Engineering Application Research on the Integrated Purification Treatment of High-Concentration Aquaculture Tailwater from Greenhouse Soft-Shelled Turtles Utilizing the A^2/O Process

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Abstract Between July and August 2023, juvenile soft-shelled turtles were stocked at a density of 60 individuals/m^2 in the Yangcun Soft-Shelled Turtle Breeding Base operated by Anhui Xijia Agricultural Development Co., Ltd., amounting to a total of 3.3 million juveniles. A specialized powdered feed containing 42% – 46% protein was utilized, which was processed into soft pellets for feeding. The pellet size was adjusted in accordance with the turtles' growth stages. By May to June 2024, the annual commercial turtle yield reached 1.3 million kg, accompanied by an annual tailwater discharge exceeding 50 million m^3. Analysis of the tailwater revealed concentrations of ammonia nitrogen (NH_3-N) at 30.9 mg/L, nitrite nitrogen (NO_2^--N) at 7.3 mg/L, suspended solids (SS) at 363.7 mg/L, and chemical oxygen demand (COD) at 151.7 mg/L. This study investigated and optimized the design of an integrated purification system for soft-shelled turtle aquaculture tailwater, based on the municipal wastewater anaerobic-anoxic-oxic (A^2/O) process. The system combined chemical flocculation, multi-stage biological treatment, the A^2/O process, and advanced filtration using zeolite and activated carbon. The engineering application demonstrated that the purification system occupied an area of 200 m^2 and treated 200 t of tailwater per day. The effluent concentrations were as follows: TN at (4.9 ± 0.7) mg/L, TP at (0.26 ± 0.16) mg/L, SS at (26 ± 5.3) mg/L, and COD at (11.4 ± 2.1) mg/L. The corresponding pollutant removal efficiencies were 98.1%, 99.8%, 91.2%, and 92.2%, respectively. The effluent quality complied with and surpassed the Class II limits outlined in the *Discharge Standard of Water Pollutants for Aquaculture* in Anhui Province. The treatment cost of water amounted to 2.08 yuan/t, and annual reductions of 8.89 t of TN and 3.9 t of TP were achieved. The system demonstrated robust resistance to load fluctuations, and its modular design facilitated rapid installation and efficient operation within aquaculture facilities.

Key words Greenhouse soft-shelled turtle; Aquaculture tailwater; Integrated purification; Chemical-biological coupling; Resource utilization
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Research Background

Anhui Province is a major region for soft-shelled turtle aquaculture in China and has experienced substantial expansion in its greenhouse-based soft-shelled turtle farming industry. The total greenhouse area amounts to 2.37 million m^2, yielding an annual production of 55 000 t of soft-shelled turtles. The annual discharge of aquaculture tailwater exceeds 1.54×10^6 m^3. In 2024, the greenhouse breeding area in Bengbu City reached 570 000 m^3, with an annual output exceeding 14 300 t and a production value of more than 450 million yuan. Under the high-density aquaculture model, the accumulation of uneaten feed, excrement, and metabolic byproducts results in a substantial increase in pollutant concentrations in the tailwater. Monitoring data indicate that during the later stages of greenhouse soft-shelled turtle breeding, total nitrogen (TN) levels in the tailwater range from 231.9 to 287.4 mg/L, total phosphorus (TP) from 90.3 to 137.6 mg/L, suspended solids (SS) from 224.7 to 363.7 mg/L, and chemical oxygen demand (COD) from 135.5 to 157.1 mg/L. These concentrations significantly exceed the water body's self-purification capacity, thereby posing a considerable threat to the ecological security of the surrounding aquatic environment. Currently, the majority of aquaculture units continue to utilize the direct discharge method, whereas a minority employ conventional tailwater treatment technologies, such as the "three-pond two-dam" or "four-pond three-dam" systems. These methods depend on multi-stage physical sedimentation, biological oxidation, and plant absorption. However, when treating high-concentration tailwater, these approaches often fail to achieve the desired treatment efficacy and are constrained by substantial land requirements, high energy consumption, and low operational efficiency. Furthermore, with the intensive development of the aquaculture industry, the pressure exerted by environmental protection policies has intensified. Notably, Anhui Province has implemented a mandatory discharge standard for aquaculture tailwater starting in 2024, stipulating that aquaculture farms with an area of no less than 0.67 hm^2 must ensure their tailwater meets the prescribed standards. Non-compliance will result in economic penalties. Consequently, there is an increasing urgency to address the treatment of high concentrations of nitrogen, phosphorus, and organic matter in aquaculture tailwater. The research and development of efficient and intensive treatment

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technologies and processes for aquaculture tailwater have thus become a central focus within the industry.

During the research and development of the "Integrated Purification Process", key parameters including volume, aeration volume, sludge production, and energy consumption of treatment units such as flocculation, sedimentation, anaerobic, anoxic, and aerobic biological reactors were calculated based on the pollutant concentration and load of aquaculture tailwater. Chemical flocculation, sedimentation, multi-stage biological reaction tanks, and deep filtration tanks were designed and integrated into a 350 m^3 engineering module. Through the flocculation effect of PAC/PAM, suspended solids and phosphorus-containing organic matter rapidly aggregate into flocs with particle sizes exceeding 50 μm, along with colloidal phosphorus, which subsequently settle. The "clarified water" then passes through the anaerobic zone, where anaerobic bacteria degrade large-molecule organic matter and release ammonia nitrogen. In the anoxic zone, denitrifying bacteria convert nitrate nitrogen into nitrogen gas (N_2). Within the aerobic zone, aerobic bacteria in the activated sludge complete nitrification and oxidize residual COD through multi-stage biological reactions. Subsequently, deep filtration using zeolite and activated carbon adsorbs and retains remaining ammonia nitrogen and trace organic substances, including antibiotics[1]. The pollutant concentrations in the effluent fully comply with the Class II limits specified in the *Discharge Standard of Water Pollutants for the Aquaculture Industry* in Anhui Province.

Research Advances

In recent years, domestic research on the anaerobic-anoxic-oxic (A^2/O) wastewater treatment process has primarily concentrated on structural optimization, process coupling, and operational control. Key areas of focus include the pre-positioning of the anoxic zone, the implementation of parallel influent diversion to anoxic and anaerobic sections, the incorporation of a biological selector within the aerobic zone to facilitate granular sludge formation, and the establishment and precise regulation of sludge return and mixed liquor return ratios tailored to various wastewater treatment types. Additionally, engineering application studies have explored the A^2/O-MBR process, which integrates the A^2/O process with membrane bioreactor (MBR) technology. These investigations have yielded notable engineering outcomes by mitigating nitrate nitrogen inhibition on anaerobic phosphorus release, enhancing carbon source availability, and balancing the reduction of carbon source competition with resistance to hydraulic shock. Collectively, these advancements have significantly improved the efficiency of nitrogen and phosphorus removal in the treatment process.

This study seeks to develop an integrated purification system that combines chemical flocculation, multi-stage biochemical reactions, and deep filtration to address the challenges posed by the high pollution levels and centralized discharge of tailwater from greenhouse soft-shelled turtle breeding. The objective is to overcome the technical limitations of conventional methods, which include extensive land requirements, prolonged treatment durations, and low efficiency. The chemical flocculation sludge undergoes anaerobic digestion to produce slow-release organic fertilizer, which is subsequently applied to farmland, thereby achieving "waste treatment with waste". The developed integrated modular structure eliminates the need for large-scale civil engineering works, and the processing unit's capacity can be flexibly adjusted to accommodate varying breeding scales. This system is particularly well-suited for high-density breeding farms with limited land availability, and the equipment can be constructed and installed within 15 d[2]. The ratio of dissolved phosphorus to dissolved organic matter (S-P/S-BOD) in the influent of the anaerobic tank is maintained at approximately 0.06 through the intelligent regulation of the low-speed propeller and aeration device. Additionally, the sludge loading rate of organic matter should exceed 0.10 kg BOD_5/kg MLSS · d. Essential nutrients, including carbon, nitrogen, and phosphorus sources required by the microorganisms in the anaerobic tank, are effectively managed. The hydraulic retention time (HRT) in the anoxic section ranges from 0.5 to 1.5 h, ensuring that the dissolved oxygen (DO) concentration remains below 0.5 mg/L. In the aerobic tank, the DO concentration should be maintained between 1.8 and 2.2 mg/L, with the sludge loading rate kept below 0.18 kg BOD_5/kg MLSS · d. Through a modular design approach, the integration of "chemistry, biology, and filtration" processes is effectively realized. Engineering practice has demonstrated that a device with a daily tailwater treatment capacity of 200 t requires only 200 m^2 of land, representing a reduction in land use of over 98% compared to traditional methods. The removal efficiencies for TN, TP, SS, and COD are 98.1%, 99.8%, 91.2%, and 92.2%, respectively, with effluent quality consistently meeting established standards. Furthermore, the equipment features online water quality monitoring, an AI-based early warning system for abnormal operating conditions, and is integrated with a "fishery-photovoltaic complementary" system. This technical process is adaptable for treating tailwater from aquaculture operations characterized by high nitrogen and phosphorus loads, such as those involving eels and sea bass.

Materials and Methods

Overview of the breeding base

The Yangcun Breeding Base of Anhui Xijia Agricultural Development Co., Ltd. (hereinafter referred to as Yangcun Base) comprises 56 greenhouses spanning a total area of 55 000 m^2 and employs a high-density, intensive breeding model. Between July and August 2023, approximately 3.3 million juvenile soft-shelled turtles were released at a density of 60 individuals/m^2. From April to June 2024, commercial soft-shelled turtles were harvested sequentially, resulting in an annual production of 1.3 million kg and a yield of approximately 23.6 kg/m^2. During the breeding process, a specialized powdered feed formulated for soft-shelled turtles, containing 42% to 46% protein, was utilized. Feed particles were processed to sizes ranging from 1.0 to 5.0 mm in accordance with the turtles' growth stages. The daily feeding rate was maintained at 3.3% to 5.5% of the turtles' body weight. Regarding tailwater discharge, the annual volume of aquaculture tailwater exceeded 3.85×10^4 m^3, with daily discharge volumes reaching 300 to 500 m^3 during the later stages of breeding. Concentrations of major pollutants in the tailwater significantly surpassed regulatory standards (Table 1). Specifically, the average concentrations of TN, TP, SS, and COD were 259.7, 114.0, 294.2, and 146.3 mg/L, respectively, identifying these as the primary limiting pollutants.

Table 1 Characteristics of tailwater pollution in greenhouse soft-shelled turtle breeding (n = 20)

Indicator	Range of concentration // mg/L	Mean ± standard deviation // mg/L
TN	231.9 – 287.40	259.7 ± 39.3
TP	90.3 – 137.60	114.0 ± 33.5
SS	224.7 – 363.72	94.2 ± 98.3
COD	143.5 – 160.00	146.3 ± 15.3

Technological process of integrated purification device

The device employs a three-stage treatment process comprising "chemical flocculation, biochemical synergy, and deep filtration" (Fig. 1). The specific procedure is detailed as follows.

In the chemical flocculation zone, polyaluminium chloride (PAC, containing 10% – 12% Al_2O_3) and anionic polyacrylamide (PAM) were introduced. The dosage of PAC ranged from 80 to 120 mg/L, while that of PAM was between 0.5 and 1.0 mg/L. Mechanical stirring was conducted at a speed of 60 to 120 r/min. Following the promotion of floc formation, the mixture proceeded to the inclined tube primary sedimentation tank. In the multi-stage biological reaction zone, the HRT in the anaerobic zone ranged from 6 to 8 h, with DO levels maintained below 0.2 mg/L. During this phase, methanogenic bacteria facilitated the degradation of large molecular organic substances. In the anoxic zone, the HRT was between 4 and 6 h, with DO levels below 0.5 mg/L, where denitrifying bacteria converted nitrate nitrogen to N_2. The aerobic zone exhibited an HRT of 8 to 10 h, with DO levels approximately 2.0 mg/L, enabling activated sludge to oxidize ammonia nitrogen and residual COD. The sludge return ratio varied from 50% to 100%, while the mixed liquor return ratio ranged from 300% to 400%. The secondary sedimentation zone further separated SS, operating at a surface loading rate of 1.0 to 1.2 $m^3/(m^2 \cdot h)$. In the disinfection and filtration zone, sodium hypochlorite with an effective chlorine concentration of 5 to 10 mg/L was applied, ensuring a water contact time of at least 30 min. The zeolite filter tank, containing particles sized 2 to 4 mm, adsorbed the residual ammonia nitrogen, while the activated carbon filter tank, characterized by an iodine value of at least 900 mg/g, retained trace organic compounds.

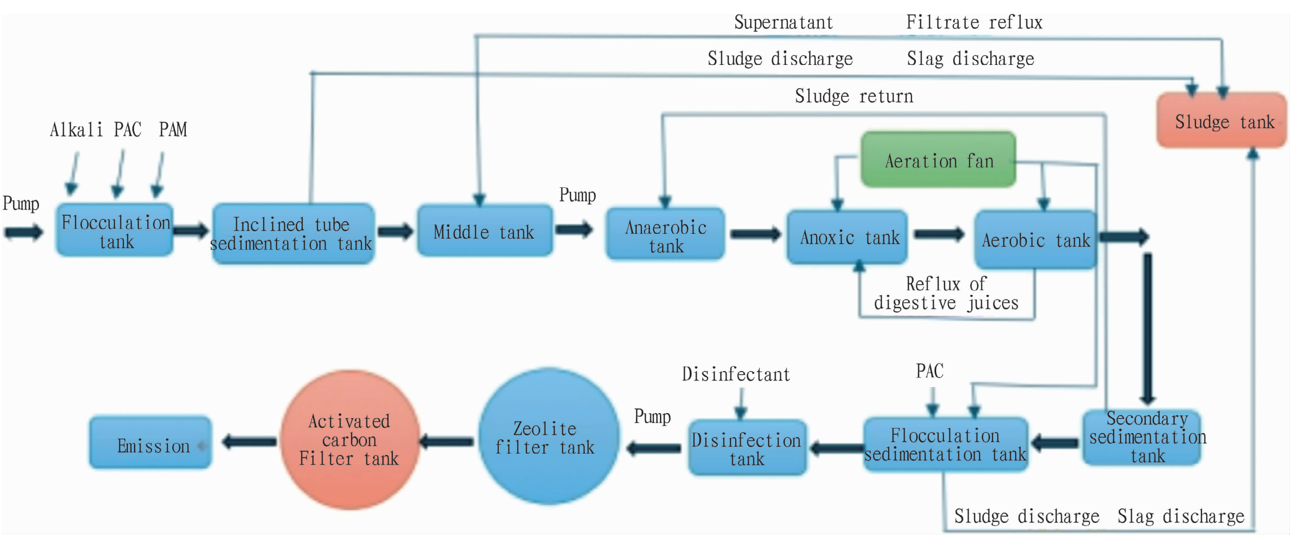

Fig. 1 Technological process of integrated purification device

Design basis, parameter calculation and detection methods

Design basis The effluent from the aquaculture tailwater at the Yangcun base was discharged into a temporary storage pool with a capacity of 1 100 m^3. The pH of the supernatant water in the storage pool was measured at 6.5, with SS at 87.2 mg/L, TN at 533.4 mg/L, TP at 291.6 mg/L, and COD at 966.3 mg/L. Based on a daily sewage treatment capacity of 200 t, the effluent complied with the design requirements corresponding to the Class II limits outlined in the *Discharge Standard of Water Pollutants for Aquaculture* in Anhui Province, which guided the parameter calculations and structural design.

Parameter calculation The volumes of the anaerobic, anoxic, and aerobic tanks were determined using the equation $V = Q \times HRT$, where V represents the volume of each respective tank

(m^3), Q denotes the designed water flow rate (m^3/d), and HRT corresponds to the hydraulic retention time of the anaerobic, anoxic, and aerobic zones (h). The calculations for nitrate reduction, phosphorus release, organic matter removal, and nitrification were performed using the formula $\Delta Z = (C_{influent} - C_{effluent}) \times Q/1\ 000$, where $C_{influent}$ and $C_{effluent}$ represent the concentrations of nitrate nitrogen, phosphate, chemical oxygen demand, and ammonia nitrogen (mg/L) in the influent and effluent, respectively. The sludge return ratio was calculated using the formula $R = Vr/Vw$, where Vr represents the sludge return flow rate (m^3/d) and Vw denotes the daily sludge discharge volume (m^3/d). The mixed liquor reflux ratio is typically established according to process requirements and operational performance, generally varying between 100% and 400%. Utilizing the determined reflux ratio alongside the water output of the corresponding area, the necessary reflux volume can be computed. The sludge age was determined using the equation $\theta c = Vp/(Qw + Ql)$, where Vp denotes the total volume of sludge in the reactor (m^3), Qw represents the sludge discharge volume (m^3/d), and Ql corresponds to the system loss volume (m^3/d). Monitoring and controlling mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) are critical parameters for ensuring the stable operation of the process. These parameters must be regularly measured and adjusted in accordance with actual operational conditions[3].

Structural design The cabinet body was constructed from carbon steel treated for corrosion resistance, with dimensions of 20 m (length) ×5 m (width) ×3.5 m (height), resulting in an effective volume of 350 m^3. It incorporated a flocculation tank, primary sedimentation tank, anaerobic, anoxic, and aerobic tanks, as well as pump sets, agitators, aeration fans, and an automatic control system. A dosing flocculation zone with a volume of 6 m^3 was designed and calculated. This zone was capable of automatically adjusting the dosage of PAC in real time based on fluctuations in influent COD, thereby ensuring stable flocculation efficiency. The inclined tube primary sedimentation zone had a volume of 30 m^3, with the inclined plate tubes positioned at an angle of 60°. The anaerobic tank had a capacity of 37 m^3, and the pH of the anaerobic treatment unit was maintained between 6.5 and 7.5. The anoxic tank had a volume of 48 m^3, with the pH controlled between 7.0 and 8.0. The combined aerobic tanks (aerobic 1 and aerobic 2) had a total volume of 88 m^3, and the pH in the aerobic treatment unit was maintained between 6.5 and 8.5. A conventional sludge loading rate of 0.3 – 0.6 kg BOD_5/kg MLSS was applied. Additionally, the secondary sedimentation tank had a volume of 43 m^3, the disinfection pool was 18 m^3, and the electrical equipment room occupied 80 m^3. Two DOTO 15 low-speed submersible propellers were selected. The agitator in the anaerobic and anoxic zones required a stirring energy of 5 W/m^3, resulting in a total energy demand of 1.8 kW for these zones. The air supply volume for each aeration head was measured to be 2.3 m^3/h[4].

Water quality determination methods pH was measured using the glass electrode method (GB6920). Dissolved oxygen was determined via the electrochemical probe method (GB11913). Chemical oxygen demand was assessed by the dichromate method (GB11914), while five-day biochemical oxygen demand was evaluated using the dilution and inoculation method (GB7488). Total nitrogen (TN) was quantified through the alkaline potassium persulfate digestion ultraviolet spectrophotometry method (GB11894). Ammonia nitrogen (NH_4^+-N) was measured by salicylic acid spectrophotometry (GB7481), total phosphorus (TP) by ammonium molybdate spectrophotometry (GB11893), and suspended solids (SS) by the gravimetric method (GB11901). The pollutant removal efficiency (removal rate) was calculated based on the difference in pollutant concentrations between the influent and effluent.

Results and Analysis

Pollutant removal effect

The integrated greenhouse soft-shelled turtle high-concentration breeding tailwater purification device, which was designed and manufactured for this study, operated continuously for over 180 d. The removal efficiency of various pollutants was systematically monitored (Table 2). The results indicated that the TN concentration in the influent was 259.7 mg/L. Following anaerobic-aerobic synergistic denitrification, the average effluent concentration decreased to 4.9 mg/L, corresponding to a removal rate of (98.1 ± 1.2) %. Additionally, the nitrification-denitrification process achieved an ammonia nitrogen conversion efficiency of 98%. Through the coupling of chemical flocculation and biological phosphorus removal processes, the influent TP concentration was reduced from 114.0 mg/L to an average of 0.26 mg/L, corresponding to a removal efficiency of 99.8%. Notably, the addition of PAC accounted for approximately 85% of the phosphorus adsorption capacity. The influent SS concentration was 294.2 mg/L. Following treatment with PAC/PAM flocculation combined with inclined plate sedimentation, the effluent SS concentration was reduced to a median value of 26 mg/L, achieving a removal rate of (91.2 ± 1.1) %. These results indicate that chemical flocculation exerts a significant and rapid interception effect on particulate matter. Additionally, the COD concentration decreased from 146.3 mg/L to an intermediate value of 11.4 mg/L after multistage treatment, corresponding to a removal efficiency of (92.2 ± 2.4) %.

Table 2 Comparison of pollutant removal effects ($n = 30$)

Indicator	Influent concentration // mg/L	Effluent concentration // mg/L	Removal rate // %
TN	59.7 ±39.3	4.90 ±0.70	98.1 ±1.2
TP	114.0 ±33.5	0.26 ±0.16	99.8 ±0.1
SS	294.2 ±98.3	26.00 ±5.30	91.2 ±1.1
COD	146.3 ±15.3	11.40 ±2.10	92.2 ±2.4

Equipment efficacy

This study presented a research design and engineering application that integrated chemical flocculation, multi-stage biological reactions, and deep filtration processes to develop an integrated purification device for high-concentration aquaculture tailwater from greenhouse soft-shelled turtle farming. The device demonstrated significantly higher removal efficiencies for TN, TP, SS, and COD compared to the conventional "four-pond and three-dams" treatment process. Furthermore, by incorporating multi-stage reaction units within the steel cabinet, the land utilization rate was enhanced by over 95%. The modular design decreased the cost of tailwater treatment to 2.08 yuan/t, representing a savings of 35% to 58% compared to traditional processes. Additionally, it demonstrated strong resistance to load shocks. At the Yangcun base alone, annual reductions of 8.89 t of TN, 3.9 t of TP, 9.39 t of SS, and 4.72 t of COD emissions were achieved, effectively mitigating the pressure of water eutrophication pollution[4]. The integrated processing device occupies an area of merely 200 m^2 and requires an installation period of 15 d. It is not constrained by the breeding season and can be designed, manufactured, and installed at any time according to the scale of breeding operations. This device is well-suited for high-density breeding environments with limited land resources and offers an engineering model conducive to large-scale implementation.

Water quality compliance rate and operational cost-effectiveness of equipment

The effluent quality fully complied with the Class II limits outlined in the *Discharge Standard of Water Pollutants for Aquaculture* (DB 34/4722-2024) in Anhui Province (Table 3). Specifically, the concentrations of TN at 4.9 mg/L, TP at 0.26 mg/L, SS at 26 mg/L, and COD at 11.4 mg/L were 18.3%, 67.5%, 69.4%, and 54.4% lower than the respective standard limits. Furthermore, the equipment demonstrates potential for further enhancement.

Table 3 Comparison of effluent quality with standard limit values

Indicator	Influent concentration//mg/L	Secondary standard limit//mg/L	Compliance rate//%
TN	4.90±0.7	≤6.0	100
TP	0.26±0.1	≤0.8	100
SS	26.00±5.3	≤85.0	100
COD	11.40±2.1	≤25.0	100

The cost of chemicals for water treatment was 0.20 yuan/t for PAC and 0.15 yuan/t for PAM. Electricity consumption amounted to approximately 1.53 yuan/t (corresponding to 1.8 Kw/h), while equipment maintenance costs were 0.20 yuan/t, resulting in a total cost of 2.08 yuan/t. The total investment in equipment was 1.2 million yuan, with an annual operating cost of approximately 72 800 yuan. The annual reductions achieved were 8.89 t of TN, 3.9 t of TP, 9.39 t of SS, and 4.72 t of COD, thereby effectively mitigating the risks of eutrophication and pollution in regional public water bodies[5]. It is anticipated that the costs can be fully recovered within 3–5 years through the reduction of environmental fines and the improvement of breeding sustainability. This device is particularly suitable for the purification treatment of high-concentration aquaculture tailwater, especially in specialized aquaculture operations such as those involving soft-shelled turtles and eels, which generate high loads of ammonia nitrogen and phosphorus. Furthermore, integrating this system with "fishery-photovoltaic complementary" projects and implementing a solar power supply system can further reduce electricity consumption costs.

Conclusions and Discussion

The tailwater from high-density greenhouse soft-shelled turtle breedinging was initially treated using PAC/PAM for chemical flocculation to improve treatment efficiency. This process facilitated the rapid aggregation of phosphorus-containing organic matter into flocs exceeding 50 μm in particle size, which can capture more than 80% of suspended solids and colloidal phosphorus. These flocs subsequently settled in an inclined tube primary sedimentation tank, thereby decreasing the load on downstream biological treatment processes. The clarified water from the primary sedimentation tank entered the anaerobic zone, where the HRT ranged from 6 to 8 h to facilitate the release of ammonia nitrogen. In the subsequent anoxic zone, the HRT ranged from 4 to 6 h, during which denitrifying bacteria converted nitrogen into N_2. Finally, in the aerobic zone, the HRT was maintained between 8 and 10 h, allowing nitrifying bacteria in the activated sludge to further oxidize NH_4^+-N to NO_3^--N. The chemical-biological coupling mechanism effectively removed nitrogen and phosphorus from high-concentration aquaculture tailwater. The adsorption efficiency of the zeolite-activated carbon filtration system for trace pollutants, including antibiotic residues, exceeded 90%, thereby ensuring the long-term operational stability of the equipment. Continuous sampling tests conducted over a three-month period by the Environmental Protection Department of Bengbu City demonstrated that the influent concentrations of TN, TP, SS, COD and other indicators fluctuated by more than 35%, whereas the effluent concentrations of these parameters varied by less than ±5%[6]. These results indicate that the system possesses a strong capacity to withstand load shocks.

To comprehensively enhance the tailwater treatment technology for greenhouse soft-shelled turtle breeding and promote the sustainable development of the industry, a dynamic PAC/PAM dosing algorithm based on real-time water quality monitoring—utilizing techniques such as UV-Vis spectroscopy and ammonia nitrogen sensors—can be developed. By integrating artificial intelligence and machine learning to predict pollutant loads[7], chemical usage costs may be reduced by 20%–30%. The incorporation of solar photovoltaic panels coupled with energy storage systems to power aeration and pumping equipment can decrease electricity consumption and carbon emissions. The combination of the reactor HRT and the filtration unit is adjusted to accommodate the varying aquaculture densities across different regions. For example, in aquaculture areas where the tailwater exhibits high phosphorus concentrations, a calcium-based adsorption layer may be incorporated. In cases where the tailwater contains elevated levels of suspended solids, a microfiltration unit with a pore size of no more than 50 μm can be employed as a pretreatment step to extend the operational lifespan of the core equipment. Additionally, chemical flocculation sludge, which is rich in nitrogen and phosphorus, can be processed through anaerobic digestion to produce slow-release organic fertilizer, subsequently returned to the agricultural system. The purified tailwater can thus be recycled within a closed-loop system, thereby reducing water resource consumption[8].

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