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Reduction of Cr (VI) into Cr (III) by organelles of *Chlorella vulgaris* in aqueous solution: An organelle-level attempt

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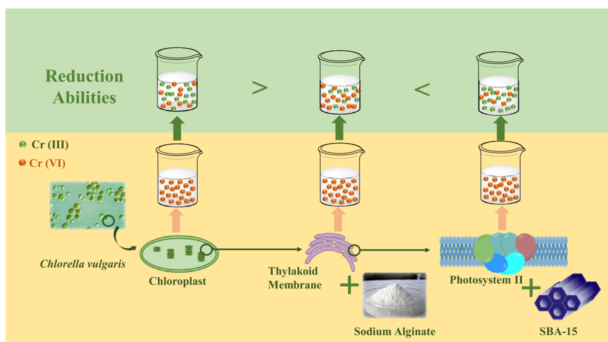
HIGHLIGHTS

- Organelle roles in Cr (VI) reduction by algae were still unclear.
- Organelles of Chlorella vulgaris were extracted for Cr (VI) reduction tests.
- Isolated thylakoid membrane (ITM) and photosystem II (PSII) were further decorated.
- The order of Cr (VI) reduction ability is PSII@SBA-15 > Chloroplast > ITM@SA.
- Organelle level study is helpful to detect mechanism of Cr (VI) reduction by algae.

GRAPHICAL ABSTRACT



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ABSTRACT

The priority pollutant chromium (Cr) was ubiquitous and great efforts have been made to reduce Cr (VI) into less-toxic Cr (III) by alga for the convenient availability and low expense. However, the functional role of organelle inside the algal cell in Cr (VI) reduction was poorly understood. In this study, organelles in green algae *Chlorella vulgaris* were extracted and further decorated for Cr (VI) reduction tests. Results showed that the chloroplast exhibited not only adsorption ability of total Cr (21.18% comparing to control) but also reduction potential of Cr (VI) (almost 70% comparing to control), whose most suitable working concentration was at 17 μg/mL. Furtherly, the isolated thylakoid membrane (ITM) showed better Cr (VI) reduction potential with the presence of sodium alginate (SA), even though the Hill reaction activity (HRA) was inhibited. As for photosystem II (PSII), the addition of mesoporous silica SBA-15 enhanced the reduction ability through improving the light-harvesting complex (LHC) II efficiency and electron transport rate. On the whole, the reduction ability order of the three kinds of materials based on chloroplast in *C. vulgaris* was PSII@SBA-15 > Chloroplast > ITM@SA. The attempt made in this study to reduce the Cr (VI) with *C. vulgaris* organelles might not only offer basement to detect the potential action mechanism of Cr (VI) reduction by *C. vulgaris* but also provide a new sight for the scavenge of heavy metal with biological materials.

1. Introduction

Nowadays, heavy metals are research hot spots because they are not biodegradable and the accumulation of heavy metals throughout the food chain may threaten to health of human beings (Ran et al., 2016;

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Singh et al., 2010; Wen et al., 2015). Especially, the pollutant chromium was paid special attention for the frequent appearances in soil and groundwater at abandoned industrial sites, which was attributed to the wide usage in tanning of leather as well as dyes and paints (Liu et al., 2014; Mahmood et al., 2015). Generally, chromium is present in the environment with two stable oxidation states, the hexavalent (Cr (V)) and trivalent (Cr (III)) states. Comparatively, the Cr (VI) is known to be more dangerous in aspects of toxicity, teratogenicity, carcinogenicity, mutagenicity and mobility than Cr (III) (Hayat et al., 2012; Velma et al., 2009). Therefore, great efforts were made into reduce Cr (VI) to less-toxic Cr (III), where methods based upon the utilization of microorganism and chemical reductants were widely applied (Cheung and Gu, 2007; Lu et al., 2006). However, the application of chemical methods was limited due to expensive cost and poor removal efficiency for meeting regulatory standards (Park et al., 2007).

Recently, the photo-reduction methods were frequently explored to reduce Cr (VI) into Cr (III) by various materials (Yang et al., 2013; Zhao et al., 2013). The Cr (VI) cannot directly reduce by photochemical effect, which needs other metals, like Fe (II) and Cu (II), to transfer the photo-excited electrons (Li et al., 2014; Xu et al., 2015). Previous study has reported that Cr (VI) could be photo-reduced into Cr (III) with the presence of algae *Chlorella vulgaris* under UV/visible light condition (Deng et al., 2006), which provided a new sight to remove Cr (VI) with microorganisms. In fact, a great number of investigations have focused on the algal reduction potential for Cr (VI) because of the convenient availability and low cost (Gupta and Rastogi, 2009; Gupta et al., 2001; Pagnanelli et al., 2013). However, unfortunately, previous studies have performed the Cr (VI) reduction only by alga individual without further detection of the function of organelles, leading the exact mechanism involved poorly understood to some extent. As we know, alga was a microorganism living under the coordination function of different organelles inside, an organelle-level research can provide with not only the information about the functional roles but also the possible action mechanism of Cr (VI) reduction by algae, which are helpful to explore the high ability and efficiency scavengers of pollutants. Therefore, the organelle-level research is of great importance and necessity. However, the functional role of organelles in Cr (VI) reduction was poorly understood.

Among the organelles, chloroplast is one of the main members in plants and algal cells. The main role of chloroplast is to conduct photosynthesis, where the photosynthetic pigment chlorophyll captures the energy from sunlight and converts it and stores it in the energy-storage molecules ATP and NADPH while freeing oxygen from water (Greer et al., 1986). The slim (5–10 nm), fast (picosecond level) and efficient (almost 100% conversion rate of photons to electrons) photoelectron-reactor consists of reaction centers photosystem I and II (PSI & PSII) (Lee and Greenbaum, 1995). In addition, it was reported that except for ferricyanide ($[Fe(CN)_6]^{3-}$), hexachloro-platinum ($[PtCl_6]^{2-}$) and hexachloro-osmate ($[OsCl_6]^{2-}$) can also be reduced to Pt and Os with the electrons supplied by PSI, respectively (Hill, 1937). Furthermore, PSII can also offer electrons to reduce variable valent metals such as platinum chloride ($PtCl_4$) and ruthenium chloride ($[RuCl_6]^{2-}$) (Lee and Greenbaum, 2004). Therefore, electrons generated during photosynthesis have shown potential to reduce heavy metal pollutants in the environment.

In the present study, the Cr (VI) reduction by *Chlorella vulgaris* was investigated in an organelle-level. Firstly, the sensitivity of *C. vulgaris* to Cr (VI) solution was detected to avoid severe phytotoxicity. The chloroplasts were extracted and followed the integrity examination and Hill reaction activity (HRA) measurements. The isolated thylakoid membrane (ITM) and photosystem II (PS II) were furtherly produced and decorated with sodium alginate (SA) and mesoporous silica SBA-15 to keep the stability. Then the Cr (VI) reduction experiments based on materials produced by *C. vulgaris* chloroplasts were performed (Fig. 1). Results obtained from this study will compare the Cr (VI) reduction abilities of organelles in *C. vulgaris*, and furthermore, the organelle-level attempt will provide the basement to detect the potential action mechanism of Cr (VI) reduction by algae. Moreover, this investigation will also provide a new sight for scavenge of heavy metals with biological materials.

2. Material and methods

2.1. Chemical reagents

Potassium dichromate ($K_2Cr_2O_7$), sodium alginate (SA), cellulase, macerozyme, ascorbic acid and bovine serum albumin (BSA) were supplied by National Medicines Corporation Ltd., China. Mesoporous silica SBA-15 ($SiO_2/Al_2O_3 \geq 500$; pore diameter: 6–8 nm; BET: 400–600 m^2/g) was obtained from Nanjing XFNano Material Tech Co. Ltd., China and 1,5-Diphenylcarbazide, mannitol, 2-Morpholinoethanesulfonic acid (MES), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and triton X-100 were purchased from Aladdin Industrial Corporation, China. Double distilled water was used throughout the study and all glassware was sterilized in an autoclave.

2.2. Algae cultivation

The freshwater microalgae *Chlorella vulgaris* was obtained from the Institute of Hydrobiology, Chinese Academy of Science (Wuhan, China). The algae were cultivated in HB-4 medium according to previous study (Wen et al., 2010) and the detailed cultivation process was shown in Text S1 in Supporting Information. To find the most suitable concentration of Cr (VI) for the reduction experiments and avoid severe phytotoxicity of Cr (VI), the sensitivity of *C. vulgaris* to Cr (VI) using $K_2Cr_2O_7$ was performed as shown in Supporting information (Text S2).

2.3. Extractions of chloroplast, thylakoid membrane and photosystem II (PSII)

The chloroplast of *C. vulgaris* was extracted after previous studies with slight modification (Braun and Aach, 1975; Nishimura et al., 1982). The detailed processes as well as the buffer A–D used were all depicted in Supporting information (Text S3). The quantification of

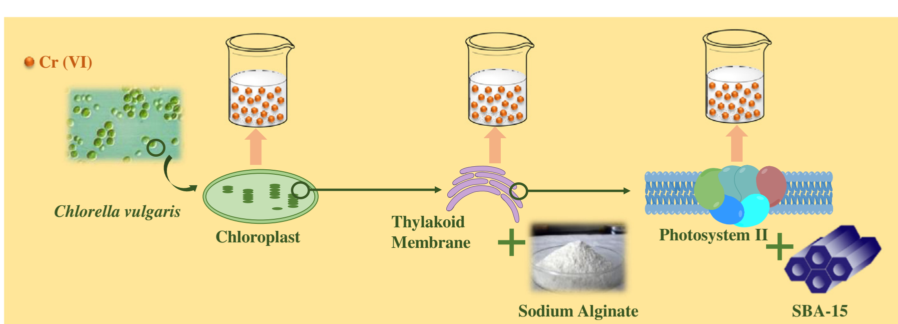

Fig. 1. The purpose of the present study: Attempt to reduce Cr (VI) by materials based on *Chlorella vulgaris* organelles.