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RESEARCH ARTICLE

Photocatalytic Dye Degradation Potential of Silver Nanocomposite from Exo-polysaccharide of *Bacillus sp.*, An Associative Bacterium of *Sargassum wightii*

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Abstract

Objectives: Screening, production, and characterization of exopolysaccharides (EPSs) from *Bacillus* sp., an associative bacteria isolated from marine seaweed *Sargassum*. To synthesize and characterize silver nanocomposites using EPS isolated from *Bacillus* sp. and to evaluate their application in dye degradation. **Methods:** Seaweed-associated bacteria were isolated from *Sargassum wightii* by serial dilution and pour plate method. The colonies capable of producing exopolysaccharides on YMG agar plates were purified and identified up to the Genus level by Bergy's Manual of Determinative Bacteriology. The EPS-mediated silver nanoparticles (EPS-AgNPs) obtained from partially purified EPS were characterized by chemical analysis, UV-visible spectroscopy, FT-IR, SEM, and Powder XRD and evaluated for photocatalytic dye degradation. **Findings :** A seaweed-associated bacterium, *Bacillus* sp. was isolated from *Sargassum wightii*, and the extracted EPS was confirmed by the presence of amide, hydroxyl, and carboxyl groups in FTIR. Further, Exopolysaccharide-silver nano-composite was prepared. The characterization studies show a specific absorbance peak at 217 nm in the UV-Vis spectrum and the presence of hydroxyl and carboxyl groups in FT-IR spectroscopy. This confirms that EPS is responsible for the reduction of silver-to-silver nanoparticles. The SEM analysis reveals the formation of AgNPs with sizes ranging from 80-100 nm in a spherical shape. Under sunlight irradiation, the photocatalytic dye degradation of organic dyes like Congo red and methylene blue was studied with the synthesized exopolysaccharide-mediated silver nanoparticle (EPS-AgNPs). The percentage of degradation efficiency was calculated as 97% for Congo red

and 95.6% for Methylene blue after 48 hours of exposure time. **Novelty :** The novel idea of the study was the utilization of potential associative bacterial isolate from seaweed *Sargassum wightii* which is capable of producing an EPS for the synthesis of silver nanocomposite. A significant achievement of this study is that the EPS component from the new bacterial isolate was suitable for the synthesis of silver nanocomposite which helps in the gradual photocatalytic degradation of textile dyes by providing a longer half-life and better degradation efficiency.

Keywords: EPS; SEM; AgNPs; FTIR; Sargassum

1 Introduction

One of the major groups of pollutants in wastewater is organic dyes. They have been a major concern and are now a threat to the environment due to significant pollution problems. Severe damage to aquatic organisms due to obstruction of sunlight, eutrophication and reduced reoxygenation capacity is caused by the accumulation of these dyes in water bodies [(1)]. Photocatalytic dye degradation via irradiation generates electron-hole pairs, which then undergo a redox reaction on the nanoparticle surface, resulting in the generation of hydroxyl radicals. These radicals have the ability to degrade harmful dyes by acting as oxidizing agents [(2)]. Besides, nanoparticle synthesis through a biogenic process is eco-friendly, cost-effective, and can also act as renewable materials that can be used to reduce metals and stabilize nanoparticles [(3)].

When compared to bulk metals, metal nanoparticles particularly silver nanoparticles have been the focus of research over the last two decades because they have been discovered to have different and enhanced properties [(4)]. The high reactivity of these nanoparticles is due to their crystallographic surface structure and large surface area as well as low diffusion resistance, faster adsorption equilibrium and higher adsorption capacity making them excellent adsorbents [(5)]. These properties enable metallic nanoparticles to be used in a variety of applications including health care, medicine, electronics, environment, agriculture and pharmaceutical products. Recently, for the degradation of toxic dyes there has been a lot of interest in the use of silver nanoparticles. There is a lot of potential in dye remediation for Nano-adsorbents because of their unique properties such as high catalytic activity, quick recovery and large specific surface area [(6)].

In the synthesis of silver nanoparticles, purified polysaccharides derived from microbial sources were used as reducing and stabilising agents. Carboxyl, hemiacetal, and hydroxyl groups play critical roles in reduction and stabilisation, resulting in a wider range of applications. It enhances the eco-friendliness of nanoparticles in order to circumvent the use of harmful chemicals in arising technological processes [(7)]. Exopolysaccharides (EPSs) are often found in the surrounding as the outermost structure of both prokaryotic and eukaryotic microbial cells. They exist in a wide range of distinct and frequently complex chemical structures and are thought to provide self-protection against anti-microbial agents. By surrounding the cells and their proximity, EPS may help microorganisms to survive in harsh conditions such as very high salinity and low food availability [(8)]. EPS can be produced by several types of microorganisms such as bacteria (extremophiles and marine bacteria) [(9–11)], fungi [(12)], cyanobacteria [(13)] and microalgae [(14)].

Extracellular polysaccharides can be produced by bacterial flora associated with marine algae such as unicellular diatoms and marine planktonic algae [(15)]. Seaweeds, particularly brown algae provide protected and nutrient-rich environments for bacterial growth. *Sargassum* sp. is a brown alga with a diverse community of beneficial

microorganisms[16]. Sun et al., isolated and cultured the alginate lyse excreting bacterial flora from *Sargassum*[17]. Bacterial flora associated with the larger multicellular marine algae is capable of synthesizing good quality of exopolysaccharide. Hence, the present study focused on the screening of new bacterial strain producing higher quantity of EPS isolated from the brown algae *Sargassum wightii* and synthesis of silver nanoparticles from bacterial EPS, characterization of EPS-AgNPs and evaluation of its photo catalytic dye degradation efficiency.

2 Methodology

2.1 Screening and EPS production

The seaweed-associated bacterium, *Bacillus* sp. isolated from the brown algae *Sargassum wightii* capable of producing exopolysaccharide was used in this study. This bacterium was streaked on YMG agar plates (Glucose -5 g**,** Yeast extract-1.4 g, Malt extract- 6 g, Peptone- 2.5 g**,** Monosodium glutamate- 0.5 g**,** Sucrose - 15 g, Agar- 9 g, Seawater -250 ml, Distilled water - 250 ml) for EPS production and the plates were incubated at 37 $\pm$ 2° C for 2 days. The production of EPS was indicated by the oozing out of gummy substances on the periphery of the bacterial colonies.

The pre-inoculums were prepared in YMG broth by incubating at 37 $\pm$ 2° C for 48 hours and 1ml of this liquid culture medium was inoculated into 100 ml of YMG broth and incubated for 5 days in an orbital shaker at 120 rpm at 37 $\pm$ 2° C. The culture broth was centrifuged at 10,000 rpm for 20 minutes after 5 days of incubation, and the supernatant was collected. After that, three volumes of ethanol were added to the supernatant to precipitate the EPS and kept at 4°C for 20 min. After centrifugation, the precipitate was collected and dried at 60°C. It is stored at 25- 30°C and used for further analysis.

2.2 Synthesis of EPS-mediated silver nanoparticles (EPS - AgNPs)

To form a uniform dispersion, the partially purified EPS (10 mg) was dissolved in 10 ml of double distilled water. To this 9 mM $AgNO_3$ was added while stirring and the mixture was stored at room temperature in a dark place. After 24 hours, the colourless solution changed to yellowish-brown colour which indicates the formation of polymeric silver nanoparticles. Furthermore, to increase the concentration of the solution it was incubated for 15 days. To track the progress of nanoparticle formation, samples were collected at regular intervals. The solution was then centrifuged for 15 minutes at 19200xg. The pellet is collected and air dried at room temperature for further analysis.

2.3 Characterization of EPS and EPS-mediated AgNPs

2.3.1 UV – VIS spectroscopy

The reduction of Ag^+ ions with EPS to form EPS-AgNPs was observed on 0, 5, 10, and 15 days of incubation in the range of 300 to 800 nm by using UV Visible spectroscopy.

2.3.2 FT-IR Spectroscopy

Fourier Transform Infrared spectroscopy (FTIR) analysis was performed on Perkin Elmer L1600300 (ATR) spectroscopy. The FT-IR spectrum was recorded in the range of 4000 – 400 cm^{-1} region.

2.3.3 SEM analysis of EPS - AgNPs

The SEM images of the silver nanoparticles synthesized from bacterial EPS were obtained using a Scanning Electron Microscope.

2.4 Degradation of organic dyes by EPS – AgNPs in aqueous solution

Dyes such as Congo red and Methylene blue were prepared at 10 ppm concentration and used as stock solutions. In 10 ml of Congo red and methylene blue dye solution, 1mg of EPS – AgNPs was added and mixed thoroughly. A control was maintained without the addition of silver nanoparticles. Then the dispersion was exposed to sunlight and monitored from morning to evening sunset. To evaluate the photocatalytic dye degradation, aliquots of 2-3 mL suspension were filtered at various time intervals and the absorbance was measured by using a UV-Visible spectrophotometer. The concentration of dye during degradation was calculated by the absorbance value at 660 nm.

The formula is used to estimate the percentage of dye degradation:

% of Decolorization = 100 X (Co – C) / Co

where, the initial concentration of the dye is represented as Co and the concentration of dye after photocatalytic degradation is represented by C.

3 Results and Discussion

3.1 Screening and production of EPS

The seaweed-associated was screened for EPS production in YMG medium. Out of 5 bacteria isolated from *Sargassum wightti*, *Bacillus* sp. produced good quality of gummy secretions either around the colonies identified by the periphery or by the whole colony (Figure 1). Huang-Lin et al., isolated *Bacillus xiamensis* capable of producing EPS from river sediment of Spain[18]. Similarly, Minimol et al., isolated *Bacillus cereus* from *Sargassum wightti* which is capable of producing higher amounts of EPS[19].

Fig 1. Exopolysaccharide produced by the bacterium

3.2 Characterization of EPS

3.2.1 UV – VIS spectroscopy analysis of exopolysaccharides

UV – VIS spectroscopy analysis of exopolysaccharides produced was recorded between 200 nm and 800 nm. The maximum absorption spectral wavelength area was found between 200 – 230 nm, which exhibits its characteristics of functional group such as ester, carbonyl, carboxyl, and amine (Figure 2). The results are in coincidence with previous studies of marine cyanobacterium, *Gloeocapsa gelatinosa*[20].

Fig 2. UV – Vis spectrum of EPS

3.2.2 FTIR analysis of exopolysaccharides

FTIR spectra of the crude EPS produced by *Bacillus* sp. showed two O–H stretching at 3745 cm^{-1} and 3274 cm^{-1} (3200 – 3800 cm^{-1} range) and are the typical characteristic of a carbohydrate group similar to *Gloeocapsa gelatinosa*[20]. A weak C –

H stretching band was absorbed in 2931 cm^{-1} can be attributed to the C-H stretching of methyl groups, which are typically found in hexoses such as glucose or galactose or deoxyhexoses such as rhamnose or fucose in reference to previous studies by *Bacillus xiamensis*[18]. The peak at 1640 cm^{-1} corresponds to C = O stretching vibration of the carboxylic acid. Also, a peak at 1525 cm^{-1} corresponds to the secondary amide group, peak at 1226 cm^{-1} represents O – S – O group of sulphate esters. The absorption peak at 1030 cm^{-1} is an anomeric region and it was attributed to C – O – C and C – O groups in polysaccharides which represents the monosaccharide in the EPS made of pyranose ring (Figure 3), similar to *Bacillus enclensis*[21]. In the present study, the FT- IR spectra of EPS produced by *Bacillus* sp. revealed the presence of –OH (3274 cm^{-1}) and COOH (1640 cm^{-1}) groups and hence confirms the presence of exopolysaccharide[22] similar to studies by Costa et al.,[14].

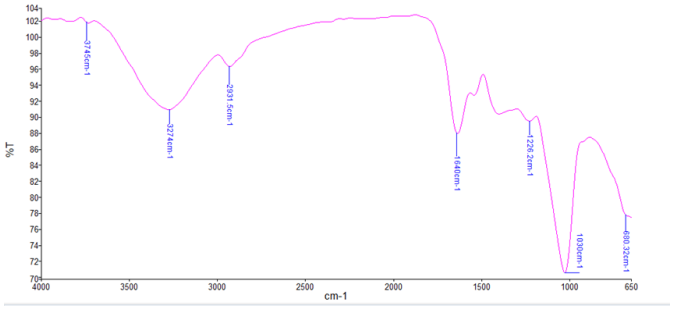

Fig 3. FT-IR Spectra of EPS

3.3 Characterization of silver nanoparticles

3.3.1 UV – Visible spectroscopy analysis of EPS-AgNPs

Through the reduction of Ag^{+} into Ag° from $AgNO^{3}$, EPS – AgNPs were synthesized. As a result of the formation of AgNPs, the colourless solution turned to dark yellowish-brown colour (Figure 4), which was observed by using UV – Visible spectrophotometer (300 – 800 nm). The band's intensity was increased by storing it in a dark room for up to 15 days. Similarly, Jaast et al.,[23] has indicated that incubation time is important in nanoparticle synthesis and AgNPs were synthesised after 12 hours of incubation in dark. In this study, the UV - Visible absorption spectra for AgNPs exhibited absorption maxima at 445 nm. The silver nanoparticles continued to display absorbance maxima at 445 nm with the identical absorption strength even after 30 days of storage in the dark. Similar results were observed in the previous studies[24]. Moreover, EPS-stabilized nanoparticles such as AgNPs displayed broad peaks at 400–550nm as in the previous reports[7].

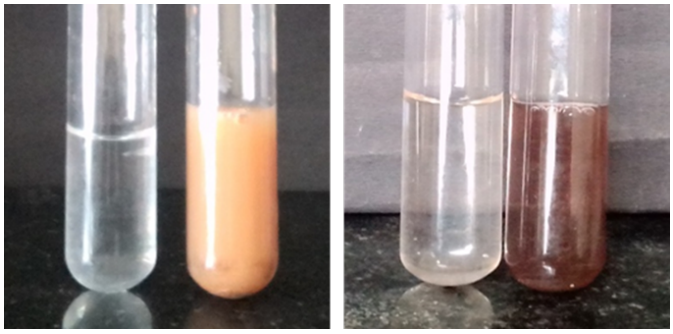

Fig 4. Synthesis of EPS – AgNPs

3.3.2 FTIR analysis of EPS-AgNPs

The FTIR spectrum of the EPS-mediated silver nanoparticles (**Fig.5**) revealed various characteristic peaks. The absorption peak observed at 2985 cm^{-1} indicates the frequency of C – H stretching. It shows an intense peak at 1725cm^{-1} indicating C – C stretching (non – conjugated), Carbonyl (CO) stretching frequency and carboxyl group. The intense and strong peak at 1283cm^{-1} shows the presence of the C – O group. The absorption band at 1129cm^{-1} showed the presence of C – O – C and

C – O stretching. These results indicated that, the EPS was responsible for the synthesis of silver nanocomposite as reported in previous studies [18,19]. These results suggest that the exopolysaccharide from *Bacillus* sp. performs a dual function of silver nanoparticle synthesis and stabilisation. EPSs have a variety of functional groups in their structure that can act as reductive and stabilising agents in the chelating and capping process used to create metal nanoparticles [25–27].

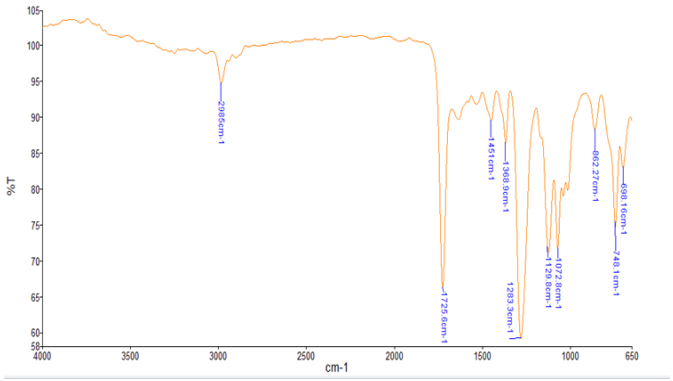

Fig 5. FTIR spectrum of EPS mediated AgNPs

3.3.3 Scanning electron microscopy (SEM) analysis of EPS-AgNPs

SEM is a topographical imaging method capable of determining a wide range of particle sizes, nanomaterial shapes and size distributions. The SEM images of silver nanoparticles synthesized from EPS showed spherical and rod-shaped nanoparticles with 80 to 100 nm in size (Figure 6). The morphology and characteristics of the AgNPs in this study is similar to the silver nanoparticle produced by *Bacillus stratosphericus* [28]. From these results, it was observed that the EPS produced from the algae-based bacteria has played as stabilizing and reducing agents. In addition, Fernando et al., reported that the range of physicochemical properties of EPS makes it an effective stabilizing or capping agent for protecting the primary structure of metal nanoparticles with an encapsulation film in order to separate the nanoparticle core from the composite mixture [25]. Due to the presence of various functional groups in the back bone of bacterial EPS such as amino, sulphate, phosphate and hydroxyl substituents as well as N-acetylamino sugars and hemiacetal ends makes them excellent candidates for the reduction, complexation, and stabilization of metal nanoparticles during their synthesis [29]. However, there are several challenges in using bacterial EPS to aid in the synthesis of metallic nanoparticles. The most significant of which is the wide variability of the EPS chemical composition. In addition, the morphological properties of the nanoparticles produced have a direct impact on all of the synthesis parameters [25].

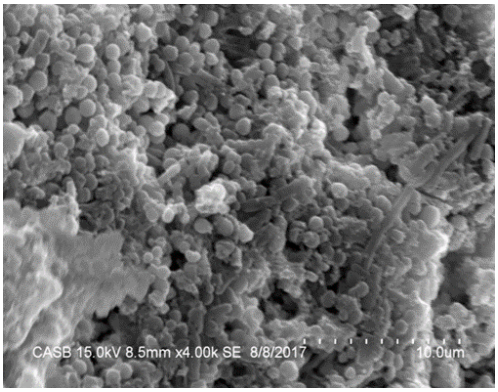

Fig 6. SEM image shows the presence of silver Nanoparticles produced from EPS

3.4 Photocatalytic degradation of organic dyes with EPS – AgNPs

The photocatalytic degradation of EPS – AgNPs was performed by using organic dyes such as Congo red and methylene blue. At various time intervals in the visible region, dye degradation was performed in the presence of silver nanoparticles. The change in colour of the reaction mixture to colourless was initially used to identify dye degradation. Initially, the dark red color of Congo red was changed into light red and the dark blue color of methylene blue was changed into light blue after 12 hours of incubation with EPS – AgNPs under solar light. The process of decolorization was completed at 36 h for both the dyes and it takes 48 hours for complete degradation in the absence of sunlight (Figure 7). Similar color change was noted in green synthesized silver nanoparticle [23,30].

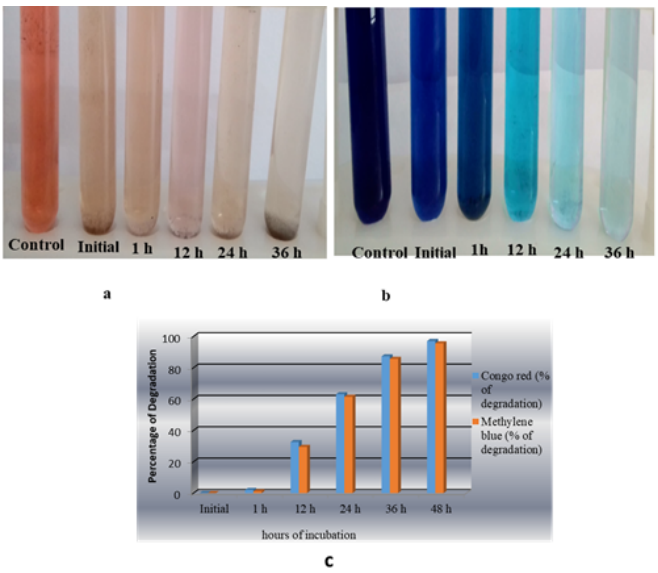

Fig 7. Photocatalytic degradation of a) Congo red, b) Methylene blue by EPS – AgNPs and c) percentage of degradation

At various time intervals, the absorption spectrum revealed decreased peaks for the dyes. The dyes' colour intensity gradually decreased as the exposure time increased, indicating a photocatalytic degradation reaction. The indication of completed photocatalytic degradation was indicated by the gradual decrease in the absorbance value of dyes approaching the baseline. The percentage of decolorization efficiency of EPS-AgNPs was calculated as 97% for Congo red and 95.6% for Methylene blue at 48 h. EPS-AgNPs have good degradation rate, better stability, better half-life when compared to the green synthesised silver nanoparticles from *Camellia sinensis* [31] and *Plantago ovata* [32,33]. This supports the novel characteristic features of EPS-Silver nanocomposite synthesized from the extracted EPS of *Bacillus* sp., new seaweed associative bacteria isolated from marine sea weed *Sargassum wightti*.

4 Conclusion

The outcomes of the current study concluded that, the marine sea weed *Sargassum wightti* associative bacterial isolate *Bacillus* sp. is capable of producing extracellular polysaccharide. This could serve as a promising biomolecule for the biosynthesis of silver nanoparticles. The EPS-AgNPs synthesised from *Bacillus* sp show promising photocatalytic degradation, better stability and longer half-life. This makes it exploitable in the textile industry for the degradation of dyes. The remarkable achievement of the present study is environmentally friendly, cheaper and renewable nature of EPS-AgNPs open up new horizon in the textile effluent process.

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