

Biotransformation of hexavalent chromium into extracellular chromium(III) oxide nanoparticles using *Schwanniomyces occidentalis*

Pallavi T. Mohite · Ameeta Ravi Kumar · Smita S. Zinjarde

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Abstract

Objectives To demonstrate biotransformation of toxic Cr(VI) ions into Cr₂O₃ nanoparticles by the yeast *Schwanniomyces occidentalis*.

Results Reaction mixtures containing *S. occidentalis* NCIM 3459 and Cr(VI) ions that were initially yellow turned green after 48 h incubation. The coloration was due to the synthesis of chromium (III) oxide nanoparticles (Cr₂O₃NPs). UV–Visible spectra of the reaction mixtures showed peaks at 445 and 600 nm indicating ⁴A_{2g} → ⁴T_{1g} and ⁴A_{2g} → ⁴T_{2g} transitions in Cr₂O₃, respectively. FTIR profiles suggested the involvement of carboxyl and amide groups in nanoparticle synthesis and stabilization. The Cr₂O₃NPs ranged between 10 and 60 nm. Their crystalline nature was evident from the selective area electron diffraction and X-ray diffraction patterns. Energy dispersive spectra confirmed the chemical composition of the nanoparticles. These biogenic nanoparticles could find applications in different fields.

Conclusions *S. occidentalis* mediated biotransformation of toxic Cr(VI) ions into crystalline extracellular Cr₂O₃NPs under benign conditions.

Keywords Cr₂O₃ nanoparticles · Hexavalent chromium biotransformation · *Schwanniomyces occidentalis*

Introduction

A variety of metal ions enter aquatic environments via natural processes and human activities. Chromium is one such toxic metal that is regarded as a priority pollutant. In nature, chromium exists in different oxidation states and the trivalent [Cr(III)] and hexavalent [Cr(VI)] forms are the most prevalent. Hexavalent chromium [Cr(VI)] negatively affects human health and there is a need to treat this effectively. In addition to the conventional physical and chemical treatment methods that are available, biological protocols for addressing this problem have also been described (Wang et al. 2008; Yong et al. 2015). Development of mild processes for detoxifying Cr(VI) ions with simultaneous production of value-added products such as Cr₂O₃ nanoparticles could be a promising way of tackling this issue.

Cr₂O₃ nanoparticles (NP) are extensively used as catalysts, coating material and pigments. They are usually synthesized by physical and chemical methods. There are recent reviews highlighting the significance of microorganisms in synthesizing metal nanoparticles (Park et al. 2015; Yadav et al. 2015). However, there are very few studies on the production

P. T. Mohite · A. R. Kumar · S. S. Zinjarde (✉)
Institute of Bioinformatics and Biotechnology, Savitribai
Phule Pune University, Pune 411007, India
e-mail: smita@unipune.ac.in

of Cr₂O₃NPs by microbial systems (Dong et al. 2013; Annamalai et al. 2014; Wang et al. 2013). These reports describe the production of “cell associated” Cr₂O₃NPs by bacterial isolates. To the best of our knowledge there are no reports on extracellular synthesis of Cr₂O₃NPs by yeasts. In the current investigation, we describe (i) biotransformation of toxic hexavalent potassium chromate (K₂CrO₄) to extracellular Cr₂O₃ nanoparticles by the yeast *Schwanniomyces occidentalis* (ii) detail the characteristics of these biogenic nanoparticles by a variety of analytical techniques and (iii) propose their eventual use in different fields.

Materials and methods

Biotransformation of hexavalent chromium ions

Schwanniomyces occidentalis NCIM 3459 was inoculated in 20 ml Burkholder’s mineral medium (Ksheminska et al. 2006) containing 10 mM K₂CrO₄ and incubated under static conditions at 30 °C for 10 days. A color change (yellow to deep green) indicated the biotransformation of Cr(VI) ions and the probable formation of Cr₂O₃ nanoparticles. The effect of temperature (10–50 °C) and salt concentration (2.5–12.5 mM) on nanoparticle synthesis was monitored. After incubation, the reaction mixtures were centrifuged at 6000×g for 10 min and Cr₂O₃NPs in the supernatant were characterized.

For all experiments, the source of Cr(VI) ions was K₂Cr₂O₄ in distilled water. The total Cr content in the cell free extract (CFE) and cells was determined by using model SpectrAA220 Atomic Absorption Spectrometer (AAS). The content of residual Cr(VI) ions in the CFE was determined spectrophotometrically by the diphenyl carbazide method. Differences between total chromium and Cr(VI) concentrations were used to determine the Cr(III) contents.

Fourier transform infrared (FTIR) analysis

The functional groups involved in nanoparticle synthesis were detected by FTIR analysis. The IR spectra of KBr-pelleted nanoparticle samples were recorded in the range of 500–4000 cm⁻¹ at a resolution of 4 cm⁻¹ on a Jasco FTIR-6100 model.

Characterization of the biogenic nanoparticles

Properties of the extracellular nanoparticles were determined by different analytical tools. UV–Visible spectra were recorded at a resolution of 1 nm. transmission electron microscope (TEM) observations were made on FEI model, Tecnai G2 20 TEM equipped with selected area electron diffraction (SAED) analyzer. Samples were prepared by coating nanoparticles on carbon-coated copper grids (200 μm × 200 μm mesh size) and drying them prior to observation. X-ray diffraction (XRD) patterns were obtained by using D8 Advanced Bruker X-ray diffractometer with Ni-filtered Cu–Kα X-rays of wavelength (λ) 1.540 Å. Thin films of the nanoparticles were coated on glass slides and X-ray diffraction patterns were recorded in the 2θ range of 20–80° with the step size of 0.05 and step time of 1 s in the transmission mode. The energy dispersive spectrometer (EDS) attachment present with the model FEI Nova NanoSEM 450 field emission scanning electron microscope (FESEM) was used to obtain EDS profiles.

All experiments were carried out in triplicate with two biological replicates and representative spectra, data and images are presented here.

Results and discussion

Biotransformation of hexavalent chromium ions by *Schwanniomyces occidentalis*

During our preliminary investigations on biotransformation of Cr(VI) ions, we identified the ability of this strain of *S. occidentalis* in biotransforming potassium chromate. *S. occidentalis* has been used in beer-making and production of single cell protein (Horn et al. 1992; Anupama and Ravindra 2000). Considerable biomass of the yeast is thus generated and this can be used for the application described in the current investigation. To the best of our knowledge, there are no reports on the use of the biomass of this yeast for preparation of chromium oxide nanoparticles in an extracellular manner and hence this study was taken up.

When *S. occidentalis* was incubated with 10 mM K₂CrO₄ under static conditions, the reaction mixtures turned from bright yellow to deep green (Fig. 1a, tubes C and T, respectively) indicating the reduction of the

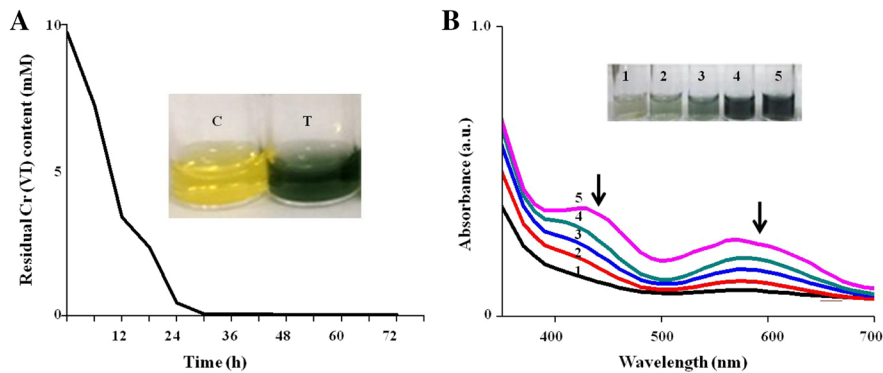


Fig. 1 Biotransformation of Cr(VI) ions by *Schwanniomyces occidentalis*. **a** Residual Cr(VI) ions in reaction mixtures over a period of time after biotransformation by *S. occidentalis* [Inset tube C (10 mM K_2CrO_4 without yeasts) and T (10 mM K_2CrO_4 with yeasts)]. **b** UV–Visible spectra and visual observations (insets) of reaction mixtures depicting effect of K_2CrO_4

hexavalent form into the trivalent form. This color change typically depicts the presence of Cr_2O_3 NPs (Willis et al. 2007). When these reaction mixtures were centrifuged, color was associated with the CFE and not with cell pellets indicating the extracellular nature of the nanoparticles. Cr(VI) ions in the reaction mixtures decreased in a time-dependent manner and were below detectable limit after 30 h of incubation (Fig. 1a). AAS analysis revealed that 98 % of the initially added chromium was present in the cell free extract (CFE). Since Cr(VI) ions were not detected in the CFE, it was clear that the cells mediated the reduction of the hexavalent form into the trivalent form as Cr_2O_3 NPs. There is a report on a *Bacillus* species bringing about reduction of Cr(VI) ions to the Cr(III) form into without being accumulated in the cell (Elangovan et al. 2006). In a similar manner, *S. occidentalis* seems to be mediating the chromium reduction reactions in an extracellular manner.

The intensity of the color increased with increasing content of K_2CrO_4 (Fig. 1b, inset tubes 1–5). Control experiments (containing medium and K_2CrO_4) did not show any color change indicating that the yeast cells were mediating the reductive reactions (Fig. 1a, tube C). UV–Visible spectra of the reaction mixtures were also obtained (Fig. 1b, lines 1–5). Peaks were observed in the vicinity of 445 and 600 nm (Fig. 1b, black arrows) corresponding to the ${}^4A_{2g} \rightarrow {}^4T_{1g}$ and ${}^4A_{2g} \rightarrow {}^4T_{2g}$ transitions in Cr_2O_3 , respectively (Li et al. 2006). The peak at around 445 nm is associated

concentration on nanoparticle synthesis [lines and tubes 1 2.5, 2 5.0, 3 7.5, 4 10 and 5 12.5 mM]. Intended for color reproduction on the Web (free of charge) and in print or to be reproduced in color on the Web (free of charge) and in black-and-white in print

with the inter-band transition of core electrons of chromium to chromium oxide (Ramesh et al. 2012). These results validate the earlier observations on the conversion of Cr(VI) ions into Cr_2O_3 that were based on AAS quantification of total chromium and spectrophotometric detection of Cr(VI) ions. Waste-waters often contain high contents of Cr(VI) ions. Incubation of such wastewaters with this yeast could thus (i) mediate effective detoxification and (ii) generate value-added products in the form of Cr_2O_3 NPs.

The effect of temperature on nanoparticle synthesis was also determined. When reaction mixtures were incubated at 10, 20, or 30 °C, color transition was observed after 10 days. Incubation at 40 and 50 °C decreased the reaction times to 6 and 2 days, respectively. In the current study, synthesis of nanoparticles was achieved in shorter time durations at higher temperatures.

It is hypothesized that incubation at 50 °C favored the release of biomolecules involved in the reduction and stabilization of nanoparticles. *S. occidentalis* is known to grow in the presence of Cr(VI) ions and produce higher contents of riboflavin (Fedorovych et al. 2001). The yeast thus has the necessary set up to tolerate and detoxify Cr(VI) ions. As discussed in the following section, TEM images showed the presence of an organic layer [that could be derived from the extracellular polymeric substance (EPS)] capping the nanoparticles. Capping agents naturally restrict nanoparticles from growing further. Higher temperatures probably favored

enhanced synthesis of the EPS and allowed the capping to occur more rapidly.

FTIR analysis

To identify the functional groups involved in nanoparticle synthesis, FTIR analysis was carried out. FTIR spectra of control (untreated with K_2CrO_4) and test reactions (treated with K_2CrO_4) are depicted in Fig. 2 (lines 1 and 2, respectively). Arrows and wave numbers in the figure highlight important peaks involved in nanoparticle synthesis. Stretching of the peaks at 3400, 1590–1600, 1395–1440 and 1078 cm^{-1} could be assigned to the –OH bond, primary amide bond, carboxylic acid residues and C–O–C and C–O–P ring vibrations of polysaccharides, respectively (Omoike and Chorover 2004). Peaks at 885–895 corresponded to the –C–H stretching and the peak at around 800 cm^{-1} indicated the role of amine groups. FTIR spectra thus suggested the role of carbohydrate and protein moieties in the synthesis and stabilization of nanoparticles.

Characterization of extracellular Cr_2O_3 NP nanoparticles

TEM images of the nanoparticles at different magnifications are depicted in Fig. 3a, b. The Cr_2O_3 NP varied from 10 to 60 nm. The nanoparticles (Fig. 3a, b, single black arrow) were embedded in an organic matrix

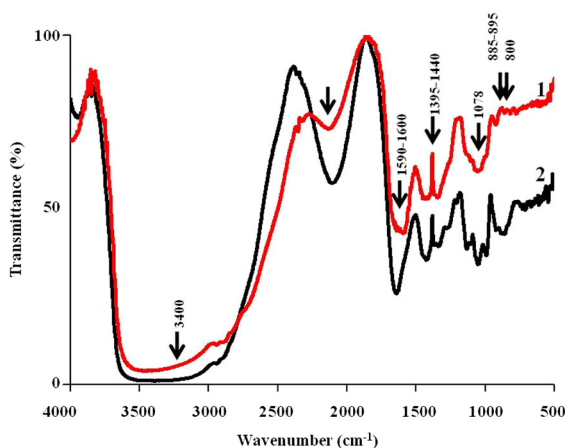


Fig. 2 Representative FTIR profiles of cell free extracts [line 1 control (before interaction with K_2CrO_4); line 2 test (after interaction with K_2CrO_4); arrows and numbers indicate important peaks]

probably derived from the EPS (Fig. 3a, b, double black arrows). The role of EPS in formation of silver nanoparticles has also been described with respect to *Exiguobacterium mexicanum* (Padman et al. 2014). SAED patterns of the Cr_2O_3 NP (Fig. 3c) showed the presence of diffraction rings and spots indicating the polycrystalline nature of the nanoparticles.

To further characterize the crystal structure of the nanoparticles, XRD studies were taken up. Sharp peaks were obtained at 2θ values of 24.65°, 41.46° and, 50.28° (Fig. 4a). These peaks could be indexed to the (0 1 2), (1 1 3) and (0 2 4) crystalline planes of Cr_2O_3 NPs, respectively (Tavares et al. 2014). A representative spot-EDS profile of Cr_2O_3 NPs displayed in addition to other peaks, distinct signals for chromium and oxygen (Fig. 4b, black arrows).

S. occidentalis thus effectively biotransformed toxic Cr(VI) ions into value-added products in the form of extracellular Cr_2O_3 NPs. As stated earlier, there are very few reports on the synthesis of Cr_2O_3 NPs by microbial systems. There are reports on bacteria such as *Shewanella oneidensis*, *Bacillus cereus* and *Bacillus subtilis* converting Cr(VI) ions to Cr_2O_3 NPs that are cell associated (Wang et al. 2013; Dong et al. 2013; Annamalai et al. 2014). Since such nanoparticles are closely linked to cells, their applications are limited. To the best of our knowledge, there are no reports on yeasts mediating such reactions. The yeast culture used in the current study offered a major advantage by synthesizing nanoparticles extracellularly. FTIR data suggested the role of carbohydrate and protein moieties in mediating the synthesis and capping of nanoparticles (Fig. 2). Moreover, TEM images also revealed the presence of a polymeric layer around the nanoparticles. The yeast thus produced EPS that mediated the reductive reactions and stabilized the nanoparticles. The significance of EPS in chromium reduction reactions has also been highlighted in a recent review (Thatoi et al. 2014).

The advantages associated with the current biogenic protocol are two-fold. The yeast cells could transform Cr(VI) ions present in high concentrations (as in wastewaters) under static and benign conditions thus making the technique cost-effective. Moreover, the end products generated as a result of the biotransformation process were value-added products. It must be noted that nanostructured Cr_2O_3 materials offer high surface areas and are thus used in catalysis, as green pigments and as coating material.

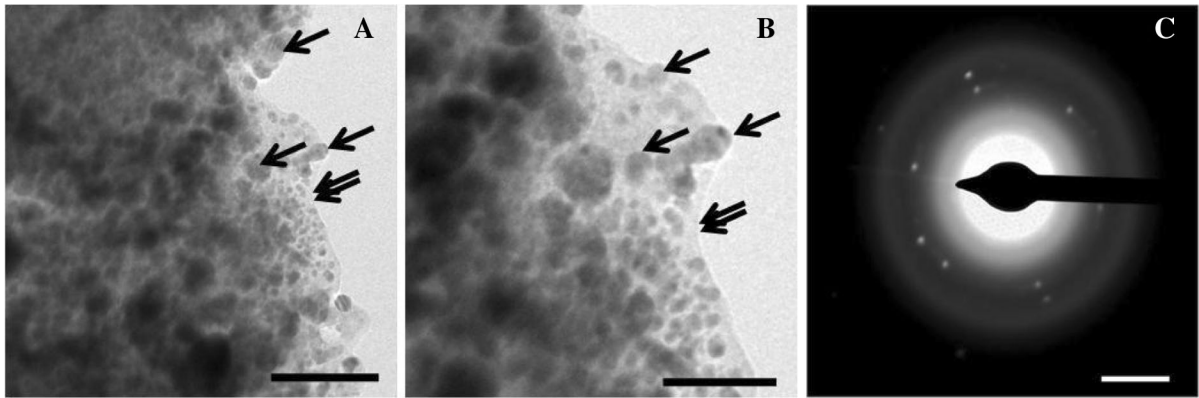


Fig. 3 Representative TEM images of the Cr_2O_3 NPs synthesized by *Schwanniomyces occidentalis* at **a** low and **b** high magnifications. **c** Representative SAED pattern depicting the

polycrystalline nature of the nanoparticles [scale bars equivalence **a** 100 nm, **b** 50 nm, **c** 5.00 1/nm]

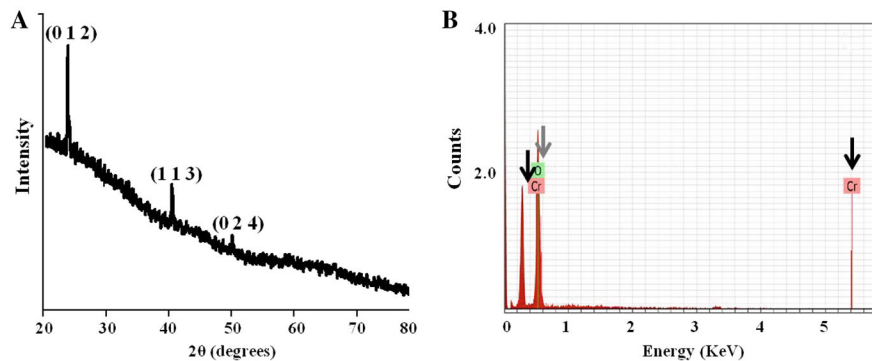


Fig. 4 Characterization of Cr_2O_3 NPs synthesized by *Schwanniomyces occidentalis*. **a** Representative X-ray diffraction profile [sharp peaks at 2θ values of 24.65° , 41.46° and 50.28° were indexed to the $(0\ 1\ 2)$, $(1\ 1\ 3)$ and $(0\ 2\ 4)$ crystalline planes

of Cr_2O_3 NPs, respectively]. **b** Representative energy dispersive spectrum showing in addition to other peaks, distinct signals for chromium and oxygen [indicated by *black* and *gray* arrows, respectively]

Conclusion

A simple eco-friendly method of biotransforming toxic hexavalent chromium ions into Cr_2O_3 NPs by using the yeast *Schwanniomyces occidentalis* is described. The yeast formed extracellular crystalline Cr_2O_3 NPs under mild conditions without the use of toxic or expensive chemicals or high-end equipment. This process could be made cost-effective and could be scaled-up by employing yeast biomass obtained from industrial processes and by using Cr(VI) ions-containing wastewaters. Work on characterization of the EPS is on-going.

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