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ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN: 0976-3376

Asian Journal of Science and Technology
Vol. 08, Issue, 05, pp.4740-4747, May, 2017

RESEARCH ARTICLE

COMPARATIVE STUDY OF BIODEGRADATION OF AZO DYES FROM NANOPARTICLES SYNTHESIZED BY USING PLANT (*TRIDAX PROCUMBENS*) AND BACTERIA (*B. SUBTILIS*)

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ARTICLE INFO

Article History:
Received 07th February, 2017
Received in revised form
01st March, 2017
Accepted 29th April, 2017
Published online 17th May, 2017

Key words:

Biodegradation,
Azo dyes,
Nanoparticles,
Tridax procumbens,
B. subtilis.

ABSTRACT

Release of textile azo dyes from industries to the environment is an issue of health concern while the use of bacteria and plants has proved to be the best choice for remediation. The present study reveals that the comparative study of biodegradation of azo dyes from nanoparticles synthesized by using plant (*Tridax procumbens*) and bacteria (*B. subtilis*). This study investigate an efficient eco-friendly and sustainable route of silver nanoparticles preparation from 1mM aqueous solution of silver nitrate using leaf extract of *T. procumbens* and supernatant of *B. subtilis* (extracellular).After 48 hrs of incubation at room temperature, the color of the solution intensified to brown indicating the formation of silver nanoparticles. The silver nanoparticles were further characterized by UV-Visible Spectrophotometer and Scanning Electron Microscope (SEM). The degradation of the dye congo red by AgNPs synthesized from *T. procumbens* and *B.subtilis* after 120 minutes of illumination were found 67.56% and 68.80 % respectively and for crystal violet, biodegradation were showed 17.54% and 20.33 % respectively. The degradation of Brilliant greenby AgNPs synthesized using *T. procumbens* and *B.subtilis*were found to be48% and 62.16% respectively. The degradation of Malachite green by AgNPs synthesized using *T. procumbens*was found to be 27.45% whereas by AgNPs synthesized using *B. subtilis*was found to be 48.88%. Comparatively the degradation of azo dyes was highest by *B. subtilis* (35.48%) as compared to*T. procumbens* (28.36%). As biodegradation is microaerophilic or aerobic process completely detoxified all the selected textile azo dyes, further efforts should be made to implement such methods for large scale dye wastewater treatment technologies.

INTRODUCTION

Dyes are a chemical which bind to material and imparts color to that material. The color of a dye is due to the presence of chromophore group. They are widely used to color the substrate like textile fiber, paper, leather, hair, fur, plastic material, wax, a cosmetic base and food stuff. Based on Chemical structure of chromophore there are 20 -30 different groups of dyes. Azo (Monoazo, diazo, triazo, polyazo), anthraquinone, phthalocyanine and triarylmethane dyes are the most important groups. The majority of industrial important azo dyes belong to the following classes: Acid dyes, Basic dyes, Direct dyes, Disperse dyes, Mordant dyes, Reactive dyes and Solvent dyes. The Acid, Basic, Direct and Reactive azo dyes are ionic dyes. Dyes contain at least one nitrogen-nitrogen (N=N) double bond, however many different structures exist, For example, in the azo dyes, monoazo dyes have only one N=N double bond, while diazo and triazo dyes contain two and three N=N double bonds respectively.

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The azo groups are generally connected to benzene and naphthalene rings. These side groups are necessary for imparting the color of the dye, with many different shades and intensities being possible. These dyes have different absorption spectrum and associated with electronic transition between molecular orbital of synthetic dyes are produced annually worldwide. During dying process, a substantial amount of azo dye is lost in waste water reported that about 10- 15% of dyes were lost in effluent during dyeing process (Sudha *et. al.,* 2014). Environmental pollution due to urbanization and rapid growth of industries has a detrimental effect on human health and ecology. Textile dyes constitute a major source of pollution. Textile industries consume a major share of dyes in India. Further, the textile industry of India also contributes nearly 14% of the total industrial production of the country (Lavanya *et. al.,* 2014). Dye effluents discharged in rivers contaminate water and surrounding environment. Aquatic animal and the biological processes in river get affected due to this contamination (Sudha M. *et. al.,* 2014). Most of the azo dyes are water soluble and readily to absorb through the skin contact and inhalation leading to the risk of cancer and allergic reactions, an irritant for the eyes and highly

toxic, if inhaled or consumed (Suresh T. *et.al.,* 2013). Thus the removal of dyes from industrial effluents is of great significance in connection with environmental and human health safety. Since the dyes are chemically and photolytically stable, they maintain the same colour for a longer period of time in the natural environment. Hence, these non treated dyes are potentially carcinogenic, mutagenic and genotoxic (Madhan Raja *et al.,* 2015). The azo group of dyes binds to an aromatic ring. Through mineralization, these dye can be broken down into an aromatic amine, an arylamine that is suspected to be carcinogenic. Most of the azo dyes are water soluble and readily to absorb through skin contact and inhalation leading to the risk of cancer and allergic reactions, an irritant for the eyes and highly toxic, if inhaled or consumed. Toxic compounds of azo dyes readily mix with water bodies and enter into aquatic organisms through food chain and ultimately reach man and cause physiological disorders such as hypertension, sporadic fever, renal damage and cramps. Some azo dyes are carcinogenic and mutagenic. They reduce the efficiency of seed germination and plant growth. Untreated effluents with higher concentration of dyes inhibit the elongation of shoot and roots. Malachite green causes serious public health hazards and environmental problem. So far through various experimental observations it is revealed that malachite green is a multiorgan toxin; it decreases food intake, growth, fertility rates; and causes damage to liver, spleen, kidney and heart (Sudha *et al.,* 2014).

Due to this there arises the need to degrade this harmful dyes. Azo dyes may be toxic to aquatic organisms and are considered as xenobiotic compounds, very resistant to natural biological degradation. Many physiochemical methods like coagulation, coagulation electro oxidation, adsorption, electrolysis, photolysis and ozonation are promising in terms of performance, while the economic aspect has become the most challenging problem. But, azo linkages are easily reduced under anaerobic conditions, yielding colorless aromatic amines and are readily degraded aerobically. Therefore, a combination of anaerobic and aerobic conditions is proposed for azo compounds mineralization (Sudha *et al.,* 2014).Conventional chemical and physical dye degradation techniques are either expensive, highly time consuming, result in sludge formation, or result in the formation of by-product. The extraordinary properties possessed by nanoparticles make dye degradation more effective than conventional methods of dye degradation (Madhan Raja *et al.,* 2015).

Biodegradation is an effective cleanup technology used for the removal of organic and inorganic contaminants from soil (Lilyprava Dash 2013).Only few aerobic bacterial strains that can utilize azo dyes and heavy metals simultaneously as growth substrates have been isolated. Microbial population proliferates when soil is amended with substances capable of sorption and inactivation of growth inhibiting substances (Samavia Batool *et. al.,* 2014). Many microorganisms like bacteria, fungi have been reported to solve the problems regarding environmental remediation .But as the load of pollutants in environment is very high, the bioremediation alone is not sufficient. So some technology has been developed to enhance the bioremediation process like Nano-bioremediation. Nano-bioremediation technology includes the application of nanoparticles or nanotechnology to enhance the process of bioremediation (Shreya Modi *et al.,* 2015).

A different nanoparticles play significant role in our daily life as several commercial products are made for human beings (Archana Yadav*et.al.,* 2015).

MATERIALS AND METHODS

Collection of soil sample and *Tridax procubens*

The soil sample was collected in sterile container and plant *Tridax procubens* from surrounding area of P.G.T.D of Microbiology, RTMNU, Nagpur. The plant was dried and authentified from P.G. Department of Botany, RTMNU, Nagpur.

Isolation and identification of *Bacillus subtilis*

Soil sample were collected and were allowed to air dry. The samples were serially diluted in sterilized distilled water. 100 µl from the dilution was spreaded on Hichrome Bacillus Agar plates Plates were incubated at 37^0 C for 24 hours. After 24 hours plates were observed for colonies.

Identification of *B. subtilis*

The isolated colonies were identified on the basis of morphology by performing Gram staining and motility, biochemical by testing sugar fermentation using Glucose, Lactose, Mannitol , Maltose, Sucrose, IMViC Test, Catalase test, Oxidase test, Triple Sugar Iron(TSI) test, Urease test and cultural characteristics by inoculating bacteria on Hichrome Bacillus Agar.

Synthesis of silver nanoparticles

From *Bacillus subtilis*

The bacterium *B. subtilis*was grown in a freshly prepared, sterilized nutrient broth and incubated at 37^oC for 24 h. After the incubationtime the culture was centrifuged at 12,000 rpmand the supernatant was used for the synthesis ofAgNPs. *B. subtilis* culture supernatant was separately added to the reaction vesselscontaining silver nitrate solution (1 mM) and incubated at room temperature until the colorchanges were observed (Gopalappa H*et.al.,* 2012).

From *Tridax procumbens*

For the synthesis of silver nanoparticles using *T. procumbens*, 10ml of plant extract was added to 50 ml of silver nitrate. The solution was then subjected to microwave irradiation for 3 min and the color change was observed from light yellow to brown color. The solution was then centrifuge at 3000 rpm for 20 min. The silver nanoparticles were isolated and concentrated by repeated centrifugation of the reaction mixture at 3,000g for 20 min. The supernatant was replaced with distilled water each time. The nanoparticles were washed well to remove any residue particles that were not the capping agents. The suspension was dried and stored as a crystalline powder for characterization studies and biological assay.(Kholoud *et al.,* 2010)

Characterization studies

The biosynthesis of SNPs was monitored periodically by scanning the aliquot sample in a wavelength range of 200-1100 nm and recording the absorption maxima in UV Visible

spectrophotometer at a resolution of 1nm. The nanoparticles produced were primarily characterized by UV-Visible spectrophotometer, SEM analysis was carried out to understand the topology of SNPs. For scanning electron microscope, the sample was prepared with a drop of colloidal solution of nanosilver on a carbon-coated copper grid and a setting completely dried by vacuum desiccators. The maxima of the UV-Visible spectra of the solution occurred at 420 nm and the following image was obtained in the SEM analysis. (Madhusudhana *et al.,* 2012)

Biodegradation studies

The dyes used for the study of biodegradation studies were Congo red, Crystal violet, Methyl orange, Malachite green, Brilliant green. The photocatalytic degradation of methyl orange was evaluated by biosynthesized Ag nanoparticles. A suspension was made by adding 1ml of silver nanoparticles to 100 ml of azo dyes. The absorption spectrum of the suspensionmixture was measured periodically using a colorimeter to ensure the degradation of azo dyes solution (Saranya *et al.,* 2016)

Formula of percent decolorisation

$$\text{Percentage of Decolorisation} = \frac{C_0 - C}{C_0} \times 100$$

Where

C_0 is the initial concentration of dye solution and

C is the concentration of dye solution after photocatalytic degradation.

RESULTS AND DISCUSSION

In the present study *Bacillus subtilis* were isolated fromsoil sample collected from P.G.T.D of Microbiology, RTMNU, Nagpur. On HiChrome Bacillus Agar plates after incubation of 24 hours four species of *Bacillus* were isolated. There four species were identified on the bases of the colour of the colonies: *Bacillus thuringeinsis* – pink, *Bacillus subtilis* – green, *Bacillus megaterium* - yellow and *Bacillus cereus* – blue (Figure 1). The biochemical test also performed for identification of *Bacillus subtilis* was represented in Table no 2 and 3.

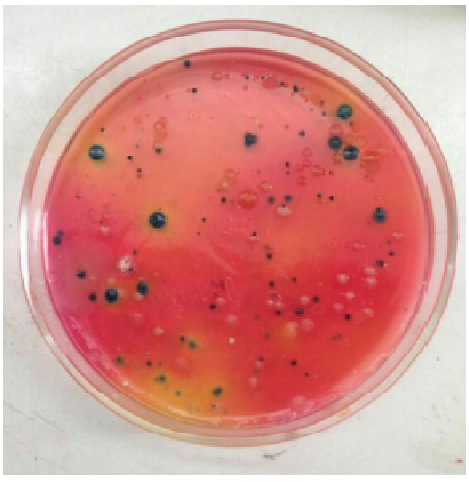


Figure 1. Isolated colonies of *Bacillus species*

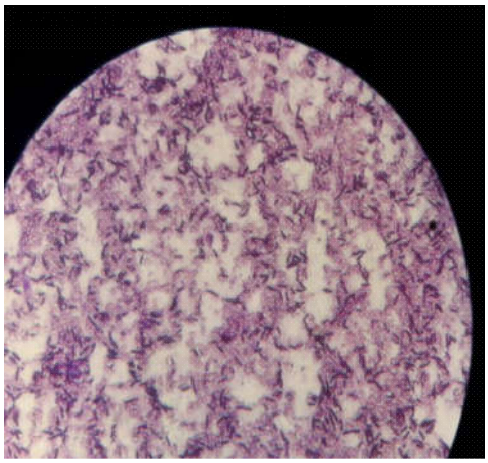
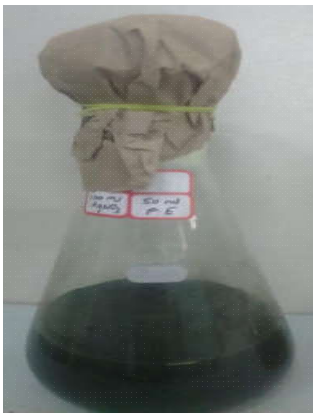


Figure 2. Gram staining of *Bacillus subtilis* on Hichrome Bacillus Agar



At Zero Time

After 48 Hours

Figure 3. Synthesis of silver nanoparticles from *T.procumbens*

The extracellular biosynthesis of silver nanoparticles (AgNPs) were achieved by the culture supernatant of bacteria – *Bacillus subtilis* and leaf extract of plant – *Tridax procumbens.* This study investigate an efficient eco-friendly and sustainable route of silver nanoparticles preparation from 1mM aqueous solution of silver nitrate using leaf extract of *T. procumbens* and supernatant of *B. subtilis* (extracellular).After 48 hrs of incubation at room temperature, the color of the solution intensified to brown indicating the formation of silver nanoparticles.

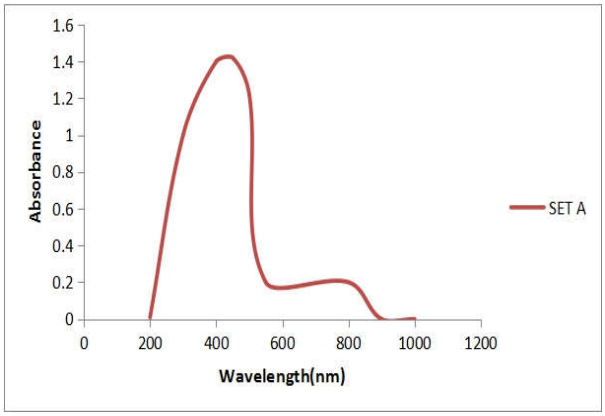
Table 1. Observation of biochemical test of *Bacillussubtilis*

Strain	Indole	MR	VP	Citrate	Urease	TSI		
						Acid	Gas	H_2S
B. subtilis	-ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve

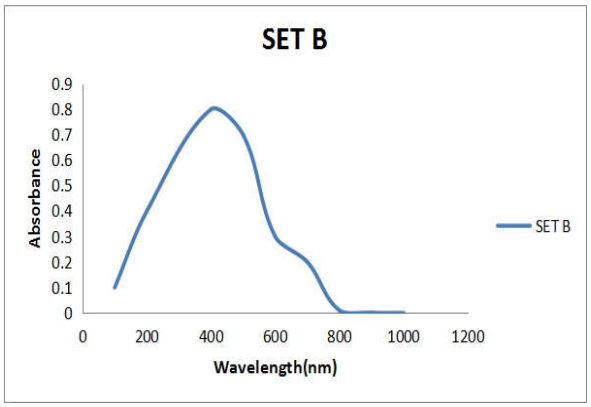
Table 2. Observation of sugar fermentation of *Bacillus subtilis*

Strains	Glucose		Sucrose		Mannitol		Lactose	
	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas
B. subtilis	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve

Figure 4. Samples of *T.procumbens* before and after centrifugation



Graph 1. Set A (For 1 mM $AgNO_3$, *Bacillus subtilis)*



Graph 2. Set B (For 1mM $AgNO_3$, *Tridax procumbens*)

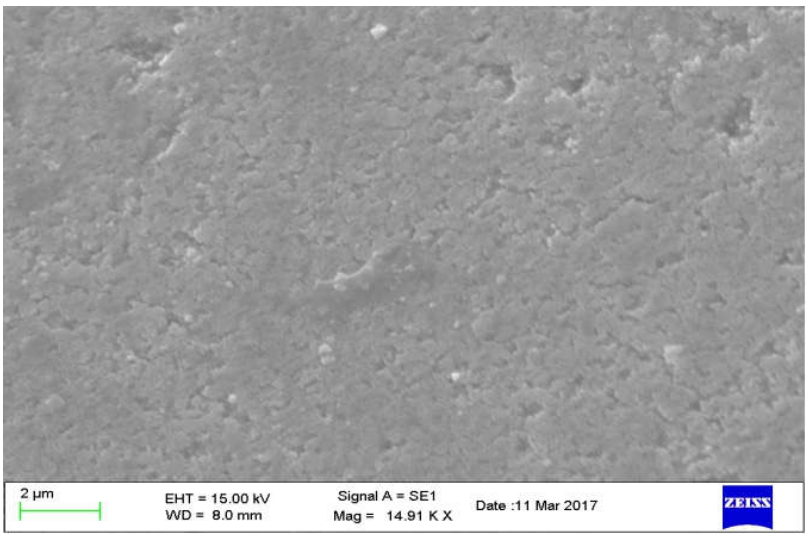


Figure 5. SEM analysis of synthesized silver nanoparticles using *T. procumbens*

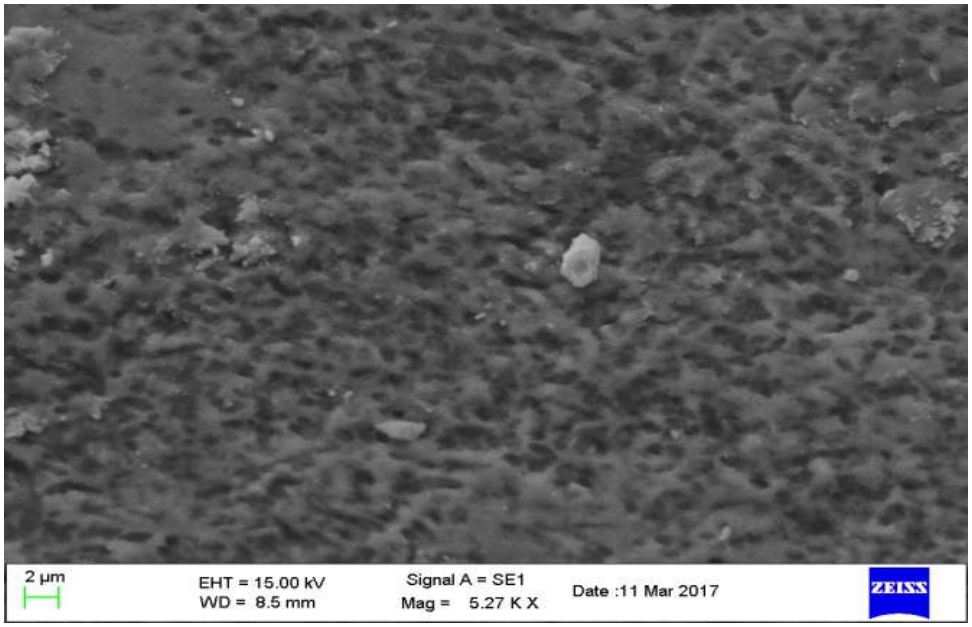
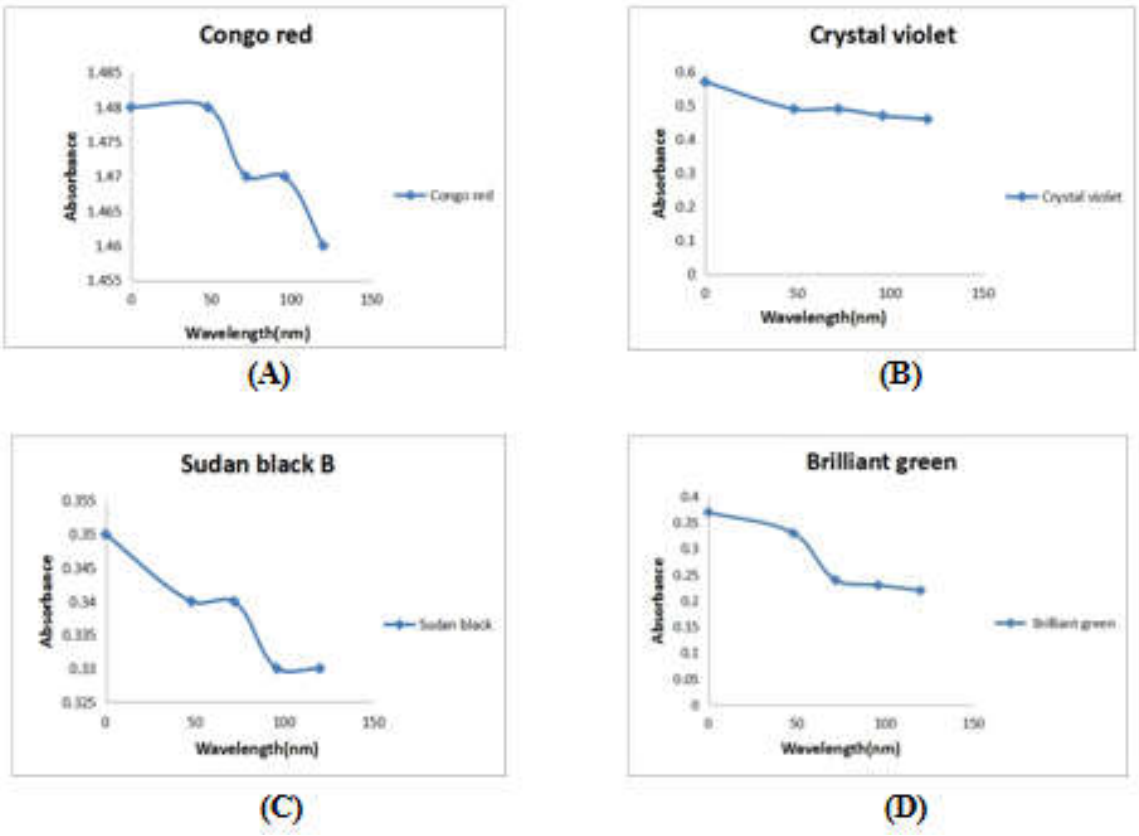


Figure 6 No. : SEM analysis of synthesized silver nanoparticles using *B. subtilis*

Table No. 3. Degradation of azo dyes by *T. procumbens* nanoparticles

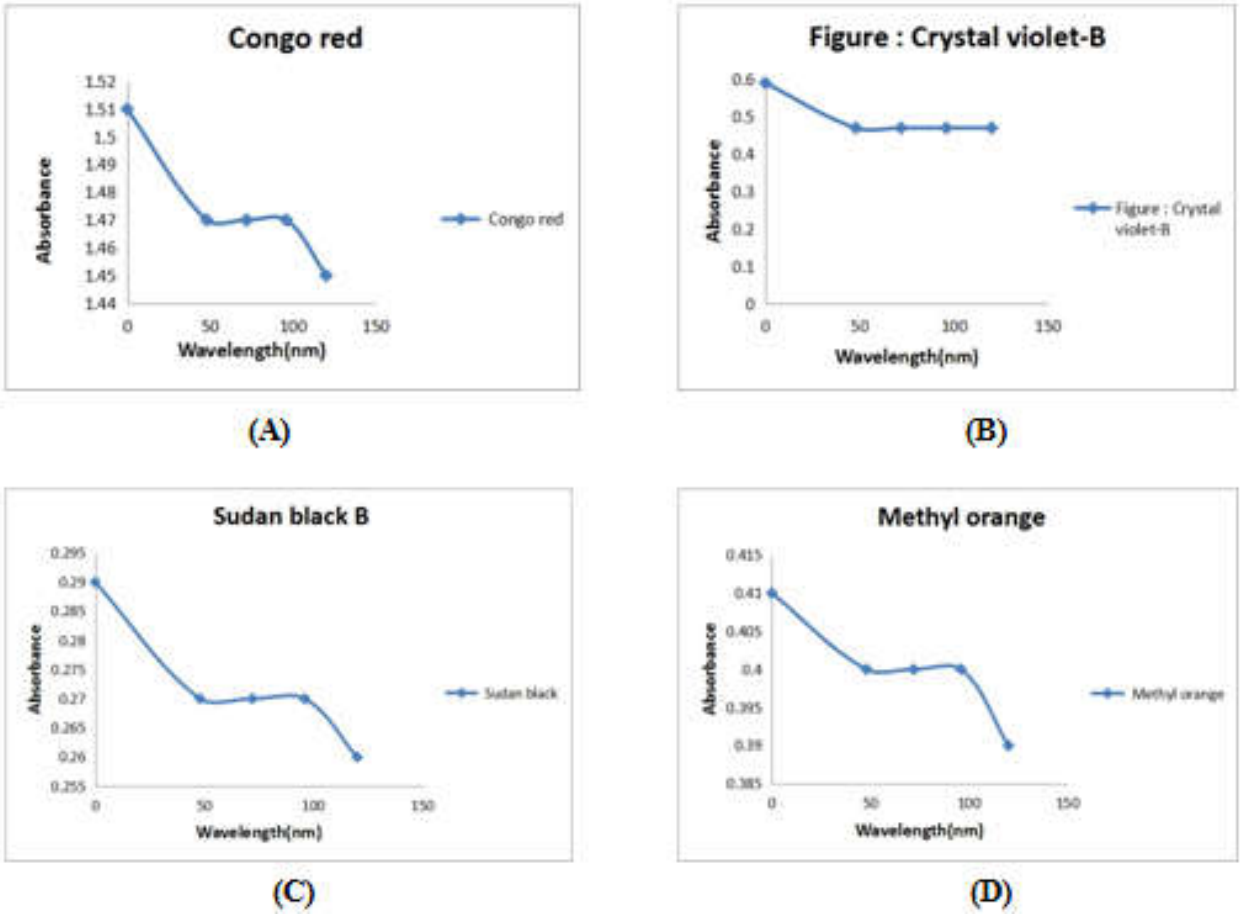
Azo dyes	Zero time	After 48 hrs	After 72 hrs	After 96 hrs	After 120 hrs	Total % degradation
Congo red	1.48	1.48	1.47	1.47	1.46	67.56
Crystal violet	0.57	0.49	0.49	0.47	0.46	17.54
Methyl orange	0.51	0.49	0.50	0.50	0.50	3.90
Sudan black B	0.35	0.34	0.34	0.33	0.33	5.70
Brilliant green	0.37	0.33	0.24	0.23	0.22	48.00
Malachite green	0.51	0.43	0.44	0.33	0.30	27.45



Graph 3: Photocatalytic degradation of (A) Congo red (B) Crystal violet (C) Sudan Black B (D) Brilliant Green

Table 4. Degradation of azo dyes by *B.subtilis* nanoparticles

Azo dyes	Zero time	After 48 hrs	After 72 hrs	After 96 hrs	After 120 hrs	Total % degradation
Congo red	1.51	1.47	1.47	1.47	1.45	68.80
Crystal violet	0.46	0.47	0.47	0.47	0.47	20.33
Methyl orange	0.41	0.40	0.40	0.40	0.39	2.40
Sudan black B	0.29	0.27	0.27	0.27	0.26	10.30
Brilliant green	0.37	0.16	0.17	0.13	0.12	62.16
Malachite green	0.45	0.22	0.24	0.24	0.23	48.88



Graph 4. Photocatalytic degradation of (A) Congo red (B) Crystal violet (C) Sudan Black B (D) Methyl orange

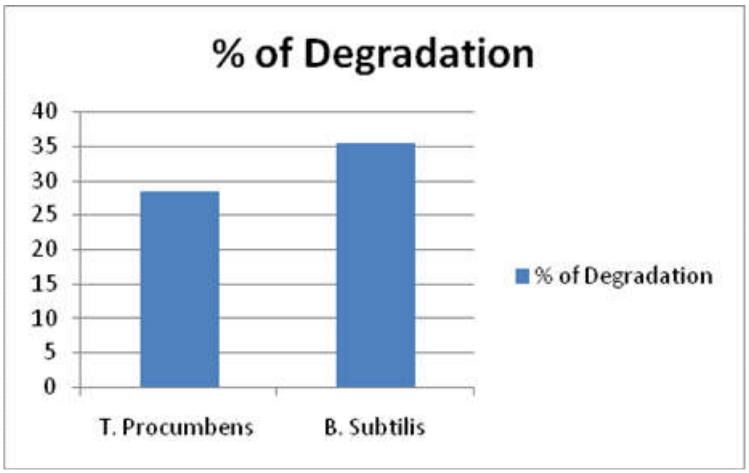


Figure 7. Comparative total degradation of azo dyes by Silver Nanoparticles produced by *T.procumbens* and *B.subtilis*

The silver nanoparticles were further characterized by UV-Visible Spectrophotometer and Scanning Electron Microscope (SEM). UV–visible spectroscopy is a convenient tool for measuring the reduction of metal ions based on optical properties called Surface Plasmon Resonance (SPR). The reaction mixture of leaf extract *Tridax procumbens* and supernatant of *Bacillus subtilis* has an absorption maximum of 420 nm and 430 nm respectively, suggesting the formation of silver nanoparticles (Graph 1 and 2). The SEM analysis confirm that silver nanoparticles synthesized using *Bacillus subtilis* were spherical in shape and size is 210 nm and using *Tridax procumbens* were spherical in shape and size is 100nm. K. Vithiya observed the mono-dispersed silver nanoparticles in the range of 50 to 120 nm was synthesized using culture supernatant of newly isolated *Bacillus species*(K. Vithiya*et.al.,* 2014). R. sangeetha study the characterization of AgNPs using *Tridax procumbens* and observed that the SEM analysis confirm the particles are spherical in shape and rang in size from 26-30nm (R. Sangeetha*et.al.,* 2016).

The degradation of the dye congo red by AgNPs synthesized from *T. procumbens* and *B.subtilis* after 120 minutes of illumination were found 67.56% and 68.80 % respectively (Table No.3,4 & Graph No. 3A,4A). This shows that degradation of congo red by *B. subtilis* AgNPS is higher as compared to the *T. procumbens* AgNPs. Arun Kumar*et.al.,* study the degradation of azo dye-congo red by *Bacillus subtilis* 'RA20' and observed that degradation of congo red was 98.23% (Madhusudhana *et al.,* 2012). In the present study degradation of congo red by nanoparticles showed 68.80%. Shreya modi.*et.al* observed the degradation of congo red dye by nano-based remediation was 13% efficient than microbial remediation (Shreya Modi *et al.,* 2015). Haradhan koyla .*et.al* study the degradation of azo dye (Congo red) using *Amaranthus gangeticus* Linn leaf extract and found that only AgNPs have no considerable effect on degradation of Congo red solution. It is obvious that the presence of a strong reducing agent $NaBH_4$ reduced the Congo red solution very slowly (Archana Yadav *et al.,* 2015). Nithya *et al*., showed that the *Pleurotus sajor caju* silver nanoparticle effectively decolorized the dye congo red within 24 hours of incubation when compared with its plain culture (*Pleurotus sajor caju)* which takes more than 48 hours for the same process (Nithya *et al*., 2011).

For dye crystal violet degradation by AgNPs synthesized using *T. procumbens* and *B.subtilis* after 120 minutes of illumination were showed 17.54% and 20.33 % respectively (Table No.3,4 & Graph No. 3B,4D). This shows that degradation of crystal violet by *B. subtilis* AgNPS is higher as compared to the *T. procumbens* AgNPs. In the present study degradation of methyl orange dye by nanoparticles using *Tridax procumbens* (3.90%)was higher than using *Bacillus subtilis*(2.30%) (Table No.3,4 & Graph No.4B). Madhan raja.*et.al.,*showed 0.99% of the photocatalytic degradation of methyl orange using magnetic nanoparticles by biological method (Madhan Raja *et.al.,* 2015). Studies conducted by Eman Alzahrani*et.al.* shows that the rate of degradation of the dye methyl orange by AgNPs synthesized from extract of tangerine peel was effective but degradation occur only in the presence of sodium borohydride or hydrogen peroxide . It was observed that degradation of dye was rapid and monitored by UV-Visible spectrophotometer (Eman Alzahrani2015). P. Kumar *et.al.,* study the degradation of methyl orange dye biometrically by using AgNPs synthesized from *Ulva lactuva* and observed the absorption peak of 430 nm. The Degradation of methyl orange was visualized by decrease in peak intensity within 12 hr incubation time (P. Kumar*et.al.,* 2013). The degradation of Brilliant greenby AgNPs synthesized using *T. procumbens* and *B.subtilis* after 120 minutes of illumination were found to be48% and 62.16% respectively. The degradation of Malachite green by AgNPs synthesized using*T. procumbens*was found to be 27.45% whereas by AgNPs synthesized using *B. subtilis* was found to be 48.88%. Comparatively the degradation of azo dyes was highest by *B. subtilis* (35.48%) as compared to *T. procumbens* (28.36%) (Figure No. 7). Suresh *et al*., studied the photocatalytic degradation of malachite green dye using titanium dioxide nanoparticles and found that the method was low, cost effective process in the removal of toxic dyes (T. Suresh *et al.,* 2013). In the present study the degradation of malachite green and sudan black B by nanoparticles synthesized using *T.procumbens* shows lower photocatalytic activity than that using *B.subtilis.* Degradation of brilliant green dye shows higher photocatalytic effect in case of *Bacillus subtilis* as compare to *Tridax procumbens*.

Conclusion

The present study comes to conclusion that degradation can be carried out in the absence of reducing agent sodium borohydride or hydrogen peroxide. The results showed that the silver nanoparticles synthesized using *Bacillus subtilis* have exhibited higher photocatalytic activity than that synthesized using *Tridax procumbens*- 35.48% degradation was recorded for AgNPs (*Bacillus subtilis*) and 28.36% for AgNPs (*Tridax procumbens*). Thus silver nanoparticles can be used for treatment of textile effluents. The development of such particles may be considered a breakthrough in the field for the efficient clean up of the dyes on large scale process since they are easy to synthesize on large scale and cost effective.

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