

Article

A Teaching Reform Case for *Water Quality Engineering* Based on C@CaCO₃ Enhanced Biological Nitrification of Low-Alkalinity Digestate Wastewater

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Abstract

Undergraduate *Water Quality Engineering* courses rely on verification experiments lacking real engineering scenarios, limiting undergraduate participants' comprehensive problem-solving skills. To remedy this issue, this study constructs an inquiry-oriented experiment using carbon-coated nano-calcium carbonate (C@CaCO₃) to enhance biological nitrification for digested wastewater with low alkalinity and high ammonia. It involves wastewater characterization, sequencing batch reactor (SBR) operation, quantification of chemical oxygen demand (COD), ammonium nitrogen (NH₄⁺-N) removal, mechanism discussion on alkalinity slow release and microbial carrier role of C@CaCO₃, and high-throughput analysis of microbial communities. Experimental results show that C@CaCO₃ increases NH₄⁺-N removal by 94.5 ± 4.8% in low-alkalinity water, while COD removal improves from 5.7 ± 3.0% to 29.5 ± 5.2%. These findings demonstrate that C@CaCO₃ effectively mitigates alkalinity limitation, facilitates microbial attachment, and promotes functional microbe enrichment, offering a practical solution for sustainable nitrogen management in nutrient-rich waste streams. Pedagogically, the case links water-quality monitoring, reactor control, and solid-waste resource recovery, enabling undergraduate participants to trace the full engineering workflow problem identification through quantitative design, operation, and mechanistic interpretation. Post-laboratory surveys and performance assessments indicate significant gains in experimental competence, data-analysis skills, and engineering judgment. By embedding a real-world, low-alkalinity wastewater challenge within the curriculum, the module advances sustainable water-resource management education and provides a replicable model for reforming laboratory teaching in *Water Quality Engineering* and related environmental-engineering courses.

Keywords: C@CaCO₃; experimental teaching reform; low-alkalinity and high-ammonia wastewater; biological nitrification; *Water Quality Engineering*



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1. Introduction

Water Quality Engineering is one of the core professional courses for undergraduates majoring in Water Supply and Drainage Science and Engineering. It covers wastewa-

ter characteristics, as well as the theories and technologies of municipal and industrial wastewater treatment and reuse [1]. As an essential component of this course, laboratory teaching should not only consolidate theoretical knowledge but also cultivate undergraduate participants' engineering reasoning, practical skills and ability to analyze complex problems. In this way, laboratory teaching can promote integration of theory and practice and improve their capacity to solve real-world engineering problems [2]. However, many laboratory sessions remain predominantly verification-oriented and rely on fixed operating procedures, leaving limited room for inquiry-based exploration [3,4]. Undergraduate participants often mechanically follow predetermined protocols, making it difficult for them to develop a deep understanding of core concepts such as water quality variation, process control mechanisms, and multi-parameter interactions [5]. Moreover, their innovative thinking and practical problem-solving abilities are not effectively stimulated, highlighting the need for reform in experimental teaching [6,7]. Cutting-edge studies now combine experimental performance evaluation with molecular simulations (e.g., density functional theory (DFT)) to reveal pollutant-removal mechanisms, as shown by Attia et al. for chloramphenicol [8]. This integrated approach implies that lab teaching should move beyond mere verification and encourage students to explore the fundamental principles behind observed phenomena.

With the expansion of large-scale livestock farming, the increasing generation of livestock and poultry manure has posed growing environmental risks, including nitrogen (N) and phosphorus (P) losses, water pollution, and eutrophication [9,10]. Anaerobic digestion provides an important pathway for manure resource recovery and its conversion into clean energy in the form of methane (CH_4) [11]. However, large volumes of high-concentration digestate produced during anaerobic digestion face multiple constraints in land application, transportation, and high-value utilization, making its treatment and resource recovery an important issue [12]. Anaerobic digestion effluent typically contains concentrations of chemical oxygen demand (COD) and ammonia nitrogen ($\text{NH}_4^+\text{-N}$), with a low C/N ratio and poor biodegradability [13]. After physicochemical treatment, the effluent often exhibits low alkalinity. In particular, electrodialysis dilute treated by calcium precipitation and electrochemical methods shows limited biological nitrification capacity [14]. Achieving stable nitrogen transformation under such complex water quality conditions is a common engineering challenge and is closely related to the core contents of *Water Quality Engineering*, including biological treatment, nitrification, denitrification, nutrient removal, and process operation regulation [15,16]. Introducing this practical problem into laboratory teaching can help undergraduate participants connect isolated theoretical concepts with engineering applications and develop stronger problem-solving and practical abilities. Recent advances in wastewater treatment have increasingly focused on sustainable and high-performance technologies, including engineered functional materials for contaminant capture, microbially enhanced biological processes for refractory wastewater treatment, and integrated strategies for improving treatment efficiency and environmental safety [17–19]. Against this background, introducing anaerobic fermentation-related wastewater treatment problems into laboratory teaching can help students understand the practical significance of biological nitrogen removal within the broader context of emerging water treatment technologies.

For actual wastewater characterized by refractory organic matter, high ammonia nitrogen, and low alkalinity, alkalinity supplementation is often required during biological nitrification to enhance ammonia removal [20,21]. Theoretically, complete nitrification consumes 7.14 g of alkalinity as CaCO_3 per gram of ammonia nitrogen, whereas denitrification generates 3.57 g of alkalinity as CaCO_3 per gram of nitrate nitrogen reduced [22,23]. Thus, sufficient alkalinity is essential for effective ammonia oxidation by nitrifying bacteria [24,25]. In conventional nitrification processes, chemicals such as NaOH , Na_2CO_3 ,

and $\text{Ca}(\text{OH})_2$ are commonly added to increase alkalinity [26]. Compared with the direct addition of soluble alkalis such as Na_2CO_3 or $\text{Ca}(\text{OH})_2$, C@CaCO_3 is hypothesized to provide superior nitrification performance by acting as both a slow-release alkalinity source and a microbial carrier, thereby sustaining a more stable microenvironment for ammonia oxidation, microbial attachment, and sludge floc formation. Carbon-coated nano calcium carbonate (C@CaCO_3), developed from digestate resource recovery [27], can gradually release CaCO_3 from its carbon matrix, thereby supplying alkalinity for nitrification in low-alkalinity wastewater and improving ammonia removal [28]. Meanwhile, the carbon layer provides a support matrix for microbial attachment and sludge floc formation, while the released Ca^{2+} can serve as nucleation sites to further promote activated sludge flocculation. At present, practical teaching cases focusing on enhanced nitrification and denitrification of low-alkalinity and high-ammonia wastewater remain insufficient. In particular, the application of solid-waste-derived C@CaCO_3 as a water treatment agent has rarely been incorporated into the experimental teaching of *Water Quality Engineering*.

Therefore, this study introduces solid-waste-derived C@CaCO_3 as a wastewater treatment agent into the laboratory teaching of *Water Quality Engineering*. An experimental case was developed to investigate enhanced biological nitrification in low-alkalinity and high-ammonia wastewater using a sequencing batch reactor (SBR) activated sludge system. Firstly, the influent wastewater SBR system were designed, and control system without C@CaCO_3 addition was established to evaluate the effect of C@CaCO_3 on COD, $\text{NH}_4^+\text{-N}$, and TN removal. Secondly, the effects of C@CaCO_3 addition on activated sludge morphology and microbial community composition were analyzed to clarify its mechanism in enhancing biological nitrogen treatment. Finally, the educational value of this inquiry-based experimental teaching case was discussed in terms of guiding undergraduate participants to apply fundamental theories to analyze and solve complex engineering problems, while improving their experimental skills and engineering practice capabilities. Guided by real-world engineering problems, this case provides a useful reference for the reform of experimental teaching in *Water Quality Engineering*.

2. Experimental Design

2.1. Experimental Objectives

‘ C@CaCO_3 strengthened biological nitrogen removal derived from digestate with high $\text{NH}_4^+\text{-N}$ and low alkalinity concentration’ is incorporated as an experimental teaching case in this experiment. The case addresses existing issues in the practical teaching of *Water Quality Engineering*, such as insufficient theoretical understanding of biological nitrogen removal, inadequate training in the analysis of engineering problems, and a lack of practical operational skills, by developing an inquiry-based experimental project with a real-world engineering context [29].

The wastewater used in the experiment is derived from the effluent of the electro-dialysis desalination chamber following anaerobic digestion, solid–liquid separation and nutrient recovery of digestate liquid [12,14]. Through this experiment, undergraduate participants are able to understand the key limiting factors in the biological treatment of wastewater with high ammonia nitrogen and low alkalinity within a realistic water quality context, and to master core knowledge in areas such as biological nitrification, denitrification, alkalinity control and reactor operation management.

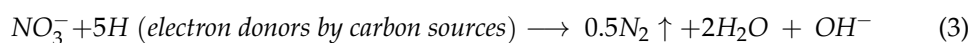
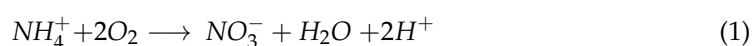
From a practical standpoint, undergraduate participants are expected to master the fundamental operating procedures and control strategies of SBR system. Additionally, they must become proficient in measuring key water quality parameters, including chemical oxygen demand (COD), ammonium ($\text{NH}_4^+\text{-N}$), total nitrogen (TN), pH, alkalinity, hardness, conductivity, and UV_{254} [30]. And they can analyze the performance of the reactor by

analyzing patterns in water quality changes in terms of theoretical understanding of the role of alkalinity in the biological denitrification process, and recognize the impact of low alkalinity conditions on nitrification, ammonia nitrogen removal efficiency and system operational stability.

This experiment utilizes solid-waste-recycled C@CaCO₃ as a water treatment material to help undergraduate participants clarify its mechanism for enhancing nitrification in low-alkalinity refractory wastewater. The carbon layer of C@CaCO₃ provides attachment sites for microorganisms and facilitates sludge floc formation. Its CaCO₃ component gradually releases alkalinity to satisfy the need for nitrification, thereby improving NH₄⁺-N removal efficiency [31]. The released Ca²⁺ also acts as nucleation centers and further strengthen the flocculation property of activated sludge [32]. C@CaCO₃ was provided by instructors, and students focused on reactor operation, water-quality monitoring, data analysis, and mechanism discussion rather than material synthesis or advanced characterization. This practice enables undergraduate participants to consolidate the theoretical principles of biological nitrogen removal, while upgrading their experimental operation, data analysis and engineering problem-solving abilities based on professional knowledge.

2.2. Experimental Principles

The nitrogen component of digestate liquid is predominantly present in the form of NH₄⁺-N. During the aerobic phase of the SBRs system, NH₄⁺-N is oxidized to nitrite (NO₂[−]) and nitrate (NO₃[−]) by ammonia oxidizing bacteria (AOB) and nitrite bacteria (NOB). This nitrification and its procedure leads to the consumption of alkalinity and may induce a reduction in pH; in theoretical terms, approximately 7.14 g of alkalinity calculated as CaCO₃ is expended for each gram of NH₄⁺-N that is oxidized, as shown in Equation (1) [23]. Under anaerobic conditions, nitrate is reduced to nitrogen gas by denitrifying bacteria, thereby removing nitrogen from the wastewater, namely denitrification [33]. During denitrification, the reduction of 1 mol of nitrate to nitrogen gas produces 1 mol of alkalinity; in other words, the reduction of 1 g of nitrate yields 3.57 g of alkalinity (expressed as CaCO₃), as shown in Equations (2) and (3) [23]. As a result, the denitrification process is only capable of recovering around half of the alkalinity demanded for nitrification operations, and the residual alkalinity must be provided from exogenous sources.



Aiming at low-alkalinity wastewater treatment, the activity of AOB and NOB decreases significantly, which impedes the nitrification process [21]. The elimination of NH₄⁺-N may be partially uncompleted and the activity of sludge may turn out to be unsteady.

C@CaCO₃ is used as a wastewater treatment material because it offers surface positions for microorganisms' attachment and growth, and its CaCO₃ can gradually release alkalinity to enhance nitrification reaction in low-alkalinity wastewater. Its calcium ions can facilitate the cross-linking of extracellular polymeric substance to accelerate formation of sludge flocs for improving sludge settling. Thus, the material was used to illustrate its functional roles rather than to emphasize material synthesis.

2.3. Experimental Procedures

2.3.1. Wastewater Characteristics

The experimental teaching case used low-alkalinity wastewater sourced from original and diluted effluents of the pilot-scale electrodialysis (ED) desalination cells [14]. Their

nitrogen removal performances was then compared. The experimental water quality parameters are listed in Table 1. The alkalinity of raw ED effluent was $1020.89 \pm 480.36 \text{ mg}\cdot\text{L}^{-1}$ as CaCO_3 , while that of diluted ED effluent was $65.70 \pm 14.92 \text{ mg}\cdot\text{L}^{-1}$ as CaCO_3 .

Table 1. Wastewater sourced from raw and diluted effluents of electrodialysis (ED) desalination cells.

Water Quality Parameter	Unit	Raw ED Effluent	Diluted ED Effluent
pH	-	8.02 ± 0.28	6.71 ± 0.13
conductivity	$\text{uS}\cdot\text{cm}^{-1}$	4.22 ± 0.24	-
alkalinity	$\text{mg}\cdot\text{L}^{-1}$	1020.89 ± 480.36	65.70 ± 14.92
hardness	$\text{mg}\cdot\text{L}^{-1}$	-	27.82 ± 9.45
$\text{NH}_4^+\text{-N}$	$\text{mg}\cdot\text{L}^{-1}$	285.88 ± 7.82	155.30 ± 7.50
TN	$\text{mg}\cdot\text{L}^{-1}$	307.25 ± 7.28	160.00 ± 13.80
COD	$\text{mg}\cdot\text{L}^{-1}$	881.12 ± 32.07	363.50 ± 15.40
TP	$\text{mg}\cdot\text{L}^{-1}$	2.23 ± 0.27	-
Cl^-	$\text{mg}\cdot\text{L}^{-1}$	769.33 ± 26.21	407.47 ± 42.11
UV_{254}	cm^{-1}	8.24 ± 0.73	3.85 ± 0.25

2.3.2. Apparatus

Four 1 L cylindrical plastic SBRs were used in the experiment and labeled R1 to R4. R1 was set as the control reactor for treating raw ED effluent with activated sludge (Figure 1). R2 treated raw ED effluent via activated sludge combined with C@CaCO_3 . R3 served as the reference reactor using activated sludge to treat diluted ED effluent, and R4 adopted activated sludge together with C@CaCO_3 for diluted effluent treatment. This experimental design was applied to explore the effects of wastewater alkalinity and functional material dosage.

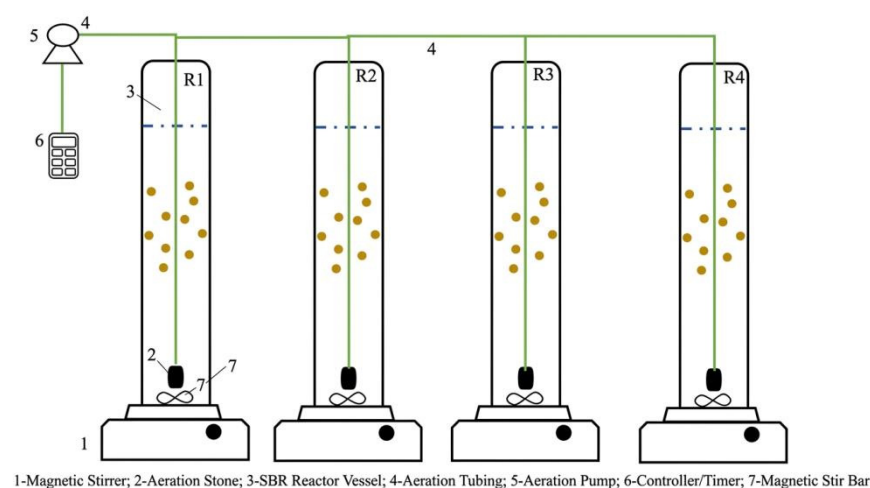


Figure 1. SBR experimental set-up (R1: activated sludge treated wastewater from electrodialysis dilution chamber effluent (CK); R2: C@CaCO_3 treated wastewater from electrodialysis dilution chamber effluent; R3: activated sludge treated wastewater from electrodialysis dilution chamber effluent diluted with deionized water (CK); R4: C@CaCO_3 treated wastewater from electrodialysis dilution chamber effluent diluted with deionized water).

The reactor was agitated with a magnetic stirrer at 500–600 rpm, and aeration was performed using a microporous aeration disk. A standard operation cycle was 60 min, consisting of 40 min of aerobic aeration phase and 20 min of aeration cessation with settling. The entire reactor system operated continuously for 23 h daily, with the remaining 1 h allocated to sludge settling, effluent discharge, and influent feeding. Based on the daily

batch replacement, the nominal HRT was approximately 1 day. The aeration pattern was therefore intermittent, with repeated aeration on/off cycling during reactor operation. The active sludge inoculum had a mixed liquor suspended solids (MLSS) concentration of approximately $2280 \text{ mg}\cdot\text{L}^{-1}$ and a mixed liquor volatile suspended solids (MLVSS) concentration of roughly $1130 \text{ mg}\cdot\text{L}^{-1}$.

The C@CaCO₃ particles used in this study were synthesized from biogas slurry pre-treatment by-products according to the method detailed in our previous study [27]. Briefly, C@CaCO₃ is a carbon-based composite carrier that supports biofilm attachment and gradually releases alkalinity during nitrification. To accommodate the limited class hours and avoid safety hazards associated with high-temperature thermal treatment, the material was pre-fabricated by lab instructors rather than prepared by students in situ. Thus, it was provided by the instructors for the undergraduate experiment; students handled dosing, SBR operation, water-quality monitoring, data processing, and mechanism interpretation, but not material synthesis.

For the experimental group using raw ED permeate, 800 mL of raw ED permeate and 200 mL activated sludge were added to bioreactors R1 and R2, respectively. At the start of the experiment, a single dosage of C@CaCO₃ was added to R2 at $3 \text{ g}\cdot\text{L}^{-1}$. For the diluted ED effluent experiment, R3 served as the control group containing only activated sludge, while C@CaCO₃ was sequentially dosed into R4 at 0.250, 0.625, and $1.000 \text{ g}\cdot\text{L}^{-1}$ to investigate the effect of dosage on system performance. With daily effluent exchange (nominal HRT of $\sim 1 \text{ d}$), most of the dissolved alkalinity and Ca²⁺ from previous runs was flushed out. Furthermore, the C@CaCO₃ dosage was increased stepwise; carryover from the preceding lower dose was unlikely to confound the results of subsequent higher runs. Throughout the entire operation period, researchers recorded pH, conductivity, mixing conditions, sludge settling characteristics, and effluent appearance at predetermined intervals. pH was monitored but not externally adjusted, allowing the system to reflect the buffering limitations of low-alkalinity digestate wastewater.

2.3.3. Test Methods

COD was determined via the potassium dichromate method. NH₄⁺-N was measured by Nessler reagent spectrophotometry, and TN was tested using alkaline potassium persulfate digestion coupled with UV spectrophotometry. UV₂₅₄ was performed by UV spectrophotometry. Alkalinity was carried out via acid-base titration with methyl orange as the indicator, while hardness was implemented by EDTA titration. pH and conductivity was detected using standard instruments. Depending on the specific requirements of the experimental course, NO₂[−], NO₃[−] and dissolved oxygen (DO) were also analyzed to better monitor the nitrification and denitrification processes.

Experimental data were processed by comparing influent and effluent concentrations, calculating pollutant removal efficiencies and analyzing parameter variation trends during reactor operation. The removal efficiencies of COD, NH₄⁺-N and TN were calculated, and the temporal variations in pH, alkalinity, NH₄⁺-N, TN, and COD were plotted. Results of R1–R2 and R3–R4 were further compared to assess the performance of C@CaCO₃ composite.

Scanning electron microscopy (SEM) was used to observe the micromorphology of C@CaCO₃, activated sludge flocs and attached microorganisms. High-throughput 16S rDNA sequencing with primers 338F and 806R was applied to characterize the microbial community structure. SEM and microbial sequencing were used to characterize carrier morphology, sludge flocs, microbial attachment, and nitrification-related mechanisms. In undergraduate teaching, these advanced procedures are performed by trained instructors, technical staff, or qualified service providers. Students observe SEM imaging under supervision and analyze representative SEM images and sequencing datasets supplied

by instructors, with emphasis on data interpretation, mechanism discussion and report writing rather than independent instrument operation.

2.3.4. Experimental Safety Precautions

Effluent from anaerobic digesters, activated sludge and biofilm-attached materials must be treated as bioactive samples. All participants must wear laboratory coats, disposable gloves and protective goggles throughout the experiment and avoid direct contact with waste liquid and sludge sediment. Used wastewater, residual sludge and biofilm-loaded C@CaCO₃ should be centrally collected and disposed of in compliance with laboratory biosafety and waste discharge standards.

The potassium dichromate method for COD determination uses toxic and corrosive reagents (potassium dichromate, concentrated acids), with chromium-containing waste collected separately. Notably, Nessler's reagent contains mercury (Hg), posing severe acute and bioaccumulative toxicity risks. It must be handled with extreme care under the direct supervision of an instructor, preferably within a certified fume hood; all mercury-containing liquid waste must be strictly segregated, captured in dedicated heavy-metal waste containers, and disposed of through authorized hazardous waste channels. For C@CaCO₃ preparation teaching, all high-temperature operations are restricted to qualified laboratory staff, conducted in well-ventilated areas with heat-resistant gloves and face protection. SEM sample preparation and microbial sample handling also require supervision. Undergraduates are strictly prohibited from direct contact with untreated biological residues. Electrical equipment (aerators, magnetic stirrers, analytical instruments) must be kept away from splashed liquids to prevent short circuits. Adherence to these safety rules ensures safe, orderly experimental conduct.

2.4. Teaching Implementation and Assessment

This teaching case is intended for senior undergraduates majoring in Water Supply and Drainage Science and Engineering or Environmental Science and Engineering. Students are expected to have completed courses in Water Quality Engineering, Environmental Microbiology, Environmental Chemistry, and Water and Wastewater Analysis, and to possess basic laboratory skills, including pH measurement, spectrophotometric analysis, titration, and safe handling of wastewater and activated sludge samples.

To accommodate different student backgrounds and task complexity, a modular design is adopted. The basic 4–6 h module covers wastewater characterization, SBR principles, alkalinity calculations, water-quality monitoring, and pollutant-removal analysis. An optional advanced 8–12 h module further includes C@CaCO₃ dosage design, reactor-performance comparison, SEM interpretation, and microbial community analysis. C@CaCO₃ was prepared and provided by the instructors before class, so students focused on process operation and engineering analysis rather than material synthesis.

Students should work in groups of 4–6, with each group assigned a specific reactor or data task. Pre-lab preparation should cover nitrification/denitrification, alkalinity, SBR operation, and carrier materials, ideally verified with a short quiz. During the lab, students conduct water quality tests, track reactor parameters, calculate removal efficiencies (COD, NH₄⁺-N, TN), and compare control versus C@CaCO₃-enhanced systems. Advanced techniques such as SEM and 16S rDNA sequencing are conducted under instructor supervision. Trained instructors, technical staff, or qualified service providers perform sample preparation, instrument operation, and sequencing procedures, while students observe SEM imaging, record key features, and analyze representative images and sequencing datasets. This approach maintains safety and feasibility while exposing students to advanced methods for mechanism analysis.

Assessment encompasses pre-lab preparation, lab performance, data processing, group discussion, and a final report (Table 2). The report must detail wastewater characteristics, reactor operations, removal efficiencies, alkalinity demands, SEM/microbial findings, and the engineering implications of C@CaCO₃-enhanced nitrification. This approach evaluates both practical skills and the ability to apply theory to engineering problems. Finally, the modular design allows flexible teaching. Basic courses can focus on water quality and SBR operations, while advanced courses can include material characterization, microbiology, and resource recovery.

Table 2. Suggested teaching implementation and assessment plan.

Teaching Stage	Main Contents	Participant Tasks	Assessment Focus
Pre-lab preparation	Digestate wastewater characteristics; Nitrification and denitrification; Alkalinity demand; SBR operation	Literature reading; Pre-lab quiz; Calculation of theoretical alkalinity demand	Understanding of basic theory and engineering background
In-lab operation	SBR setup; C@CaCO ₃ addition; Water-quality monitoring	Measure COD, NH ₄ ⁺ -N, TN, pH, alkalinity; conductivity and other indicators; Record reactor operation	Experimental operation and data reliability
Data analysis	Removal efficiency calculation; Comparison between control and C@CaCO ₃ -enhanced systems	Plot variation trends; Analyze dosage–response relationships; Evaluate nitrification performance	Quantitative analysis and process evaluation
Mechanism discussion	C@CaCO ₃ alkalinity release; Microbial attachment; SEM and microbial community interpretation	Interpret SEM images and microbial data provided by instructors; Explain possible enhancement mechanisms	Mechanistic reasoning and interdisciplinary integration
Final report and discussion	Engineering significance limitations, and optimization strategies	Submit laboratory report; Present group findings; Propose process improvement measures	Engineering judgment and problem-solving ability

3. Results and Discussion

3.1. Effects of C@CaCO₃ on COD, and Biological Nitrification

Figure 2 shows that the influence of C@CaCO₃ on the elimination of COD, NH₄⁺-N and TN. The results indicated that the addition of C@CaCO₃ clearly increased the removal of COD and NH₄⁺-N, particularly in low-alkalinity wastewater sourced from diluted effluents of electrodialysis (ED) desalination cells (Table 1). For the raw effluents of ED permeate, mean COD was at 881.12 ± 32.07 mg·L^{−1} (Figure 2a). Over the period of days 2 to 11, reactor R2 was added into 3 g·L^{−1} C@CaCO₃ and obtained mean removal efficiency of COD at 22.04 ± 4.19%, maximum value at 31.59 ± 0.18% on the 5th day (Figure 2a). But the activated sludge in reactor R1 exhibited an average removal efficiency of COD at 19.53 ± 3.77%, with a highest value only at 27.18 ± 0.35% (Figure 2a). Hence, C@CaCO₃ strengthened COD elimination by roughly 2.51% under raw effluents of ED permeate.

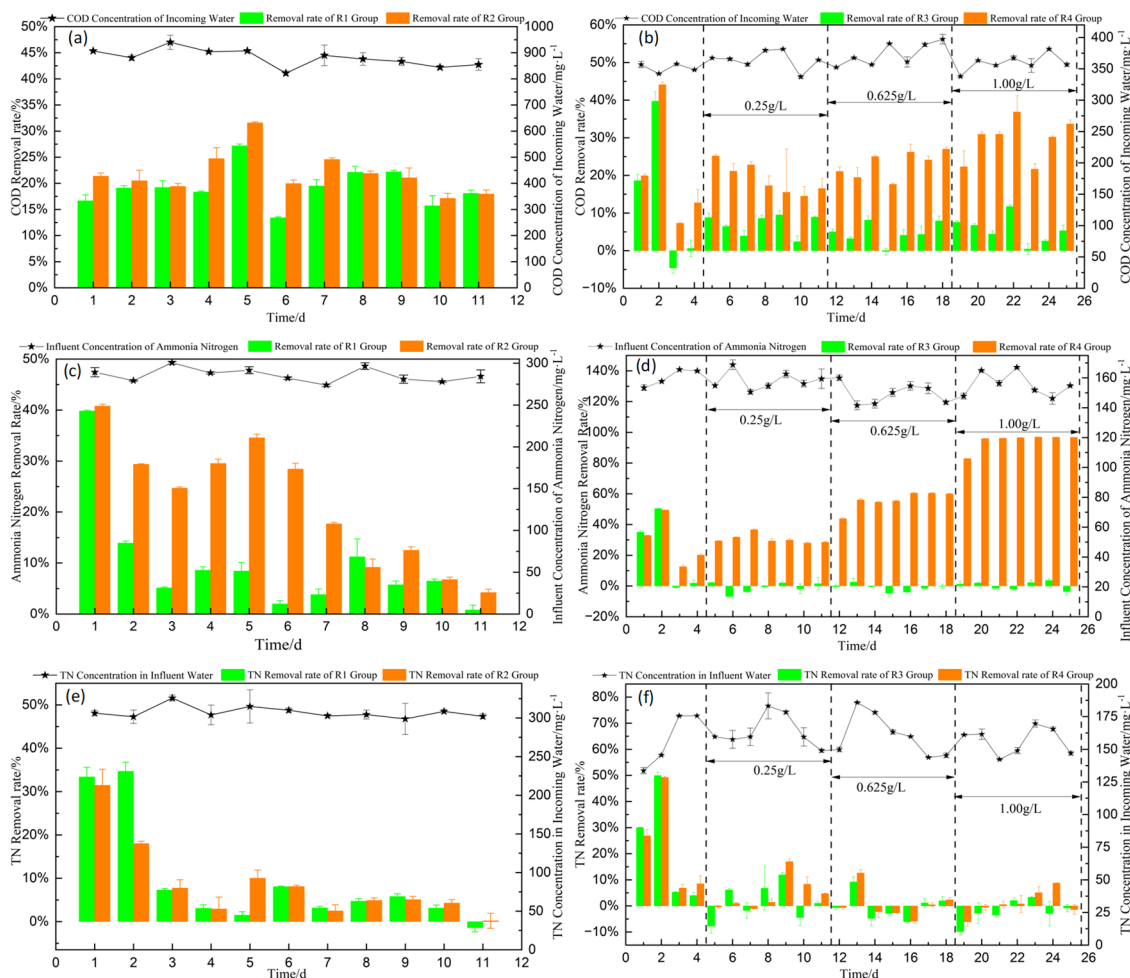


Figure 2. COD, $\text{NH}_4^+\text{-N}$ and TN degradation of wastewater from electro dialysis dilution chamber by nano C@CaCO_3 : (a) comparison of COD of wastewater from electro dialysis dilution room treated in R1 and R2; (b) comparison of COD of wastewater from electro dialysis dilution chamber effluent diluted with deionized water treated in R3 and R4; $\text{NH}_4^+\text{-N}$ degradation of wastewater from electro dialysis dilution chamber by nano C@CaCO_3 ; (c) comparison of $\text{NH}_4^+\text{-N}$ of wastewater from electro dialysis dilution room treated in R1 and R2; (d) comparison of $\text{NH}_4^+\text{-N}$ of wastewater from electro dialysis dilution chamber effluent diluted with deionized water treated in R3 and R4; TN degradation of wastewater from electro dialysis dilution chamber by nano C@CaCO_3 ; (e) comparison of TN of wastewater from electro dialysis dilution room treated in R1 and R2; (f) comparison of TN of wastewater from electro dialysis dilution chamber effluent diluted with deionized water treated in R3 and R4.

Within the diluted effluent of ED permeate, the removal efficiency of COD by C@CaCO_3 was significantly strengthened and enhanced (Figure 2b). In reactor R3 with only activated sludge, the mean COD removal rate was at $5.70 \pm 3.05\%$, with the maximum value merely at 11.74% (Figure 2b). In comparison, reactor R4 was added into C@CaCO_3 , and its COD removal rate obtained an obvious increase. When the addition amounts of C@CaCO_3 were at 0.25, 0.625, and $1.00 \text{ g}\cdot\text{L}^{-1}$, the average removal efficiency of COD were at $18.98 \pm 3.76\%$, $22.92 \pm 3.31\%$, and $29.51 \pm 5.20\%$, respectively (Figure 2b). These results indicated that the addition of C@CaCO_3 played a role in COD removal in low-alkalinity wastewater.

For the raw effluent of ED permeate, the mean incoming $\text{NH}_4^+\text{-N}$ content reached $285.88 \pm 7.82 \text{ mg}\cdot\text{L}^{-1}$ (Figure 2c). An average removal efficiency of $\text{NH}_4^+\text{-N}$ was at merely $5.80 \pm 3.14\%$ in control group R1, while the removal rate in R2 supplemented with

C@CaCO₃ was at $18.62 \pm 10.45\%$, with the maximum value of $34.56 \pm 0.71\%$ on the 5th day (Figure 2c). The removal rate of NH₄⁺-N was higher by 12.85% in R2 than in the control group R1. For the diluted effluent of ED, the mean NH₄⁺-N concentration was at $155.30 \pm 7.50 \text{ mg}\cdot\text{L}^{-1}$ (Figure 2d). R3 displayed an average NH₄⁺-N removal efficiency of $-0.64 \pm 2.71\%$, signifying that activated sludge in isolation was unable to sustain efficient nitrification under low alkalinity circumstances (Figure 2d). Notably, the diluted ED wastewater in R3 had an alkalinity of only $65.70 \pm 14.92 \text{ mg}\cdot\text{L}^{-1}$ as CaCO₃ (Table 1). But NH₄⁺-N elimination performance in R4 with C@CaCO₃ dosage increment reached $30.48 \pm 2.74\%$, $50.82 \pm 5.14\%$, and $94.55 \pm 4.78\%$, respectively. These results demonstrate that the improvement of removal efficiency of NH₄⁺-N is tightly associated with C@CaCO₃ due to its alkalinity-releasing capability and supporting carrier role [28,32].

The average influent TN concentration in reactors R1 and R2 was $307.25 \pm 7.28 \text{ mg}\cdot\text{L}^{-1}$ (Figure 2e). During the stable operation phase (days 3–11), the control group CK (R1, activated sludge only) achieved an average TN removal of only $3.92 \pm 2.77\%$, with maximum value of $8.05 \pm 0.15\%$ on day 6. For R2 with addition of $3 \text{ g}\cdot\text{L}^{-1}$ C@CaCO₃, the mean TN removal was $5.07 \pm 2.92\%$ and the maximum value at $10.02 \pm 1.88\%$. In the diluted wastewater runs (R3 and R4, influent TN of $160 \pm 13.8 \text{ mg}\cdot\text{L}^{-1}$) (Table 1), the TN removal efficiency was essentially negligible, and the average removal rate of TN was $-0.24 \pm 5.38\%$ for R3 and $1.99 \pm 5.38\%$ for R4 (Figure 2f). These results indicate that while C@CaCO₃ supplemented alkalinity in R4 and significantly enhanced biological nitrification (as evidenced by high NH₄⁺-N removal), it had negligible effects on TN removal.

The negligible TN removal observed in this study was mainly due to carbon limitation, dissolved oxygen (DO) carryover, and unfavorable reactor cycling. No external carbon source was supplied during SBR operation. Although the influent contained COD, it was mainly refractory and could not provide enough readily biodegradable electron donors for denitrification [34]. In addition, the SBR cycle consisted of 40 min of aerobic aeration followed by 20 min of settling, without an anoxic mixing phase. Residual DO carried over into the settling period, creating a micro-aerobic environment that suppressed denitrifying activity. Under these conditions, the autotrophic nitrifiers supported by C@CaCO₃ successfully converted ammonia to nitrate, which subsequently accumulated in the system. Crucially, while C@CaCO₃ releases alkalinity and offers a physical carrier for biofilm growth, it cannot supplement organic electrons for heterotrophic pathways. This metabolic mismatch, coupled with minor biomass decay and cell lysis, explains the flat or slightly negative TN removal rates. These results show that C@CaCO₃ primarily promoted ammonium oxidation, whereas TN removal remained limited due to insufficient denitrification.

Pedagogically, the stark contrast between efficient ammonium oxidation and negligible TN removal serves as an excellent troubleshooting and active-learning exercise for undergraduate participants. Traditional verification experiments often present wastewater treatment as a highly idealized process with synchronous pollutant removal, shielding students from real-world engineering failure. This discrepancy challenges participants to perform stoichiometric calculations to quantify the systemic carbon deficit using theoretical C:N ratio. Such calculations force learners to distinguish between autotrophic nitrification (which demands DO and alkalinity) and heterotrophic denitrification (which requires strictly anoxic conditions and organic electrons). To resolve this problem, students must assume the role of process engineers and propose concrete, evidence-based optimization strategies. These proposals include redesigning SBR cycle configurations to incorporate dedicated anoxic mixing periods and calculating supplemental dosages of external carbon sources such as sodium acetate. Integrating these real-world data deviations into lab coursework effectively bridges fundamental chemistry, biological kinetics, and SBR process control. Ultimately, this approach transitions students from passive protocol-following

to active engineering diagnostics, cultivating critical problem-solving skills necessary for professional practice.

3.2. Microbial Morphology and Community Analysis

Figure 3 shows the SEM morphology of C@CaCO₃ and the digested effluent from the ED permeate before and after treatment with activated sludge. Prior to the reaction, the surface of C@CaCO₃ consisted of layered nanosheets, forming a petal-like porous structure with a large specific surface area, which provides abundant sites for microbial attachment (Figure 3a). Following nanoscale modification, the increase in surface defects and unsaturated bonds further facilitates the formation of a dense, ordered particle structure. After the reaction, a large number of microorganisms were observed uniformly attached to the surface of C@CaCO₃, forming spherical aggregates (Figure 3b), indicating that its porous structure is conducive to microbial enrichment and growth. In contrast, prior to the reaction, the activated sludge exhibited a rough, loose, floc-like surface (Figure 3c); although it formed clump-like aggregates after the reaction, it had fewer pores overall and a denser structure (Figure 3d), resulting in a lower enrichment efficiency compared to C@CaCO₃. The results indicate that the porous surface of nanoscale C@CaCO₃ facilitates microbial attachment and enrichment and can enhance mass transfer efficiency and microbial metabolic activity within the system.

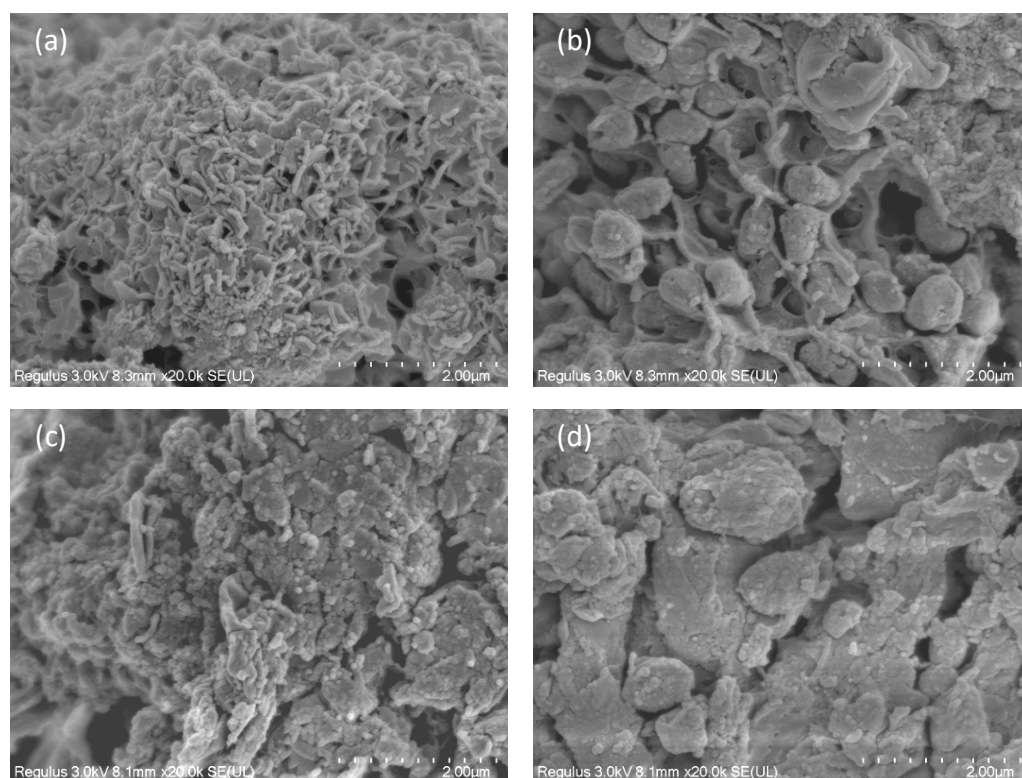


Figure 3. Electron microscope scans picture from nano C@CaCO₃ and activated sludge for treating wastewater from electrodialysis dilution chamber effluent diluted with deionized water. (a) Nano C@CaCO₃ pre-reaction (20,000×); (b) nano C@CaCO₃ post-reaction (20,000×); (c) activated sludge pre-reaction (20,000×); (d) activated sludge post-reaction (20,000×).

Table 3 demonstrates the microbial community sequencing results for the raw inoculated sludge group (N), blank control group (CK), and C@CaCO₃ addition group. Coverage for all three groups exceeded 0.99, indicating that the sequencing depth was sufficient to fully reflect the microbial community composition of the samples. Community abundance indices (Sobs, Ace, and Chao) showed that the CK group had the highest values, followed

by the C@CaCO₃ group, with the N group being the lowest. The Shannon and Simpson indices exhibited the same trend, suggesting that both community abundance and diversity in the CK and C@CaCO₃ groups were higher than in the raw sludge, with a more distinct increase in the CK group. These results indicate that both reactor operation and C@CaCO₃ addition can promote the enrichment and diversification of the sludge microbial community to a certain extent.

Table 3. Microbial Alpha diversity index statistics.

Sample No.	Richness Index			Diversity Index		Coverage Index
	Sobs	Ace	Chao	Shannon	Simpson	
N	256 ± 4	258 ± 3	257 ± 3	3.74 ± 0.40	0.09 ± 0.06	0.9992 ± 0.0001
CK	403 ± 27	405 ± 26	404 ± 26	4.87 ± 0.21	0.03 ± 0.01	0.9993 ± 0.0002
C@CaCO ₃	346 ± 4	347 ± 3	346 ± 3	4.50 ± 0.21	0.05 ± 0.02	0.9996 ± 0.0004

Figure 4a presents the microbial community composition at the phylum level for the raw inoculated sludge (N), blank control (CK), and C@CaCO₃-added group. A total of 28 bacterial phyla were detected across the three groups, 12 of which were dominant (relative abundance > 0.01), mainly including *Proteobacteria*, *Chloroflexi*, *Patescibacteria*, *Bacteroidota*, *Actinobacteriota*, and *Nitrospirota*. *Proteobacteria* was the dominant phylum, with relative abundances of 12.81%, 32.01%, and 43.03% in groups N, CK, and C@CaCO₃, respectively. Previous studies have shown that *Proteobacteria* is a common dominant phylum in biological wastewater treatment systems. Many functional microorganisms involved in organic matter degradation, denitrification, and phosphorus removal, such as AOB, NOB, denitrifying bacteria, and polyphosphate accumulating organisms (PAOs), belong to this phylum [35,36]. The relative abundances of *Chloroflexi* in the three groups were 15.25%, 15.56%, and 31.71%, respectively. This phylum can degrade complex organic compounds (e.g., polysaccharides and proteins) and plays a structural supporting role in biofilm or granular sludge formation. For *Nitrospirota*, its relative abundances in groups N, CK, and C@CaCO₃ were 0.037%, 0.039%, and 7.09%, respectively. Some members of this phylum are NOB and can oxidize NO₂[−] to NO₃[−].

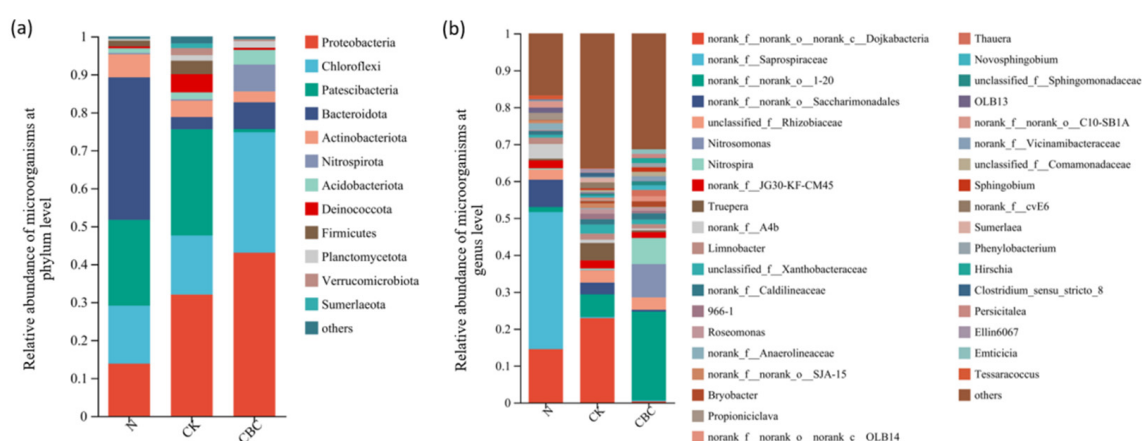


Figure 4. (a) Structural composition of microbial communities at phylum level; (b) structural composition of microbial communities at genus level.

Figure 4b further illustrates the distribution of dominant bacterial genera across the three sample groups. The predominant genera in the N group were *Saprospiraceae* (37.10%), *Dojkabacteria* (14.49%) and *Saccharimonadales* (7.43%). The CK group was dominated by *Dojkabacteria* (22.90%), 1–20 (6.12%) and *Truepera* (4.71%), whilst the C@CaCO₃ group

was featured by 1–20 (24.06%), *Nitrosomonas* (9.01%) and *Nitrospira* (7.09%). Compared with the original sludge, the dominant bacterial genera in both the CK and C@CaCO₃ groups underwent significant succession. In the CK group, Dojka bacteria became the most dominant genus, whilst in the C@CaCO₃ group, 1–20 exhibited the highest relative abundance. 1–20 belongs to the *Anaerolineae* class within the *Chloroflexi* phylum. Previous studies have shown that an increase in the relative abundance of *Anaerolineae* is beneficial for nitrate removal. The addition of C@CaCO₃ promoted the enrichment of 1–20, which may be a key factor in its improved COD and TN removal efficiency. Furthermore, in the C@CaCO₃ group, the relative abundances of *Nitrosomonas* and *Nitrospira* increased to 9.01% and 7.09%, respectively. These genera are closely associated with the ammonia oxidation and nitrite oxidation processes, which helps to enhance the removal of NH₄⁺-N. Overall, C@CaCO₃ promoted attached-growth and nitrifying taxa, particularly *Nitrosomonas* and *Nitrospira*, explaining the enhanced NH₄⁺-N removal. However, TN removal remained limited because denitrification was constrained by carbon deficiency and the lack of a dedicated anoxic phase.

Mechanistically, the petal-like porous structure and carbon coating of C@CaCO₃ provided excellent colonization sites, favoring the retention of slow-growing nitrifiers (*Nitrosomonas* and *Nitrospira*) and biofilm-associated *Chloroflexi* (*Anaerolineae*). Furthermore, gradual CaCO₃ dissolution supplied localized alkalinity, creating a buffered pH microenvironment that relieved nitrification inhibition under low-alkalinity conditions. Concurrently, released Ca²⁺ promoted extracellular polymeric substance (EPS) cross-linking, stabilizing sludge flocs and attached biomass. These carrier-associated microhabitats promoted functional taxa enrichment, directly explaining the improved NH₄⁺-N and COD removal. However, complete TN removal remained constrained by carbon deficiency and the lack of a dedicated anoxic phase.

3.3. Alkaline Requirement and C@CaCO₃ Addition

The correlation between C@CaCO₃ dosage and NH₄⁺-N removal is a critical instructional link between environmental stoichiometry and reactor operation. Nitrification has a specific alkalinity requirement. Approximately 7.14 mg of alkalinity (as CaCO₃) is needed to oxidize 1 mg of NH₄⁺-N [23]. When the initial alkalinity of wastewater is insufficient, nitrification depletes the system's buffering capacity, reduces pH, and inhibits nitrifying microorganisms.

The CaCO₃ component in C@CaCO₃ acts as a slow-release alkalinity source, gradually replenishing alkalinity during the reaction and improving the microenvironment for nitrification. In instructional practice, this correlation can assist educators in roughly calculating the theoretical C@CaCO₃ dosage based on influent NH₄⁺-N concentration, target removal efficacy, initial wastewater alkalinity, and the available CaCO₃ component of the used medium. The basic calculation formula is as follows:

$$A_{nit} = 7.14 \times \Delta NH_4^+ - N \quad (4)$$

$$A_{denit} = 3.57 \times \Delta NO_x^- - N \quad (5)$$

$$A_{add} = A_{nit} - A_{denit} - A_{initial} \quad (6)$$

where A_{nit} is the alkalinity requirement for nitrification, A_{denit} is the alkalinity recovered via denitrification reactions, $A_{initial}$ is the initial alkalinity of the influent wastewater, and A_{add} is the theoretical demand for external alkalinity supply. All measured values are expressed in CaCO₃ units. Using this calculation method, learners can understand that the dosage of substances should not be determined by empirical judgment, but based on pollutant load, reaction stoichiometric relationships, and process operation targets.

During SBR trials with the diluted ED effluent, nitrification in R4 rebounded sharply with C@CaCO₃ supplementation, with the NH₄⁺-N removal efficiency from 30.48 ± 2.74% to 94.55 ± 4.78% as the dosage increased from 0.25 to 1.00 g·L⁻¹ (Figure 2d). Despite this sequential dosing in the same reactor, carryover effects were likely minimal. Daily batch replacement (nominal HRT of ~1 d) consistently flushed out dissolved Ca²⁺ and alkalinity from prior runs. Because the dosage was increased stepwise, residual chemicals could not artificially inflate subsequent performance trends. Furthermore, the soluble carbon skeleton served primarily as an inert physical scaffold for biofilm growth rather than a soluble reactant, exerting little direct chemical influence on the bulk water quality. Still, future studies should utilize parallel reactors or mandate distinct washout periods to establish a more rigorous dose–response profile. COD removal also showed an upward trend with increasing C@CaCO₃ dosage, indicating that its role is not limited to alkalinity adjustment but is also be associated with microbial adhesion, and local mass transfer.

Therefore, the appropriate dosage should balance the alkalinity requirement for nitrification, microbial adhesion, effluent quality, and operational costs. This section trains learners to convert stoichiometric knowledge into engineering regulation strategies, reflecting the connection between theoretical calculation and process implementation in *Water Quality Engineering*.

3.4. Cultivation of Comprehensive Knowledge Application Ability

This experiment uses digestate effluent treatment as its engineering context, organically integrating water quality analysis, material resource recovery, SBR operation control, and microbial mechanism analysis to guide undergraduate participants in comparing pollutant removal efficiencies and analyzing material dosages. By measuring parameters including COD, NH₄⁺-N, TN, pH, and alkalinity, undergraduate participants compare the treatment performance of different systems. Combined with raw effluent characteristics, reactor operation results, SEM morphology, and microbial community changes, undergraduate participants analyze how C@CaCO₃ enhances denitrification, gradually developing an analytical approach from phenomenon observation to engineering judgment.

The experimental content covers alkalinity control, biological denitrification, carrier material roles, and functional microbial community succession. It spans interdisciplinary fields, including environmental chemistry, materials science, microbiology, and water treatment engineering, helping undergraduate participants establish systematic connections between different knowledge areas and improve their ability to comprehensively analyze and solve complex engineering problems [37]. C@CaCO₃, prepared from digestate pretreatment by-products and used in subsequent biological treatment, embodies the principles of resource recycling and low-carbon management. It also strengthens undergraduate participants' awareness of engineering practice and understanding of resource recovery.

3.5. Advantages and Limitations of Experimental Teaching Case

This experimental case aligns closely with *Water Quality Engineering* by SBR operation, biological nitrification and denitrification, and microbial community shifts. Rather than using synthetic wastewater, the design introduces an authentic engineering challenge of high-ammonia and low-alkalinity digestate treatment. Using waste-derived C@CaCO₃ produced from digestate pretreatment, undergraduate participants can conduct stoichiometry calculations, assess dosage–response relationships, and analyze nitrogen transformation, bridging the gap between classroom theory and practical process engineering.

A major limitation of this study is the lack of direct undergraduate learning-outcome data. The current results were obtained from pilot-scale engineering trials; therefore, this work should be regarded as the development of an inquiry-based laboratory case based

on real engineering data, rather than a completed classroom intervention with systematic pedagogical evaluation. A carbon-only control was not included in the original trial. Future work will compare carbon-only, CaCO_3 -only, and C@CaCO_3 treatments to distinguish the roles of the carbon skeleton, alkalinity release, and their combined effect. The present case demonstrates enhanced nitrification, not complete nitrogen removal, because effective denitrification was not achieved under the current SBR conditions. At this stage, direct evidence from pre/post-tests, student questionnaires, and long-term learning-outcome assessment is not yet available. In future teaching practice, this module will be implemented in *Water Quality Engineering* laboratory courses, and its effectiveness will be evaluated using pre/post-tests, operational-performance rubrics, student feedback, group presentations, and final laboratory reports. These data will be used to quantitatively assess student learning gains and further optimize the experimental module.

Given the complexity of SBR operation, SEM characterization, and microbial sequencing, full implementation within a single standard lab session is challenging. We therefore propose a modular approach where basic labs can cover SBR operation and water chemistry assays, while advanced or research-oriented tracks can interpret SEM images and sequencing datasets provided by instructors. For future classroom implementation, teaching effectiveness should be evaluated via a structured framework including pre-lab quizzes on theoretical concepts, in-lab operational assessments, and post-lab reports or presentations evaluating the students' ability to interpret water quality changes and propose process-optimization strategies.

As shown in Table 4, this case differs from conventional verification-type labs by requiring undergraduates to analyze multi-dimensional datasets rather than passively follow protocols. Notably, the experimental results serve as an excellent educational tool—for instance, where $\text{NH}_4^+\text{-N}$ was nearly eliminated but TN removal remained negligible due to carbon limitation. This discrepancy prompts undergraduate participants to distinguish nitrification from complete denitrification, emphasizing the necessity of process optimization (e.g., carbon supplementation and anoxic-phase control) in real-world wastewater engineering.

Table 4. Comparison of the present teaching case with conventional and reported wastewater-treatment laboratory teaching cases.

Teaching Case Type	Engineering Context	Wastewater Matrix	Main Experimental Process	Resource Recovery Concept	Material-Assisted Treatment	Microbial Mechanism Analysis	Main Learning Outcomes
Conventional verification-type wastewater experiments [1,35]	Limited; Mainly textbook-based	Simulated or simplified wastewater	Fixed water-quality tests or standard operation procedures	Rarely included	Not involved	Generally absent	Basic experimental operation and verification of known principles
Biological nitrogen-removal experiments using synthetic wastewater	Moderate; Focused on process principles	Synthetic ammonium or nitrate wastewater	Activated sludge batch test or SBR operation	Not emphasized	Usually not involved	Limited or absent	Understanding nitrification, and nitrogen transformation
SBR-based wastewater treatment experiments [21,23]	Related to municipal wastewater treatment	Domestic wastewater or simulated wastewater	SBR operation and pollutant-removal evaluation	Rarely included	Not involved	Usually limited to process performance	Reactor operation, process control, and removal-efficiency calculation

Table 4. Cont.

Teaching Case Type	Engineering Context	Wastewater Matrix	Main Experimental Process	Resource Recovery Concept	Material-Assisted Treatment	Microbial Mechanism Analysis	Main Learning Outcomes
Material-based wastewater treatment experiments [11,19]	Related to pollutant removal by functional materials	Dye, heavy-metal or nutrient-containing wastewater	Adsorption, coagulation, or catalytic treatment	Sometimes involved when waste-derived materials are used	Included, but mainly physicochemical	Generally not included	Material characterization and pollutant-removal performance evaluation
Ecological or constructed-wetland experiments [38]	Related to decentralized wastewater treatment	Domestic or agricultural wastewater	Plant-substrate-microbe combined treatment	Sometimes involved	Substrate-assisted treatment may be included	Often treated as a black-box process	Understanding ecological treatment and system operation
Present study	Real digestate wastewater treatment and resource utilization	Raw and diluted electrodialysis diluted with high $\text{NH}_4^+\text{-N}$, low alkalinity, refractory organics, and high salinity	SBR-activated sludge system enhanced by C@CaCO_3	Included; C@CaCO_3 is derived from digestate-treatment by-products	Included; C@CaCO_3 provides slow-release alkalinity microbial attachment sites	Included; SEM and 16S rDNA sequencing are used to explain microbial adhesion and functional community enrichment	Integration of water-quality analysis, alkalinity calculation, reactor operation, material function, microbial mechanism, and engineering judgment

4. Conclusions

This study has developed a comprehensive experimental teaching case based on C@CaCO_3 for enhanced biological nitrification removal from digestate effluent, while aiming to address the lack of engineering context, fragmented content and insufficient comprehensive skill training in the practical teaching of *Water Quality Engineering II*. Using raw and diluted electrodialysis permeate as the target wastewater, this case integrates water quality analysis, SBR operation, material function, and microbial community analysis, forming a complete experimental chain from problem identification to mechanism interpretation. The results showed that the addition of C@CaCO_3 markedly enhanced the nitrification under low alkalinity conditions, with $\text{NH}_4^+\text{-N}$ removal increasing from less than 6% in the control system to as high as 94.55%, while TN removal remained negligible because of carbon limitation and the lack of an effective anoxic phase. Mechanistically, C@CaCO_3 improved the treatment performance by supplying slow-release alkalinity, promoting microbial attachment and sludge floc formation, and enriching functional microorganisms related to biological ammonium removal. By integrating resource recovery, wastewater treatment, and engineering practice, this teaching case provides a useful framework for helping undergraduate participants to develop the ability to analyze and solve complex engineering problems.

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Conflicts of Interest: The authors declare no conflicts of interest.

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