



## CHARACTERIZATION OF MICROBIALLY SYNTHESIZED SILVER NANOPARTICLES AND ITS APPLICATION IN DYE DECOLORIZATION.

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### ABSTRACT

Biological approach for synthesis of nanoparticles using microorganisms received great interest because of their potential to synthesize nanoparticles of different sizes, shape, and morphology. Microbial synthesis is known as green synthesis because neither hazardous chemical are required for the synthesis nor any hazardous by product is generated. Silver nanoparticles gain more attention because of their wide application in various fields like medical, agriculture, bio-remediation and industrial. The present study is focused on synthesis of silver nanoparticles by microorganism isolated from stressed environment of industrial effluents. Among 7 isolates 4 isolates were screened positive for synthesis of nanoparticles. Color changes from pale yellow to brown due to reduction of silver nitrate with culture supernatant indicates formation of silver nanoparticles. Characterization of synthesized nanoparticles were carried out by UV-visible spectrophotometer which showed silver surface plasmon vibration band near 420nm. Average size of synthesized particles ranged from 134-207 nm. FTIR (Fourier transform infrared spectroscopy) analysis showed 3 major peaks at 3433.90 cm, 2062.28 cm, 1633.64 cm indicative of the synthesized particles with the bacterial isolates. Photo catalytic degradation of dyes viz. methylene blue and brilliant red was studied using the microbially synthesized nanoparticles. Methylene blue decolorization was observed to be 64% and 49% decolorization of brilliant red within 24 hours of exposure. These microbially synthesized silver nanoparticles could be an effective alternative for the treatment of textile effluent.

### KEY WORDS

biological, characterization, plasmon, silver, spectroscopy, dye decolorization.

### INTRODUCTION

The term 'nanotechnology' was first defined by Tokyo science university in 1974. The development of nano science and technology got an impetus in 1980 with the invention of scanning tunneling microscope. Amidst the many applications of nanotechnology, one of the major breakthroughs is in the changed approach in the disease diagnosis and treatments. Nanotechnology refers to the science and engineering involved in designing, characterization and application of structure and system by controlling shape and size at nanoscale. Nanotechnology is the growth of experiment processes for the synthesis of nanoparticles of different size,

shape and dispersity.<sup>1</sup> Their size makes those material superior and essential in many areas of human activity.<sup>2</sup> Nanoparticles can be synthesized by three methods: Physical, Chemical, Biological. Chemical synthesis of nanoparticles make many hazardous by products as compared to the microbial synthesis which is clean, non-toxic, cheaper and eco-friendly method for synthesis of nanoparticles.<sup>3</sup> Microbial synthesis of nanoparticles are of two types (1) Extracellular Synthesis (2) Intracellular Synthesis. Studies shows that extracellular synthesis of nanoparticles using cell filtrate is beneficial over intracellular synthesis.<sup>4</sup> Among various metals silver is metal of choice in many fields of biological system, living organisms and in medicine. reduction of silver ions leads

to the formation of silver atoms followed by agglomeration into cluster which leads to formation of the colloidal silver nanoparticles solution having yellow color due to surface plasmon absorption and intense band in the 380-400 nm range.<sup>5</sup> Microorganisms have versatile metabolism and these metabolites can be successfully incorporated for the synthesis of nanoparticles, among which *Bacillus spp* were used to produce silver nanoparticles.<sup>6</sup> Silver nanoparticles have various applications in non-linear optics, solar energy absorption bio-labeling, drug delivery, food industry, antiseptic agent in waste water treatment and pesticides mineralization.<sup>7</sup> Silver is an effective antibacterial agent and possess antibacterial activity against bacteria, virus, fungi but also reported to have an effective anticancerous activity which has changed the scenario of disease diagnosis and treatment<sup>8</sup>. Silver nanoparticles are also used widely in the treatment of industrial effluent in degradation of various dyes.<sup>9,10</sup> Silver nanoparticles shows activity against detoxification and removal of dye in the presence of sun light is called photo catalytic decolorization.<sup>11</sup> Silver nanoparticles are stable and rapid photo catalyst under proper temperature, they are cheaper and environment friendly.<sup>12</sup>

## MATERIALS AND METHODS

### Sample collection, isolation and production of biomass

Effluent samples were collected from industrial regions of Vapi and transported to the laboratory and stored till use. Isolation of bacteria was carried out from the effluent samples by 'serial dilution technique'. Samples were serially diluted and then from each respective dilution tube 0.1 ml sample was spread on nutrient agar plates and incubated at 37°C for 24 hours. All the bacterial isolates were cultured in Luria-Bertani broth and 1% inoculum was used for biomass production.

### Synthesis of silver nanoparticles

For synthesis of silver nanoparticles, culture was harvested after 24 hours of incubation. Culture was taken in sterile centrifuge tube and centrifuged at 10,000 rpm for 10 minutes. After centrifugation, culture supernatant was used for synthesis of silver nanoparticles. In 100 ml of supernatant 1mM of silver nanoparticles was added and mixed well. The reaction between supernatant and silver nitrate was carried out in bright condition for 24 hours. A change in color from

pale yellow to brown indicated the synthesis of silver nanoparticles.

### Characterization of silver nanoparticles

**UV-visible spectral analysis:** UV-visible spectra were recorded as a function of wavelength using UV-vis spectrophotometer from 380-680 nm. Formation of silver nanoparticles can be easily detected by spectroscopy because the silver nanoparticles shows atypical absorbance peak near 400 nm.

**Size distribution:** Dynamic light scattering which is based on laser diffraction method with multiple scattering techniques was employed to study the average particle size distribution of silver nanoparticles. DLS were performed of synthesized nanoparticles for determination of average size distribution of particles using Malvern software.

### FTIR analysis:

Fourier transform infra-red spectroscopy is a sensitive technique used for identification of functional groups present. Most molecules absorb light in the infra-red region of the electromagnetic spectrum and frequency range measured as wave number typically over the range 400-600 nm.

### Photocatalytic decolorization:

Synthesized nanoparticles were used to study their efficiency for decolorization of dye. In this study 0.05% of methylene blue and 0.05% brilliant red were prepared in distilled water. For catalytic degradation, in 20 ml of dye 5 ml of silver colloidal was added. Control was prepared without addition of silver nanoparticles and incubated in sun light for 24hours. After incubation, centrifugation was carried out at 10000 rpm for 5 minutes. UV-vis spectra was recorded to ensure photocatalytic decolorization of dye and % decolorization of dye was calculated. Methylene blue have maximum absorbance at 609 nm, brilliant red has maximum absorbance at 425 nm. The % decolorization was calculated as below:

$$\% \text{ Decolorization} = \frac{\text{initial O.D} - \text{final O.D}}{\text{initial O.D}} \times 100$$

## RESULTS AND DISCUSSION

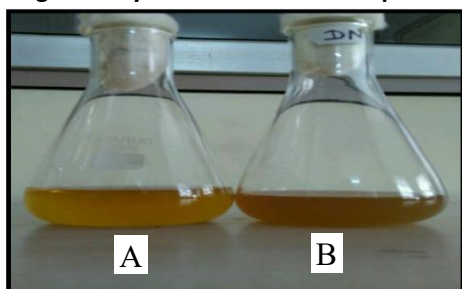
### Isolation and Screening of bacteria for synthesis of silver nanoparticles

Total 7 bacterial isolates were successfully isolated from industrial sample. All these isolates were preserved on nutrient agar slant till further study. In this study, bacterial culture were grown in Luria Bertani medium and incubated for 24 hours. Bacterial culture

supernatant was mixed with silver nitrate crystals and incubated at 37°C for 24 hours. Four isolates were screened positive for synthesis named as N11, N21, N31, N41. The figure (1) showing no color change of mixture was bacterial supernatant without addition of

silver nitrate and incubated in same condition. The color changes from pale yellow to brown indicates the synthesis of silver nanoparticles (figure 1). Brown color of the medium could be due to excitation of surface plasmon vibration of silver nanoparticles.

**Figure 1: Synthesis of Silver nanoparticles**



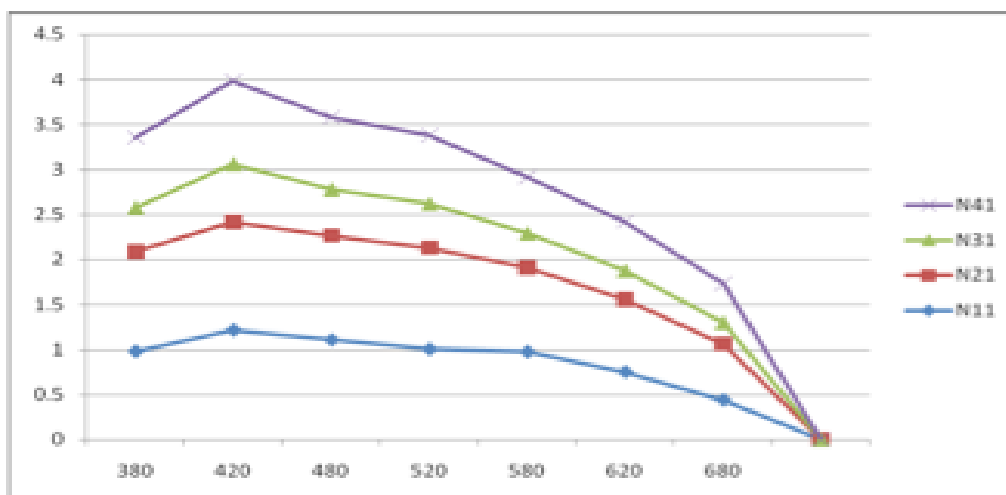
**A: culture supernatant without silver nitrate (control) B: synthesized silver nanoparticles**

#### UV-visible spectra analysis

The synthesized nanoparticles was primarily characterized by UV-vis spectrophotometer. The UV-vis spectra of synthesized nanoparticles were recorded at different time intervals. The strongest peak was

obtained at 420 nm which is typical absorbance peak of silver nanoparticles. There was increase in absorbance with the increase in incubation time. The microbially synthesized nanoparticles from all the cultures showed a maximum absorption at 420nm

**Graph 1: Absorption spectra of synthesized silver nanoparticles**



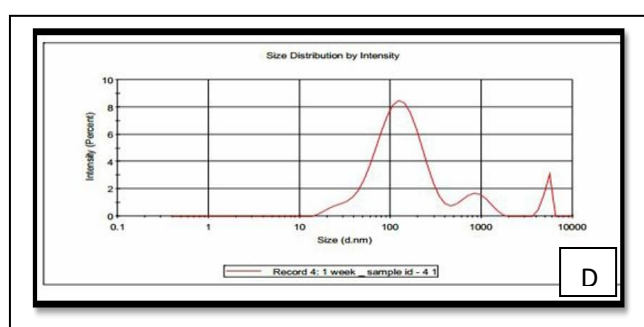
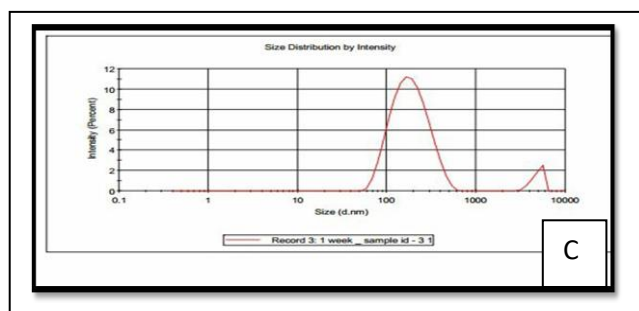
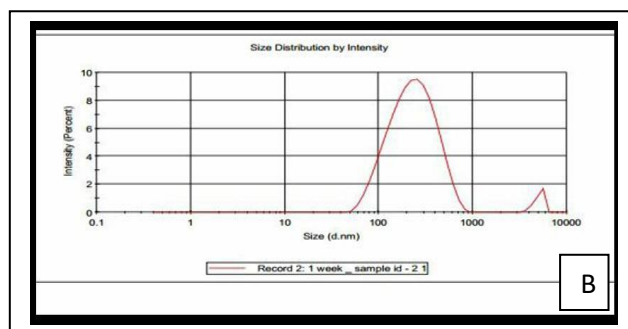
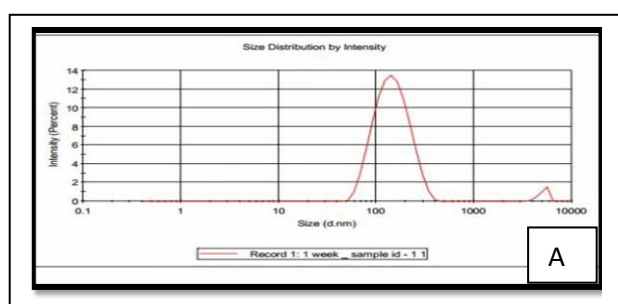
#### Particles size distribution:

Particle size distribution of synthesized silver nanoparticles were carried out by dynamic light scattering. Graphs (1,2,3,4) shows the particles size

distribution of silver nanoparticles synthesized by isolates N11, N21, N31, N41. Z-average (d.nm) found to be 139, 207, 178, 134 respectively. PDI is poly dispersity index, it should be less than 0.5 (Table 1).

**Table 1: z-average of synthesized silver nanoparticles**

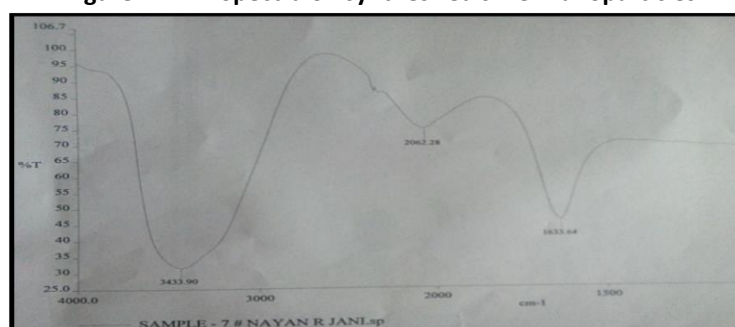
Particles	Z-average (d.nm)	Pdl
<b>N11</b>	139	0.256
<b>N21</b>	207	0.354
<b>N31</b>	178	0.341
<b>N41</b>	134	0.552

**Graph 2: particles size distribution of Nanoparticles A) particles synthesized by culture NJ11, B) synthesized by culture NJ21, C) synthesized by culture NJ31, D) synthesized by culture NJ11.**


### FTIR Analysis

The FTIR spectra of synthesized nanoparticles by N41 shown in figure. FTIR band at 3443 indicates OH stress, presence of alcohols and phenols. Band observed at 2062 indicates transition metal and carbonyl

compound. Peak obtained at 1633 indicates presence of amine, carbonyl compound group frequency (Figure 2). From the spectra obtained it can be considered as amine groups and carbonyl compounds are responsible for synthesis and stabilization of the particles.

**Figure 2: FTIR Spectra on synthesized silver nanoparticles**


### Photocatalytic decolorization

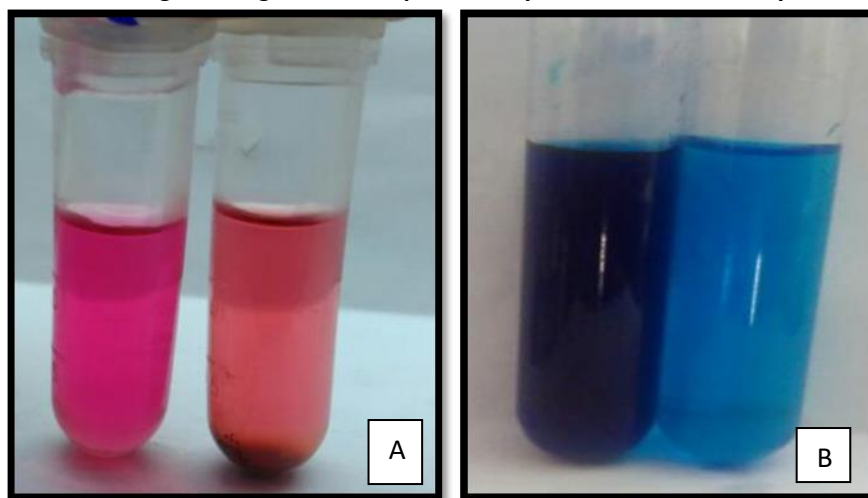
Catalytic activity of the AgNPs was determined by decolorization of methylene blue and brilliant red under sunlight. Decolorization of these dyes were determined

by the decreasing intensity of absorbance. 64% decolorization was observed after 24 hours in methylene blue and 49% decolorization observed in brilliant red after 24 hours (Table 2, Figure 3).

**Table 2: UV-visible spectra of dyes.**

Dye	Initial absorbance	Final absorbance
Methylene blue	0.615	0.217
Brilliant red	0.440	0.221

**Figure 3: AgNP induced photocatalytic decolorization of dyes**



**A: brilliant red before and after decolorization, B : methylene blue before and after decolorization**

### CONCLUSION

The study concluded with the isolation of 7 bacteria from effluent sample among them 4 screened positive for synthesis of silver nanoparticles. Color changes from yellow to brown indicated formation of silver nanoparticles further characterization was done by UV-vis spectroscopy and particle size analysis which showed stabilized AgNPs after storage. Also, FTIR analysis of synthesized nanoparticles shows peak for metal. In summary, a simple microbial reduction method has been developed for synthesizing silver nanoparticles. This methodology could be used for synthesizing a number of metallic nanoparticles involving other metals with good size and shape morphology which shall become an effective alternative for the dye reduction from the industrial textile effluents.

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Received: 04.05.18, Accepted: 07.06.18, Published: 01.07.2018

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