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Synthesis of silver nanoparticles using bacterial exopolysaccharide and its application for degradation of azo-dyes

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ABSTRACT

In this study, the synthesis and characterization of exopolysaccharide-stabilized sliver nanoparticles (AgNPs) was carried out for the degradation of industrial textile dyes. Characterization of AgNPs was done using surface plasmon spectra using UV–Vis spectroscopy, X-ray diffraction (XRD) and Raman spectroscopy. The morphological nature of AgNPs was determined through transmission electron microscopy (TEM), scanning electron microscopy (SEM) and atomic force microscopy (AFM), which indicated that the AgNPs were spherical in shape, with an average size of 35 nm. The thermal behaviour of AgNPs revealed that it is stable up to 437.1 °C and the required energy is 808.2J/g in TGA-DTA analysis. Ability of EPS stabilized AgNPs for degradation of azo dyes such as Methyl orange (MO) and Congo red (CR) showed that EPS stabilized AgNPs were found to be efficient in facilitating the degradation process of industrial textile dyes. The electron transfer takes place from reducing agent to dye molecule via nanoparticles, resulting in the destruction of the dye chromophore structure. This makes EPS-AgNPs a suitable, cheap and environment friendly candidate for biodegradation of harmful textile dyes.

1. Introduction

Synthesis of diverse nano-materials are the keystone of nanotechnology for its application in different fields such as medicines (drug delivery, drug targeting, cell imaging and biosensors), food sciences (nano-composites, nano-emulsions, nano-encapsulation etc.) and environmental sciences (bio-flocculant, microbial monitoring and detection, and chemical degradation [1,2]. Food-grade micro-particles and nanoparticles are synthesized from different range of ingredients such as biopolymers, surfactants, minerals and lipids and they own the ability to alter the functional behaviour of foods that is they can make the foods suitable for human health [4]. Many microflora can produce nanoparticles through both intra and extracellular levels. Silver nanoparticles (AgNPs) are used as a nanomaterial most commonly in various consumer products [3]. Synthesis of AgNPs can be performed in various parts of the microbial cells [5]. The purified polysaccharides from plants, animals and microflora sources were used as reducing and stabilizing agents for the synthesis of nanoparticles [6,7]. Polysaccharides have hydroxyl and hemiacetal groups, which plays a vital role in reduction and stabilization that generate vast chances for their application and probable mass production. It increases the eco-friendly approach characteristics of nanoparticles to avoid using toxic chemicals in the demand of growing technological processes [8]. Several lactic acid bacteria (LAB) such as *Lactobacillus spp.*, *Pediococcus pentosaceus* and *Enterococcus faecium* are able to reduce silver ions to silver nanoparticles. LAB produces diverse categories of exopolysaccharides containing different monomers (glucose, galactose, mannose and fructose) those are known to involve in redox reaction to synthesize silver nanoparticles (AgNPs) [10]. Recently, it was found that the AgNPs with a high surface area have more reactivity towards chemicals compounds and are effective tools in treatment of waste water within a short time period [11]. Chitosan-stabilized AgNPs combined with advanced oxidation process (AOP) showed good results in the degradation of various dyes [12].

Recently, AgNPs are extensively used to degrade the organic dyes through redox potential techniques and photocatalytic reaction under solar radiation [13,14]. In this study, we characterized the exopolysaccharide stabilized AgNPs for degradation of Methyl orange (MO) and Congo red (CR).

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2. Materials and Methods

2.1. Bacterial strain and chemicals

The EPS producing strain *Leuconostoc lactis* was isolated from idli batter (an acidic fermented food) and has been characterized through 16 s rRNA level (NCBI Gene bank submission ID KC117496) which further used as the source of exopolysaccharide in this work [15]. All reagents used were of analytical grade.

2.2. Growth condition for L. lactis KC117496

For preparation of inoculum, a loopful of *L. lactis* was transferred into 5 mL MRS medium supplemented with 2% sucrose. For EPS production, 10 mL of culture was used as an inoculum in 100 mL of MRS broth (2% sucrose) and incubated under shaking conditions (rpm) for 48 h at 30 °C [15].

2.3. Extraction and purification of EPS

The fermented broth was harvested after 48 h and the cell suspension was heated to 100 °C for 10 min to inactivate the enzymes. Further, the suspension was cooled to room temperature and centrifuged at 4100 x g for 20 min to remove the biomass. The crude solution was further treated with Sevage reagent (chloroform: n-butanol at 5:1 v/v) three times to remove the proteinaceous materials. EPS was precipitated with cold ethanol (thrice volume) and left overnight at 4 °C. The precipitate was collected through centrifugation at 19200 x g for 15 min and dissolved in Milli Q water. Afterwards, it was encased in a dialysis bag (12–14 KDa) and dialyzed at 4 °C with Milli Q water for 48 h for partial purification. The sugar content of EPS was analysed using phenol sulphuric acid method [16]. The EPS was characterized by using FT-IR, HPTLC, NMR, AFM, SEM, TGA and XRD analysis which revealed the presence of only glucose monomers, indicating the glucan nature of EPS consisting α-(1→6) and α-(1→3) glycosidic linkages [15].

2.4. Synthesis of polymeric silver nanoparticles (EPS-AgNPs)

The partially purified EPS (10 mg) was dissolved in 10 mL of Milli Q water to form a uniform dispersion and 9 mM $AgNO_3$ was added under stirring condition. Subsequently, this solution was stored in a dark place at room temperature. After 24 h, the colourless solution changed to yellow, indicating the formation of polymeric sliver nanoparticles. Furthermore, to increase the concentration of solution it was further kept under incubation for 1 month. Samples were taken at various intervals and in between to know the progress of nanoparticle formation. Afterwards, the solution was centrifuged at 19200 x g for 15 min. The pellet was collected and air dried at room temperature for further analysis [17].

2.5. Characterization of sliver nanoparticles

2.5.1. UV–vis Spectroscopy

The reduction of Ag^+ ions with EPS to form sliver nanoparticles was observed after 1, 5, 10, 20 and 30 days of incubation under UV–Vis spectroscopy (UV-1800, Shimadzu) in the range of 300 to 800 nm [17].

2.5.2. TEM and SEM analysis

A drop of EPS-AgNPs was distributed onto a carbon copper grid and dried completely using a vacuum desiccator. The images were obtained using a transmission electron microscope (TEM) and a scanning electron microscope (SEM-Hitachi, Model: S-3400N) [18].

2.5.3. AFM analysis

About 5–10 μL of EPS-AgNPs was distributed on a mica disc (Pelco mica disc 10 mm) by a spin rotating plate and absolute ethanol was dropped over the sample to fix it on the mica disc. Then, the mica sheet was air-dried to remove the residual ethanol. The AFM images were captured using a scanning probe microscope (Brukers MM8) in tapping mode. The cantilever oscillated at its appropriate frequency (158 kHz) and ambitious amplitude (0.430 V) [19].

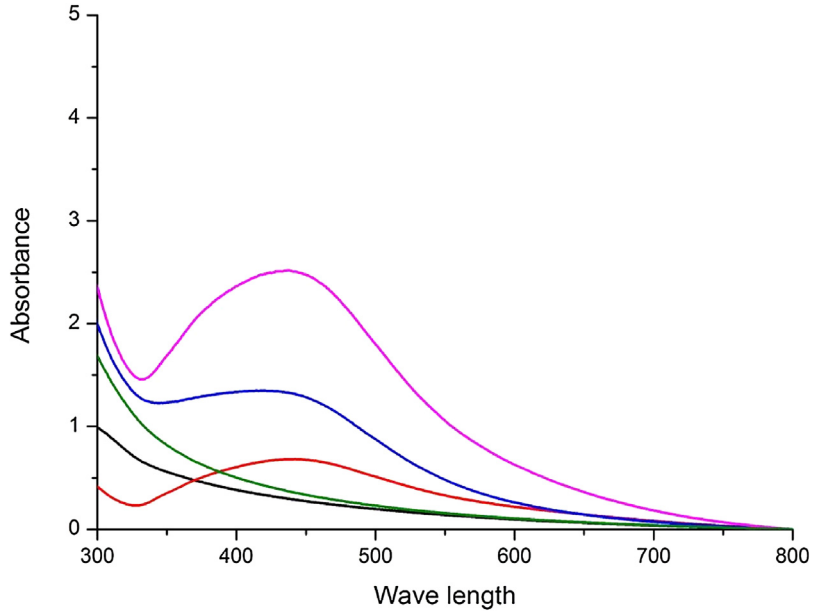

Fig. 1. Uv-Vis spectroscopy indicating the synthesis of EPS-AgNPs in increasing order during different storage period (Black–0 h, Green- 1 day, Red-10 days, Blue-20 days, Violet-30 days).