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Eco-friendly synthesis and characterization of gold nanoparticles using *Klebsiella pneumoniae*

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Abstract

The thrust to develop eco-friendly procedures for the production of nanoparticles arises from the extremely recent nanotechnology research. Extracellular biosynthesis of gold nanoparticles was achieved by an easy biological procedure using *Klebsiella pneumoniae* as the reducing agent. After exposing the gold ions to *K. pneumoniae*, rapid reduction of gold ions is observed foremost to the formation of gold nanoparticles in colloidal solution. UV-vis spectrum of the aqueous medium containing gold nanoparticles showed a peak around 560 nm. The crystalline nature of the particles was confirmed from an X-ray diffractometer. Transmission electron microscopy (TEM) micrograph analysis of the gold nanoparticles indicated that they were well dispersed and ranged in sizes 35 to 65 nm. The high crystalline in the FCC phase is evidenced by bright circular spots in a selected-area electron diffraction pattern and clear lattice fringes in the high-resolution TEM image. Fourier transform infrared spectroscopy revealed possible involvement of reductive groups on the surface of nanoparticles. The method exploits a cheap and easily available biomaterial not explored so far for the synthesis of metallic nanoparticles.

Keywords: Gold nanoparticles, Extracellular synthesis, Characterization, *K. pneumoniae*, TEM

Background

Currently, nanoparticles (NPs) have drawn marvelous consideration because of their valuable properties on various fields such as medical, sensor, optical, electronic, and catalytic application [1]. Green-mediated synthesis and characterization of nanoparticles have emerged as a significant division of nanotechnology in the last decade, particularly for noble metals such as gold, silver, platinum, and palladium. Moreover, the chemical or physical approaches are usually employed to synthesize metal nanoparticles because of their intrinsic advantage in producing well-defined NPs with quite controllable shapes and sizes [2]. However, the former methods involve tedious treatments, such as inert gas condensation, pyrolysis [3], laser ablation, and hydrothermal and solvothermal synthesis [4]. Sol-gel method is based on inorganic polymerization reactions. The sol-gel process includes four steps: hydrolysis, polycondensation, drying, and thermal decomposition [5]. Ambitious by the growing momentum of green nanotechnology, the synthesis of NPs utilizing biological materials could be an improved alternative to toxic chemicals and the expensive physical methods. In general, biological organisms, such as bacteria and fungi majorly referred to as microorganisms [6], plants [7], and algae [8] represent a development of an eco-friendly and cost-effective approach [9]. Using of bacteria for the biosynthesis of metal nanoparticle process is the easiest method.

Following the revolutionary study of [10] on bacteria-mediated biosynthesis of silver NPs, many efforts have been directed toward this area with the use of bacteria, such as *Thermomonospora* sp. [11], *Lactobacillus strains* [12], *Shewanella algae* [13], *Actinobacter* sp. [14], *Plectonema boryanum* [15], *Rhodopseudomonas capsulate* [16], *Shewanella algae* [17], *Geobacter sulfurreducens* [18], *Morganella* sp. [19], *Bacillus subtilus* [20], *Staphylococcus aureus* [21], *Escherichia coli* [22], and *Serratia nematodiphila* [6]. These bacteria were demonstrated to be responsible for the bacterial synthesis and stabilization of metal nanoparticles. One of the chief applications of nanotechnology is the use of gold nanoparticles in biomedical research like X-ray computed tomography

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and magnetic resonance imaging [23], cancer nanotechnology [24], drug delivery applications [25], and its optical properties for cancer diagnosis and photo thermal therapy.

Herein, an eco-friendly method for the bacteria-mediated synthesis of gold nanoparticles by the reduction of $HAuCl_4$ ions using the broth of *Klebsiella pneumoniae* is reported. The bioreduction process was monitored by the UV-visible spectroscopy, and the crystalline structure was investigated by the X-ray diffraction (XRD) technique. The nanostructure and size of the synthesized gold nanoparticles were characterized by transmission electron microscopy (TEM) and Fourier transformation infrared spectroscopy (FTIR) was used to understand the biomolecules responsible for the biosynthesis.

Results and discussion

Isolation and identification

The bacterial strain is used for the synthesis of gold nanoparticles which is isolated from the saltpan soil from Tuticorin. The isolate strain MAA was morphologically and biochemically identified as *K. pneumoniae* that produces a red pigment. *K. pneumoniae* MAA was a gram-positive rod-shaped non-motile bacterium that has to be identified and maintained at the Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh.

Synthesis of gold nanoparticles

Visual identification of gold nanoparticles

The detailed study on extracellular biosynthesis of gold nanoparticle by *K. pneumoniae* was carried out in this work. Figure 1a shows the biomass of *K. pneumoniae* without the addition of $HAuCl_4$, and Figure 1b,c exhibits the $HAuCl_4$-treated biomass incubated at 6 to 24 h. In the biomass of *K. pneumoniae*, the gold nanoparticle synthesis process was completed at 24 h. The color change from yellow to dark purple indicates the formation of gold nanoparticles at 24 h incubation time. The appearance of a dark-purple color at 24 h of incubation time confirms the reduction of gold chloride into gold NPs using the *K. pneumoniae* culture supernatant. The pink color in the solution at 6 h indicates that the gold nanoparticle synthesis process has started. After 24 h, there was no absorbance. The color of the colloidal solution reaction turned from yellow to purple which indicated the formation of gold nanoparticles upon using *K. pneumoniae* observed in the UV-vis spectra and also confirmed the completion of gold nanoparticle synthesis reaction in both bacteria. The color change is dependent upon the incubation time (6 to 24 h) and the shape of the nanoparticles. Similarly, the gold nanoparticles were synthesized by *P. aeruginosa* [26] and *R. capsulate* [27]. The colloidal solutions of gold nanoparticles show a very intense color, which is absent in the bulk material as well as for individual atoms. The origin is attributed to the collective oscillation of free conduction electrons by an interacting electromagnetic field. These resonances are called surface plasmon resonances [28].

UV-vis spectra of gold nanoparticles

The production and stabilization of the reduced gold nanoparticles in the colloidal solution was monitored by

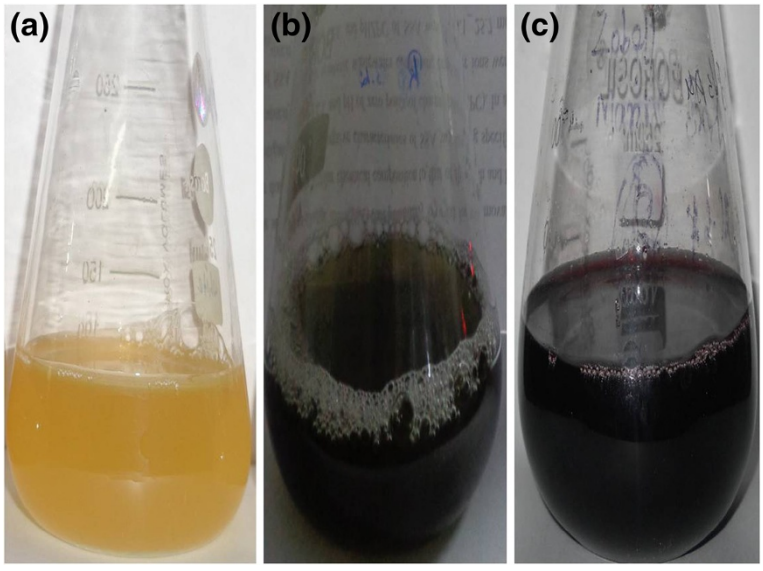

Figure 1 Extracellular synthesis of *K. pneumoniae* biomass and gold chloride mixed with biomass. *K. pneumoniae* biomass (**a**), 1 mM gold chloride mixed with biomass at the beginning of reaction showing a greenish-brown color change (**b**), and after 1 day of reaction showing a dark-purple in color change (**c**).

UV-vis spectrophotometer analysis. UV-vis spectroscopy is one of the most important techniques to identify the formation and stability of the gold nanoparticles in aqueous solution. Gold nanoparticles are known to exhibit at maximum in the range of 400 to 700 nm. The synthesis of gold nanoparticles was monitored at different time intervals such as 6, 12, 18, 24, 36, and 48 h. The gold nanoparticles synthesized by *K. pneumoniae* are positioned at 560 nm (Figure 2). Initially, at 6 h, the gold nanoparticle was absorbed slowly, and the absorbance was gradually increased up to 48 h. After 48 h, there was no absorbance which indicated that the gold NP synthesis process was completed. Similarly, Skirtach et al. (2005) reported that the gold NPs were absorbed at 560 nm, and it was synthesized using *P. aeruginosa.* The reduction of gold ions occurs comparatively slowly, but the gold nanoparticles are found to be very stable in the colloidal suspension. The spectrum shows three bands: one in the visible region and the other two in the NIR region, which is characteristic for the formation of anisotropic nanoparticles [29,30]. It is well known that spherical nanoparticles of Au should exhibit single-surface plasmon bands whereas anisotropic particles should exhibit two or three bands, corresponding to the quadrupole and higher multipole plasmon excitations [31,32].

XRD spectrum of gold nanoparticle

The crystalline nature of the gold nanoparticles was confirmed from X-ray diffraction analysis. The XRD pattern clearly shows that the extracellular synthesis of gold nanoparticles formed by the reduction of gold chloride ions using *K. pneumoniae.* Gold nanoparticles exhibited four prominent Bragg reflections at around 38.2°, 44.7°, 64.5°, and 78.3° (Figure 3) for *K. pneumoniae.* The fraction between the intensity of the (200), (220), and (311) diffraction peaks is much lower, suggesting that the (111) plane is the predominant orientation. The XRD facets of the gold nanoparticle match with standard gold which was published by JCPDS (file no. 04-0784). The mean size of gold nanoparticles was calculated using the Debye-Scherer equation by determining the width of the (111) and the similar Bragg reflection [33,34]. *R. capsulata* was found to have an average size of 38 nm which matched with the particle sizes obtained from TEM results discussed later.

FTIR

The FTIR measurements were carried out to identify the possible biomolecules responsible for the reduction and capping of the reduced gold nanoparticles synthesized by *S. nematodiphila* and *K. pneumoniae*. The FTIR spectra of gold nanoparticles with absorption peaks 1,716, 1,670, 1,630, 1,526, 1,389, 1,239, and 1,037 cm^{-1} for *K. pneumoniae* are seen in Figure 4. The strong peak at 1,630 cm^{-1} are identified as C=O stretching vibrations due to the carbonyl stretch in proteins of the respective amide I group of proteins [35,36]. The peak at 1,526 cm^{-1} shows the characteristic of N-O asymmetric stretching vibrations of nitro compounds. The small band at 1,670 cm^{-1} arises from the -C=C stretching vibrations corresponding to the -C=O stretching vibrations due to carboxylic acids and carbonyl groups [37,38]. The small peak at 1,239 cm^{-1} can be assigned to the C-N stretching vibrations of aliphatic amine groups. Gold nanoparticles can bind to proteins through free amine groups or carboxylate groups in the protein. The presence of the intense

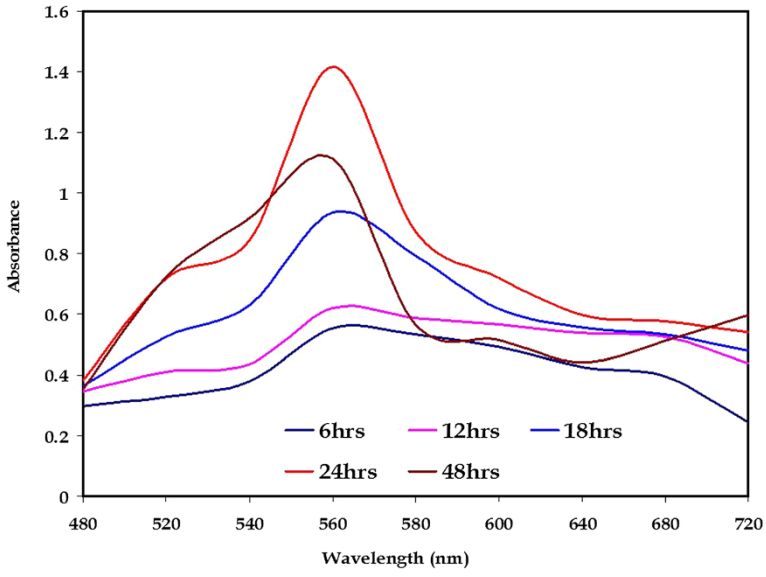

Figure 2 UV-visible absorption spectra of reduction of gold ions to gold nanoparticles using *K. pneumoniae.*

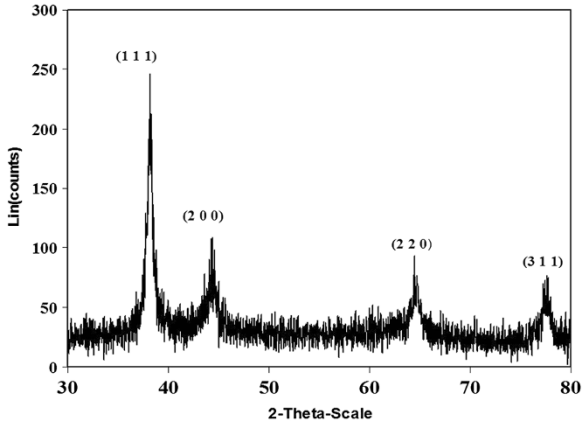

Figure 3 X-ray diffraction pattern of gold nanoparticles using *K. pneumoniae*.

peak at C=O stretching mode indicates the presence of carboxylic groups in the material bound to gold nanoparticles [39].

TEM and SAED pattern of gold nanoparticle

TEM analysis is performed to examine the size and shape of the biosynthesized gold nanoparticles using *K. pneumoniae*. Interestingly, the *K. pneumoniae*-derived gold nanoparticles exhibit a predominantly spherical shape (Figure 5). Such different sizes of gold nanoparticles are extracellularly synthesized by the bacteria *R. capsulate*, [34], fungus *Sclerotium rolfsii*, and plant *Coriandrum sativum*. The spherical-shaped nanoparticles are formed at the beginning of the reaction, and after that, the spherical-shaped nanoparticles are aggregated with each other. Due to the presence of enough amount of reducing agent in the biomass, the initially synthesized gold nanoparticles are stablilized in spherical shape, similar to the shape of nanoparticles synthesized by the bacteria *K. planticola* [40].

The single-crystalline nature of *K. pneumoniae* of these nanoparticles was further confirmed by their corresponding selected-area electron diffraction (SAED) analysis for *K. pneumoniae*. The SAED pattern of the spherical nanoparticle showed clear lattice fringes with bright circular rings corresponding to (111), (200), (220), and (311) planes. There are three sets of spots that could be identified from this diffraction pattern as shown in Figure 6. *K. pneumoniae*-derived gold nanoparticles also showed strong intensity at (111) plane of gold nanoparticles. Similarly, four concentric rings are observed in the gold nanoparticles indicating their crystalline nature, as reported by [39]. TEM analysis revealed that the synthesized gold nanoparticles are stable in solution.

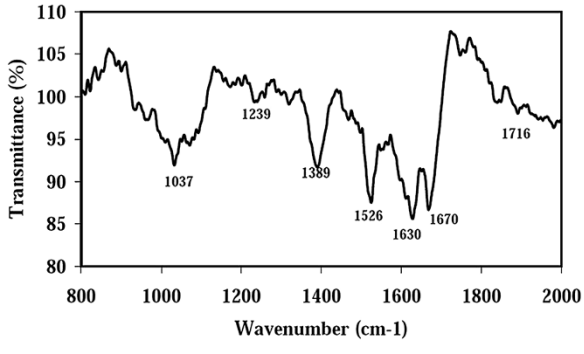

Figure 4 Typical FTIR absorption spectra of the bio-moieties of the macerated extracellular solution.

Conclusion

The microbe-mediated biosynthesis of gold nanoparticles is non-toxic compared to all other reported methods. Also, the method has advantages: the process is easy since it can be scaled, and it is also economically possible. Reduction and surface accretion of metals may be processed, by which bacteria keep themselves from the toxic effects of metallic ions. In the present investigation, the gold nanoparticles were synthesized using *K. pneumoniae* which was isolated from saltpan soil and identified by MTCC. The synthesis of gold nanoparticles was confirmed by color change of the liquid medium from yellow to intense dark purple, and it exhibited its maximum absorbance at 560 nm which played a prominent role in the reduction of gold chloride to gold nanoparticles. The X-ray diffractometer showed the crystalline nature of nanoparticles. The Fourier transform infrared spectroscopy showed that the functional groups of gold nanoparticles can bind to proteins through free amine groups or carboxylate groups in the protein. The morphology of the gold nanoparticles are found to be spherical in shape and stable in water for 3 months that can be attributed to the surface binding of stabilization materials secreted by the bacteria. Gold colloidal solution is biologically well suited and has the potential to be used in medical and pharmaceutical applications due to their homologous size distribution.

Methods

Isolation and identification of microorganism

The organism was isolated from saltpan soil, and the sample was collected from Tuticorin. The collection of samples was serially diluted, and the isolates were morphologically and microbiologically characterized as *Klebsiella pneumoniae*. The isolated culture was identified as *Klebsiella pneumoniae* (MAA) from MTCC and maintained it by a subculture process for the synthesis of gold nanoparticles.

Extracellular synthesis of gold nanoparticle

K. pneumoniae (MAA) was grown in 100 ml nutrient broth and incubated at 35°C for 24 h. The overnight

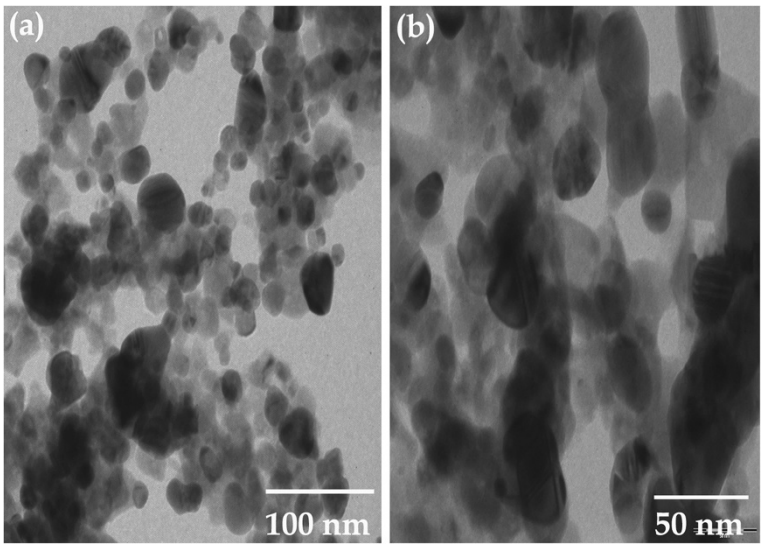

Figure 5 The Transmission electron microscopy and photo of gold nanoparticles prepared by using K. pneumoniae (a) 100 nm and (b) 50 nm. TEM images of gold nanoparticles using *K. pneumoniae* (**a**) and (**b**).

culture broth was centrifuged at 6,000 rpm for 10 min. The cell-free supernatants were collected, 1 mM of gold chloride was added and mixed, and the solution was incubated at 35°C for 24 h. The spectrum of the sample was measured using a UV-visible spectrophotometer.

Characterization of gold nanoparticle

UV-vis spectrophotometer

The bioreduction of pure $HAuCl_4$ are monitored using UV-vis spectroscopy at regular intervals. During the reduction, 0.1 ml of samples was taken and diluted several times with millipore water. After dilution, it was centrifuged at 800 rpm for 5 min. The supernatant was scanned using a UV-300 spectrophotometer (UNICAM, York Street, Cambridge, Cambridgeshire) for a UV-vis 1601 Schimodzu spectrophotometer (Kyoto, Japan), operated at a resolution of 420 nm.

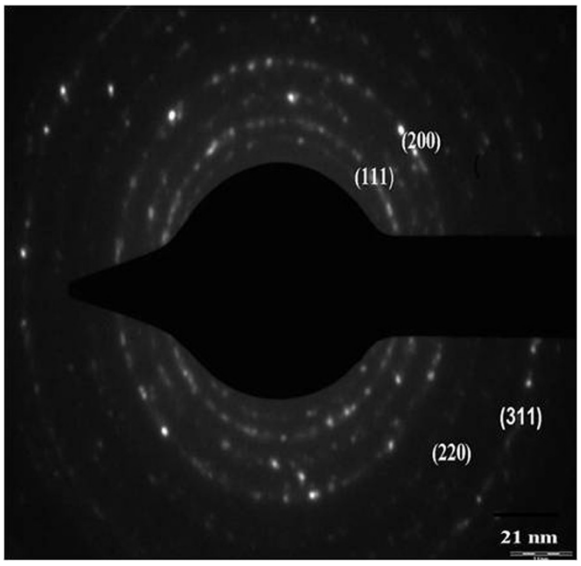

Figure 6 SAED pattern of gold nanoparticles synthesized using *K. pneumoniae*.

X-ray diffractometer

X-ray diffraction (Bruker, Karlsruhe, Germany) is one of the important techniques for the structure characterization of the crystalline material. Prepared nanoparticles can be analyzed by this instrument using the lynx eye detector (silicon strip detector technology).

Transmission electron microscopy

Transmission electron microscopy (TEM) analysis of the sample was done using a Philips CM 200 instrument (Philips, Amsterdam, The Netherlands) operated at an accelerating voltage of 200 kV with a resolution of 0.23 nm. A drop of the solution was placed on carbon-coated copper grid and later exposed to infrared light (45 min) for solvent evaporation.

FTIR

A known weight of sample (1 mg) was taken in a mortar and pestle and ground with 2.5 mg of dry potassium bromide (KBr). The powder so obtained was filled in a 2-mm internal diameter microcup and loaded onto the FTIR set at 26°C ± 1°C. The samples were scanned in the range of 4,000 to 400 cm^{-1} using a Fourier transform infrared spectrometer (Thermo Nicolet Model-6700, Waltham, MA, USA). The spectral data obtained were compared with the reference chart to identify the functional groups present in the sample.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
CM and SR carried out the nanoparticle synthesis. MV and KP carried out the manuscript preparation. GG carried out the antimicrobial activity. All authors read and approved the final manuscript.

Authors' information
CM completed her B.Sc. in Zoology and M.Sc. Environmental Biotechnology in Sri Paramakalyani Centre for Environmental Sciences, Manonmaniam Sundaranar University, Alwarkurichi, Tirunelveli. She achieved her Ph.D. degree under the guidance of GA in the field of nanotechnology. Her research interests include synthesis of semiconductor nanomaterials and metal nanoparticles and its biomedical applications. SR completed his M.Sc. Biotechnology in Periyar University and obtained his Ph.D. degree in Nanotechnology under the guidance of GA. He published seven research articles in nanoparticle synthesis using algae. He is interested in the metallic nanoparticle synthesis using algae and algae-derived compounds and their potential applications in the biomedical field. VM obtained her M.Sc. in Environmental Biotechnology in Sri Paramakalyani Centre for Environmental Sciences, Manonmaniam Sundaranar University, Alwarkurichi, Tirunelveli. She obtained her Ph.D. degree under the guidance of GA in the field of nanotechnology. Her research interests include eco-friendly synthesis of nanoparticles and its application in the environment. KP received his B.Sc. (2005) and M.Sc. in Biotechnology (2007) from Madurai Kamaraj University and Periyar University, India, respectively. He is currently working to obtain his Ph.D. degree at Manonmaniam Sundaranar University in India. He is interested in the green and immobilized microbe-mediated synthesis process of nanoparticles and nanocomposites and their biomedical and textile industry applications. GG completed her M.Sc. in Biotechnology in Periyar University and obtained her Ph.D. degree in Nanotechnology under the guidance of GA. Her research interests include green-meditated synthesis of nanoparticles and its agricultural applications for controlling plant diseases. GA received his M.Sc. in Applied Chemistry (1992) and Ph.D. in Environmental Biotechnology (1997) from Anna University, India. He had ten years (1999 to 2008) post-doctoral experiences from the National Taiwan University in Taiwan, National Institute of Advanced Industrial Science and Technology in Japan, and National Central University in Taiwan. He has received many research awards from Indian and other country governments. He is an associate editor in five international journals. At present, he is an associate professor of the Environmental Biotechnology and the leader of Environmental Nanobiotechnology Division at Sri Paramakalyani Centre for Environmental Sciences, Manonmaniam Sundaranar University, India. His main research interest is on the biosynthesis of nanoparticles and nanomaterials, nanobiocatalyst, and environmental chemistry.

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