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Note

Fabrication of PVA-Silver nanoparticle composite film for elimination of microbial contaminant from effluent

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The effluent contains many harmful microbes which should be eliminated before it is discharged into a water body. Silver nanoparticles (AgNPs) being high-quality significance and have a great impact on this research field as it inhibits microbial proliferation and infection. Therefore, it may use for Bioremediation purposes, our laboratory is fascinated by the production of polymer matrix entrapment silver nanoparticles for *in situ* bio-remediation purposes. The AgNPs was prepared from sawdust by decoction method. The yellowish solution turns into dark brown colour indicating the formation of AgNPs. A sharp SPR (Surface Plasmon Resonance) band formation in UV-vis spectroscopy scan establishes the formation and stability of silver nanoparticles in an aqueous solution. SEM microphotograph indicated roughly spheroidal structure with (63±3) nm average diameters of newly synthesized AgNp. Polyvinyl alcohol (PVA) is eco-friendly and non-toxic to the environment was chosen for the preparation of polymeric matrix. The non-toxic concentration (1 μg/mL) of AgNp was dispersed into PVA solution followed by cross-linked with maleic acid. PVA- maleic acid is cross-linked by the formation of an ester bond, whereas silver nanoparticles physically entrap into the cross-linked matrix. The silver nanoparticles were released from the matrix nearly after 10 min of swelling of the composite film. In a microbial assay using *E. coli* agar medium, PVA-AgNp composite film shows the significant killing of microorganisms. Microbial elimination is measured indirectly by pH measurement and dissolved oxygen concentration measurement of the effluent *in situ* against RO- water, taken as control. The dissolved oxygen concentration from RO water and effluent water was measured on Day “0” followed by treatment and incubation at the BOD chamber. The treatment with PVA-AgNp composite film reduced the BOD Level and increase dissolved oxygen level simultaneously increasing the quality of water.

Keywords: Bioremediation, BOD, Dissolved Oxygen, PVA-AgNp composite film, Silver nanoparticle

Nowadays, there has been a great demand for ‘Green Polymers’ those which are actually derived from pure and natural resources. It concludes that green polymers are more environmentally accepted than biologically produced polymers. ‘Green polymers’ refers to the production of polystyrene and elastomers from inexhaustible resources because they are biodegradable, environmental-friendly with low energy consumption[1]. Nanotechnology is an interdisciplinary science comprising materials science, chemistry, and biology at the molecular level. It involves the manipulation of particles smaller than 100 nm as well as involving technology for developing materials or devices within that size invisible to the human eye[2]. AgNp is an important antibacterial agent that kills resistant microbes also. AgNp has a greater affinity to attach with microbial surfaces because of AgNp has a large specific surface area. AgNps have broad-spectrum antimicrobial activities, low cytotoxicity and anti-inflammatory characteristics[3].

Our research lab synthesizes silver nanoparticles from sawdust by decoction method. Polyvinyl alcohol (PVA) is a bio-compatible, eco-friendly, non-toxic, highly crystalline, biodegradable, water-soluble polymer. PVA provides a promising semi-crystallization property for some applications such as packaging, drug delivery, *etc*. AgNps, which were blended with PVA bio- polymer show greater adsorbent property and antimicrobial efficacy, more over AgNps enhance the stability of polymer which has been confirmed by FTIR followed by DAC in our past published experiment[3, 4]. Therefore, our targeted thin bio-composite film may show a bioremediation effect.

Materials and Methods

Preparation of the extract by decoction method

Sawdust was collected from *Shorea robusta* (commonly known as Sal tree) tree bark. It is naturally grown but well maintained in the University Campus area. 10 gm of sawdust was added into 200 mL methanol (Merck). This mixture was kept for overnight. The next day, this solution was stirred on a magnetic stirrer at room temperature with warm heat. The color of the solution was changed to golden yellow. This solution was filtered and stored at 4°C for further use.

Preparation of silver nanoparticle (AgNP)

$AgNO_3$ (Sigma-Aldrich) was weighted and the proportionate amount was dispersed into fresh extract, the solution was stirred with applying heat

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until the color turned from golden yellow to dark brown color. Color changes indicated the formation of nascent silver nanoparticles (AgNps). The newly synthesized AgNps were precipitated by a high-speed cooling centrifuge (Remi) at 14000 rpm for 20 min. The supernatant was discarded and the pellets were taken for further experiment.

UV-Spectroscopy and SEM microphotograph of AgNps

One drop of concentrated AgNp was dispersed into distilled water for possible dilution. It was then scanned over UV-Spectroscopy (Agilent) for obtaining its SPR band. Similarly, SEM microphotograph was obtained with this diluted sample.

Preparation of PVA-AgNPs composite film

The composite film was prepared as per our previously published article[4]. Briefly, PVA film was made by using polyvinyl alcohol (PVA) (Loba- chemie) and Maleic acid (Loba-chemie). The appropriate amount of PVA is mixed with distilled water and PVA hydrogel is obtained when PVA is cross-linked with 10% by weight of Maleic acid. This hydrogel was dried to obtain a thin PVA Film. It was taken as control. Similarly, the same percentage of PVA hydrogel was prepared. The non-toxic 1 g/mL concentration of nascent AgNps was dispersed over PVA film followed by its cross-linking with Maleic Acid (equivalent amount as before). The solution was poured over a Petri plate and dried for obtaining PVA-AgNp composite film.

Measurement of the swelling property of PVA-AgNp composite film

The dried PVA-Composite film was taken and cut into three pieces having weighed same. It was dipped into distilled water and taken its weight with our predetermined time intervals of 5, 15, 30, 45 and 60 min.

Microbial assay

Escherichia coli culture was prepared in agar media by pour plate technique and then incubated at 37°C as per standard microbiological study protocol[3]. The next day, the Petri plate was divided into four quadrants where water was kept in 1st quadrant, soaked PVA film was put into 2nd quadrant as a control, Antibiotic (tetracycline) was put into 3rd quadrant (taken as positive Control) finally soaked PVA-AgNp composite film was put into 4th quadrant (sample treatment). It was incubated at 37°C overnight.

BOD & dissolved oxygen measurement

The RO water and fresh pond water were taken as control and standard respectively. The sample effluent was collected from a nearest industrial area. All the water sample was collected in a 300 mL BOD bottle. The dissolved oxygen amount was measured by The Winkler Method, briefly, Winkler's A ($MnSO_4$ Solution) and Winkler's B (Alkaline KI solution) was added to all labeled bottle (Control, Standard, Effluent Sample) preciously for avoiding possible air contact. It was mixed by inverting the bottle 3-4 times. A precipitate appeared and allowed it to settle down. Then concentrated H_2SO_4 was added and closed the bottle followed by shaking till the precipitate dissolved. It was kept in dark till the color change was observed. It was titrated with 0.005 (N) $Na_2S_2O_3$ solution using starch as an indicator. The colored solution turned colorless at the endpoint. Repeat the titration to get 2 or 3 concurrent readings.

After measuring the dissolved oxygen (DO) amount in the water, and the water was treated with 0.5gm of PVA-AgNp composite film. Then all of them were kept in a BOD incubator for 5 days for further measurement of DO. After 5 days, water colour was observed and the titration experiment was performed the same as before.

Data management and statistical analysis

All experiment was carried out thrice and data were electronically captured into a Microsoft excel sheet. One-way ANOVA was performed for the analysis of data. Group analyses were also performed. Data were represented as Mean ± SD value followed by significancy were written alphabetically upon it. The same letter represented non-significant and different letters assured significancy.

Results and Discussion

The UV-vis spectroscopic analysis (Fig. 1) of the nascent silver nanoparticle showed SPR (Surface Plasmon Resonance) peak at 435 nm. Silver nanoparticle forms when silver nitrate is reduced by a natural reducing agent like $NADPH_2$. Our synthesized silver nanoparticles (AgNps) give SPR band at 435 nm wavelength and form a sphere-like structure due to its dielectric potential and interband transition, which starts from 380 nm. SEM microphotograph analysis (Fig. 2) revealed a roughly spheroidal structure with (63±3) nm average diameters. The PVA-AgNp composite film was prepared where AgNp is entrapped into the cross-linked PVA.

The FTIR analysis of our previous work[4] indicates the formation of the ester bond. The alcoholic group of PVA reacts with the carboxylic group of Maleic acid to form ester bond. Silver nanoparticles are physically linked with the polymer matrix. Therefore, AgNps are released from the film when the film was swelled up.

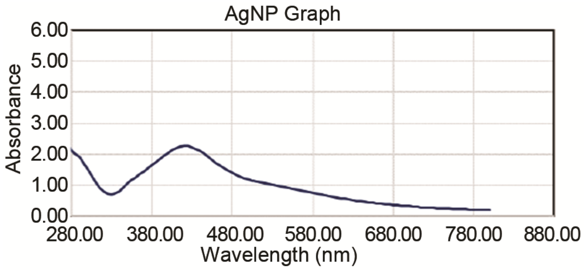

Fig. 1 — UV- spectroscopy analysis of nascent AgNp

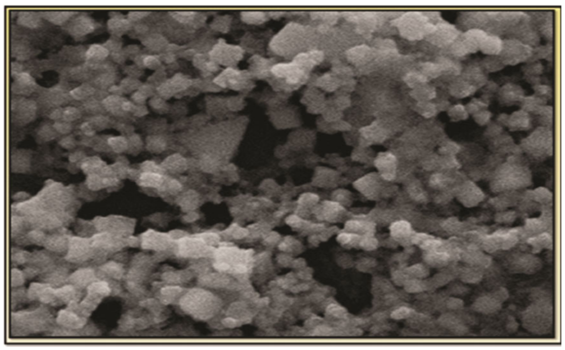

Fig. 2 — SEM Microphotograph of AgNp

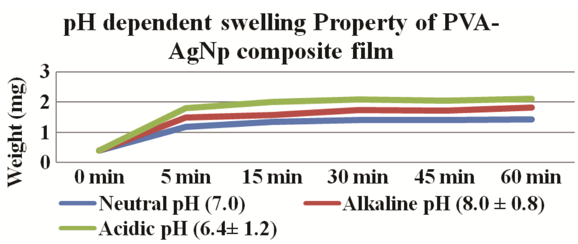

Fig. 3 — pH dependent swelling Study of PVA-AgNp film

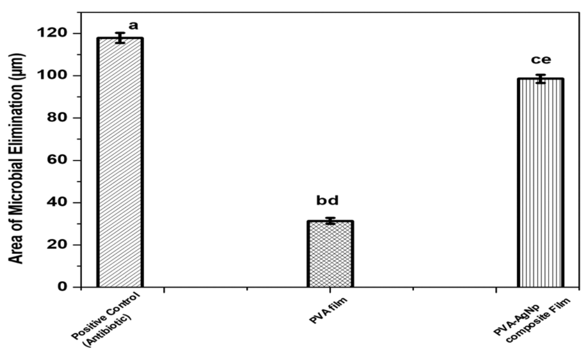

Fig. 4 — Area of microbial elimination, data represneted as mean ± SD

pH-dependent swelling study (Fig. 3) indicates maximum water absorption occurred at acidic pH (6.4 ± 1.2) followed by alkaline pH (8.0 ± 0.8) and neutral pH at 7. In acidic pH the physical entrapment bond between PVA and AgNps are breakdown hence AgNp released out. The maximum release was found to be taken place at or near 30 min.

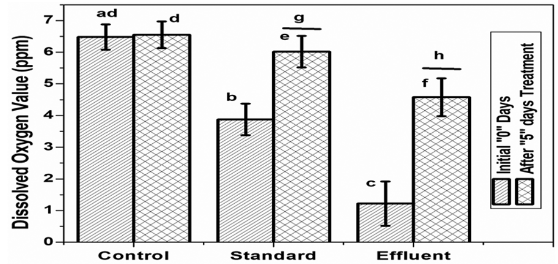

Fig. 5 — Dissolved Oxygen value, data represneted as mean ± SD

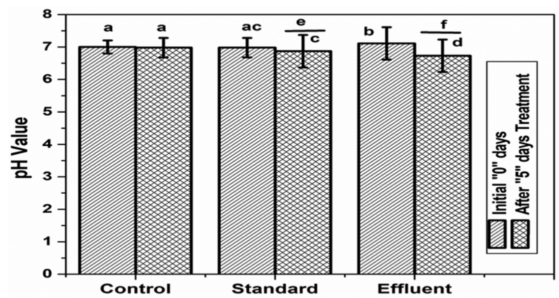

Fig. 6 — pH value, data represneted as mean ± SD

In a microbial assay using *E. coli* agar medium PVA-AgNp composite film shows a significant killing of micro-organisms than PVA film. PVA film showed less/no toxicity to *E. coli* strain. The soaked composite film kills microorganisms nearly similar to positive control antibiotic, therefore, PVA-composite film was considered for further experiment (Fig. 4).

For *in situ* bioremediation, we found that 5 days of treatment with PVA-AgNp films reduced the BOD level of effluent significantly compared to standard (Pond water) and control RO water. It significantly increased the DO level from day 0 to day 5 both in standard and effluent water (Fig. 5). pH value significantly reduced in effluent water after treatment (Fig. 6). Slightly alkaline water promotes microbial growth as it contains minerals, whereas, in an acidic environment AgNPs disrupt the integrity of the bacterial cell wall and membrane, promoting the permeability of the membrane and the leakage of the cell constituents, and eventually induce cell death. Therefore, BOD level decrease and DO increase[3,4].

Conclusion

Bioremediation is a process of elimination of harmful bacteria and pathogens from effluents or sewage water. Purification of effluent is required for the welfare of the aquatic ecosystem. There are many processes of killing bacteria and harmful pathogens in the effluent. The silver nanoparticle is one of the procedures. But raw use of silver nanoparticles creates many environmental problems as a high concentration of AgNp is toxic to the environment as well as human health. Hence we prepared a composite film entrapped with AgNp. It contains a fixed proportion of AgNp moreover it is very easy to carry and does not require any specific storage condition. A test experiment showed a significantly better result. Therefore, PVA-AgNp composite film may be suitable for commercialization.

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Conflict of interest

The authors declare there is no conflict of interest regarding this manuscript. It is also informed that the reported work is original and it has not been published elsewhere, either in part or in full, including the illustrations. Moreover, the proper citations to the previously reported works have been given.

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