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Efficient Solar-Powered Bioremediation of Hexavalent Chromium in Contaminated Waters by *Chlorella* sp. MQ-1

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Abstract: Microalgae are known for their efficient removal of hexavalent chromium (Cr(VI)) through biosorption and bioaccumulation, yet the subsequent release of Cr(VI) upon cell death remains a challenge. The reduction of Cr(VI) to the less toxic trivalent chromium [Cr(III)] is another critical remediation strategy that mitigates the risk of Cr(VI) re-release, but research on microalgal reduction of Cr(VI) is scarce. In this study, a microalgal strain designated as MQ-1 was isolated from chromium-contaminated mine effluent, demonstrating the capability to tolerate and remove Cr(VI). Phylogenetic analysis revealed that MQ-1 is closely related to the genus *Chlorella*; hence, it is classified as *Chlorella* sp. MQ-1. This strain exhibited robust growth at Cr(VI) concentrations below 2 mg/L, achieving a removal rate higher than 82% for initial Cr(VI) concentrations between 0.5 and 1 mg/L after a 5-day incubation period. Mechanistic studies revealed that MQ-1 promoted the removal of Cr(VI) mainly through intracellular bioreduction and bioaccumulation processes, in which more than 60% of Cr(VI) was reduced to the less toxic Cr(III) and stocked in the cells. A two-stage cultivation strategy, involving initial biomass accumulation followed by Cr(VI) treatment, significantly enhanced the removal efficiency, which was further accelerated under illuminated conditions. Notably, MQ-1 cultures with initial OD_{680} values of 4 and 6 accomplished 84.28% and 91.31% Cr(VI) removal from 2 mg/L solutions, respectively, within 30 hours under light exposure. These findings highlight the potential of MQ-1 to utilize renewable solar energy to reduce Cr(VI) and to mitigate the risk of its re-release into the environment. This characteristic positions MQ-1 as a potentially sustainable and cost-effective solution for Cr(VI) remediation and suggests its significant potential for large-scale implementation in bioremediation strategies aimed at Cr(VI)-contaminated waters.

Keywords: hexavalent chromium; microalgae; Cr(VI) remediation; bioreduction; bioaccumulation

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1. Introduction

Heavy metals, particularly in their ionic forms, pose significant environmental and health risks due to their high toxicity and the ease with which they can be absorbed by living organisms [1]. Among these, hexavalent chromium [Cr(VI)] is of particular concern

due to its widespread presence in industrial wastewaters from sectors such as electroplating, leather tanning, and textile manufacturing [1–3]. The United States Environmental Protection Agency (USEPA) has classified Cr(VI) as a Group A human carcinogen, owing to its high solubility, strong oxidizing properties, and potential for inducing severe genetic damage, including chromosomal aberrations, as well as acute health effects such as gastrointestinal distress and hemorrhage [4,5]. In response to these hazards, stringent regulations have been established, limiting the total chromium discharge to surface waters to 2 mg/L, with a specific limit of 0.05 mg/L for Cr(VI) [6].

To comply with these stringent emission standards, conventional physico-chemical methods, including chemical reduction [7,8], electrodialysis [9], and activated carbon adsorption [10,11], are widely used for the removal of Cr(VI) from wastewater. However, these traditional methods come with several drawbacks, such as high consumption of chemicals and energy, harsh operational conditions, and a notable decrease in efficiency when the Cr(VI) concentration falls below 10 mg/L [12,13]. In contrast, certain live microorganisms, such as microalgae, have shown remarkable potential for heavy metal removal, suggesting a more economical, environmentally friendly, and sustainable approach for treating Cr(VI)-contaminated wastewater [13–15]. Previous studies have employed various autotrophic microalgal strains for the removal of Cr(VI), including *Chlorella vulgaris* [16,17], *Scenedesmus* sp. [15,18], *Chlamydomonas reinhardtii* [19], and other species (all with removal efficiencies above 40%) [14,20]. Microalgae's adaptability to a wide range of environmental conditions, including varying temperatures, pH levels, and ion concentrations, along with intrinsic tolerances to different heavy metals, further underscores their potential as effective bioremediation agents [21].

The primary mechanisms by which microalgae remove Cr(VI) are biosorption and bioaccumulation [22]. Biosorption involves the binding of metal ions to the cell surface, irrespective of whether the biomass is alive or dead, through the attachment to negatively charged functional groups on microalgal polysaccharides or via ion exchange with cations on the cell wall [23]. Bioaccumulation, on the other hand, involves the active transport of toxic metals into the microalgal cells, driven by the concentration gradient across the cell membrane [24]. Although these mechanisms show promising results, a significant challenge remains: once Cr(VI) is adsorbed or bioaccumulated by the microalgae, it remains associated with the biomass and can be re-released upon the death of the microalgae. For complete chromium removal from water, the microalgal biomass must be harvested and removed, adding to the overall cost and complexity of the treatment process and making large-scale application more challenging [23].

Considering that the toxicity of Cr(VI) is approximately 100 times that of Cr(III), the reduction of Cr(VI) to Cr(III) is also an important means of chromium pollution remediation [25]. Even if the reduced Cr(III) is re-released into the water, its toxicity is significantly lower. However, research on this process has primarily focused on bacteria [26,27], with only a few reports having mentioned the chromium-reducing ability of microalgae [28,29]. Unlike bacteria, which require a carbon source for growth and Cr(VI) reduction, microalgae can utilize light energy for growth and reproduction, making them more suitable for large-scale applications. Considering the significant role of chromium-reducing ability in chromium pollution remediation and the current research gap in microalgae chromium reduction, it is advantageous to explore the screening of microalgae with chromium-reducing capabilities and to investigate their underlying mechanisms.

In this study, we report the isolation of a microalgal strain, designated MQ-1, from chromium-contaminated mine effluent. This strain exhibits both high tolerance and efficient removal capacity for Cr(VI). Through the analysis of chromium distribution and valence states, we found that the removal of Cr(VI) by MQ-1 is predominantly mediated by bioreduction and bioaccumulation, with most of the absorbed Cr(VI) being reduced to Cr(III) and subsequently accumulated within the cells. Furthermore, we investigated the effect of initial biomass concentration and light conditions on the Cr(VI) removal efficiency. Our results show that higher biomass concentrations lead to increased removal

rates and efficiencies, with intracellular chromium mainly present in the form of Cr(III). This suggests that the removal of Cr(VI) by MQ-1 at high biomass concentrations is largely dependent on the bioreduction of Cr(VI) to Cr(III). Moreover, the removal efficiency under light conditions was significantly higher than under dark conditions, indicating that the bioreduction of Cr(VI) by MQ-1 is likely light-dependent. These findings highlight the potential of microalgae, specifically strain MQ-1, as a viable and sustainable solution for the treatment of Cr(VI)-contaminated waters.

2. Materials and Methods

2.1. Isolation of MQ-1

Samples were collected from chromium-contaminated mine effluent in Meizhou, China. A 2 mL aliquot of the sample was added to 50 mL of sterile modified BG-11 medium [30] supplemented with 2 g/L Tris base as a pH buffer (BG-11-T), with an initial pH of 7 and a Cr(VI) concentration of 2 mg/L. The inoculated medium was incubated under continuous illumination at 8000 lx from LED lamps in a phototrophic cultivation chamber for 5 days, followed by a transfer every 5 days with a 5% inoculum. Microalgae were isolated using the dilution plating method. Plates were incubated at 30 °C under continuous illumination at 4000 lx for 8 days. Single colonies were streaked on BG-11 solid plates and incubated under the same conditions until pure cultures of microalgae MQ-1 were obtained.

2.2. Identification of MQ-1

Pure cultures of MQ-1 were grown in BG-11-T medium until the logarithmic phase. A 20 mL sample of logarithmic phase algal culture was centrifuged at 8000 r/min for 5 min to collect the cells. The cells were ground in liquid nitrogen and DNA was extracted using the Ezup column fungal genomic DNA extraction kit (Shanghai Bioengineering Co., Ltd., Shanghai, China). The 18S rRNA gene was amplified using universal primers NS1 and NS6 (Shanghai Bioengineering Co., Ltd.) [31]. The PCR amplification system consisted of 12.5 μL of 2×Tap mix, and the PCR program was as follows: initial denaturation at 95 °C for 5 min; 30 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s; final extension at 72 °C for 10 min. The PCR products were analyzed by agarose gel electrophoresis to confirm the presence of the target band, and the products were sequenced by Bioengineering (Shanghai) Co., Ltd. The 18S rDNA sequence of the MQ-1 strain has been deposited in the NCBI database with the accession number PQ474650. To establish its phylogenetic position, we conducted a BLAST search against the NCBI database and subsequently constructed a phylogenetic tree using the neighbour-joining method in MEGA software (version 11.0). The robustness of the tree was evaluated with bootstrap support values obtained from 1000 replicates [32].

2.3. Scanning Electron Microscopy (SEM) Analysis of MQ-1

A 2 mL sample of microalgal culture was centrifuged in a centrifuge tube at 6000 r/min for 5 min. The pellet was subsequently resuspended in 1% phosphate-buffered saline (PBS) and centrifuged once more to remove the supernatant. The cells were fixed overnight at 4 °C in a 2.5% glutaraldehyde solution prepared in 0.1 M phosphate buffer (pH 7.4). After fixation, the samples were rinsed with 0.15% glutaraldehyde in phosphate buffer to remove excess fixative. Dehydration was carried out through an ethanol gradient, increasing in concentration from 30% to 100% at intervals of 15 min for each step. Following dehydration, the samples were infiltrated with tert-butanol to replace the ethanol, allowed to stand for 15 min, and then centrifuged at 8000 r/min for 5 min to pellet the cells. The resulting microalgal pellets were subjected to freeze-drying for 2 h to remove residual moisture. Prior to SEM analysis, the samples were sputter-coated with a thin layer of gold to enhance conductivity and facilitate imaging. The coated samples were then examined using a Phenom Pro X scanning electron microscope [33].

2.4. Cr(VI) Removal Capacity of MQ-1

Seed cultures in BG-11-T medium were prepared by inoculating with 5% of the stock culture and grown for 6 days under an illumination intensity of 8000 lx. BG-11-T medium was prepared with the addition of $K_2Cr_2O_7$ stock solution to achieve Cr(VI) concentrations of 0, 0.5, 1, 2, and 4 mg/L. Logarithmic phase MQ-1 seed culture was inoculated into the medium at a 5% volume ratio and incubated at 30 °C under continuous illumination at 8000 lx for 5 days. Daily samples were collected for the measurement of optical density at 680 nm (OD_{680}). These samples were subsequently filtered through a 0.45 μm membrane, and the resulting filtrate was utilized for the determination of Cr(VI) concentration.

2.5. Cr(VI) Removal Capacity of Different Microalgae

Microalgae strains *Chlorella vulgaris* FACHB-2338 (F2338), *Chlorella* sp. FACHB-9 (F9), *Scenedesmus* sp. FACHB-1420 (F1420), and *Parachlorella kessleri* FACHB-4 (F4) were obtained from the Freshwater Algae Collection of the Chinese Academy of Sciences, Wuhan, China. These strains were cultivated in BG-11-T medium, and cultures in the logarithmic growth phase were inoculated into fresh BG-11-T medium containing Cr(VI) concentrations of 0.5, 1, 2, and 4 mg/L to achieve an initial OD_{680} = 0.2. Cultures were incubated at 30 °C under continuous illumination at 8000 lx for 5 days, with OD_{680} and Cr(VI) concentrations being measured.

2.6. Cr Content in MQ-1 Extracellular Polymeric Substances (EPSs)

The EPS of microalgae was divided into soluble EPS (S-EPS) and bound EPS (B-EPS). S-EPS and B-EPS were extracted using the method described by Chen et al. [34]. After the MQ-1 culture solution was filtered through a 0.45 μm membrane, the algae cells remaining on the filter membrane were resuspended in 0.6% sodium chloride solution to maintain osmotic equilibrium and cell integrity. The resuspended cells were centrifuged at 4 °C, 4000× *g* for 15 min to collect the supernatant, which was filtered through a 0.45 μm cellulose acetate membrane. The filtrate was considered as the S-EPS. The remaining cells were resuspended in 0.6% sodium chloride solution, re-centrifuged at 10,000× *g* for 15 min at 4 °C, and the supernatant, after filtration, was considered the B-EPS. The Cr(VI) and Cr(III) content in S-EPS and B-EPS were measured after extraction.

2.7. Intracellular Cr Content in MQ-1

After extraction of S-EPS and B-EPS, MQ-1 algal cells were resuspended in distilled water to the original culture volume, and cells were disrupted using a grinder instrument (MP Fastprep 24 5G, Santa Ana, CA, USA) with the addition of 1 mm steel beads [35]. The disrupted cell suspension was filtered through a 0.45 μm filter, and the filtrate was used for Cr content determination.

2.8. Cr(VI) Removal Capacity of MQ-1 Biomass

MQ-1 was cultured in BG-11-T medium to achieve an OD_{680} of 2, 4, and 6. Algal cells were collected and resuspended in distilled water with the Cr(VI) concentration of 2 mg/L. The suspensions were incubated under 8000 lx illumination and in the dark at 30 °C, and OD_{680} and Cr(VI) concentration were monitored. The intracellular and extracellular Cr(VI) concentrations and Cr(III) concentrations were measured after 30 h.

2.9. Determination of Cr(VI) and Cr(III)

The determination of Cr(VI) was performed according to the diphenylcarbazide spectrophotometric method [36]. A 500 μL aliquot of the filtrate and 1.1 mL of ddH_2O were transferred to a 2 mL colorimetric tube. To this, 100 μL of 1:1 diluted sulfuric acid and 100 μL of 1:1 diluted phosphoric acid solution were added, and the mixture was thoroughly mixed. Subsequently, 200 μL of the color-developing agent was added, and the solution was mixed well to ensure complete reaction of Cr(VI) with the color-developing agent. After 5–10 min, the absorbance was measured at 540 nm using a spectrophotometer, with

water as the reference to eliminate the influence of the solvent itself on the absorbance. The absorbance measured in the blank test was subtracted, and the Cr(VI) content in the sample was calculated using the standard curve. The removal rate of Cr(VI) was calculated using the following equation: Removal rate = (C0 − Ci)/C0 × 100%, where C0 and Ci are the initial and final Cr(VI) concentrations in mg/L, respectively [37].

The determination of total chromium was performed according to the potassium permanganate oxidation-diphenylcarbazide spectrophotometric method [38]. An appropriate amount of the filtrate sample was placed in a 2 mL colorimetric tube, and the pH was adjusted to neutral with ammonium hydroxide or sulfuric acid solution to ensure the subsequent reaction. Then, 100 μL of 1:1 diluted sulfuric acid solution and 100 μL of 1:1 diluted phosphoric acid solution were added and mixed well. Then, 2 drops of 40 g/L potassium permanganate solution were added. If the purple color faded, potassium permanganate solution was added to maintain the purple color. And then 100 μL of 200 g/L urea solution was added and mixed well to reduce the excess potassium permanganate. Sodium nitrite solution was added dropwise until the purple color of potassium permanganate faded to remove the excess potassium permanganate, which could interfere with the subsequent absorbance measurement. Then, 200 μL of the color-developing agent was added (ddH_2O was added to 2 mL). After 5–10 min, the absorbance was measured at 540 nm, with water as the reference to eliminate the influence of the solvent itself on the absorbance. The absorbance measured in the blank test was subtracted, and the total chromium content in the sample was calculated using the standard curve.

The content of Cr(III) was calculated as the difference between the total chromium content and the Cr(VI) content.

3. Statistical Analysis

Each experiment was performed in triplicate. One-way ANOVA with SPSS 24.0 was used to perform statistical analysis of the data.

4. Results and Discussion

4.1. Screening and Characterization of MQ-1 Strains with Cr(VI) Tolerance Ability

A microalgae with Cr(VI) tolerance, designated as MQ-1, was isolated from chromium-contaminated mine effluent through targeted enrichment and purification. NCBI BLAST comparison revealed that the 18S rRNA sequence of MQ-1 showed the highest similarity (99.70%) to *Chlorella sorokiniana* SAG 211-8k. To further confirm the taxonomic position of MQ-1, a phylogenetic tree was constructed using the 18S rRNA gene sequences of MQ-1 and several other highly similar algal strains (Figure 1b). Phylogenetic analysis confirmed the close relation of MQ-1 with the genus *Chlorella*, as it clustered with *Chlorella* spp. within the same clade; hence, it is classified as *Chlorella* sp. MQ-1 (Figure 1b). Scanning electron microscopy (SEM) was employed to examine the morphology of the MQ-1 microalgal cells. The SEM images revealed that the cells were spherical in shape with a diameter ranging from approximately 2.5 to 4 μm (Figure 1a). The surface of the cells appeared relatively rough and had extracellular secretions similar to EPS structures (Figure 1a). These EPS structures may play a role in the adsorption and accumulation of Cr(VI).

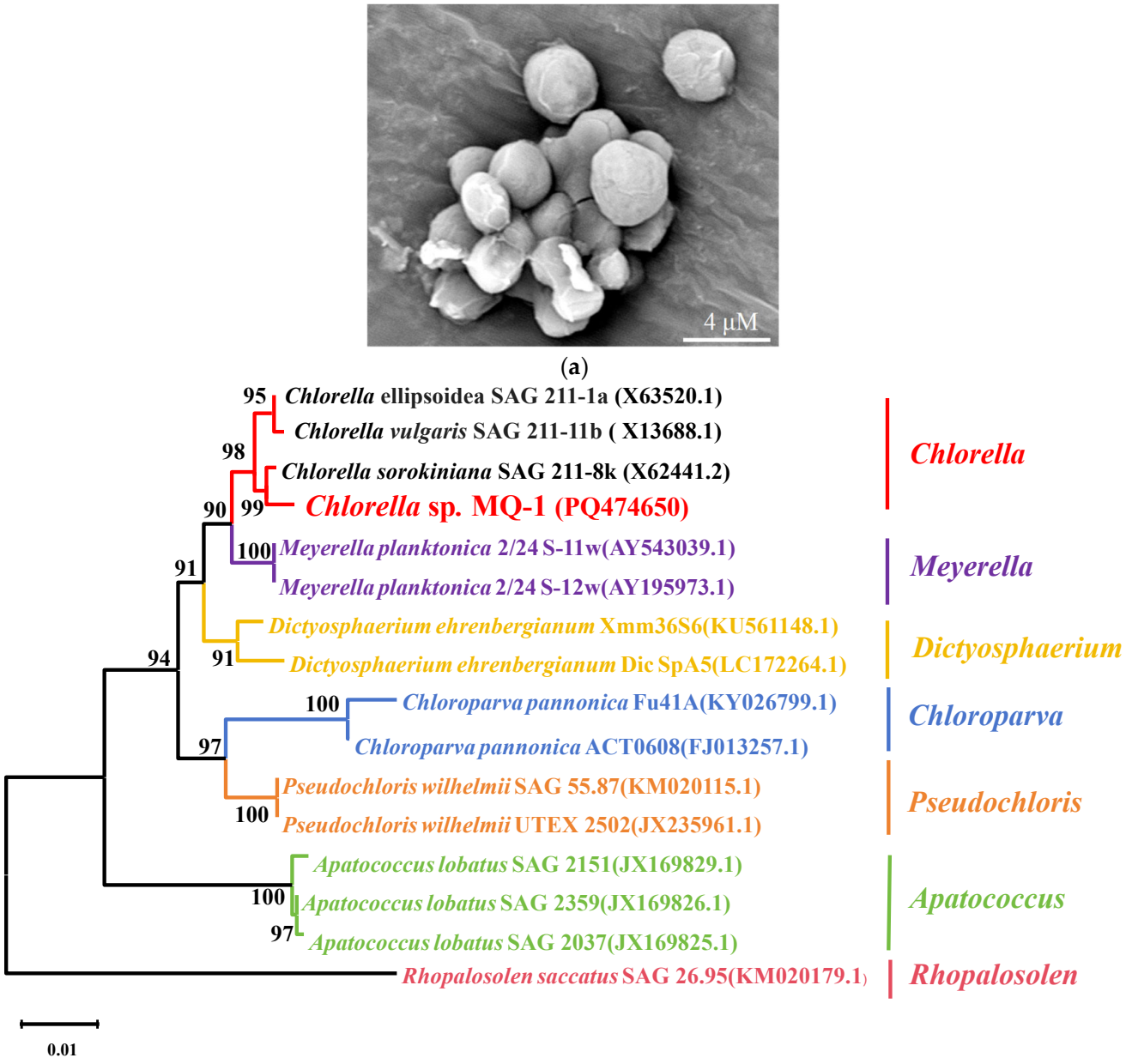

Figure 1. SEM characterization of MQ-1 (**a**) and its phylogenetic tree based on 18S rRNA gene sequences (**b**).

4.2. Removal Efficiency of Cr(VI) by MQ-1

The growth of MQ-1 under various initial Cr(VI) concentrations is illustrated in Figure 2a. When the initial Cr(VI) concentration was between 0.5 and 1 mg/L, the growth of MQ-1 was slightly inhibited (with the final biomass being approximately 80% of the control group). At an initial Cr(VI) concentration of 2 mg/L, the growth of MQ-1 was significantly suppressed, with the final biomass reaching only 44.44% of that of the control group, indicating that the EC50 value of Cr(VI) (the concentration that resulted in a 50% reduction in growth) was estimated to be approximately 2 mg/L. At an initial Cr(VI) concentration of 4 mg/L, the growth of MQ-1 was severely inhibited, but it was still able to grow slowly. After 5 days of cultivation, the biomass of MQ-1 could increase twofold; thus, it is considered that MQ-1 could tolerate a Cr(VI) concentration of 4 mg/L.

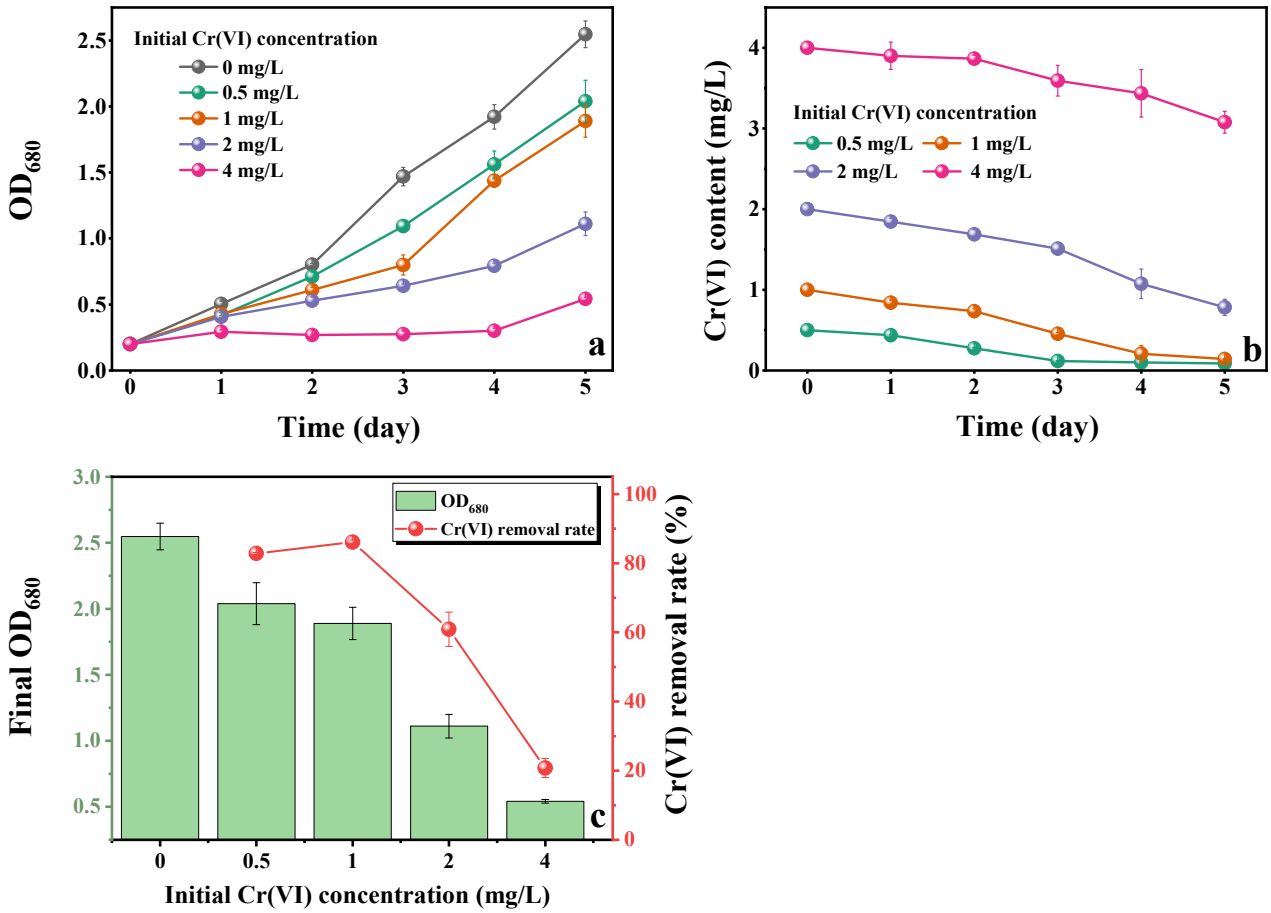

Figure 2. Growth of MQ-1 under varying initial concentrations of Cr(VI) (**a**,**c**) and its Cr(VI) removal efficiency (**b**).

MQ-1 demonstrated a certain capacity for Cr(VI) removal across the range of initial concentrations tested (Figure 2b). The removal efficiency was found to be inversely proportional to the growth of the microalgae (Figure 2c). At initial Cr(VI) concentrations of 0.5 and 1 mg/L, MQ-1 exhibited relatively high Cr(VI) removal efficiencies, with final removal rates of 82.82% and 86.13%, respectively (Figure 2c). The enhanced Cr(VI) removal at an initial concentration of 1 mg/L compared to 0.5 mg/L may be attributed to a lower removal efficiency of MQ-1 at the extremely lower concentration. For instance, at an initial Cr(VI) concentration of 0.5 mg/L, the Cr(VI) concentration on day 3 was 0.12 mg/L, corresponding to a removal rate of 76.35%, and by day 5, the Cr(VI) concentration had decreased to 0.088 mg/L, with a removal rate of 82.86%, marking an increase of only 6.01% (Figure 2b). This suggests that when the Cr(VI) concentration approaches 0.1 mg/L, the removal efficiency of MQ-1 significantly declines. In summary, the suitable Cr(VI) concentration range for MQ-1 growth and treatment is between 0.5 and 2 mg/L. Within this concentration range, MQ-1 could achieve significant Cr(VI) removal while ensuring its own growth.

4.3. Removal Efficiency of Cr(VI) by Different Microalgae

Microalgae MQ-1, alongside other documented microalgae capable of thriving in Cr(VI)-polluted environments and mitigating Cr(VI) contamination, is detailed in Table 1. Given the requirement to proliferate under Cr(VI)-enriched conditions, the Cr(VI) concentrations addressed by these microalgae are typically modest, seldom exceeding 1–5 mg/L. Furthermore, due to the inhibitory effects of chromium contamination, the growth duration for microalgae often spans over ten days. In contrast, MQ-1, when subjected to Cr(VI) concentrations between 1 and 2 mg/L, can attain Cr(VI) removal rates between 86.13% and

60.90% after five days of cultivation. Collectively, these observations suggest that MQ-1 possesses certain advantages in terms of growth kinetics in the presence of Cr(VI) and the efficiency of Cr(VI) remediation.

Nevertheless, the culture media, growth conditions, and analytical techniques employed across various studies are not uniform, and the intrinsic Cr(VI) removal capabilities of microalgae can fluctuate markedly under differing cultivation parameters [39,40]. To rigorously establish the Cr(VI) removal proficiency of MQ-1, a selection of representative Cr(VI)-mitigating microalgae were grown under uniform experimental conditions. As depicted in Figure 3, with an initial OD_{680} of 0.2, MQ-1 demonstrated superior growth velocity across all tested Cr(VI) concentrations. At a hexavalent chromium concentration of 0.5 mg/L, all other tested microalgal strains were able to grow and showed the ability to remove Cr(VI), with removal rates between 43.38% and 67.34% after 5 days of cultivation; however, this removal rate was lower than the 83.52% of MQ-1 (Figure 3a). At a Cr(VI) concentration of 1 mg/L, MQ-1 not only grew robustly but also achieved a remarkable Cr(VI) removal rate of 88.63%, whereas the growth of the other microalgae strains was substantially impeded, culminating in final Cr(VI) removal rates below 40% (Figure 3b). At a more challenging Cr(VI) concentration of 2 mg/L, MQ-1 still managed to maintain a Cr(VI) removal rate of 62.94%, while the other microalgae strains hardly grew, with final Cr(VI) removal rates plummeting below 20% (Figure 3c). At the highest Cr(VI) concentration of 4 mg/L, all assessed microalgae strains exhibited diminished Cr(VI) removal capabilities, attributable to restricted growth (Figure 3d). These findings underscore the enhanced Cr(VI) tolerance and removal potential of MQ-1 in comparison to other microalgae. Certainly, since MQ-1 was isolated from a Cr(VI)-containing medium, it has already undergone stress acclimation to Cr(VI) pollution. Therefore, it is possible that MQ-1 may have pre-adapted to Cr(VI)-polluted environments, which could account for its superior performance compared to other strains that have not undergone similar environmental acclimation.

Table 1. Removal efficiency of Cr(VI) by different microalgae and their mechanisms.

Microalgae	Cr(VI) ppm	Removal (%)	Time	Mechanism	References
Chlorella minutissima	1.7	92%	12 day	Biosorption and bioaccumulation	[37]
Chlorella thermophilia	0.2–0.6	50–65%	15 day	Biosorption and bioaccumulation	[41]
Desmodesmus subspicatus	1	95%	15 day	Biosorption and bioaccumulation	[42]
Scenedesmus dimorphus	0.1	94.24%	5 day	Biosorption and bioaccumulation	[43]
Parachlorella kessleri R-3	5	81.50%	9 day	Biosorption and bioaccumulation	[20]
Spirulina platensis	5	65.20%	28 day	Biosorption and bioaccumulation	[44]
Chlorella vulgaris FACHB-2338	0.5	67.34%	5 day	-	Present study
Chlorella sp. FACHB-9	0.5	43.38%	5 day	-	Present study
Scenedesmus sp.FACHB-1420	0.5	48.44%	5 day	-	Present study
Parachlorella kessleri FACHB-4	0.5	65.03%	5 day	-	Present study
Chlorella sp. MQ-1(growth)	1–2	86.13–60.90%	5 day	Bioreduction and bioaccumulation	Present study
Chlorella sp. MQ-1(high biomass)	2	91.31%	1.5 day	Bioreduction and bioaccumulation	Present study

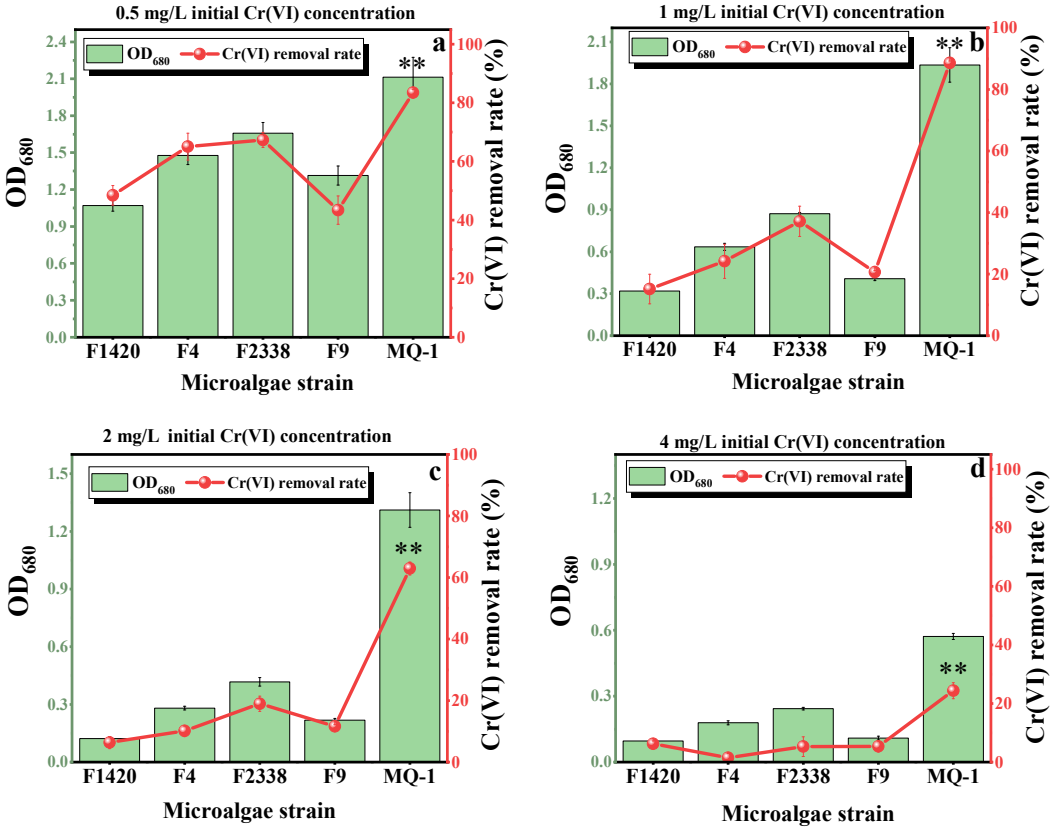

Figure 3. The growth and Cr(VI) removal efficiency of various microalgae at Cr(VI) concentrations of 0.5 mg/L (**a**), 1 mg/L (**b**), 2 mg/L (**c**), and 4 mg/L (**d**). F1420: *Scenedesmus* sp. FACHB-1420; F4: *Parachlorella kessleri* FACHB-4; F2338: *Chlorella vulgaris* FACHB-2338; F9: *Chlorella* sp. FACHB-9. ** the Cr(VI) removal rate of MQ-1 is significantly different from that of other microalgae strains ($p < 0.05$).

4.4. Mechanism of Cr(VI) Removal by MQ-1

The potential mechanisms of Cr(VI) removal by microorganisms are multifaceted and can be broadly categorized as biosorption, bioreduction, and bioaccumulation [45]. Biosorption: this mechanism involves the adsorption of Cr(VI) onto the surface of microbial cells or their EPS. Bioreduction: Reduces Cr(VI) to the less toxic and more stable form of Cr(III) through enzymatic reactions or non-enzymatic reactions. The reduced Cr(III) is then often adsorbed onto cell surfaces or released outside the cell. Bioaccumulation: This refers to the active transport of Cr(VI) across the cell membrane and its subsequent accumulation within the cell. Once inside the cell, Cr(VI) can be converted to Cr(III) and may be sequestered into vacuoles or bound to intracellular components, thereby reducing its bioavailability and toxicity [45].

To elucidate the Cr(VI) removal mechanisms of MQ-1, we assessed the concentrations of Cr(VI) and Cr(III) in different compartments of MQ-1 after 5 days of growth in medium containing 1 mg/L Cr(VI). As shown in Figure 4a, the extracellular concentration of Cr(VI) in MQ-1 was 0.14 mg/L, with a Cr(III) concentration of 0.097 mg/L, totaling 0.235 mg/L and accounting for 23.53% of the total Cr element content. This indicates that the majority of the Cr elements were adsorbed or absorbed by MQ-1. By extracting the extracellular EPS of MQ-1, we measured a total Cr(VI) concentration of 0.022 mg/L and a Cr(III) concentration of 0.068 mg/L in the S-EPS and B-EPS, totaling 0.09 mg/L and representing 9.03% of the total Cr element content. Typically, microalgae secrete increased amounts of EPS when exposed to toxic substances [46]. These EPSs can mitigate the toxic effects on microalgae by adsorbing the toxins [47]. The low concentration of Cr(VI) in MQ-1's EPS suggests that the

removal of Cr(VI) by MQ-1 is not solely a biosorption process dependent on EPS, indicating a more complex mechanism at play in the detoxification process.

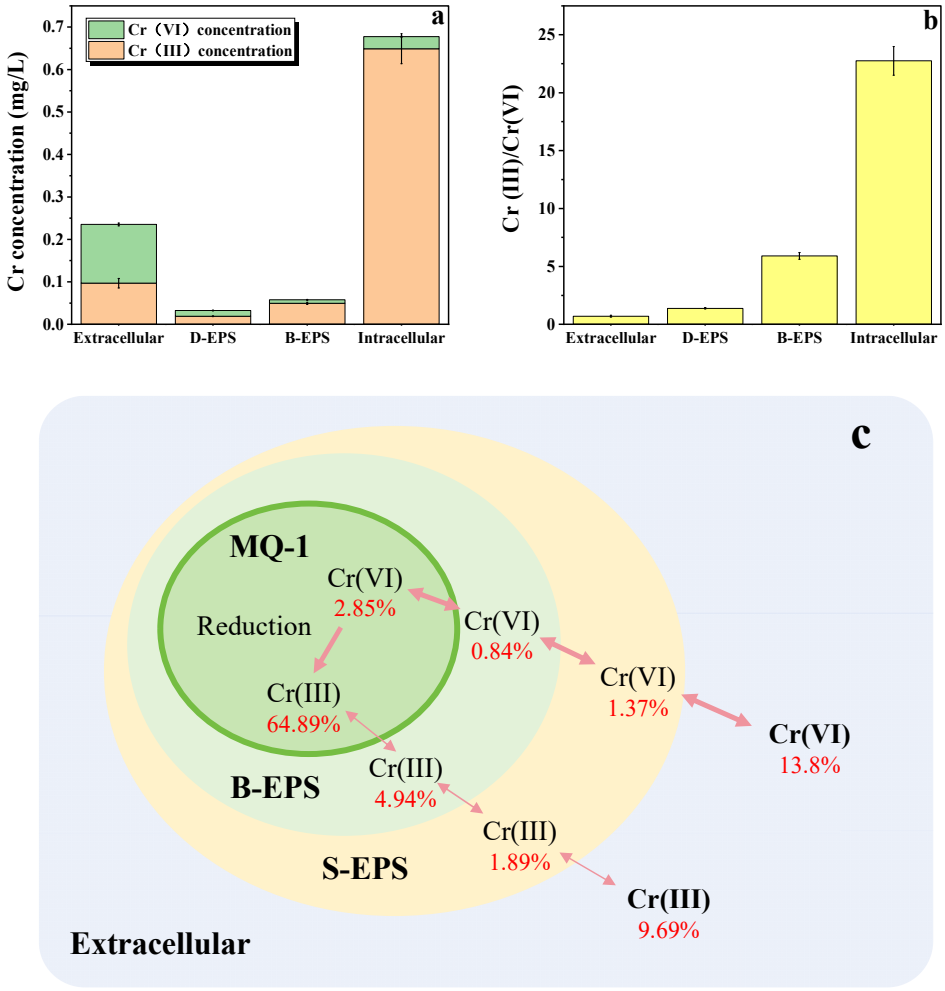

Figure 4. Quantification and distribution of Cr(III) and Cr(VI) within distinct cellular compartments of MQ-1 (**a**), along with the ratio (**b**) and percentage composition (**c**).

Upon disrupting the cells of MQ-1, the intracellular concentrations of Cr(VI) and Cr(III) were found to be 0.028 mg/L and 0.649 mg/L, respectively, totaling 0.68 mg/L and constituting 67.74% of the total Cr element content. This suggests that a substantial portion of Cr(VI) was absorbed by MQ-1, converted to Cr(III), and thereafter amassed within the cell, thereby confirming that MQ-1's elimination of Cr(VI) encompasses both bioaccumulation and bioreduction processes.

Upon determining the Cr(III)/Cr(VI) ratio across various cellular compartments of MQ-1, it was observed that the ratio peaks intracellularly, followed by B-EPS, S-EPS, and the extracellular environment (Figure 4b). This finding unequivocally points to the intracellular space as the primary site for Cr(VI) reduction by MQ-1, where Cr(VI) is reduced to Cr(III) and subsequently incrementally released into the extracellular milieu. Synthesizing these observations, the fundamental mechanism for Cr(VI) removal by MQ-1 can be delineated as follows: MQ-1 transports Cr(VI) into cells through passive/active transport mechanisms, which is then followed by the bioreductive conversion of Cr(VI) to Cr(III). Significantly, the majority of the generated Cr(III) is sequestered within the cell, while a portion is progressively released to the cell's exterior (Figure 4c).

As introduced in the introduction, the primary mechanisms by which microalgae remove Cr(VI) are biosorption and bioaccumulation, with Cr remaining in the form of Cr(VI) either inside the cells or in the extracellular polymeric substances (EPSs). To completely

eliminate Cr(VI) from the water, the collection of microalgae would be necessary, which entails a substantial cost. In contrast, MQ-1 exhibits a robust Cr(VI) reduction capability. Given that Cr(VI) is approximately 100 times more toxic than Cr(III), the reduction of Cr(VI) to Cr(III) effectively detoxifies the chromium pollution and could be considered as the initial completion of the purification of water contaminated with Cr(VI). This process negates the need for the separation of microalgal biomass. Of course, the Cr(III) within MQ-1 cells could also be separated from the water body through physical means such as biomass harvesting. Moreover, due to the lower toxicity of Cr(III), the operation is relatively safer and more manageable.

4.5. Removal of Cr(VI) by MQ-1 Biomass

In the domain of microalgal Cr(VI) removal research, a substantial body of work has adopted a two-stage methodology [48]. The initial phase is dedicated to cultivating microalgae to achieve a predefined biomass, which is subsequently utilized in the second phase for Cr(VI) removal experiments [49]. This approach circumvents the inhibitory effects of Cr(VI) on microalgal growth, thereby enhancing the efficiency of Cr(VI) removal. Leveraging this strategy, our study extends the investigation to assess the efficacy of a two-stage process for Cr(VI) removal by MQ-1. We initially cultivated MQ-1 to attain varying biomass levels (OD_{680} = 2, 4, 6) and then evaluated their Cr(VI) removal performance under both dark and illuminated conditions.

As presented in Figure 5, the two-stage method significantly enhanced both the rate and efficiency of Cr(VI) removal by MQ-1. Previously, a 60.9% removal rate was achieved by MQ-1 cultured for 5 days in 2 mg/L Cr(VI). In contrast, MQ-1 with an initial OD_{680} of 2 reached a 63.72% removal rate under illuminated conditions within a mere 30 h (Figure 5a). Furthermore, increasing the initial biomass of MQ-1 resulted in a further improvement in Cr(VI) removal efficiency, with initial OD_{680} values of 4 and 6 attaining removal rates of 84.28% and 91.31%, respectively (Figure 5a). Consistent with the results of other microalgae studies, the two-step approach for Cr(VI) removal with MQ-1 allows MQ-1 to grow rapidly and produce higher biomass in the first step through a chromium-free environment, which improves the efficiency of Cr(VI) removal in the subsequent steps [41,42,48]. However, the method is more costly due to the need for pre-cultivation of MQ-1 biomass, making it suitable for centralized large-scale treatment of Cr(VI) pollution, such as in wastewater treatment plants. Conversely, the one-step method involves inoculating MQ-1directly into low Cr(VI)-contaminated environments. Although growth may be constrained, MQ-1 still effective in removing Cr(VI), making this approach suitable for treating water bodies with low levels of Cr(VI) contamination.

The Cr(VI) removal rate under dark conditions was markedly lower than that under illuminated conditions, further suggesting that Cr(VI) removal by MQ-1 is not solely attributed to biosorption (Figure 5a). Measurements of Cr(III) and Cr(VI) within and outside the cells revealed that the removed Cr(VI) predominantly existed as Cr(III) within the cells of MQ-1 (Figure 5d), indicating that the removal of Cr(VI) by MQ-1 is primarily reliant on bioreduction and bioaccumulation processes.

Even under dark conditions, the removal of Cr(VI) by MQ-1 is predominantly dependent on intracellular reduction. The majority of the removed Cr(VI) is stored within the cells as Cr(III) (Figure 5d). This suggests that MQ-1 cells contain stored nutrients that could be utilized for the synthesis of reductive materials, which in turn reduce Cr(VI). Thus, even in the absence of light, the cells retain a certain capacity for Cr(VI) absorption and reduction.

At low biomass levels (initial OD_{680} = 2), the removal rates of Cr(VI) by MQ-1 in dark and illuminated conditions were 15.48% and 63.72%, respectively, demonstrating a significant difference. While at high biomass levels (initial OD_{680} = 6), the removal rates in dark and illuminated conditions were 76.37% and 91.31%, respectively, indicating a much smaller difference. This suggests that at low biomass levels, the nutrients contained within the MQ-1 biomass are insufficient to meet the demand for Cr(VI) reduction, requiring light

energy to synthesize a sufficient amount of reductive materials for Cr(VI) reduction. At high biomass levels, the reductive materials synthesized by MQ-1 could largely satisfy the demand for Cr(VI) reduction, although illumination still enhances and accelerates this process.

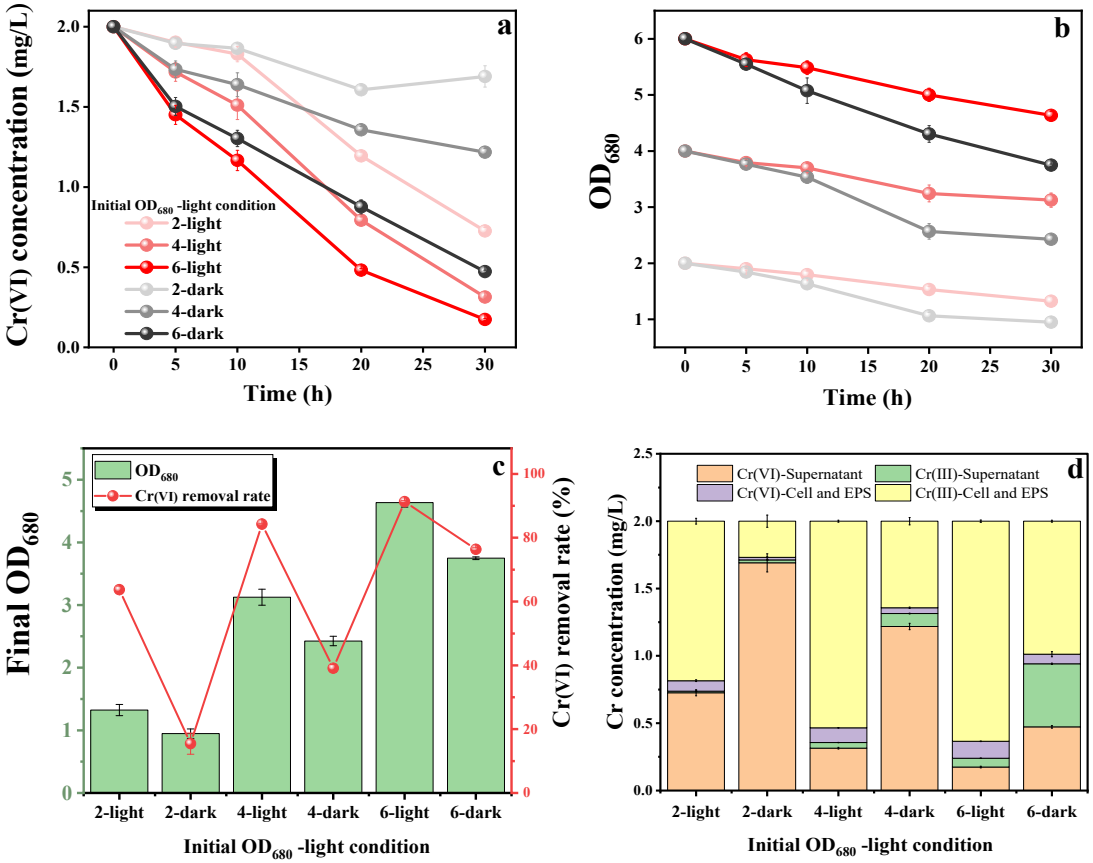

Figure 5. The Cr(VI) concentration (**a**), biomass (**b**), final removal rate (**c**), and the content of Cr elements inside and outside the cells (**d**) during the Cr(VI) removal experiment with MQ-1 of different initial biomass.

Under dark conditions, the content of Cr(III) in the extracellular supernatant was higher than under illuminated conditions (Figure 5d). This may be attributed to the fact that under dark conditions, MQ-1 cells, limited by energy, exhibit a significantly higher cell mortality rate compared to illuminated conditions (Figure 5b), leading to the release of Cr(III) that was originally accumulated within the cells into the extracellular environment.

In conclusion, these findings demonstrate that the biomass of MQ-1 could be effectively employed for the proficient elimination of Cr(VI). Notably, the primary mode of Cr(VI) removal by MQ-1 involves intracellular reduction, which could be intensified by exposure to light. This implies that MQ-1 possesses the capability to harness renewable solar energy for the reduction and bioaccumulation of Cr(VI). In contrast to other microalgae that primarily rely on adsorption for Cr(VI) removal, MQ-1 has the distinct advantage of converting Cr(VI) into the less harmful Cr(III), thereby mitigating the risk of Cr(VI) being re-released into the environment. Additionally, when compared to bacteria capable of Cr(VI) reduction, the utilization of solar energy by MQ-1 offers substantial cost savings and augments its potential for large-scale implementation.

5. Conclusions

In this study, a *Chlorella* strain, designated as MQ-1, with the capability to remove Cr(VI) was isolated from chromium-contaminated mine effluent through targeted enrich-

ment and purification. MQ-1 exhibited the ability to grow in Cr(VI) concentrations below 4 mg/L, with removal efficiencies reaching 82.82% and 86.13% at initial concentrations of 0.5 to 1 mg/L after a 5-day cultivation period. Further mechanistic studies revealed that over 60% of the total Cr content was present in the form of Cr(III) within the MQ-1 cells, indicating that the primary pathways for Cr(VI) removal by MQ-1 are bioreduction and bioaccumulation. Employing a two-stage cultivation approach, which entails initial biomass accumulation followed by Cr(VI) treatment, significantly enhanced the Cr(VI) removal efficiency of MQ-1. The process was further accelerated under illuminated conditions, suggesting that MQ-1 has the potential to utilize solar energy for the bioreduction and bioaccumulation of Cr(VI). This capability not only reduces operational costs but also enhances the potential for the large-scale application of MQ-1 in Cr(VI) remediation.

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