

Article

Bioremediation of Printing and Dyeing Wastewater by *Synechocystis aquatilis*: System Construction, Kinetics and Mechanisms

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Abstract

Actual printing and dyeing wastewater (APDW), as one of the most difficult types of wastewater to treat, has become a significant environmental risk due to its toxicity and the challenges associated with its degradation. Microalgae-based treatment of APDW is a promising, eco-friendly, and cost-effective strategy. In this study, a cyanobacterium, *Synechocystis aquatilis*, was isolated from APDW. The strain demonstrated good environmental tolerance and the capacity to remove pollutants and valorize biomass simultaneously. Under optimized conditions, it removed COD ($120.27 \text{ mg} \cdot \text{L}^{-1} \cdot \text{d}^{-1}$), $\text{NH}_4\text{-N}$ ($0.89 \text{ mg} \cdot \text{L}^{-1} \cdot \text{d}^{-1}$), and total phosphorus ($9.52 \text{ mg} \cdot \text{L}^{-1} \cdot \text{d}^{-1}$), while achieving substantial decolorization. The strain concurrently accumulated lipids (373.08 mg/g), polysaccharides (167.85 mg/g), and proteins (72.05 mg/g). Mechanistic analyses revealed that *S. aquatilis* microalgae adsorb dyes and impurities via bioadsorption and then biodegrade dyes and nitrogen and phosphorus compounds via NADPH generation, glutamate and butyrate metabolism, and oxidoreductase activity. This study presents a promising application of *S. aquatilis* as a novel and environmentally friendly treatment method for APDW, enabling simultaneous wastewater treatment and resource recovery.

Keywords: *Synechocystis aquatilis*; actual printing and dyeing wastewater; biodegradation; decolorization



Academic Editor: Hassimi Abu Hasan

Received: 7 April 2026

Revised: 11 May 2026

Accepted: 11 May 2026

Published: 12 May 2026

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1. Introduction

With the increasing demand for textiles, a large amount of printing and dyeing wastewater will be produced [1]. Actual printing and dyeing wastewater (APDW) is challenging to treat due to its complex composition, which contains various dyes, color fixatives, heavy metals, and surfactants [2]. Untreated APDW is characterized by high chemical oxygen demand (COD), elevated pH, and high toxicity, resulting in severe environmental pollution in rivers, soils, and oceans. APDW reduces water transparency and inhibits aquatic plant growth, disrupting aquatic ecosystems. Moreover, the chemical dyes in APDW containing aromatic structures are mutagenic and carcinogenic, causing serious toxicity to aquatic organisms and humans through the food chain [3,4]. Therefore, APDW must be treated before discharge.

Currently, the treatment technologies for APDW include physical adsorption [5], membrane separation [6], chemical coagulation [7], chemical oxidation, and photocatalytic oxidation [8,9]. Physical adsorption exhibits high adsorption, but the cost of adsorbents is high. Chemical flocculation and chemical oxidation can remove dyes, COD, phosphorus, and ammonia nitrogen from wastewater, but they produce large amounts of chemical sludge during treatment, causing secondary pollution [10]. Therefore, exploring low-cost and environmentally friendly methods to treat APDW has become an important research topic [11].

Biological treatments have attracted considerable attention in wastewater treatment due to their high efficiency and environmental friendliness [12]. There are many biological treatment materials, such as bacteria, fungi, and plants. Among them, microalgae are ideal candidates for wastewater treatment. Microalgae remove pollutants via bioadsorption, bioaccumulation, and biodegradation [13,14]. Microalgal cell surfaces contain functional groups such as carboxyl, hydroxyl, and amino groups, which facilitate the adsorption of pollutants [15]. Then, dye molecules and nitrogen and phosphorus compounds are transported into microalgal cells, where they can be biotransformed into non-toxic metabolites via enzymatic processes [16]. Moreover, microalgal growth yields high-value by-products, such as pigments, carbohydrates, lipids, and biomass, which can be utilized for biofuels, biofertilizers, biological feeds, and drug production, offering economic benefits [17,18]. Despite these advantages, the challenge of using microalgae for practical applications is poor tolerance in APDW. Thus, screening microalgal strains with high tolerance in APDW and optimizing conditions are important.

In this study, the APDW was treated with *Synechocystis aquatilis* (*S. aquatilis*), a strain with high tolerance in APDW that was isolated from it. The APDW treatment conditions were optimized for *S. aquatilis*, and the physicochemical changes in the microalgae before and after treating APDW were analyzed. Kinetic models for dye and COD adsorption during wastewater treatment were developed, and the mechanisms of *S. aquatilis* were investigated, including biosorption, enzyme activity profiles, and potential gene pathways involved in these processes. Transcriptome analysis further elucidated changes in the regulation of specific genes related to adaptive and degradation processes during APDW treatment. This study, thus, presents an environmentally friendly APDW treatment method using high-tolerance *S. aquatilis*.

2. Materials and Methods

2.1. Microalgae and Chemicals

The original APDW was obtained from pre-treatment, dyeing, printing, and post-treatment processes involved in cotton fabric production at a printing and dyeing factory in Shandong Province, China. The physical and chemical properties of APDW samples on 18 July 2022 and 6 August 2023 are shown in Table S1. The APDW was sterilized in the following treatment studies to eliminate the influence of other microorganisms on the experiment. Unless specifically indicated, all chemical reagents were purchased from Sinopharm Group Chemical Reagent Co., Ltd, Shanghai, China. and were of analytical grade.

S. aquatilis was isolated and purified from the original APDW, and the specific procedures are shown in Section S1. DNA sequencing was performed by Sangon Biotec, and species were identified by NCBI (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) (accessed on 3 January 2024).

2.2. The System Construction of Treating APDW with *S. aquatilis*

S. aquatilis was inoculated into sterilized APDW (18 July 2022) and cultured for 9 days. Samples were collected in two-day intervals for physicochemical and biological analyses.

The effects of key variables on decolorization efficiency and microalgal growth were systematically examined. The experimental factors included the APDW dilution ratio (1~8 fold), initial pH (6~10), light intensity (3000~12,000 Lux), and inoculation proportion (10~30%, *v/v*). Following sampling, the cultures were centrifuged at 4 °C and 4000 rpm for 10 min (Centrifuge 5804 R, Eppendorf, Germany) to separate the biomass from the supernatant before analysis.

The COD, NH₄-N, and TP values of the APDW were determined using reagent kits from Zhejiang Lohand Environment Technology. The absorbance of APDW dyes was measured on the ninth day from 300 to 700 nm at 0.5 nm increments (UV-1900; Shimadzu, Hitachi, Japan). The biomass of *S. aquatilis* was estimated based on the chlorophyll concentration [19]. Detailed information on these parameters is provided in Section S2.

2.3. The Sustainable System Treatment of APDW Under Optimized Conditions

The experiments were conducted using two parallel groups: an APDW treatment group and a control group. The sterilized APDW was diluted two-fold with deionized water and adjusted to pH 7. In the treatment group, *Synechocystis aquatilis* was inoculated at 20% (*v/v*) into diluted APDW (6 August 2023), which was maintained in 1 L flasks at 20 °C and 9000 Lux and incubated for 9 days. The control group consisted of *S. aquatilis* cultivated in BG-11 medium. Samples were collected at 2-day intervals to monitor wastewater quality parameters, including COD, NH₄-N, and TP, as well as microalgal growth dynamics.

2.4. The Proteins, Polysaccharides, and Lipids of *S. aquatilis*

The total proteins were extracted using the plant protein extraction kit (Suzhou Grace Biotechnology Co., Ltd., Suzhou, China). The total polysaccharides were extracted using the plant polysaccharides extraction kit (Suzhou Grace Biotechnology Co., Ltd.). The total lipid content was measured using the chloroform–methanol extraction method. For detailed experimental procedures, please refer to Section S3.

2.5. Water Quality Evaluation of APDW

The ecotoxicity of untreated and microalgae-treated APDW was assessed using a mung bean germination assay. Surface-sterilized seeds (2% H₂O₂; 15 min) were rinsed and soaked for 6 h in deionized water (control), untreated APDW, or microalgae-treated APDW. The seeds were then incubated between moist filter papers in Petri dishes at 25 °C in the dark for 72 h. The germination percentage and primary root length were recorded. The germination rate and root inhibition were calculated as follows: germination rate (%) = (number of germinated seeds/total number of seeds) × 100%; root length inhibition rate (%) = [(control group-experimental group)/control group] × 100% [20].

2.6. Kinetic Models

The mechanism involved in the degradation process of dyes and COD with *S. aquatilis* was determined using different kinetic models (first-order kinetic model, second-order kinetic model, pseudo-first-order kinetic model, and pseudo-second-order kinetic model) [21,22]. The degradation of dyes was determined using methylene blue as a sample.

The equations for the kinetic models are as follows:

First-order (FO) kinetic model: $\ln \frac{C_0}{C_t} = k_1 t$, where C_0 is the initial concentration, C_t is the concentration at time t , and k_1 is the rate constant.

Second-order (SO) kinetic model: $\frac{1}{C_t} - \frac{1}{C_0} = k_2 t$, where C_0 is the initial concentration, C_t is the concentration at time t , and k_2 is the rate constant.

Pseudo-first-order (PFO) kinetic model: $\ln(C_{max} - C_t) = \ln C_{max} - k_1 t$, where C_{max} is the maximal degradation concentration, C_t is the concentration at time t , and k_1 is the rate constant.

Pseudo-second-order (PSO) kinetic model: $\frac{t}{C_t} = \frac{1}{k_2 C_{\max}^2} + \frac{t}{C_{\max}}$, where $C_{\max}$ is the maximal degradation concentration, C_t is the concentration at time t , and k_2 is the rate constant.

2.7. The Mechanism of Treatment of APDW with *S. aquatilis*

2.7.1. The Morphology of *S. aquatilis*

The *S. aquatilis* samples before and after treatment were fixed in 2.5% (*v/v*) glutaraldehyde for 2 h, dehydrated with ethanol with graded concentrations from 10% to 100%, and dried with the critical point method. The scanning electron microscopy (S-3400N, Hitachi, Japan) was used to observe the morphological characteristics of *S. aquatilis*.

2.7.2. Genome Extraction and Sequencing

S. aquatilis was grown in BG-11 medium at 26 °C for 9 days to generate sufficient biomass for DNA extraction. Total genomic DNA was subsequently extracted using the E.Z.N.A. HP Plant DNA Kit (Omega Bio Inc., Norcross, GA, USA). Ligation products were verified via electrophoresis and PCR and then sequenced on an Illumina NovaSeq 6000 platform (Majorbio Biotechnology Co., Ltd., Shanghai, China). For genome assembly, SOAPdenovo2 was employed, and genome functional annotation was conducted against the NR, Swiss-Prot, Pfam, EggNOG, GO, and KEGG databases [23].

2.7.3. Transcriptome Profiling Using mRNA Sequencing

S. aquatilis was grown in BG-11 medium and APDW at 26 °C for 9 days to extract RNA. Total RNA was extracted using the Trizol RNA extraction kit (Invitrogen, Carlsbad, CA, USA). Quality control of RNA samples incorporated electrophoresis and Agilent 2100 bioanalyzer analysis. Eukaryotic mRNA was first depleted of rRNA, fragmented, and subjected to reverse transcription into cDNA using the NEBNext Ultra RNA Library Preparation Kit (NEB#7530, New England Biolabs, Ipswich, MA, USA). cDNA fragments were end-repaired, A-tailed, ligated to Illumina adapters, amplified via PCR, and sequenced on an Illumina NovaSeq 6000 platform (Majorbio Biotechnology Co., Ltd., Shanghai, China). The RT-qPCR was conducted using the SYBR qPCR Master Mix Q711 kit on the StepOne-Plus™ Real-Time PCR instrument (Thermo Fisher Scientific, Waltham, MA, USA). In this experiment, *Rnp* was selected as the internal reference gene. The RT-qPCR data were analyzed using the $2^{-\Delta\Delta C_t}$ method.

2.7.4. Determination of Enzyme Activity of *S. aquatilis*

The experiment compared the differences in superoxide dismutase, peroxidase, malonaldehyde, and total antioxidant activity in *S. aquatilis* before and after APDW treatment. These enzymes were measured using test kits (Nanjing Jiancheng Corp., Nanjing, China), and the detailed extraction procedure is provided in Section S4.

2.8. Statistical Analysis

Three parallel experiments were conducted in each experiment. All data were expressed as mean $\pm$ standard deviation (SD), statistically analyzed using Microsoft Excel and SPSS 13.0. The graphs were drawn using Origin (2018). One-way analysis of variance (ANOVA) was used to assess statistical differences, with $p < 0.05$ considered statistically significant.

3. Results

3.1. Isolation of Microalgae

Sequence homology analysis identified the isolated microalgal strain as *Synechocystis aquatilis*. Phylogenetic analysis using the MEGA 11 software further confirmed this clas-

sification, clustering the strain robustly within the *S. aquatilis* clade (Figure S1). Morphologically, the strain exhibited typical *S. aquatilis* characteristics, appearing oval-shaped with a distinct blue–green pigmentation. Previous studies have utilized *S. aquatilis* in environmental research to investigate the toxicity of tin and cadmium. However, research on its application in APDW treatment is lacking. *S. aquatilis* isolated from APDW may be a promising algal strain for biological wastewater treatment of APDW.

3.2. The System Construction of *S. aquatilis* Treatment of APDW

3.2.1. Effect of Dilution Fold of APDW on APDW Treatment

The impact of APDW dilution on treatment performance is shown in Figure 1. Increasing the dilution factor enhanced pollutant removal, with COD, $\text{NH}_4\text{-N}$, and TP removal efficiencies rising from 19.13% to 53%, 18.11% to 96.21%, and 0.53% to 17.98%, respectively. This trend paralleled improvements in the physiological status of *S. aquatilis*, as reflected in elevated chlorophyll content (Figure S2b), indicating that reduced toxicity alleviated growth inhibition and restored metabolic activity. At lower dilution ratios, APDW exerted a pronounced inhibitory effect on algal growth. In contrast, biomass accumulation in the two-, four-, and eight-fold-diluted groups converged after day 9, suggesting that beyond a threshold dilution, growth limitation was largely relieved. From an engineering perspective, higher dilution ratios also increase operational costs due to additional freshwater input. By balancing pollutant removal efficiency, biomass productivity, and economic feasibility, the two-fold dilution represents the optimal treatment condition.

3.2.2. Effect of pH of APDW on APDW Treatment

Solution pH exerted a decisive influence on both APDW remediation performance and the physiological status of *S. aquatilis*. Post-treatment analyses revealed pronounced differences in dye removal efficiency across pH conditions, with pH = 7 yielding the highest overall removal (Figure 1). Under this condition, COD and $\text{NH}_4\text{-N}$ removal increased rapidly during the first seven days, reaching maxima of 35.17% (day 5) and 47.11% (day 7), respectively, before declining slightly by day 9. Despite this decrease, removal efficiencies remained substantial, suggesting ongoing biotransformation of residual dyes and associated organic compounds rather than simple stagnation of treatment performance. Algal growth dynamics mirrored these trends. As shown in Figure S2a, biomass accumulation progressively declined with increasing pH, indicating that alkaline conditions imposed physiological stress on *S. aquatilis*. Therefore, pH 7 was determined to be the optimal condition.

3.2.3. Effect of Light Intensity on APDW Treatment

Light intensity critically regulated both pollutant removal efficiency and biomass productivity during APDW treatment. Across all treatments, COD, $\text{NH}_4\text{-N}$, and TP removal increased progressively with time. Among the tested conditions, 9 kLux yielded the highest removal efficiencies for COD (41.44%) and TP (35.16%) (Figure 2). Although $\text{NH}_4\text{-N}$ removal at 9 kLux (60.43%) was marginally lower than that observed at 3 kLux (62.38%) and 6 kLux (62.07%), the differences were not statistically significant. Biomass accumulation exhibited a positive correlation with light intensity up to 9 kLux, consistent with enhanced photosynthetic carbon fixation before the onset of photoinhibition. In contrast, exposure to 12 kLux suppressed growth, likely reflecting damage to the photosynthetic apparatus and increased reactive oxygen species (ROS) generation under excessive irradiance [24]. Due to the APDW's dark colors and low transparency, the 3k Lux, 6k Lux, and 9k Lux light intensities were found suitable for *S. aquatilis* growth. Integrating pollutant removal performance and biomass productivity, 9 kLux emerged as the optimal operational condition for *S. aquatilis*-mediated treatment of APDW.

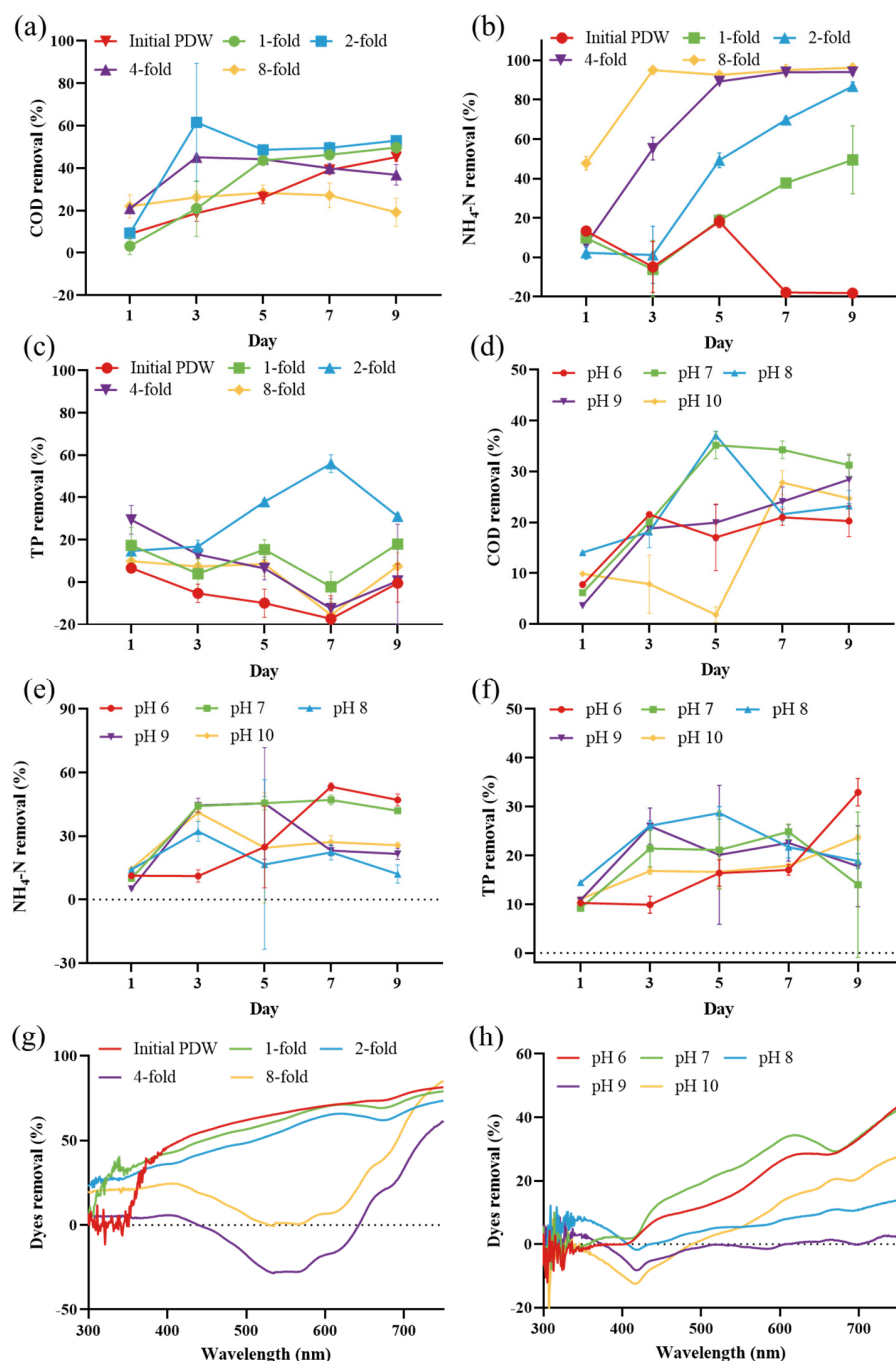


Figure 1. The influence of APDW dilution factor and pH on APDW treatment. (a–c) COD, $\text{NH}_4\text{-N}$ and TP removal rate at different dilution ratios. (d–f) COD, $\text{NH}_4\text{-N}$ and TP removal rate at different pH values. (g) Dye removal rate (on the ninth day) at different dilution ratios. (h) Dye removal rate (on the ninth day) at different pH values.

3.2.4. Effect of Algae Supplementation on APDW Treatment

The initial inoculum density of *S. aquatilis* substantially influenced APDW treatment performance. Across all groups, COD removal increased progressively and peaked on day 7 before declining (Figure 2), indicating a dynamic balance between active assimilation and subsequent nutrient limitation. The 20% inoculation group achieved the highest COD removal efficiency (47.69%), significantly outperforming the 10% and 30% groups (32.46% and 19.76%, respectively) during the later treatment phase. A similar trend was observed for TP removal, which reached 49.05% on day 7 in the 20% group. In contrast, $\text{NH}_4\text{-N}$ removal converged across treatments after day 5, suggesting that nitrogen assimilation

capacity was not strongly constrained by inoculum density under the tested conditions. Dye removal efficiencies were comparable between the 20% and 30% groups, indicating that further increases in biomass did not proportionally enhance chromaticity reduction. Biomass in the 20% group increased steadily throughout the experiment, whereas the 30% group exhibited an initial rise followed by a decline (Figure S2d). This collapse likely reflects rapid nutrient depletion under excessive cell density, resulting in metabolic stress, growth arrest, and reduced overall system stability. Therefore, 20% inoculation was determined to be the optimal condition.

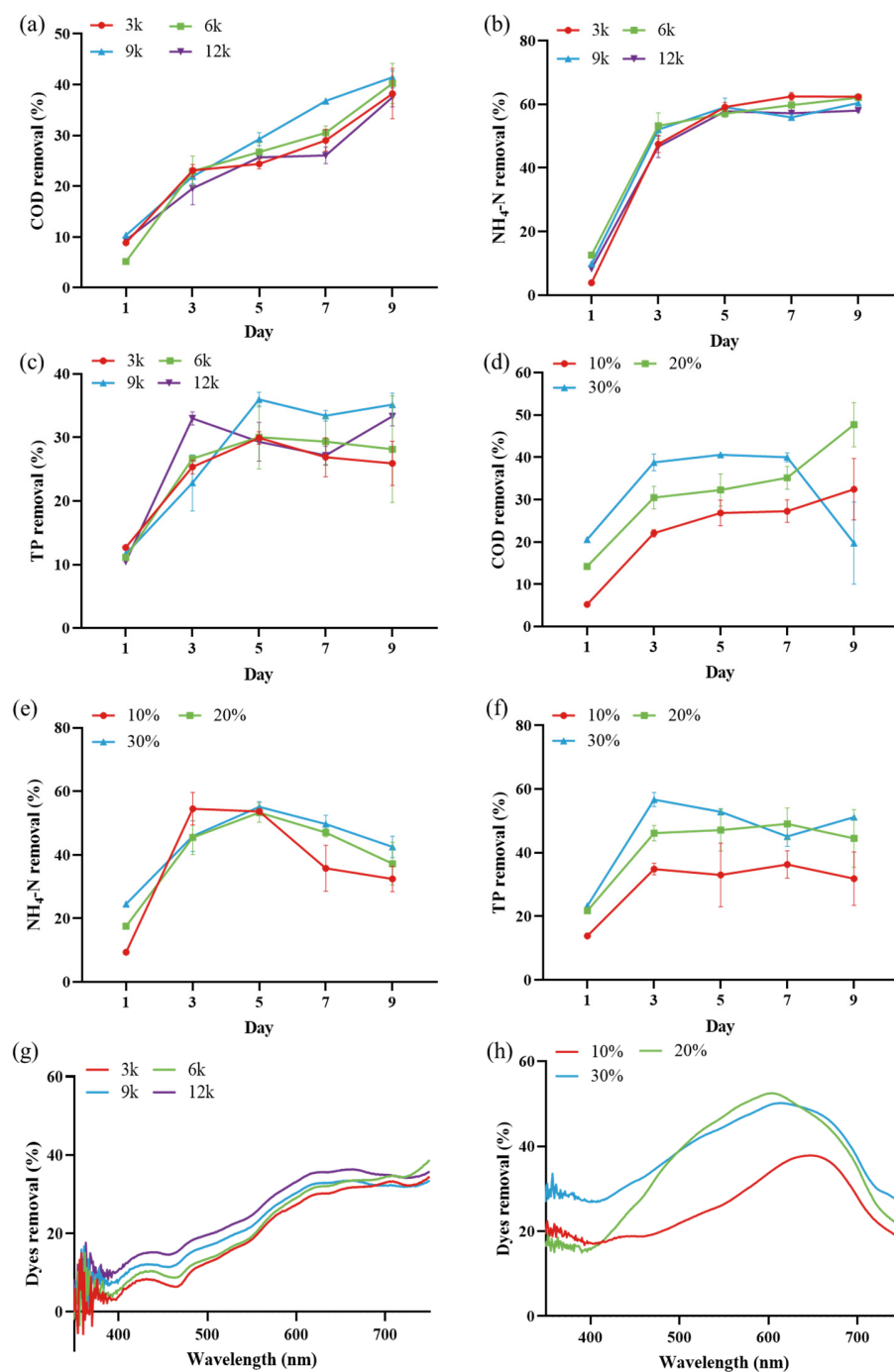


Figure 2. The influence of light intensity and algae supplementation on APDW treatment. (a–c) COD, $\text{NH}_4\text{-N}$ and TP removal rate at different light intensities. (d–f) COD, $\text{NH}_4\text{-N}$ and TP removal rate at different algae supplementation percentages. (g) Dye removal rate (on the ninth day) at different light intensities. (h) Dye removal rate (on the ninth day) at different algae supplementation percentages.

3.3. A Sustainable System for Treatment of APDW Under Optimized Conditions

The *S. aquatilis* treatment of APDW (taken on 6 May 2023) significantly improved under optimized conditions (pH 7, a light intensity of 9k Lux, and 20% *S. aquatilis* supplementation) (Figure 3a–d). The COD content in APDW decreased to 2416.5 mg/L on the seventh day, with a removal rate of $120.27 \text{ mg} \cdot \text{L}^{-1} \cdot \text{d}^{-1}$. The $\text{NH}_4\text{-N}$ and TP contents decreased to 5.50 mg/L and 364.0 mg/L, with removal rates of 0.89 and $9.52 \text{ mg} \cdot \text{L}^{-1} \cdot \text{d}^{-1}$, respectively. These reductions were accompanied by a visible color transition from blue–grey to light brown (Figure S3), consistent with substantial chromaticity attenuation. Spectral analysis further confirmed significantly higher dye removal of 55.75%, particularly within the 500–750 nm range.

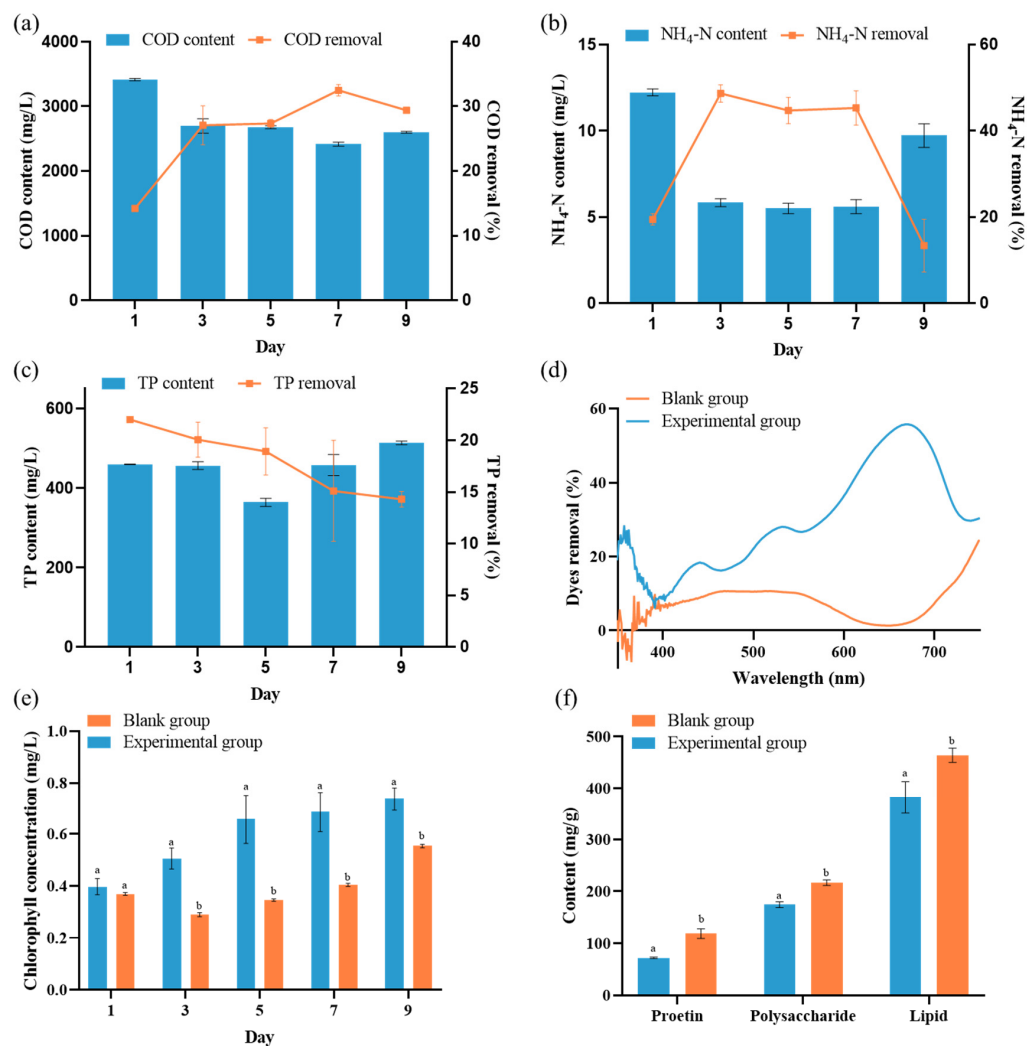


Figure 3. The treatment of APDW in optimal condition and by-products of *S. aquatilis* (a) COD, (b) $\text{NH}_4\text{-N}$, (c) TP, (d) dye removal rate on the ninth day, (e) chlorophyll concentration and (f) by-product content. a,b indicates significant difference between experimental group and blank group ($p < 0.05$).

Beyond pollutant removal, APDW supported robust algal growth. As a high-strength organic wastewater enriched in nitrogen and phosphorus, APDW provided substantial nutrient resources [25]. Biomass accumulation and chlorophyll content were significantly elevated compared with BG-11 medium (blank), with total chlorophyll reaching 0.74 mg L^{-1} in APDW versus 0.55 mg L^{-1} in BG-11 (Figure 3). This enhancement indicates efficient nutrient assimilation and metabolic activation, demonstrating that *S. aquatilis* tolerates and

exploits APDW as a growth substrate. The APDW treatment simultaneously generated substantial algal biomass enriched in bioactive compounds, which could be used as biofertilizers. Other research used *Chlorella vulgaris* as a biofertilizer, which enhanced the rates of corn and soybean growth [26].

3.4. Proteins, Polysaccharides, and Lipids Generated by *S. aquatilis* After Treatment of APDW

Beyond pollutant removal, *S. aquatilis* generated value-added biomolecules, including proteins, polysaccharides, and lipids. Protein content was higher in BG-11 medium (118.80 mg/g) than in APDW (72.05 mg/g) (Figure 3), indicating that nutrient imbalance and wastewater-associated stress reshaped intracellular carbon allocation. Although lipid content in APDW-grown cells was lower than in BG-11 controls, it remained substantial, demonstrating preserved carbon storage capacity under high-strength wastewater conditions. The comparatively reduced lipid accumulation is consistent with previous reports that excessive phosphorus availability constrains lipid biosynthesis by favoring protein and structural growth over storage metabolism [27]. Similar adaptive responses have been observed in other wastewater systems, where lipid accumulation in *Scenedesmus* sp. reached 24.89% in monosodium glutamate wastewater [28], and filamentous algae exhibited lipid increases from 12.8 to 66.4 mg/g in saline wastewater [29]. These results indicate that APDW supports algal growth and sustains appreciable lipid production, underscoring the feasibility of coupling wastewater remediation with biofuel-oriented biomass valorization.

3.5. Water Quality Evaluation

The mung bean test was used to evaluate the water quality of APDW. Mung bean seed germination was used to assess the phytotoxicity of APDW before and after treatment. The germination index (%) and root lengths of germinated mung beans were measured, as detailed in Table S2. Figure 4 depicts the effects of APDW (before and after treatment) and pure water on mung bean growth after 4 days. Mung bean in *S. aquatilis*-treated APDW exhibited root growth of 1.55 cm, greater than that in untreated APDW (0 cm) and shorter than that in the pure water group (3.4 cm).

3.6. Kinetic Models of APDW Treatment

The adsorption of methylene blue and COD onto *S. aquatilis* over time was evaluated using four distinct kinetic models: first-order (FO), second-order (SO), pseudo-first-order (PFO), and pseudo-second-order (PSO) kinetics (Figure 5). The parameters for each model are detailed in Table S3. The PSO rate constants (k_2) for methylene blue and COD were determined to be 0.0350 and 0.00013, respectively. In the case of PSO kinetics, the value of R^2 was greater than 0.95 for both methylene blue and COD adsorption, specifically 0.97 and 0.996, respectively. The R^2 values of the FO, SO, and PFO models were 0.525, 0.537, and 0.758 for methylene blue and 0.633, 0.637, and 0.596 for COD adsorption, respectively. These higher R^2 values indicated that the PSO kinetic model provided a superior fit for the adsorption data, indicating its suitability for methylene blue and COD adsorption onto the surface of *S. aquatilis*. The PSO kinetics suggested that the adsorption process for dyes (methylene blue) and COD removal followed a chemical adsorption and ion exchange mechanism, consistent with the finding of Mahmoudi et al., who reported that the date pits effectively removed methylene blue and conformed well to the pseudo-second-order model [30].



Figure 4. The phytotoxicity test of mung bean. (a) pure water, (b) untreated APDW and (c) treated APDW.

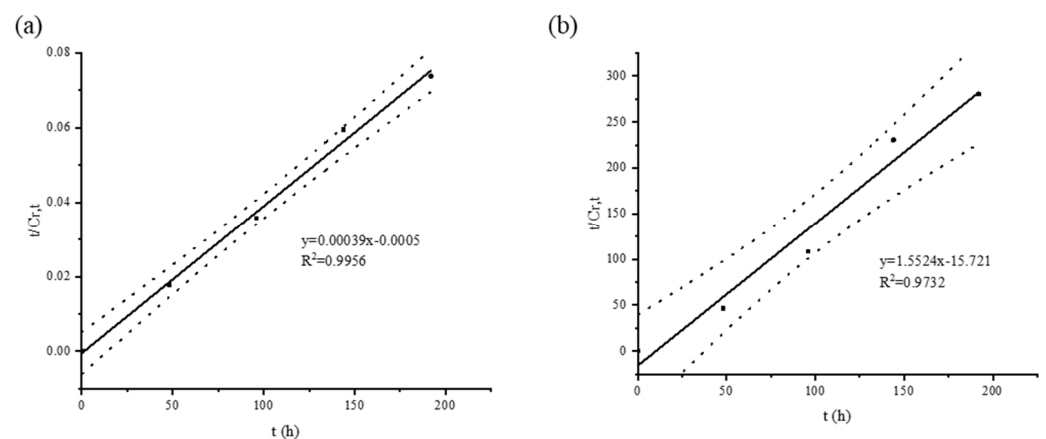


Figure 5. Kinetic models of COD and methylene blue. (a) PSO kinetics model of COD and (b) PSO kinetics model of methylene blue.

3.7. The Mechanism of *S. aquatilis* Treatment of APDW

3.7.1. Biosorption Mechanism of *S. aquatilis* Treatment of APDW

Scanning electron microscopy (SEM) revealed pronounced structural differences between cells grown in BG-11 and APDW (Figure S4). In BG-11, *S. aquatilis* cells appeared spherical, smooth, and actively dividing. In contrast, APDW-exposed cells exhibited sur-

face wrinkling and occasional membrane disruption, indicating environmental stress. Cells formed compact aggregates in APDW, with particulate matter adhering to their surfaces. These aggregates likely result from the secretion of extracellular polymeric substance (EPS) and the abundance of functional groups (carboxyl, hydroxyl, and amino moieties) on the cell wall, facilitating electrostatic and chemical interactions with dye molecules and suspended solids. This structural evidence supports biosorption as an initial and critical step in pollutant removal.

3.7.2. Biotransformation Mechanism of *S. aquatilis* Treatment of APDW

Six samples of *S. aquatilis* were sequenced using the Illumina Novaseq platform. RNA sequencing generated high-quality datasets (average clean reads: 22.02~25.50 million; Q30 > 96%), with strong reproducibility among biological replicates (Figure S5). Comparative transcriptomic analysis identified 912 differentially expressed genes (DEGs), including 471 upregulated and 441 downregulated genes in APDW-treated cells relative to BG-11 controls (Figure S6). This study randomly selected four differentially expressed genes (two upregulated genes and two downregulated genes) for validation analysis to evaluate the accuracy of transcriptome sequencing data (Figure S9).

GO enrichment revealed that downregulated DEGs predominantly involved photosynthesis, proton-transporting ATP synthase, proton channels, and membrane protein complexes. Suppression of these pathways likely restricted toxin influx and reduced energy expenditure on photophosphorylation, reflecting an adaptive stress response (Figure 6). Conversely, upregulated DEGs were significantly enriched in oxidoreductase activity, electron transfer, NAD(P)H generation, glutamate biosynthesis, butanoate metabolism, and organic substance transport. This pattern indicates a metabolic transition toward redox-intensive biodegradation. Enhanced butanoate metabolism, likely derived from glycolysis and lipid oxidation, feeds pyruvate into the TCA cycle, generating abundant NADH and ATP to sustain electron-dependent dye transformation [31]. These findings align with those reported by Chen [32]. The *Anoxybacillus* sp.-degraded azo dye was related to carbohydrate, lipid, and amino acid metabolisms. These metabolisms provided the necessary energy for dye degradation.

Enzymes involved in azo dye degradation, including monooxygenases, methyltransferases, pyrophosphatases, hydrolases, and eight oxidoreductases, were significantly induced (Figures S6 and S7; Table 1), consistent with electron-mediated cleavage and oxidation of phenolic and azo groups [33]. P450 cytochrome-related genes (*gene 1361*) and Mg chelatase-like ATPases were induced, promoting hydroxylation and epoxidation reactions that further facilitate pollutant transformation [34]. Genes encoding glycosyltransferases were upregulated, suggesting enhanced stress sensing and chemical modification of dye molecules to reduce cytotoxicity. Glycosyltransferases can bind to various hetero-substances, transferring sugar groups from donors to dye molecules via catalytic reactions. Moreover, stress-responsive regulators, including histidine kinases, were also induced, reflecting activation of adaptive signaling networks (Table 1). Meanwhile, a modest elevation in malondialdehyde (MDA) levels (Table 2) indicated limited membrane damage, consistent with the observed resilience of *S. aquatilis* under APDW exposure.

In addition, APDW contains substantial carbon-, nitrogen-, and phosphorus-bearing compounds. Genes related to carbohydrate transport and nitrogen metabolism—including 0464, 1631, and 1648—were upregulated (2.1–2.85-fold; Figure S8). These findings suggest that *S. aquatilis* actively assimilates organic nutrients from wastewater, including nitrogen compounds, utilizing them for growth and metabolism. KEGG enrichment further highlighted nitrogen metabolism and atrazine degradation pathways, suggesting functional overlap between heterocyclic compound transformation and azo dye biodegradation.

(Figure S7). Moreover, the ability of *S. aquatilis* to metabolize both organic and inorganic nutrients suggests a versatile response to the diverse components present in APDW. Further confirmation of specific metabolic pathways involved in nutrient assimilation through mass balance will be researched in the future.

Collectively, these results reveal that *S. aquatilis* responds to APDW stress through integrated transcriptional programs that couple energy generation, electron flux, and enzymatic capacity to sustain growth while efficiently degrading dyes and reducing nutrient loads.

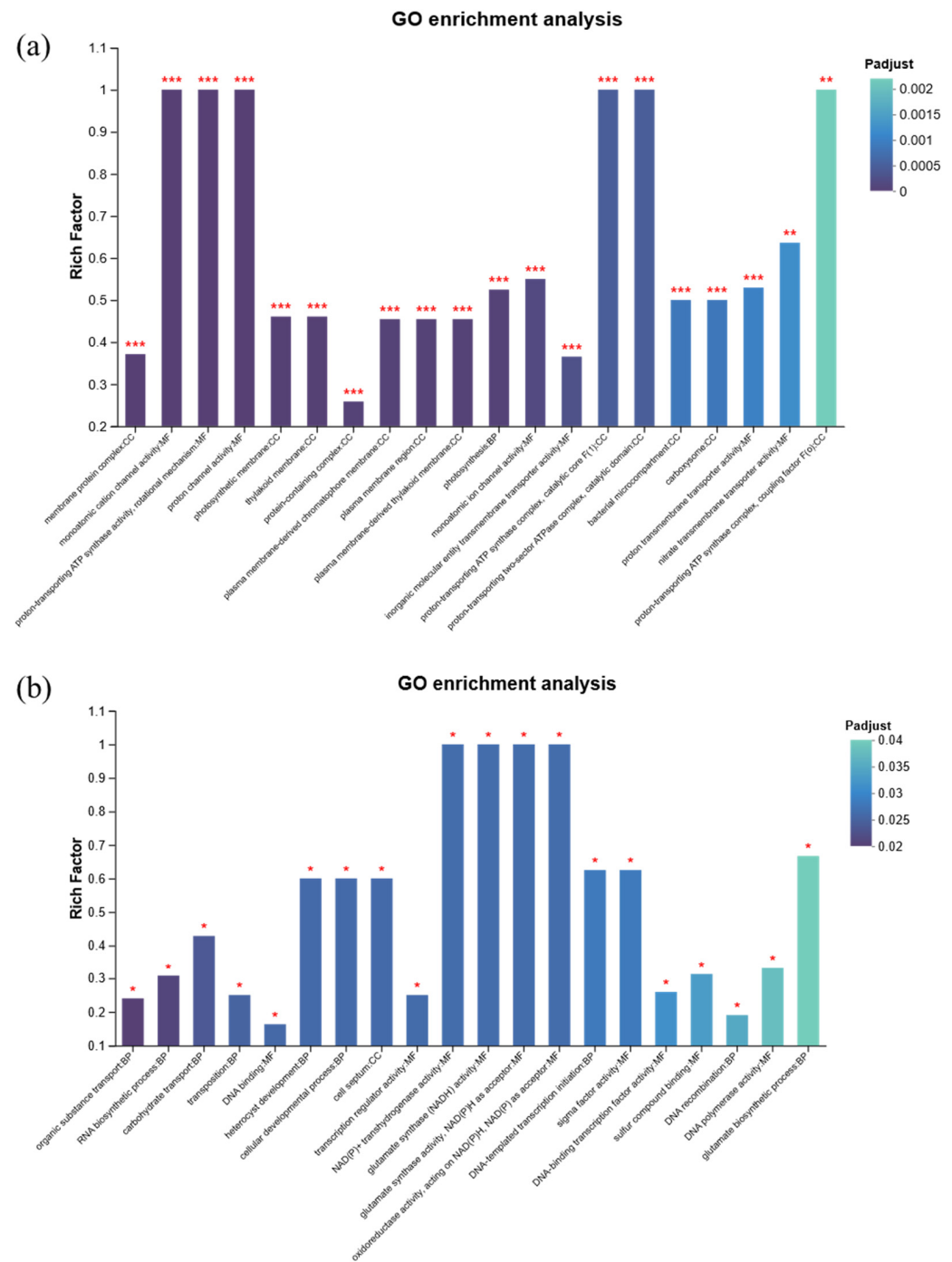


Figure 6. Cont.

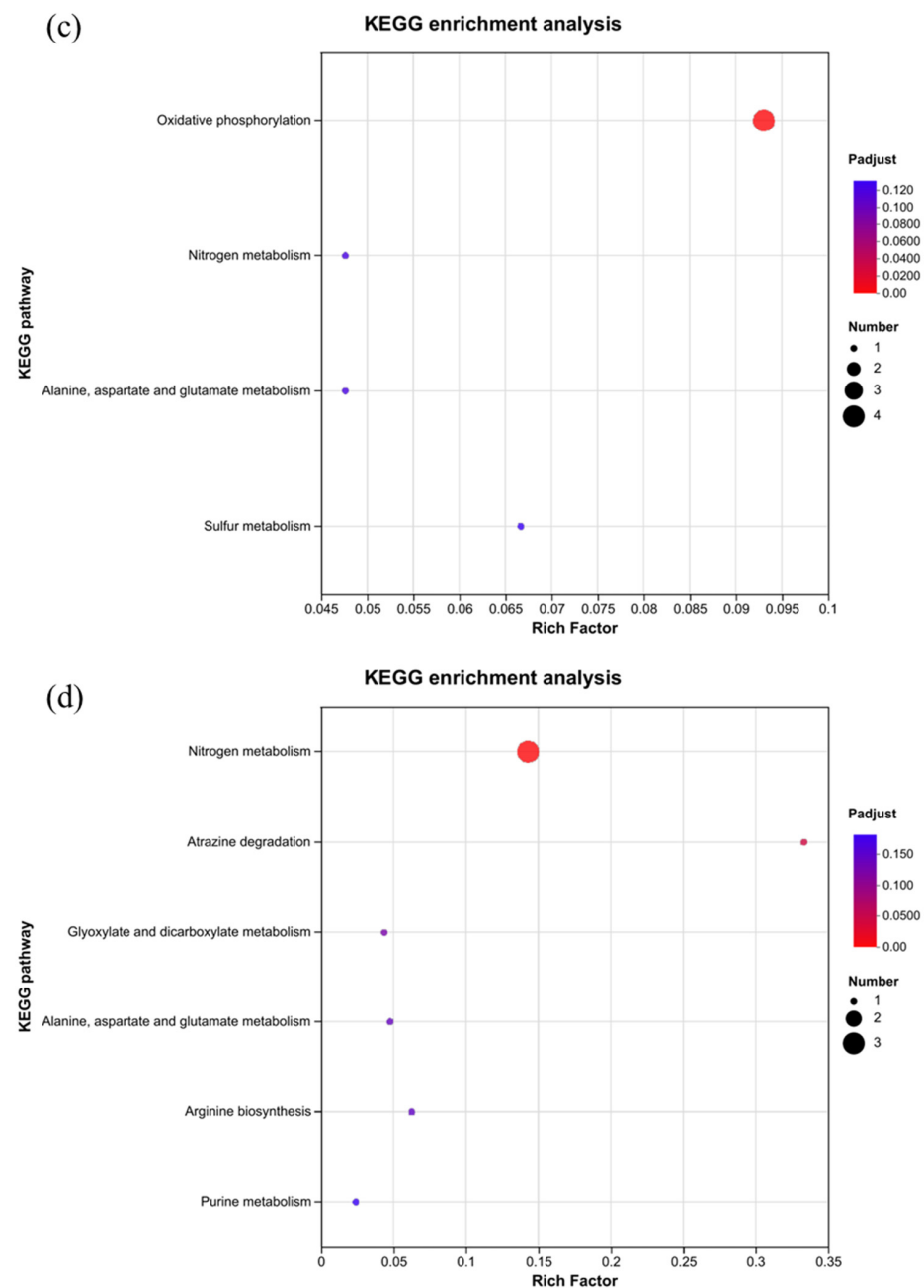


Figure 6. GO and KEGG enrichment of *S. aquatilis* in BG-11 and APDW (a) GO analysis of downregulated DEGs, (b) GO analysis of upregulated DEGs, (c) KEGG enrichment of downregulated DEGs and (d) KEGG enrichment of upregulated DEG. * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$.

Table 1. Expression of genes related to degrading APDW in *S. aquatilis*.

Gene Name (Number)	Gene ID (log2FoldChange)
Oxidoreductase (9)	<i>gene0274</i> (2.23); <i>gene0455</i> (1.02); <i>gene1506</i> (2.24); <i>gene1807</i> (1.24); <i>gene2621</i> (1.43); <i>gene3201</i> (2.19); <i>gene3202</i> (2.67); <i>gene3203</i> (1.62); <i>gene1535</i> (1.05)
Monooxygenase (1)	<i>gene1286</i> (1.48)
Hydrolase (1)	<i>gene3337</i> (1.61)
Methyltransferase (7)	<i>gene1030</i> (1.83); <i>gene1753</i> (1.23); <i>gene2156</i> (1.03); <i>gene2517</i> (1.42); <i>gene2672</i> (1.27); <i>novel0032</i> (1.87)
Aminotransferase (1)	<i>gene1263</i> (2.78)
Cytochrome oxidase (2)	<i>gene0464</i> (2.32); <i>gene2329</i> (5.14)
Pyrophosphatase (2)	<i>gene2050</i> (1.22); <i>gene0720</i> (1.55)

Table 2. The enzyme activities of *S. aquatilis* in APDW.

Enzymes Activities	Experiment Group	Control Group
POD (U/mg prot)	33.457	11.262
SOD (U/mg prot)	167.321	37.954
MDA (nmol/mg prot)	21.031	16.217
T-AOC (μ mol/g)	0.270	0.144

3.7.3. Role of Enzymes of *S. aquatilis* in Degradation Process

Transcriptomic analysis revealed pronounced upregulation of genes encoding antioxidant and oxidoreductive enzymes in *S. aquatilis* following exposure to APDW. To validate these transcriptional responses at the functional level, the enzymatic activities of peroxidase (POD), superoxide dismutase (SOD), and total antioxidant capacity (T-AOC) were quantified in cells cultivated in APDW and BG-11 medium (Table 2). All three activities were significantly elevated in APDW-treated cultures. SOD activity increased more than four-fold relative to the control, indicating a strong oxidative stress response. POD and overall antioxidant capacity were likewise enhanced, reflecting intensified redox turnover under wastewater conditions. The activation of these enzymatic systems likely serves a dual function: maintaining intracellular redox homeostasis under chemical stress and facilitating electron transfer reactions associated with dye transformation.

4. Discussion

This research investigated factors affecting APDW treatment, including the dilution fold of APDW, pH, light intensity, and algae supplementation. In terms of the dilution fold of APDW, *S. aquatilis* showed high tolerance to APDW without the need for extensive dilution or nutrient supplementation. Previous studies indicated that *Chlorella* required wastewater to be diluted four times or even over 10 times for treatment [33], whereas *S. aquatilis* exhibited far higher tolerance than *Chlorella* and other algae species. This high tolerance reduces operational complexity and treatment costs, making it a promising candidate for large-scale remediation of APDW (Table 3). The pH is vital for examining the degradation ability of *S. aquatilis*. *S. aquatilis* demonstrated higher nitrogen consumption at pH 7 compared with alkaline conditions [35]. Given that most dyes in APDW are cationic, electrostatic attraction between positively charged dye molecules and the negatively charged algal surface likely enhances adsorption under neutral conditions. At a higher pH, although the adsorption potential may have increased, cellular growth was impaired, ultimately reducing the overall treatment capacity. Maintaining pH 7 reduces the need for costly pre-treatment processes, further supporting the economic feasibility of this approach. In addition, light intensity and inoculum density further influenced degradation kinetics, reflecting the dependence of remediation on photosynthetically driven redox metabolism. The biomass generated by *S. aquatilis* could be used for multiple high-value applications. The industrial feasibility of such systems depends on several factors, including the costs associated with wastewater dilution, biomass harvesting, and the potential revenue from biomass utilization. Detailed techno-economic analyses will be required for future industrial applications to make the system economically viable on a larger scale. A transition from single-purpose wastewater treatment to resource-recovery systems was suggested. Seed germination assays using mung bean confirmed that treated APDW exhibited reduced phytotoxicity, indicating that biodegradation products were less harmful than the parent dye compounds. This ecological validation strengthens the case for environmental safety and practical reuse of treated effluent. The results were consistent with the Haq research [36].

Table 3. Comparative Summary of Algal-Based Wastewater Treatment Studies.

Algal Strain	Wastewater Type	Tolerance Performance	Reference
<i>S. aquatilis</i>	Actual printing and dyeing wastewater (APDW)	Dilution 2 times (3300 mg/L COD, 590 mg/L TP and 15 mg/L NH ₄ -N)	This research
<i>C. sorokiana</i>	Azo dye wastewater	Diluted 16–200 times (cADWS) concentrations of 112.5, 90, 67.5, 45, 22.5, and 9 mg/L)	[33]
<i>C. sorokiana</i>	Actual printing and dyeing wastewater (APDW)	Ddilution 4 times (200 mg/L COD, 10 mg/L TP and 9 mg/L NH ₄ -N)	[37]
<i>Shewanella</i>	CI Reactive Red 66	25, 50, 100, 150 and 200 mg/L	[38]
<i>Anoxybacillus</i> sp. PDR2	Actual textile effluent	1720–2170 mg/L COD	[39]
<i>A. lipolytica</i>	Congo red	25–125 mg/L Congo red	[40]

Kinetic modeling indicated that dye removal followed pseudo-second-order behavior, suggesting that adsorption rates scale with the square of available binding sites and are, therefore, governed by chemisorption rather than purely physical partitioning. The cell wall of *S. aquatilis* is enriched with imidazole, amino, carboxyl, hydroxyl, and carbonyl functional groups capable of forming electrostatic interactions and hydrogen bonds with dye molecules. This chemically active interface likely initiates contaminant capture, contributing substantially to the observed reductions in COD and chromophoric compounds.

The mechanisms of *S. aquatilis* treating APDW mainly involve biosorption, bioaccumulation, and biodegradation. The various functional groups are present on the surface of *S. aquatilis* cells, such as carboxyl, sulfate, amino, and hydroxyl groups. The negatively charged surface of algae favors binding to dye molecules with a positive charge. Latifa found that –OH and –COOH functional groups of *Fucus spiralis* bind with MB via hydrogen bonding [41]. In addition, Deb elucidated that *Bacillus* sp. secreted extracellular substances such as EPS, promoting the formation of stable cellular aggregates during the cell culture process, which presented synergistic improvement in dye degradation [42]. Many dyes are positively charged, which could combine with the electrons, causing structural changes in dye molecules. The *Shewanella*-CdS facilitated azo dye hydroxylation via electron transfer, influencing the efficiency of dye degradation [43]. Azo bonds were found to be vulnerable to electron attack and azo dye decolorization via extracellular electron transfer [44]. Transcriptomic analysis revealed that *S. aquatilis* in APDW upregulated glycolysis, the tricarboxylic acid cycle, lipid oxidation, and amino acid biosynthesis, indicating elevated generation of NAD(P)H and electrons. Azo bonds (–N=N–), known to be susceptible to electron attack, undergo reductive cleavage and hydroxylation reactions, leading to decolorization and structural modification. The progressive color fading of APDW during treatment is consistent with such electron-mediated disruption of chromophoric systems. The transcriptome results indicated that the nitrogen, phosphorus, and atrazine degradation pathway metabolism genes in *S. aquatilis* were also significantly upregulated. The *Arthrobacter aurescens* TC1 degraded atrazine via dichlorination, hydroxylation, and hydrolysis [45]. The atrazine structures were similar to those of the nitrogen-containing compounds and azo dyes in APDW, indicating that *S. aquatilis* similarly degraded these compounds. Similar conclusions can be drawn for phosphorus pathways. The mechanism of *S. aquatilis* treatment of APDW is depicted in Figure 7. In the future, gene editing technology will be used to overexpress the key biodegradation genes in *S. aquatilis* to obtain biomaterials with higher removal efficiencies.

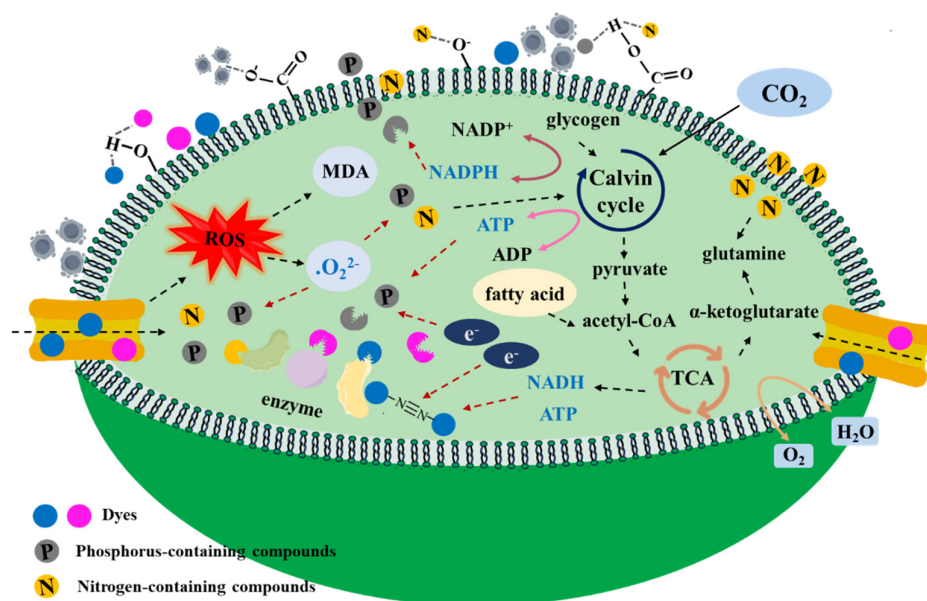


Figure 7. The mechanism of *S. aquatilis*-treated APDW.

Enzymes play a significant role in the degradation process. The transcriptome results suggested that the oxidoreductase could catalyze the reduction of azo bonds ($-N=N-$) to aromatic amine products and provide electrons for dye degradation [46]. The NADH-DCIP reductases transformed reactive Orange 16 into a colorless substance via electrons in *Bacillus* sp., as a marker enzyme in dye degradation [47]. Therefore, *S. aquatilis* used the oxidoreductase to break the nitrogen bonds in dyes and transformed them into aromatic amines, which was consistent with the increasing content of NH_4-N during the later stage of APDW treatment. Enzyme activity assays also validated the transcriptome results. Antioxidant enzymes are crucial in maintaining the stability of cells, enhancing tolerance to environmental stresses, and eliminating the toxicity of phenolic amines in the physiological and metabolic processes of microalgae [48]. POD biodegrades phenol and synthetic dyes [49]. It provides many electrons and has a high redox potential to attack the aminoxyl radical ($-N-O\cdot$) of dye compounds [50]. POD, SOD, and T-AOC were produced during the treatment, facilitated electron transfer, and accelerated the degradation of dyes and other nitrogen and phosphorus compounds. These results suggest that oxidoreductase promotes the degradation of dyes and transforms them into compounds that can promote the growth of *S. aquatilis*, which was consistent with the findings from the transcriptomic analysis. POD and SOD are also crucial in protecting cells against environmental stress [51].

5. Conclusions

This study provides a high-tolerance microalga, *S. aquatilis*, for APDW treatment. The results demonstrated that *S. aquatilis* had potential for APDW treatment. The degradation rates of COD, NH_4-N , and TP in APDW under optimized conditions were 120.27, 0.89, and 9.52 $mg \cdot L^{-1} \cdot d^{-1}$, respectively. Meanwhile, *S. aquatilis* produced several high-value by-products (a 373.08 mg/g lipid yield, 167.85 mg/g of polysaccharide, and 72.05 mg/g of protein). The mechanism of APDW degradation involved bioadsorption, bioaccumulation, and biodegradation facilitated by functional group adsorption, DNA repair pathways, oxidoreductase degradation, and electron transfer to tolerate APDW effectively. Thus, *S. aquatilis* provides an environmentally friendly method to treat APDW, with enormous potential for development. In future studies, gene-editing methods and synergistic bacteria–algae interactions will be employed to investigate the use of *S. aquatilis* in treating wastewater, aiming to enhance treatment efficiency.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/w18101167/s1>, Figure S1: *S. aquatilis* under the microscope (a) and phylogenetic tree of *S. aquatilis* (b); Figure S2: The content of Chlorophyll concentration of *S. aquatilis* in different condition (a) pH, (b) dilution fold of APDW, (c) light intensity, (d) algae supplementation; Figure S3: The change of APDW color during the *S. aquatilis* treatment; Figure S4: The surface morphology of *S. aquatilis* by scanning electron microscopy (SEM) (a,b) *S. aquatilis* in BG-11 medium, (c,d) *S. aquatilis* in APDW; Figure S5: Heat map of inter-sample correlation. Y: *S. aquatilis* after treatment of APDW; D: *S. aquatilis* in BG-11 medium; Figure S6: DEGs volcano in Y and D. Y: *S. aquatilis* after treatment of APDW; D: *S. aquatilis* in BG-11 medium; Figure S7: GO and KEGG enrichment of upregulated DEGs in *S. aquatilis* in BG-11 and APDW (a) GO analysis of upregulated DEGs in oxidoreductive enzymes, (b) GO analysis of upregulated DEGs in nitrogen, (c) KEGG enrichment of upregulated DEGs in oxidoreductive enzymes, (d) KEGG enrichment of upregulated DEGs in nitrogen; Figure S8: (a) Changes in oxidoreductive enzymes genes in *S. aquatilis* (b) Changes in nitrogen genes in *S. aquatilis*; Figure S9: RT-qPCR results for validation of RNA-seq data. (a,b) Down regulated genes. (c,d) Up regulated genes; Table S1: The physical and chemical properties of APDW samples; Table S2: The germination index (%) and root lengths of germinated mung beans; Table S3: Kinetic models of the adsorption of methylene blue and COD and statistical analysis. References [52–56] are cited in the Supplementary Materials.

Author Contributions: Conceptualization, L.W.; Methodology, X.Q. and L.W.; Investigation, X.Q. and L.W.; Writing—original draft, X.Q. and L.W.; Writing—review and editing, X.Q., M.G., Y.S., S.W., S.G., X.X., X.L., X.W., Q.F., J.Z., L.W. and G.W.; Supervision, G.W. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by Key R&D Program of Shandong Province, China (No. 2025CXGC010618; 2025LZGC037); Key Technology Research Projects in Qingdao City, China (No. 25-1-1-gjgg-54-hy); project 25-1-1-187-zyyd-jch supported by Qingdao Natural Science Foundation; National Natural Science Foundation of China (No. 42276146); Ministry of Agriculture and Rural Affairs of the People's Republic of China, China [grant numbers CARS-50] and the Research Fund for the Taishan Scholar Project of Shandong Province, China [grant numbers tspd 20210316].

Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Materials. Further inquiries can be directed to the corresponding authors.

Conflicts of Interest: Author Quancheng Fan was employed by the company Yuyue Home Textile Co., Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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