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Synthesis and characterization of monodispersed orthorhombic manganese oxide nanoparticles produced by *Bacillus* sp. cells simultaneous to its bioremediation

Arvind Sinha [a], Vidya Nand Singh [b], Bodh Raj Mehta [b], Sunil Kumar Khare [a,*]

[a] Enzyme and Microbial Biochemistry Laboratory, Department of Chemistry, Indian Institute of Technology, Delhi, Hauz Khas, New Delhi 110 016, India
[b] Thin Film Laboratory, Department of Physics, Indian Institute of Technology, Delhi Hauz Khas, New Delhi 110016, India

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ABSTRACT

A heavy metal resistant strain of *Bacillus* sp. (MTCC10650) is reported. The strain exhibited the property of bioaccumulating manganese, simultaneous to its remediation. The nanoparticles thus formed were characterized and identified using energy dispersive X-ray analysis (EDAX), high resolution transmission electron microscopy (HRTEM), X-ray photoelectron spectroscopy (XPS), powder X-ray diffraction (PXRD) and atomic force microscopy (AFM). When the cells were challenged with manganese, the cells effectively synthesized nanoparticles of average size 4.62 ± 0.14 nm. These were mostly spherical and monodispersed. The ex situ enzymatically synthesized nanoparticles exhibited an absorbance maximum at 329 nm. These were more discrete, small and uniform, than the manganese oxide nanoparticles recovered after cell sonication. The use of *Bacillus* sp. cells seems promising and advantageous approach. Since, it serves dual purposes of (i) remediation and (ii) nanoparticle synthesis. Considering the increasing demand of developing environmental friendly and cost effective technologies for nanoparticle synthesis, these cells can be exploited for the remediation of manganese from the environment in conjunction with development of a greener process for the controlled synthesis of manganese oxide nanoparticles.

1. Introduction

Synthesis of nanosize manganese oxide particles has attracted special attention because of their unique physical and chemical properties. The manganese nanoparticles have copious industrial applications in supercapacitors, catalysis, biosensors, ion sieves, molecular adsorption, high density magnetic storage media, batteries, drug delivery system and magnetic resonance imaging [1–6]. Since, the properties of nanoparticles are size and shape dependent, the synthesis process having good control on monodispersity, size and shape is an important area of research [7]. Currently, different chemical and physical methods are employed for the synthesis of nanosize manganese oxide particles. Most of these techniques require stringent reaction conditions, viz very high temperature, pressure and the use of toxic chemicals [8–10]. Also, a strong tendency to precipitate or coagulate during the synthesis renders the processes more difficult [11–14].

Biological systems have well organized and controlled physiological processes and thus, their use in the nanoparticle synthesis is rapidly gaining importance. Several strains of microorganisms are known for having metal resistance capability. They are endowed with various cellular mechanisms for metal detoxification. The synthesis of nanosized metal is one such strategy/adaptive feature [15,16]. The use of microbial systems in nanobiosynthesis have been successfully demonstrated for gold nanoparticles by *Bacillus subtilis*, *Pseudomonas* sp., *Verticellum* and *Fusarium* sp., silver nanoparticles by *Bacillus* sp., Fe_3O_4 magnetic nano crystals by *Magnetospirillum magnetotacticum*, palladium nanoparticle by sulphate reducing bacteria [17] and CdS nano crystals by *Rhodobacter spheroids* [18]. Microbes are therefore, amenable to be exploited, for the synthesis of nanoparticles with controlled shape, size and monodispersity [19].

In nature, the excess of Mn^{2+} is oxidized by both abiotic and biotic processes. The formation of Mn^{3+} and Mn^{4+} is thermodynamically favorable in the presence of oxygen and higher pH. Nevertheless, these are mostly catalyzed by microorganisms due to the higher activation energy [20]. Microbial systems, especially bacterial like *Pseudomonas putida* strains MnB1, *Bacillus* sp. SG-1, *Leptothrix discophora* SP-6 and *Leptothrix discophora* SS-1 have been used as attractive model systems for the microbial manganese oxidation [21]. However, these strains have rarely been exploited for the synthesis of manganese oxide nanoparticles [22].

Manganese rich discharge is one of the major constituent of industries involving steel and nonsteel alloy production, colorants, pigments, battery manufacture, fuel additives, catalysts, and metal coatings. Manganese at higher concentration is neurotoxic; causing neurological syndrome like Parkinson's disease [23,24]. Therefore

* Corresponding author. Tel.: +91 11 26596533; fax: +91 11 26581102.
E-mail addresses: skhare@rocketmail.com, skkhare@chemistry.iitd.ac.in (S.K. Khare).

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microbial systems having manganese bioaccumulating properties may be advantageous to serve two purposes (i) remediation and (ii) nanoparticle synthesis.

Taking advantage of the manganese bioaccumulating potential of the self isolated *Bacillus* sp. strain, the present work explores the feasibility of manganese remediation simultaneous to the synthesis of manganese oxide nanoparticles as a model system.

2. Materials and methods

2.1. Materials

$MnCl_2.4H_2O$ was a procured from Glaxo Laboratories Ltd. (Mumbai, India). The media components were procured from Hi Media Laboratories (Mumbai, India). All other chemicals used were of analytical grade. A stock solution of 1000 mg L^{-1} $MnCl_2{\cdot}4H_2O$ was prepared in Milli Q water and 0.22 μ filter sterilized $MnCl_2{\cdot}4H_2O$ solution equivalent to the respective manganese concentration was used in each experiment.

2.2. Bacterial strain and culture conditions

A metal resistant *Bacillus* sp. strain, isolated from soil was used in the present study. The samples were obtained from oil spilled soil near about IIT Delhi (India). The strain was isolated and purified by repeated streaking on nutrient agar plates. It was identified by morphological, biochemical and 16S rDNA analysis. The 16S rDNA sequence was submitted to National Center for Biotechnology Information GenBank, NCBI, USA. The NCBI GenBank accession number assigned is JF281096. The pure culture was submitted to Microbial Type Culture Collection, IMTECH Chandigarh, India, with accession no. MTCC 10650. The culture was maintained at 4 °C in agar slants and sub-cultured at monthly intervals. The purity of the laboratory culture was checked at regular time intervals by repeated streaking on nutrient agar plates.

2.2.1. Inoculum

A loopful of inoculum from the slant was introduced into the mother culture medium containing (g L^{-1}): yeast extract 3.0; peptone, 5.0; NaCl, 2.5; adjusted to pH 7.0 followed by incubation at 30 °C and 120 rpm. The 24 h grown culture having OD ~ 1.0 (A_{660} nm) was used as the mother culture.

2.2.2. Culture conditions

50 mL of culture medium containing (g L^{-1}): yeast extract, 3.0; peptone, 5.0; glucose, 5.0; NaCl, 2.5; $MgSO_4{\cdot}7H_2O$, 0.5; pH 7.5 was taken in a 250 mL Erlenmeyer flask. The culture medium was inoculated with 1% (v/v) mother culture and incubated at 30 °C with constant shaking at 120 rpm (Orbital Rotary Shaker, Orbitech, India). The culture medium composition and conditions were kept parallel in all the experiments otherwise stated. Growth of the cells was recorded at 660 nm using double beam UV–visible spectrophotometer (Specord 200, Analytikjena, Germany).

2.3. Biosynthesis of manganese oxide nanoparticles

Predetermined concentrations (as per the experimental conditions) of filter sterilized $MnCl_2{\cdot}4H_2O$ was added into the culture medium prior to inoculation. Rest of the culture conditions were kept same as described in Section 2.2.2. The samples were withdrawn periodically and processed accordingly for monitoring (i) the cell growth (ii) manganese concentration (iii) nanoparticle synthesis. Five millilitres of culture media was withdrawn aseptically at regular time intervals and centrifuged at 14,000 × *g* for 10 min at 4 °C. The supernatant was taken to estimate the residual manganese by using atomic absorption spectrophotometer (AAnalyst 100, Perkin-Elmer, USA). A part of the culture was further processed for the transmission electron microscopy. A control experiment was similarly run without inoculating with the cells to check for any abiotic precipitation of the manganese during the operational experimental conditions.

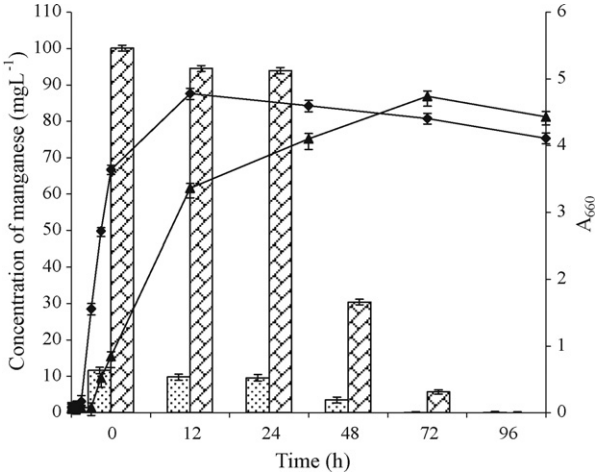

Fig. 1. Growth and manganese bioremediation by *Bacillus* sp. cells. The cells were grown in culture medium as described in materials and methods Section 2.3; [♦], bacterial growth (A_{660}) in presence of 10 mg L^{-1} manganese; [▲], bacterial growth (A_{660}) in presence of 100 mg L^{-1} manganese; [▨], residual manganese concentration in culture media (10 mg L^{-1} manganese); [▨], residual manganese concentration in culture media (100 mg L^{-1} manganese).

2.4. Effect of varying culture conditions on nanoparticle synthesis

Cells were cultivated as described in Section 2.3, except that one parameter was varied at a time. For incubation time, the cells grown in 100 mg L^{-1} manganese were harvested at different time intervals of 24, 48 and 72 h. The effect of manganese concentration was monitored at 100 mg L^{-1}, 150 mg L^{-1} or 200 mg L^{-1} manganese. The cells were harvested after 72 h of incubation. The harvested cells with different culture conditions were analyzed by transmission electron microscopy.

2.5. Analytical methods used for identification and characterization of synthesized nanoparticles

2.5.1. Transmission electron microscopy (TEM)

Cells grown in absence or presence of manganese were harvested by centrifugation at 8000 × *g* for 10 min at 4 °C. The pellets were washed thrice with phosphate buffer (0.1 M, pH 7.4) and fixed overnight in modified Karnovsky's fluid at 4 °C. Post fixation was done with 1% OsO_4 for 1 h at room temperature. Dehydration was carried out with acetone series (30, 50, 70, 90 and 100% acetone). The samples were treated for 30 min at each acetone concentrations and processed further as per the procedure of David et al. [25]. Transmission electron micrographs were recorded without regular double staining in TEM equipped with EDAX (HRTEM, Technai G^2; 200 kV, USA). High resolution transmission electron microscopy (HRTEM) and energy dispersive X-ray analysis was done on the same bacterial thin film used for taking TEM micrographs in nanoprobe mode.

2.5.2. X-ray photoelectron spectroscopy (XPS)

In a 250-mL Erlenmeyer flask, 50 mL of culture medium containing 100 mg L^{-1} manganese was inoculated and incubated as