

Photoreduction of chromium(VI) in the presence of algae, *Chlorella vulgaris*

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Abstract

In this thesis, the photochemical reduction of hexavalent chromium Cr(VI) in the presence of algae, *Chlorella vulgaris*, was investigated under the irradiation of metal halide lamps ($\lambda = 365$ nm, 250 W). The affecting factors of photochemical reduction were studied in detail, such as exposure time, initial Cr(VI) concentration, initial algae concentration and pH. The rate of Cr(VI) photochemical reduction increased with algae concentration increasing, exposure time increasing, initial Cr(VI) concentration decreasing and the decrease of pH. When pH increased to 6, the rate of Cr(VI) photochemical reduction nearly vanished. When initial Cr(VI) concentration ranged from 0.4 to 1.0 mg L⁻¹ and initial algae concentration ranged from ABS_{algae} (the absorbency of algae) = 0.025 to ABS_{algae} = 0.180, According to the results of kinetic analyses, the kinetic equation of Cr(VI) photochemical reduction in aqueous solution with algae under 250 W metal halide lamps was $V_0 = kC_0^{0.1718} A_{\text{algae}}^{0.5235}$ (C_0 was initial concentration of Cr(VI); A_{algae} was initial concentration of algae) under the condition of pH 4.

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Keywords: Photoreduction; *Chlorella vulgaris*; Cr(VI); Kinetic analyses

1. Introduction

Hexavalent chromium (Cr(VI)) is a priority pollutant and is associated with the development of cancer and various chronic health disorders including organ damage, dermatitis and respiratory impairment. While, trivalent chromium (Cr(III)) is much less toxic than Cr(VI). The chemical reduction of Cr(VI) by various methods is known to dramatically decrease the toxicity and bioavailability of this metal [1].

The prevention of heavy metal contamination in aquatic environments is often performed by conventional methods, which include the addition of chemical for metal precipitation or exchange resins to bind them. The use of microbial biomass to remove metal ions from aqueous environment has gained considerable interest as a potential alternative method to chemical treatments [2].

The observation of Kiber and Helz [3] that sunlight can initiate chromium(VI) reduction in natural waters has motivated research on chromium photoreduction [4,5]. Since chromium(VI) is not substantially reduced by direct photochem-

ical reactions, the photo-initiated electron transfer must occur via some intermediates, main metals, such as iron(II) and copper(I). Many microorganisms of several genera can biosorb while utilizing a wide range of substrates at near neutral pH [6]. Hence, biological processes may provide an alternative to the conventional technique for Cr(VI) removal. Jean-Francois et al. [7] indicate that senescent algae could photoproduce some reductive radicals that induce photodegradation of organic pollutants. It is possible that some oxidative radicals photoproduced from senescent algae can induce metal photoreduction.

However, little work has been reported on the role of algae on the photoreduction of Cr(VI) in aqueous medium. Given that algae are a significant component of environmental system, algae may play very important role in the photochemical transformation of metals in aqueous media. This work helps us to understand the photochemical behavior of heavy metal chromium in the presence of algae.

2. Experimental section

2.1. Chemicals and reagents

All reagents used in this work were analytical grade and used without any further purification. K₂Cr₂O₇ was used as Cr(VI)

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source. The water employed in the irradiation experiments was doubly distilled. The pH of the aqueous solutions was adjusted with HCl or NaOH.

2.2. Algae

The algae used in the experiments were *Chlorella vulgaris* obtained from the Wuhan Hydrobiology Institute of Chinese Academy of Sciences (Wuhan, PR China). The algae were grown in axenic culture medium at 25 °C using a 24 h light cycle in a culturing room equipped with constant temperature air-conditioner. The room light intensity was 2000 lux). The axenic culture medium (0.25 g NaNO₃, 0.075 g K₂HPO₄·3H₂O, 0.175 g KH₂PO₄, 0.025 g NaCl, 0.005 g FeCl₃·6H₂O, 0.1 g Fe-EDTA, 0.075 g MgSO₄·7H₂O, 0.025 g CaCl₂·H₂O, 0.286 mg H₃BO₃, 0.181 mg MnCl₂·4H₂O, 0.022 mg ZnSO₄·7H₂O, 0.0079 mg CuSO₄·5H₂O, 0.0039 mg (NH₄)₆Mo₇O₂₄·4H₂O, 0.5 mL soil extract in 1 L of distilled water) was adjusted to pH 7.0 by 0.1 M Na₂CO₃. Under the condition the algae were growing in a logarithmic growth phase and the density of algae was high (normally 12–14 days), the algae were taken for use in experiments after being washed.

To remove colloidal ferric hydroxide particles that might have been adsorbed on the algae cells, a modified version of the procedure [8] was used in experiments. This procedure involved washing the cells by gentle agitation for 30 min with 0.01 M aqueous ascorbic acid adjusted to pH 3.0. Then the algae were washed with double-distilled water three times. The cell counting was carried out under inverted microscope at 400× and the density of algae (cells L⁻¹) was calculated. Thus, the algae were prepared for subsequent use. Different concentrations of algae were gained through diluting washed algae with double-distilled water.

2.3. Photoreduction reaction procedure

Irradiation experiments were carried out in a cylindrical reactor (20 cm length, 10 cm diameter, 1.0 cm wall thickness), with a 250 W metal halide lamp ($\lambda \geq 365$ nm, Changzhou Shangzi Lamp Co. Ltd., China) placed in cooling trap for maintaining constant temperature at 20 ± 2 °C by water circulation. The cylindrical reactor was not sealed in the uppermost part. The cylindrical reactor was placed in a box. HCl and NaOH were used to adjust the pH values of solutions. The cylindrical reactor containing solutions was kept in the dark before and after irradiation. A desired amount of K₂Cr₂O₇ and harvested algae were added into the reactor. The reaction mixture solutions containing algae inside the cylindrical reactor were maintained in suspension by means of purging 99.999% nitrogen gas. At regular intervals, a 10 mL aliquot of the suspension was withdrawn and through centrifugation. The determined Cr(VI) concentration was compared with that of a blank filtrate from an identical suspension kept in the dark. The photobiological activity was evaluated by the decrease of Cr(VI) concentration after irradiation.

2.4. Analyses

Samples were withdrawn from the cylindrical reactor at proper intervals (e.g. 0.5, 1.0 h) by a 10 mL glass tube. Before analysis the samples were centrifuged at 3500 rpm for 20 min, and the supernatant fraction was analyzed for the remaining Cr(VI). Hexavalent chromium was determined by colorimetric technique with diphenylcarbazide (DPC) in acid solution as described in the standard methods (APHA, 1993) for the examination of water and wastewater. The absorbance was measured at 540 nm with a 722 spectrophotometer. The standard methods detection limit was 0.004 mg L⁻¹. The calibration equation for Cr(VI) was $ABS_{Cr(VI)} = 1.99C_{Cr(VI)}$, $R = 0.99948$ ($n = 6$, $R_c = 0.97406$), where $C_{Cr(VI)}$ was the concentration of Cr(VI) in the range of 0.04–0.2 mg L⁻¹. All vitreous apparatus were dipped in HNO₃/water (the volume ratio was 1:1) overnight.

Error. All the experiments were triplicate. The results presented were the mean values with a total error less than 5%.

3. Results and discussion

3.1. ABS_{algae} versus algae concentration change

The algae concentrations were determined by a 722 spectrophotometer. Fig. 1 showed ABS_{algae} versus algae concentration change. The calibration equation for algae was $n(10^9) = 0.63 + 36.49ABS_{algae}$, $R = 0.9974$ ($n = 5$, $R_c = 0.9912$, $p = 0.001$), where the algae concentrations were in the range of 8.0×10^8 to 2.25×10^{10} cells L⁻¹. One sets Dixon tests were done to evaluate the data quality, but there was no distinct variation in one sets Dixon tests. All vitreous apparatus were dipped in HNO₃/water (the volume ratio was 1:1) overnight.

3.2. Control experiments

In order to investigate the effects of algae on the photoreduction of Cr(VI), a blank test on irradiated reaction solution containing Cr(VI) of 1.0 mg L⁻¹ concentration under continuous purging nitrogen gas without algae was carried out. As

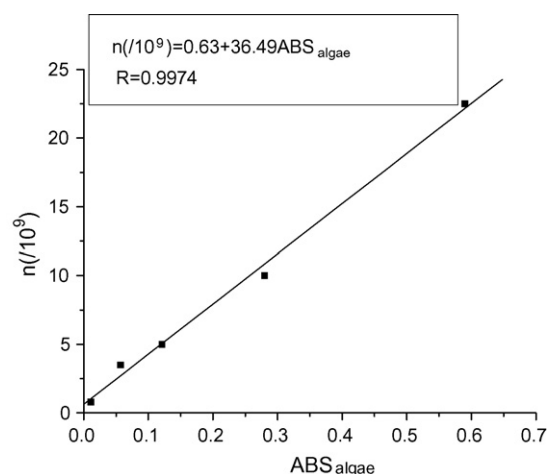


Fig. 1. ABS_{algae} vs. algae concentrations change.

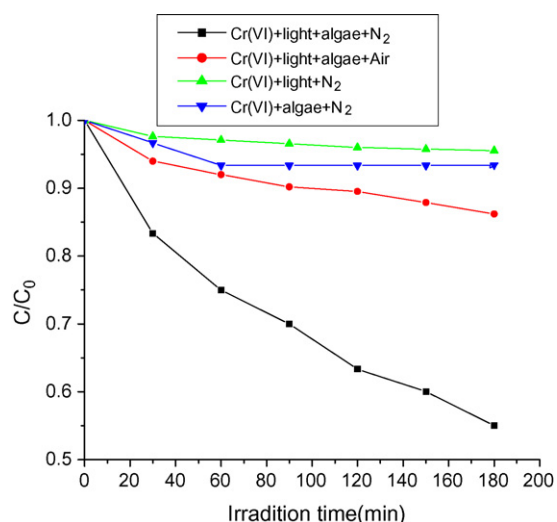


Fig. 2. Effects on the photoreduction of Cr(VI) with irradiation time ($C_0 = 1 \text{ mg L}^{-1}$, pH 4, $\text{ABS}_{\text{algae}} = 0.18$).

shown in Fig. 2, the results showed that the homogeneous photo-biological reaction of Cr(VI) after 180 min irradiation was small to be negligible, which was in agree with a previous report [9]. Another blank test on a reaction solution containing Cr(VI) of similar concentration as above with algae under purging nitrogen gas without irradiation was also carried out. A decrease of Cr(VI) concentration also could be negligible. And, still some tests on reaction solutions containing Cr(VI) concentration 1.0 mg L^{-1} with algae under purging nitrogen gas (or air) with irradiation were carried out. The experimental data also showed that a greater photoreduction was observed under purging N_2 compared to that under purging air.

Algae cell walls, mainly containing polysaccharides, proteins and lipids, offer many functional groups, which have been shown to sequester metal ions [10,11]. In addition, the cell wall structures of algae contain a large quantity of hydroxyproline and rich glycoprotein, with arabinose, mannose, galactose, and glucose being the predominant sugars [12]. The functional groups and surface properties of algae that were illuminated with metal halide lamp have a higher level of performance for reduction.

In our previous study, some free electrons and small dissolved organic matter could be induced in aqueous solution with algae. The results suggested that algae had an important influence on the environmental photoreduction of Cr(VI). The rate of photoreduction increased with increasing light exposure time and with increasing algae concentration. As could be seen, Cr(VI) photoreduction appeared to slowly decrease over time. The light irradiation of algae could have important consequences for its subsequent photoreductive reactivity, including a decrease in its ability to further participate in photoreductive reactions. Natural algae with a large number of chlorophylls will be able to absorb some photons, which increases the likelihood of photoreductive reactivity resulting in the transformation of excited species. However, as irradiation continues over time, the light irradiation of algae chlorophylls leads to less light absorbance due to the destruction of certain chlorophylls that would render it less reactive.

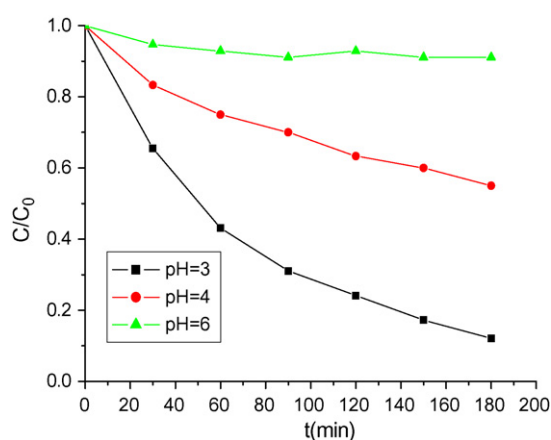


Fig. 3. PH effect on photoreduction of Cr(VI) solution with algae ($\text{ABS}_{\text{algae}} = 0.200$, $C_0 = 1 \text{ mg L}^{-1}$).

3.3. Effect of pH on the photoreduction of Cr(VI) in aqueous solution with algae

The photoreduction experiment was performed in Cr(VI) aqueous solutions at several pH values between 3 and 6. As shown in Fig. 3, there was a decrease in the photoreduction efficiency of Cr(VI) with increasing pH. At pH 3, up to 87.93% of Hg(II) was removed from the suspension after 180 min light irradiation. At pH 3, only 9.0% Hg(II) was removed from the suspension after 180 min light irradiation. Effect of pH on the photoreduction of Cr(VI) demonstrates that pH could play an important role in the photochemical reduction of Cr(VI) in aqueous solution with algae. There were two reasons. First, the redox potenti(al) of Cr(VI) increases with increasing pH and it is easy to be reduced. The reason of pH effect is due to the formation of different Cr(VI) complexes (HCrO_4^- versus CrO_4^{2-}), the chart of Cr(VI) conformation distribution is shown in the paper [13]. In contrast to Cr(III), Cr(VI) is highly soluble. At low concentrations, Cr(VI) is present in water as diprotic chromic acid (H_2CrO_4 , $\text{p}K_{\text{a}1} = 0.81$, $\text{p}K_{\text{a}2} = 6.49$). Thus, in natural water supplies two Cr(VI) oxyanion species predominate, monovalent HCrO_4^- below pH 6.5 and divalent CrO_4^{2-} above pH 6.5. An additional Cr(VI) species, dichromate ($\text{Cr}_2\text{O}_7^{2-}$), predominates at concentrations greater than 1 g L^{-1} [13]. Redox potential of HCrO_4^- is higher than that of CrO_4^{2-} , and easy to be reduced. And second, the algae releases more free electrons and acidic organic matters during the processes of irradiation on acid condition.

3.4. Effects of algae concentration on the photoreduction of Cr(VI)

To test the effects of algae on the photoreduction of Cr(VI), suspensions of algae at three initial concentrations, $\text{ABS}_{\text{algae}} = 0.025$, 0.100 and 0.180, at pH 4.0, were illuminated under metal halide lamps up to 180 min. As shown in Fig. 4, the rate of Cr(VI) photochemical reduction increased with algae concentration increasing. After 180 min irradiation, 45% of Cr(VI) was removed from the suspension when the initial algal concentration was $\text{ABS}_{\text{algae}} = 0.180$; 35% of Cr(VI)

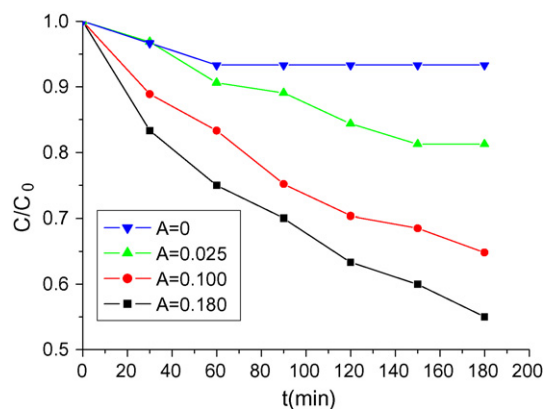


Fig. 4. Photoreduction of Cr(VI) in aqueous solution with different concentrations of algae ($C_0 = 1 \text{ mg L}^{-1}$, pH 4).

disappeared at initial algal concentration $\text{ABS}_{\text{algae}} = 0.100$ and 18.9% of Cr(VI) was removed from the suspension when the initial algal concentration was $\text{ABS}_{\text{algae}} = 0.025$. A higher algal concentration results in a faster photoproduction of free electrons and small dissolved organic matters. The dissolved organic matters, like humic and fulvic acid, can serve as photosensitizers and accelerate the photoreduction of Cr(VI) [14–17].

3.5. Effects of initial concentrations of Cr(VI) on the photoreduction of Cr(VI) in algal suspensions

The influence of initial Cr(VI) concentration on the photoreduction of Cr(VI) with the algae was examined by irradiating the suspensions of algae at pH 4.0 with initial concentrations (C_0) of Cr(VI) at 1.0, 0.8, 0.4 mg L^{-1} , respectively. The experiments were conducted to study effects of different concentrations of initial Cr(VI) on Cr(VI) photoreduction. As shown in Fig. 5, metal halide lamps (250 W) could evidently induce the photoreduction of Cr(VI) in aqueous solution with algae. The photoreduction rate of low concentration Cr(VI) decreased faster than that of the high concentration Cr(VI) in aqueous solution with algae. Under the condition, with initial concentration (C_0) of Cr(VI) increasing, the reduction rate decreased.

3.6. Kinetics analysis

Fig. 4 data were fitted by an equation $A = A_0 + a e^{-t/b}$ with the initial concentrations of suspensions of algae ranging from $\text{ABS}_{\text{algae}} = 0.025$ to $\text{ABS}_{\text{algae}} = 0.200$, at pH = 4.0. As shown in Table 1, the rate of Cr(VI) photochemical reduction increased with algae concentration increasing. When the initial photoreduction rates as listed in Table 1 were plotted as a function of

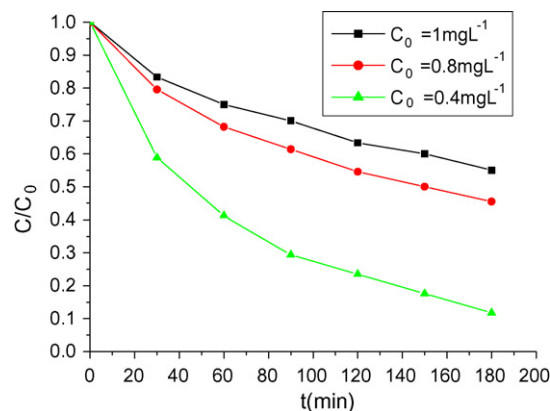


Fig. 5. Effects of initial concentrations of Cr(VI) on the photoreduction of Cr(VI) in algal suspensions ($\text{ABS}_{\text{algae}} = 0.200$, pH 4).

Table 1

Kinetics analysis of initial concentrations of algae affecting the photoreduction of Cr(VI) ($C_0 = 1 \text{ mg L}^{-1}$, pH 4)

$\text{ABS}_{\text{algae}}$	R	$A = A_0 + a e^{-t/b}$	$V_0 = a/b$
0.200	0.99218	$0.73 + 0.75 e^{-t/94.5}$	0.0079
0.085	0.99507	$0.76 + 0.59 e^{-t/113.3}$	0.0052
0.025	0.98026	$1.09 + 0.52 e^{-t/184.3}$	0.0028

$R_c = 0.9507$, $n = 7$, $\alpha = 0.001$.

Table 2

Kinetics analysis of initial concentrations of Cr(VI) affecting the photoreduction of Cr(VI) ($\text{ABS}_{\text{algae}} = 0.200$, pH 4)

C_0 (mg L^{-1})	R	$A = A_0 + a e^{-t/b}$	$V_0 = a/b$
1.0	0.99218	$0.73 + 0.75 e^{-t/94.5}$	0.0079
0.8	0.99643	$0.44 + 0.65 e^{-t/82.9}$	0.0078
0.4	0.99521	$0.049 + 0.37 e^{-t/54.3}$	0.0068

$R_c = 0.9507$, $n = 7$, $\alpha = 0.001$.

initial concentration of algae, a linear relationship was achieved. Under the condition the initial photoreduction rate of Cr(VI) could be expressed by $\ln V_0 = -3.953 + 0.5235 \ln A_{\text{algae}}$ with a correlation coefficient $R = 0.9997$. Because the initial photoreduction rate plotted as a function of initial concentration of algae could also be expressed by the equation $V_0 = k_1 A_{\text{algae}}^b$, the initial photoreduction rate of Cr(VI) under the condition could be expressed by the equation $V_0 = 0.0192 A_{\text{algae}}^{0.5235}$. Fig. 5 data were fitted by the equation $A = A_0 + a e^{-t/b}$ with the initial concentrations of Cr(VI) ranging from 0.4 to 1.0 mg L^{-1} , at pH 4.0, as shown in Table 2. When the initial photoreduction rates as listed in Table 2 were plotted as a function of initial concentration of Cr(VI), a linear relationship was achieved. Under

Table 3

Initial rate of the photoreduction of Cr(VI) kinetics analysis equation (pH 4)

Variable parameter	R	Initial rate fitted equation	R_c, n, α
A_{algae}	0.99965	$V_0 = 0.0192 A_{\text{algae}}^{0.5235}$	0.9995, 3, 0.02
C_0 (mg L^{-1})	0.98739	$V_0 = 0.00799 C_0^{0.1718}$	0.9877, 3, 0.10
A_{algae}, C_0 (mg L^{-1})	0.99595	$V_0 = 0.01881 C_0^{0.1718} A_{\text{algae}}^{0.5235}$	0.9912, 5, 0.001

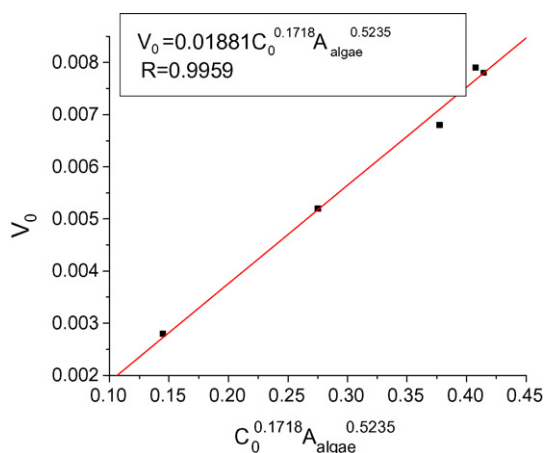


Fig. 6. Effects of initial concentrations of Cr(VI) and algae on the photoreduction of Cr(VI) (pH 4, $R_c = 0.9912$, $n = 5$, $\alpha = 0.001$).

the condition the initial photoreduction rate of Cr(VI) could be expressed by $\ln V_0 = -4.83 + 0.1718 \ln C_0$ with a correlation coefficient $R = 0.9956$. Because the initial photoreduction rate plotted as a function of initial concentration of Cr(VI) could also be expressed by the equation $V_0 = k_2 C_0^\alpha$, the initial photoreduction rate of Cr(VI) under the condition could be expressed by the equation $V_0 = 0.00799 C_0^{0.1718}$. When the initial photoreduction rates as listed in Table 3 and Fig. 6 were plotted as a function of initial concentration of Cr(VI) and algae, a linear relationship was achieved. The initial photoreduction rate of Cr(VI) under the condition studied could be expressed by $V_0 = 0.01881 C_0^{0.1718} A_{algae}^{0.5235}$ with a correlation coefficient $R = 0.9960$.

4. Conclusions

C. vulgaris existed in the natural water can be used for effective photoreduction of chromium(VI). The results of this study indicated that *C. vulgaris* were able to reduce chromium(VI) under UV/visible light illumination. A greater photoreduction was observed under purging N_2 compared to that under purging air. The rate of Cr(VI) photochemical reduction increased with algae concentration increasing, initial Cr(VI) concentration decreasing and the decrease of pH, and the velocity of the photochemical reduction also increased with alga concentration increasing, the increase of initial Cr(VI) concentration and the decrease of pH. When pH increased to 6, the process nearly vanished. When initial Cr(VI) concentration ranged from 0.4 to 1.0 mg L^{-1} and initial algae concentrations ranged from $ABS_{algae} = 0.025$ to $ABS_{algae} = 0.180$, According to the results of kinetic analyses, the kinetic equation of Cr(VI) photochem-

ical reduction in aqueous solution with alga under 250 W MHL was $V_0 = k C_0^{0.1718} A_{algae}^{0.5235}$ under the condition of pH 4.

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