

Reduction and Transformation of Hexavalent Chromium by Thermoacidophilic Cyanidiales

Yen-Lin Cho (卓宴琳)¹ and Yu-Ting Liu (劉雨庭)^{1,2*}

¹Department of Soil and Environmental Sciences, National Chung Hsing University, Taichung 40227, Taiwan

²Innovation and Development Center of Sustainable Agriculture, National Chung-Hsing University, Taichung 40227, Taiwan

yliu@nchu.edu.tw

Abstract

Due to the tolerance and withstanding for extreme stresses of heat, acid, and heavy metals, Cyanidiales could be considered as promising materials to remove heavy metals such as Cr across a wide range of environments. In this study, we aimed to develop the retention mechanisms of Cr(VI) on Cyanidiales in relation to the Cr speciation and changes of functional groups on Cyanidiales. Sorption isotherms of Cr(VI) at pH 2.0/7.0 on two species of Cyanidiales including *Galdieria partita* (Gp) and *Cyanidioschyzon merolae* (Cm) were conducted, wherein observed maximum sorption capacities were Cm (294.3 mg g⁻¹) > Gp (103.9 mg g⁻¹) at pH 2.0 and Gp (152.1 mg g⁻¹) > Cm (86.6 mg g⁻¹) at pH 7.0. By means of the transmission X-ray microscopy (TXM), X-ray absorption spectroscopy (XAS) and synchrotron-based Fourier-transform infrared spectroscopy (FTIR), the complexation with proteins and surface functional groups of algae and precipitation as Cr(OH)₃ and K₂Cr₂O₇ on algae surfaces are mainly responsible for the Cr retention on Cyanidiales.

Keywords - Cyanidiales, thermoacidophilic, Cr sorption, XAS, proteins secondary structure

Introduction

Chromium (Cr) occurs in the environments primarily in hexavalent and trivalent chromium [Cr(VI) and Cr(III)]. However, Cr(VI) pollutant is particular concerned in soil and aquatic systems due its carcinogenic and mutagenic attribute. The toxicity and mobility of Cr(VI) are higher than that of Cr(III) as Cr(III) usually occurs as insoluble compound in aqueous solutions. The red algae, Cyanidiales, might be a promising green material to remove metals due to their tolerance for a wide range of temperature (room Temp. - 56 °C) and acidity (pH 0.2 - 5). In addition, a wide range of applicable genes derived by means of horizontal gene transfer (HGT) from prokaryotic bases, such as arsenic methyltransferase genes, entrust Cyanidiales with noteworthy ability to endure in inimical environments [1]. Therefore, in this study, we aimed to determine the reduction and accumulation capacity of Cr(VI) in Cyanidiales [*Galdieria partita* (Gp) and *Cyanidioschyzon merolae* (Cm)] using the transmission X-ray microscopy (TXM), X-ray absorption spectroscopy (XAS) and synchrotron-based Fourier-transform infrared spectroscopy (FTIR).

Materials and Methods

Sorption Isotherms

Sorption isotherms of Cr(VI) on Gp and Cm were conducted with the cell density of 0.5 mg g⁻¹ at pH 2.0 and 7.0 for 6 h. The sorption data were fitted using the Freundlich isotherm models.

Spectroscopic Analysis

Red algae with sorbed Cr(VI) were analysed using TXM, FTIR and linear combination fitting (LCF) of Cr K-edge XAS. 2-D and 3-D images for selected samples collected from sorption isotherms were acquired using the TXM at beamline (BL) 01B1 of the National Synchrotron Radiation Research Center (NSRRC). The FTIR spectra were collected at the BL 14A1 of NSRRC. Collected spot size is 20 μm (H) x20 μm (V) with the resolution of 4 cm⁻¹ from 4000-650 cm⁻¹. Spectra were analysed using the OMNIC 8.0 software. The area ratio of the 1652-cm⁻¹ peak (α-helix) to the 1631-cm⁻¹ peak (parallel β-strand) was calculated. Speciation of sorbed Cr on red algae was

determined using the XAS collected at BL 17C1 of NSRRC. The linear combination fitting (LCF) of Cr X-ray absorption near edge structure (XANES) spectroscopy with reference standards including inorganic and organic Cr species was also performed. All of the XAS data were background removed, normalized, and merged using the IFEFFIT program.

Results

Chromium sorption isotherms on Gp at pH 2.0 and Cm at pH 7.0 (Fig. 1) showed that there were two stages of sorption behaviours for Cr. The observed maximum sorption capacities were Cm (294.3 mg g⁻¹) > Gp (103.9 mg g⁻¹) at pH 2.0 and Gp (152.1 mg g⁻¹) > Cm (86.6 mg g⁻¹) at pH 7.0. 2-D images showed sorbed Cr that presented as darker shadows scattered inside the cell (Fig. 2b-c, 2e-f), suggesting the plausible in vivo accumulation for Cr. In addition, the cell shrinkage upon Cr sorption was found on Gp at pH 2.0 and Cm at pH 7.0. Such cell shrinkage corresponded to the decreasing peak intensity of mannose (1076 cm⁻¹) on polysaccharide with increasing Cr sorption amounts, which implied cellular structure collapse (Fig. 3). On the contrary, Cr sorption on Gp and Cm at pH 7.0 and pH 2.0, respectively, led to an opposite trend. Fig. 4 showed the decomposition results of FTIR spectra. Wherein, peaks around 1652 and 1631 cm⁻¹ contributed from protein secondary structure (PSS) of α-helix and β-strand could indicate how Cyanidiales regulate metal homeostasis and impart defence against heavy metal toxicity. The proportion of α-helix in all samples decreased upon Cr sorption. In terms β-strand, its proportion increased only on Gp at pH 2.0 (3.5%). On Cm at pH 7.0, however, the β-strand proportion decreased 8.7%. The LCF results of the XAS data (Table 1) showed that Cr bound with organic groups dominated the Cr inventory for all samples.

Discussion

The XAS results indicated that Cr(VI) can be sorbed and reduced to Cr(III) via the complexation with Cyanidiales. Changes in the complexation degree between Cr and PSS suggested that N-terminal and C-terminal motifs joined by α-helix and β-strand participate in metal

detoxification process. Gp and Cm showed distinct sorption behaviours. While Gp showed the greater Cr sorption capacity at pH 7.0, Cm owned superior Cr sorption capability at pH 2.0. This result could be plausibly interpreted by the cell shrinkage of Gp at pH 2.0 and Cm at pH 7.0, which were also evidenced in the TXM results and the negative correlation between mannose peak intensity and Cr sorption amount. Such structure damage might hinder the retention capacity of Cr on Cyanidiales. As indicated by XAS results, the Cr complexation with organic functional groups of Cyanidiales such as cysteine lead to a significant detoxification response by transforming and reducing Cr(VI) to less mobile and toxic Cr(III). Collectively, the extremophilic Cyanidiales could serve as promising green material for the remediation of Cr pollution. Possible mechanisms for Cr retention on Cyanidiales include complexation with organic functional groups, algal uptake, and inorganic precipitation as $\text{Cr}(\text{OH})_3$ and $\text{K}_2\text{Cr}_2\text{O}_7$ (Fig 5.).

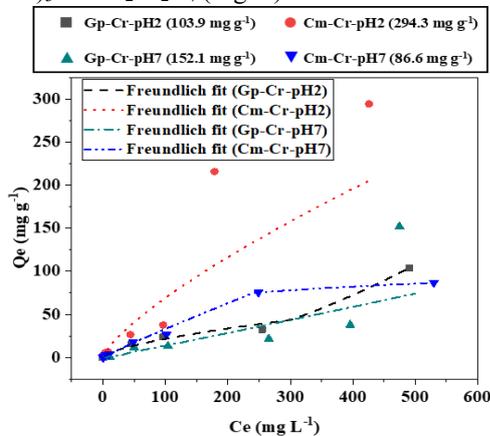


Fig. 1. Adsorption isotherms of Cr on Gp and Cm at pH 2.0 and 7.0.

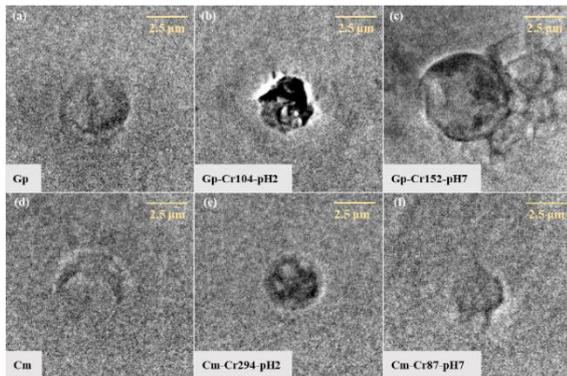


Fig. 2. The 2-D images for (a-c) Gp and (d-f) Cm with and without sorbed Cr. Sorbed Cr were (b) 104 and (c) 152 mg g^{-1} on Gp at pH 2.0 and 7.0; (e) 294 and (f) 87 mg g^{-1} on Cm at pH 2.0 and 7.0.

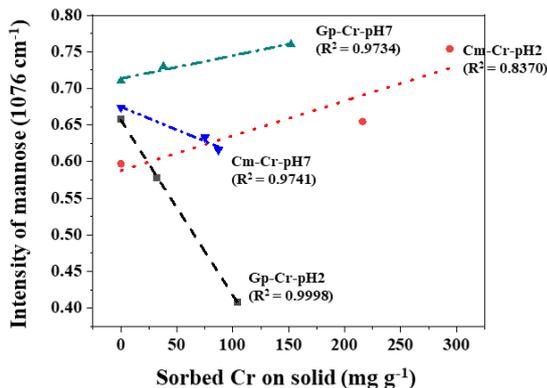


Fig. 3. Peak intensity of mannose (1076 cm^{-1}) from polysaccharide as the function of Cr sorption capacity on Gp and Cm at pH 2.0 and 7.0.

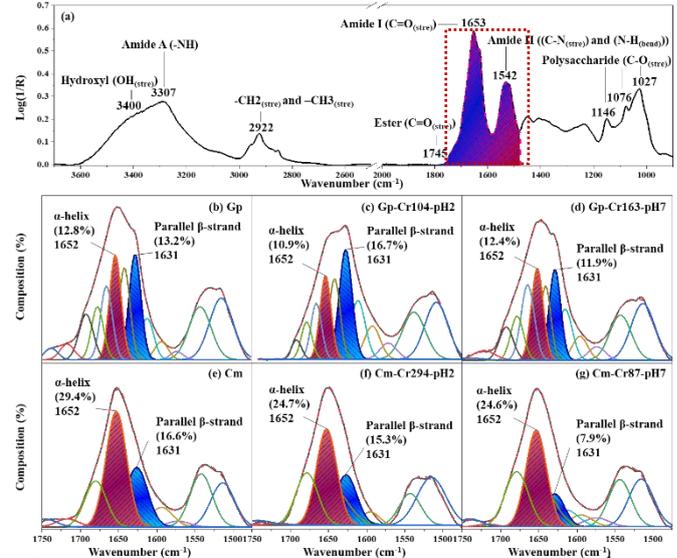


Fig. 4. (a) Representative FTIR spectra ($3800\text{--}900 \text{ cm}^{-1}$) for origin Gp. Representative decomposition results of FTIR spectra ($1465\text{--}1750 \text{ cm}^{-1}$) for (b-d) Gp and (e-g) Cm with and without sorbed Cr. Sorbed Cr were (c) 104 and (d) 152 mg g^{-1} on Gp at pH 2.0 and pH 7.0; (f) 294 and (g) 87 mg g^{-1} on Cm at pH 2.0 and pH 7.0.

Table 1. LCF results for Cr sorbed on Gp and Cm at pH 2.0 and 7.0.^a

Samples	Sorbed Cr mg g^{-1}	Organic species		Inorganic species		R-factor ^e
		Cr(III)-AH ^b	Cr(OH) ₃ ^c	K ₂ Cr ₂ O ₇ ^d	%	
Gp-Cr104-pH2	103.9	44.9(±7.9)	0.5(±7.8)	54.6(±1.3)	0.0048	
Cm-Cr294-pH2	294.3	74.2(±4.1)	17.7(±4.0)	8.1(±0.6)	0.0011	
Gp-Cr152-pH7	152.1	65.9(±4.4)	1.9(±4.3)	32.2(±0.5)	0.0015	
Cm-Cr87-pH7	86.6	44.9(±4.2)	14.0(±4.1)	41.1(±0.6)	0.0015	

^aThe fitted spectral range was between -20 and $+30 \text{ eV}$. The weighting factors on each fit were summed to $100 \pm 1\%$ and were normalized to 100%; ^bCr-acetate hydroxide [Cr(III)-AH]; ^cCr-hydroxide [Cr(OH)₃]; ^dPotassium dichromate [K₂Cr₂O₇(s)]; ^eNormalized sum of the squared residuals of the fit (R-factor = $\sum(\text{data-fit})^2/\sum\text{data}^2$); ^fMean \pm standard deviation.

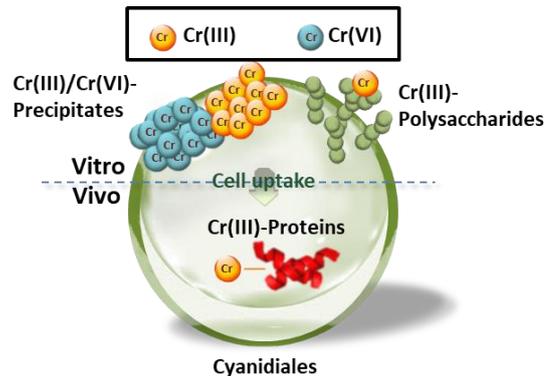


Fig. 5. Accumulation mechanisms of Cr on Cyanidiales.

Acknowledgments

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References

- [1] Cho et al., "Molecular mechanisms for Pb removal by Cyanidiales: a potential biomaterial applied in thermo-acidic conditions," *Chem. Eng. J.*, vol. 401, pp. 125828, June 2020.