H.F., García, M.G., 2020. Bioprecipitation of calcium carbonate by bacillus subtilis and its potential to self-healing in cement-based materials. *Journal of Applied Research and Technology* 18. <https://doi.org/10.22201/icat.24486736e.2020.18.5.1280>

Pulsawat, W., Leksawasdi, N., Rogers, P.L., Foster, L.J.R., 2003. Anions effects on biosorption of Mn(II) by extracellular polymeric substance (EPS) from rhizobium etli. *Biotechnology Letters* 25, 1267–1270. <https://doi.org/10.1023/a:1025083116343>

Rao, K., Kundu, T., Parker, S., 2012. Molecular modeling of mineral surface reactions in flotation. *Molecular modeling for the design of novel performance chemicals and materials* 65–105.

Rodríguez-Navarro, C., Burgos Cara, A., Elert, K., Putnis, C.V., Ruiz-Agudo, E., 2016. Direct nanoscale imaging reveals the growth of calcite crystals via amorphous nanoparticles. *Crystal Growth & Design* 16, 1850–1860. <https://doi.org/10.1021/acs.cgd.5b01180>

Rodríguez-Navarro, C., Ruiz-Agudo, E., Harris, J., Wolf, S.E., 2016. Nonclassical crystallization in vivo et in vitro (II): Nanogranular features in biomimetic minerals disclose a general colloid-

mediated crystal growth mechanism. *Journal of Structural Biology* 196, 260–287. <https://doi.org/10.1016/j.jsb.2016.09.005>

Safar, F., Whalen, J.K., 2023. Mechanical stability of newly-formed soil macroaggregates influenced by calcium concentration and the calcium counter-anion. *Geoderma* 430, 116333. <https://doi.org/10.1016/j.geoderma.2023.116333>

Sand, K.K., Rodriguez-Blanco, J.D., Makovicky, E., Benning, L.G., Stipp, S.L.S., 2011. Crystallization of CaCO₃ in water–alcohol mixtures: Spherulitic growth, polymorph stabilization, and morphology change. *Crystal Growth & Design* 12, 842–853. <https://doi.org/10.1021/cg2012342>

Sanghi, R., Bhattacharya, B., Dixit, A., Singh, V., 2006. Ipomoea dasysperma seed gum: An effective natural coagulant for the decolorization of textile dye solutions. *Journal of Environmental Management* 81, 36–41. <https://doi.org/10.1016/j.jenvman.2005.09.015>

Seifan, M., Berenjian, A., 2019. Microbially induced calcium carbonate precipitation: A widespread phenomenon in the biological world. *Applied Microbiology and Biotechnology* 103, 4693–4708. <https://doi.org/10.1007/s00253-019-09861-5>

Sengco, M.R., 2001. The aggregation of clay minerals and marine microalgal cells: Physicochemical theory and implications for controlling harmful algal blooms (Thesis).

Shi, W., Tan, W., Wang, L., Pan, G., 2016. Removal of microcystis aeruginosa using cationic starch modified soils. *Water Research* 97, 19–25. <https://doi.org/10.1016/j.watres.2015.06.029>

Srinivasan, R., 2011. Advances in application of natural clay and its composites in removal of biological, organic, and inorganic contaminants from drinking water. *Advances in Materials Science and Engineering* 2011, 1–17. <https://doi.org/10.1155/2011/872531>

Su, M., Li, W., Fang, J., Cao, T., Ai, Y., Lü, C., Zhao, J., Yang, Z., Yang, M., 2025. Effects of oxygenation resuspension on DOM composition and its role in reducing dissolved manganese in drinking water reservoirs. *Environmental Science & Technology* 59, 10498–10509. <https://doi.org/10.1021/acs.est.5c00235>

Su, M., Yu, J., Zhang, J., Chen, H., An, W., Vogt, R.D., Andersen, T., Jia, D., Wang, J., Yang, M.,

2015. MIB-producing cyanobacteria (*Planktothrix* sp.) in a drinking water reservoir: Distribution and odor producing potential. *Water Research* 68, 444–453. <https://doi.org/10.1016/j.watres.2014.09.038>
- Tebo, B.M., Johnson, H.A., McCarthy, J.K., Templeton, A.S., 2005. Geomicrobiology of manganese(II) oxidation. *Trends in Microbiology* 13, 421–428. <https://doi.org/10.1016/j.tim.2005.07.009>
- Tran, H.N., 2023. Applying linear forms of pseudo-second-order kinetic model for feasibly identifying errors in the initial periods of time-dependent adsorption datasets. *Water* 15, 1231. <https://doi.org/10.3390/w15061231>
- Verspagen, J., Visser, P., Huisman, J., 2006. Aggregation with clay causes sedimentation of the buoyant cyanobacteria microcystis spp. *Aquatic Microbial Ecology* 44, 165–174. <https://doi.org/10.3354/ame044165>
- Wang, Z., 2016. [The role of biomolecules in hydroxyapatite biomineralization](#) (PhD thesis). University of Akron.
- White, L.R., 1977. On deviations from young's equation. *Journal of the Chemical Society, Faraday Transactions 1: Physical Chemistry in Condensed Phases* 73, 390. <https://doi.org/10.1039/f19777300390>
- Wnorowski, A., 1992. Tastes and odours in the aquatic environment: A review. *Water Sa* 18, 203–214.
- Woods Jr, C.E., 2004. Examination of the effects of biosurfactant concentration on natural gas hydrate formation in seafloor porous media. Mississippi State University.
- Wu, K., Ouyang, S., Tao, Z., Hu, X., Zhou, Q., 2024. Algal extracellular polymeric substance compositions drive the binding characteristics, affinity, and phytotoxicity of graphene oxide in water. *Water Research* 260, 121908. <https://doi.org/10.1016/j.watres.2024.121908>
- Wu, T., Huang, Q., Liu, Z., Wang, Y., Lin, D., 2020. Distribution-balanced loss for multi-label classification in long-tailed datasets, in: *Computer Vision – ECCV 2020*. Springer International Publishing, pp. 162–178. https://doi.org/10.1007/978-3-030-58548-8_10

- Yetilmezsoy, K., Demirel, S., Vanderbei, R.J., 2009. Response surface modeling of pb(II) removal from aqueous solution by pistacia vera l.: Box-behnken experimental design. *Journal of Hazardous Materials* 171, 551–562. <https://doi.org/10.1016/j.jhazmat.2009.06.035>
- Yin, Z., Chu, R., Zhu, L., Li, S., Mo, F., Hu, D., Liu, C., 2021. Application of chitosan-based flocculants to harvest microalgal biomass for biofuel production: A review. *Renewable and Sustainable Energy Reviews* 145, 111159. <https://doi.org/10.1016/j.rser.2021.111159>
- Young, K.D., 2006. The selective value of bacterial shape. *Microbiology and Molecular Biology Reviews* 70, 660–703. <https://doi.org/10.1128/membr.00001-06>
- Yu, F.-C., Phalak, N., Sun, Z., Fan, L.-S., 2011. Activation strategies for calcium-based sorbents for CO₂ capture: A perspective. *Industrial & Engineering Chemistry Research* 51, 2133–2142. <https://doi.org/10.1021/ie200802y>
- Yu, J., Wang, K., Yang, P., Li, M., Dong, B., Jin, Z., Hong, S., Ma, H., 2024. Simulation of calcium carbonate nucleation processes in confined c-s-h nanopores with different calcium-silicon ratios. *Construction and Building Materials* 438, 137157. <https://doi.org/10.1016/j.conbuildmat.2024.137157>
- Yu, Z., Song, X., Cao, X., Liu, Y., 2017. Mitigation of harmful algal blooms using modified clays: Theory, mechanisms, and applications. *Harmful Algae* 69, 48–64. <https://doi.org/10.1016/j.hal.2017.09.004>
- Yu, Z., Zou, J., Ma, X., 1995. Application of clays to removal of red tide organisms III. The coagulation of kaolin on red tide organisms. *Chinese Journal of Oceanology and Limnology* 13, 62–70. <https://doi.org/10.1007/bf02845350>
- Zhang, Y., Chen, Y., Westerhoff, P., Crittenden, J., 2009. Impact of natural organic matter and divalent cations on the stability of aqueous nanoparticles. *Water Research* 43, 4249–4257. <https://doi.org/10.1016/j.watres.2009.06.005>
- Zhang, Y., Wang, Y., Cao, X., Xue, J., Zhang, Q., Tian, J., Li, X., Qiu, X., Pan, B., Gu, A.Z., Zheng, X., 2020. Effect of carboxyl and hydroxyl groups on adsorptive polysaccharide fouling: A comparative study based on PVDF and graphene oxide (GO) modified PVDF surfaces. *Journal*

of Membrane Science 595, 117514. <https://doi.org/10.1016/j.memsci.2019.117514>

Zhao, H., Yang, Y., Shu, X., Wang, Y., Ran, Q., 2018. Adsorption of organic molecules on mineral surfaces studied by first-principle calculations: A review. *Advances in Colloid and Interface Science* 256, 230–241. <https://doi.org/10.1016/j.cis.2018.04.003>

Zhao, J., Feng, J., Du, Y., Yan, Z., Li, X., Qin, J., Su, M., Yang, M., 2024. Alkalinity control in sludge propels the conversion of concrete slurry waste into micro- and nano-sized biogenic CaCO₃. *Water Science & Technology* 90, 1070–1081. <https://doi.org/10.2166/wst.2024.255>

Zhou, H., Fu, C., 2020. Manganese-oxidizing microbes and biogenic manganese oxides: Characterization, mn(II) oxidation mechanism and environmental relevance. *Reviews in Environmental Science and Bio/Technology* 19, 489–507. <https://doi.org/10.1007/s11157-020-09541-1>

Zhu, W., Chen, T., He, R., Ding, Y., Duan, T., Xiao, B., 2019. Understanding the interfacial interactions of bioinspired chitosan-calcite nanocomposites by first principles molecular dynamics simulations and experimental FT-IR spectroscopy. *Carbohydrate Polymers* 223, 115054. <https://doi.org/10.1016/j.carbpol.2019.115054>

Zhuang, D., Yan, H., Tucker, M.E., Zhao, H., Han, Z., Zhao, Y., Sun, B., Li, D., Pan, J., Zhao, Y., Meng, R., Shan, G., Zhang, X., Tang, R., 2018. Calcite precipitation induced by bacillus cereus MRR2 cultured at different Ca²⁺ concentrations: Further insights into biotic and abiotic calcite. *Chemical Geology* 500, 64–87. <https://doi.org/10.1016/j.chemgeo.2018.09.018>

Zou, H., Pan, G., Chen, H., Yuan, X., 2006. Removal of cyanobacterial blooms in taihu lake using local soils II. Effective removal of microcystis aeruginosa using local soils and sediments modified by chitosan. *Environmental Pollution* 141, 201–205. <https://doi.org/10.1016/j.envpol.2005.08.042>

Zou, Z., Habraken, W.J.E.M., Matveeva, G., Jensen, A.C.S., Bertinetti, L., Hood, M.A., Sun, C., Gilbert, P.U.P.A., Polishchuk, I., Pokroy, B., Mahamid, J., Politi, Y., Weiner, S., Werner, P., Bette, S., Dinnebier, R., Kolb, U., Zolotoyabko, E., Fratzl, P., 2019. A hydrated crystalline calcium carbonate phase: Calcium carbonate hemihydrate. *Science* 363, 396–400. <https://doi.org/10.1126/science.1264141>

