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Research Article

EXTRACTION OF EXOPOLYSACCHARIDE FROM *ENTEROCOCCUS FAECIUM* GRDAA AND THE APPLICATION OF ITS SILVER NANOPARTICLES IN DEGRADATION OF AZO DYES

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ABSTRACT

The present study was delineated on synthesis and characterization of exopolysaccharides (EPS)- stabilized silver nanoparticles (AgNPs) were carried out for the degradation of industrial textile dye. The green synthesis of AgNPs was performed using bacterial EPS as reducing and stabilizing agent. SEM analysis revealed that spherical-shaped AgNPs stabilized by EPS and AgNPs are homogenous and stable for longer duration as determined by UV-Vis spectroscopy and FTIR analysis. EDX results with high peak of Ag that signifies its presence in the suspension. The efficient degradation of textile dyes such as Methyl orange (MO), Congo red (CR) and food coloring agents Yellow and Green was attempted with EPS stabilized AgNPs and it was found to be better than bacterial degradation and AgNPs. These EPS-stabilized AgNPs can further be used as environmental friendly and a low cost strategy for degradation of harmful dyes with potential applications in textile industries and food additives.

Keywords: Exopolysaccharide, AgNPs, Characterization, Azo-dye degradation

1. INTRODUCTION

The lactic acid bacteria (LAB) are defined as a group of Gram positive, non sporing cocci and rods with non-aerobic habit but aero tolerant. LAB was first isolated from milk. They can be found in fermented products such as meat, milk products, vegetables, beverages and bakery products. LAB occurs naturally in soil, water, manure, sewage, silage and plants. They are part of micro biota on mucous membrane such as the intestines, mouth, skin, urinary and genital organs of both human and animals and have a beneficial influence on these ecosystems [1]. Exopolysacharides (EPS) are formed monosaccharide residues of sugar derivatives [2]. Bacterial exopolysaccharides are ubiquitous in marine resources and are distributed in the form of free living or associated forms such as biofilm microbial mats etc. Microbial exopolymeric substances are produced by both prokaryotes and eukaryotes. Microbial EPS generally exist in two forms depending on their locations: 1) cell bound exopolysaccharides, which closely adhere to the bacterial surface as capsular (cEPS's), 2) released EPS that release into surrounding medium [3, 4]. Synthesis of silver nanomaterials is 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 [5]. Food grade micro particles and nano particles are synthesized from different range of ingredients such as biopolymers, surfactants, minerals and lipids and owe the ability to alter the functional behavior of foods suitable for human health [6, 7]. Many micro floras can produce nano particles through both intra and extracellular levels. Silver nanoparticles (AgNp's) are used as a nanomaterial in various consumer products. Synthesis of AgNp's can be performed in various parts of the microbial cells. The purified polysaccharides from plants, animals and microflora sources were used as reducing and stabilizing agents for the synthesis of nanoparticles [8]. Several LAB such as *Lactobacillus* spp., *Pediococcuspentosaceus* 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 AgNp's. Recent research depicted that AgNp's with higher surface area have more reactivity towards

chemical compounds and are effective tools in treatment of waste water within a short time period [9]. Chitosan stabilized AgNp's combined with advanced oxidation process (AOP) showed good results in the degradation of azo dyes [10]. Recently, AgNp's are extensively used to degrade the organic dyes through redox potential techniques and photo catalytic reaction under solar radiation [11, 12]. In this study, we characterized the exopolysaccharide stabilized AgNp's for degradation of Azo dyes.

2. MATERIAL AND METHODS

2.1. Bacterial strain and chemicals

The EPS producing strain *Enterococcus faecium* GRD AA was isolated from milk sample collected and identified by 16s rRNA level (NCBI Gene Bank submission ID MH113816) which further used as s source of exopolysaccharide in this study. All reagents used were of analytical grade [13].

2.2. Growth condition for *Enterococcus faecium* GRDAA (MH113816)

For preparation of inoculum, a loop of *E. faecium* was transferred into 5 ml MRS medium supplemented with 2% sucrose. For EPS production, 10 ml of culture was used as an inoculum in 100ml of MRS broth (2%sucrose) and incubated under shaking conditions (rpm) for 48h at 30°C [14].

2.3. Extraction and purification of EPS

The fermented broth was harvested after 48h 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 4100xg for 20 min to remove the biomass. The crude solution was further treated with sewage reagent (chloroform: n butanol at 5:1v/v) three times to remove the proteinaceous materials. EPS was precipitated with cold ethanol (thrice the volume) and left overnight at 4°C. The precipitate was collected through centrifugation at 19200xg for 15 min and dissolved in MilliQ water. Further encased in a dialysis bag and analyzed at 4°C with MilliQ water for 48h for partial purification. The sugar content of EPS was analyzed using Phenol Sulphuric acid method. The EPS was characterized by using FTIR [15].

2.4. Synthesis of polymeric silver nanoparticles (EPS-AgNp's)

The partially purified EPS (10mg) was dissolved in 10 ml of MilliQ water to form a uniform dispersion and 9mM AgNO3 was added under stirring condition. Subsequently this solution was stored in dark at room temperature. After 24h, the colorless solution changed to yellow, indicating the formation of polymeric silver 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 19200xg for 15 min. The pellet was collected and air dried at room temperature for further analysis [15].

2.5. Characterization of silver nanoparticles

2.5.1. UV-Vis spectroscopy

The reduction of Ag+ ions with EPS to form silvernanoparticles was observed after 1,5,10,20 and 30 days of incubation under UV-Vis spectroscopy (HACH) in the range of 300-800nm [16].

2.5.2. SEM analysis

A drop of EPS- AgNp'sas distributed onto a carbon copper grid and dried completely using a vacuum desiccator. The images were obtained using scanning electron microscope (SEM) [16].

2.5.3. Degradation of organic azo dyes in aqueous solution by EPS-AgNp's

The catalytic degradation of organic azo dyes such as methyl orange (MO) and Congo Red (CR) were monitored using UV-Vis spectroscopy. A 3 ml of 10^{-5}M aqueous solutions of MO and CR on a quartz cuvette were taken and 5mg of EPS-AgNp's solutions were added separately. To this reaction mixture, 50 ml of 0.1 M sodium citrate was added and the decreasing absorption of maximums of azo dyes were recorded [16].

3. RESULTS AND DISCUSSION

Milk sample was selected as a source for isolating bacteriocin producing lactic acid bacteria. From more than 10 colonies screened for the antibacterial activity against the indicator strain, one colony was found to produce high inhibitory zone, which was designated as ALB. The pure cultures were maintained on MRS Agar. Nowadays there is a growing concern regarding the replacing of the use of chemical preservatives in food. Lactic acid bacteria (LAB) have been used for a long time for the fermentative preservation of several food products from dairy product to meat and vegetables. They can produce antimicrobial substances (e.g. organic

acids, hydrogen peroxide and bacteriocins) which can influence the growth of the possible harmful and/or spoilage microorganisms. Since vegetables are sources of several vitamins, dietary fibers and minerals, it is desirable that vegetable-based food products preserve their biological values during fermentation. There becomes more conspicuous the needless of heat treatment and the controlled safe fermentation process with starter cultures. Besides the traditional role of lactic acid bacteria in food preservation, they exert beneficial effects to the gut. Intestinal epithelial cells play unique role as the initial point of contact with any pathogens and other immunological challenges. In addition to their basic barrier function, they are able to interact with cells in the gut-associated lymphoid tissue. Ensuring safe and quality food has become more complex task today, than at any point of time in the history. Processed and packaged foods were luxury item in colonial times but after 1960 these food items were in great demand globally due to growing urbanization, breakdown of large families into nuclear families and increase in the number of working women. Chemical preservatives and other traditional barriers have been used in food products to inhibit microbial growth which lead to serious health disasters, thus challenging the food scientists for providing safer and healthier food. Food preservation has become a major issue because food borne pathogens can cause havoc in preserved/fresh food items at high temperature, room temperature and even at low temperature [17]. Lactic acid can be synthesized industrially by two means either through chemically or by microbial fermentation. However, the least one (fermentation through microbes) has some potential advantages e.g. pure lactic acid can be attained whereas, chemical synthesis of lactic acid always give a raceme mixture [18]. The isolates were assayed for its bacteriocin activity, had good inhibitory activity on the growth of the indicator strain *L. monocytogenes* MTCC 657.

16S rRNA genes were amplified using specific primers and amplified products were visualized by agarose gel electrophoresis. The amplicons were sequenced using 16S rDNA sequencing and the identity of the sequences were determined using NCBI blast where the sequences were searched against GenBank database were found to be *Enterococcus faeciu*m. The obtained sequences were further submitted to obtain accession number MH113816. *Enterococcus faecium* is a Gram-positive, homo-fermentative, lactic acid bacteria that is natural inhabitant of the gastrointestinal tract. Nevertheless, they are also found in fermented foods and are frequently isolated from starter cultures and cheese producers [19]. *Enterococcus faecium* also has a place in food biotechnology industry. The presence of the bacteria in certain foods helps develop flavours and aromas. The synthesis of EPS was determined by growing *Enterococcus faecium* GRD AA on MRS agar supplement with 10% (w/v) sucrose the synthesis of EPS was observed by visual appearance of creamy, mucoid colonies (plate 10). EPS was isolated, purified and the sugar content of EPS was analyzed using phenol sulphuric acid method is 0.9mg/ml.

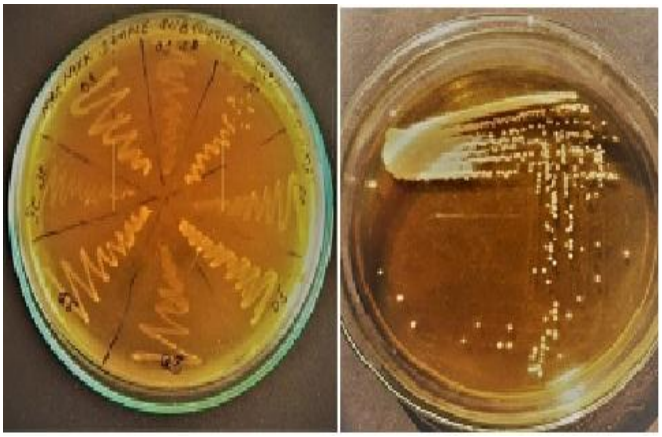

Fig. 1: Isolation and screening of lactic acid bacteria

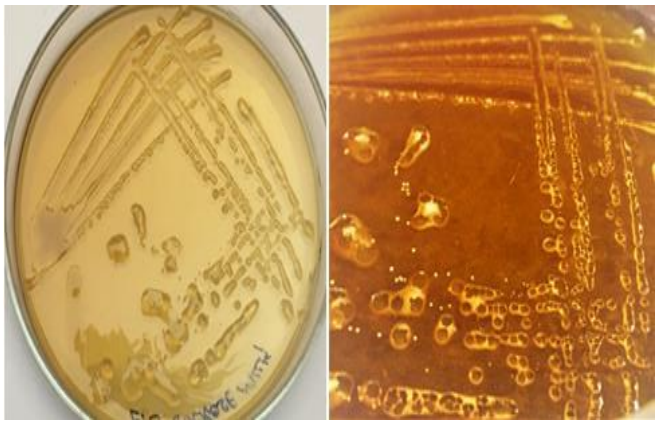

Fig. 2: Screening of exopolysaccharide

The partially purified EPS (10 mg) was dissolved in 10 mL of Milli Q water to form a uniform dispersion. 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. The first ever concept was

presented in 1959 by famous prof. of physics Dr. Richard Feynman. The term "Nanotechnology" was coined by an eminent scientist Prof. Norio Taniguchi in 1974. "Nanotechnology" is a technology of applied science which works in the field of theoretical as well as experimental changes at molecular level of compound. The word "Nano" derived from the Greek word "Nano", which means extremely small at Nano level. The prefix "Nano" means a size of 10-9. It allows the arrangement of small structures with accuracy, intelligibility and in expensive. "K. E. Drexler" is known as the father of Nanotechnology. He is the man who explains the nanotechnology in depth and popularized the subject. He is an American engineer known for increasing the value of molecular Nanotechnology. Nanotechnology has a breakthrough as a multidisciplinary scientific field and is undergoing uncontrollable development [20, 21] . The products of Nanotechnology are nanoparticles, nanotubes, and Nano rods, Nano spheres that have size below 100nm and have high surface area to volume ratio. The minimization of size brings about marked changes in their morphological properties with respect to those properties observed in massive materials. The products of nanotechnology can be of metallic nature, having mineral and polymer-based materials [22]. Nanotechnology is a big tree of research, having branches in every dimension and touches different fields like cosmetics, electronics, packaging, bio-sensor, medicine and paints, automobile, bio-engineering and catalysts. In Canada, there are 80 companies that make 150 products that use 88 different nanomaterials. The purified polysaccharides from plants, animals and microflora sources were used as reducing and stabilizing agents for the synthesis of nanoparticles. Polysaccharides have hydroxyl and hemi-acetal 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. Several lactic acid bacteria (LAB) such as *Lactobacillus* spp., *Pediococcus pentosaceus and Enterococcus faecium* are able to reduce silver ions to silver nanoparticles.

EPS-AgNPs were synth-sized through reduction of Ag+ into Ago from $AgNO_3$.The colourless solution changed to dark yellowish brown colour, indicating the formation of AgNPs, which was monitored in UV–Vis spectra ranges (300-800 nm). The synthesis of polymeric AgNPs after 1, 5, 20 and 30 days of incubation formed a broad surface plasmon resonance absorption band in between 400-550 nm. The intensity of band was increased by increasing storage period up to 1 month in a dark room, indicating the synthesis of AgNPs was increased during the storage period.

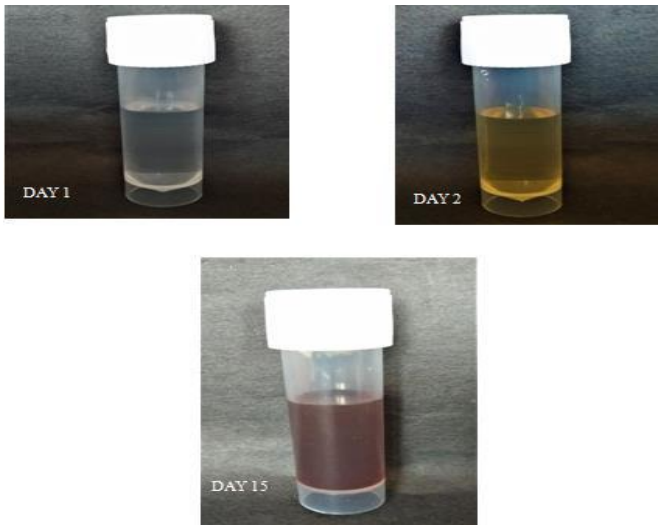

Fig. 3: Synthesis of silver nanoparticles

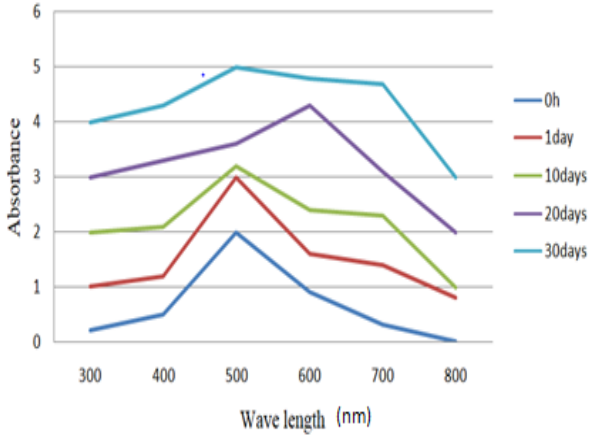

Fig. 4: UV–Vis spectrum of AgNPs synthesized from EPS of *Enterococcus faecium* GRDAA

FTIR analysis was used to identify the molecules, proteins and functional groups involved in the reduction of silver ions into silver nanoparticles (Fig. 5). The FTIR analysis showed that the absorption peaks located at 3784.34 and 3714.90 cm^{-1}(O-H stretch of alcohol), 3348.42cm^{-1}(Primary amines-asym NH2 Stretch), 3255.84cm^{-1} (Primary amines-symm NH2 Stretch), 1639.49cm^{-1} (Tertiary amides-amides bond), 1396.46cm-1 (Anthacenes ring stretch), 1280.73cm^{-1} (Epoxy deviation S-ring stretch) and 1145.72cm^{-1} (n-

alkanes CH_2 twist and rock). The other peaks found to have involvement in the EPS AgNPs adsorption were at 1203.58, 3961.79 and 3903.92cm^{-1} respectively.

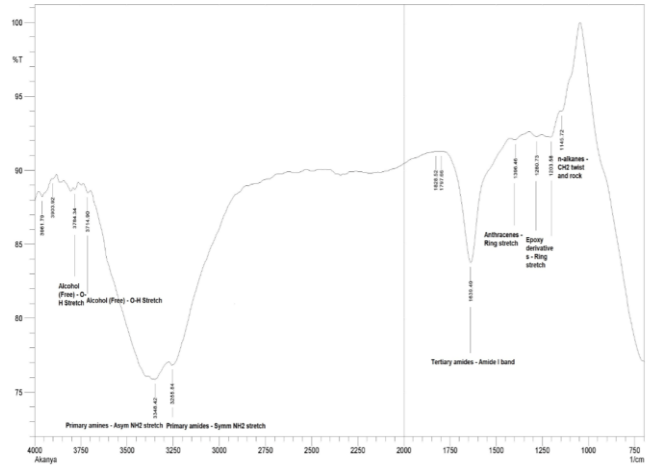

Fig. 5: FTIR analysis of silver nanoparticles synthesized from EPS of *Enterococcus faecium* GRDAA

SEM of the biosynthesized AgNPs synthesized from EPS of *Enterococcus faecium*was used to define the morphology of the AgNPs from the EPS. The aqueous solution AgNPs of synthesized was dried and subjected to scanning electron microscopy.

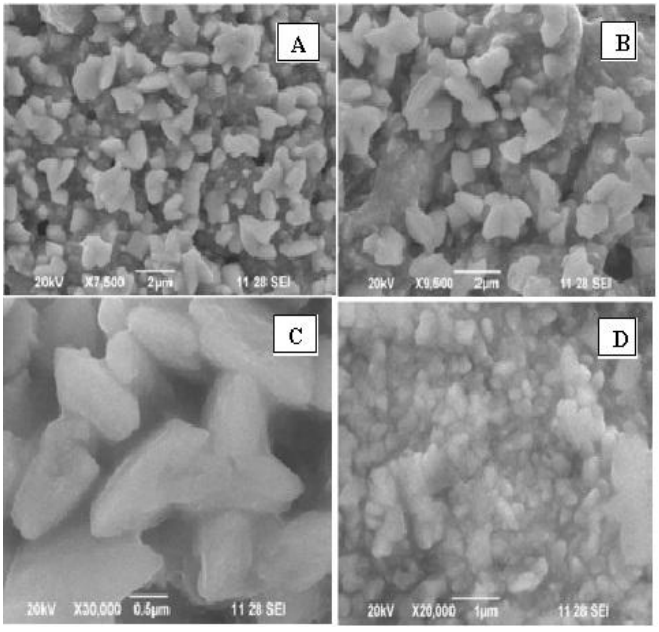

Fig. 6: A and B -Scanning electron micrograph of synthesis of AgNPs from the EPS; C and D - Scanning electron micrograph exhibit the morphology of EPS stabilized AgNPs

The element composition of the silver nanoparticle EPS from *Enterococcus faecium*was used by EDX to verify the presence of Ag in the suspension of nanoparticles. The EDX result showed a high peak of Ag NPs with no other elemental contaminants.

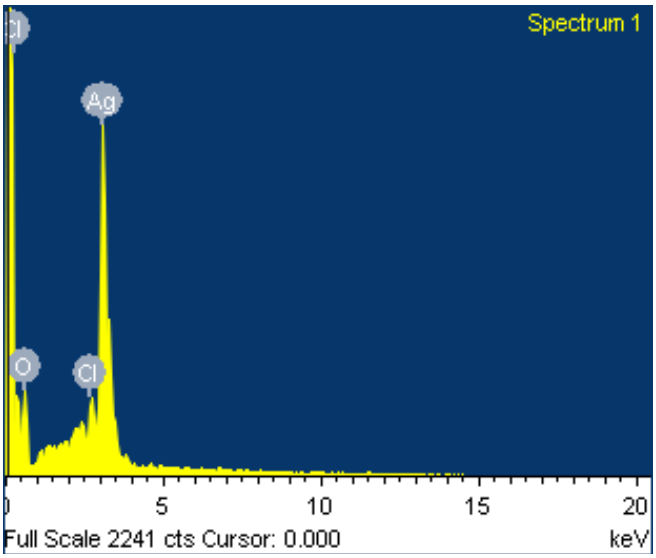

Fig. 7: Characterization of AgNPs by EDX analysis displaying the purity and chemical composition of the AgNPs

To examine the catalytic efficiency of EPS-stabilized AgNPs towards degradation of various organic dye molecules using sodium citrate as a reducing agent, commercial colors Methyl orange (MO) ,Congo red (CR), food coloring agent-yellow, green commonly used in many dyeing industries have been selected. The degradation reaction was carried out at room temperature and monitored using UV–Visible spectrometry. The catalytic degradation of these dyes was monitored by the change in absorbance at 465. The amount of EPS-AgNPs was varied keeping other parameters as constant to determine the effect of the amount of catalyst on the rate of the degradation. Experiments were performed by using 1 mg to 8 mg of EPS-AgNPs nanomaterial and the results were shown in terms of dye degradation. The degradation rate values were plotted against the amount of catalyst, as shown in Fig.8. 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). 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. Chitosan-stabilized AgNPs combined with advanced oxidation process (AOP) showed good results in the degradation of various dyes

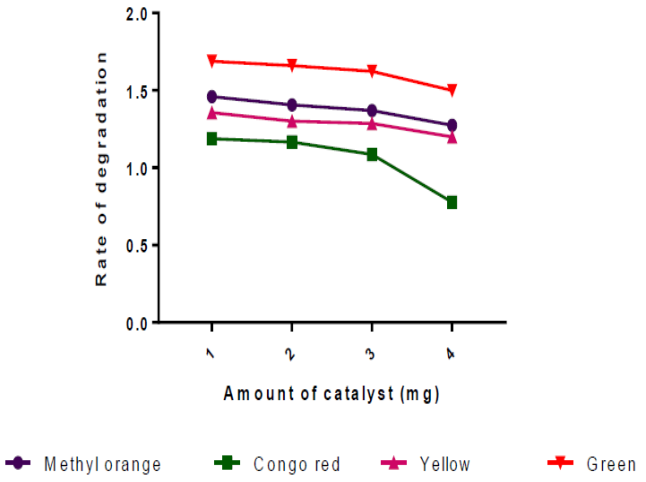

Fig. 8: degradation of organic azo-dyes in aqueous solution by EPS AGNPS

4. CONCLUSION

The green synthesis of AgNPs was performed using bacterial EPS as both reducing and stabilizing agent.SEM analysis revealed that spherical-shaped AgNPs stabilized by EPS and AgNPs are homogenous and stable for longer duration as determined by UV–vis spectroscopy and FTIR analysis.EDX results shows the high peak of Ag that confirmed its presence in the suspension.The efficient degradation of textile dyes, Methyl orange (MO),Congo red (CR) and food coloring agents Yellow and Green was attempted with EPS stabilized AgNPs and it was found to be better than bacterial degradation and AgNPs. These EPS-stabilized AgNPs can be used as environmental friendly and a low cost strategy for degradation of harmful dyes with potential applications in textile industries and food additives.

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