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GREEN SYNTHESIS OF ZINC OXIDE NANOPARTICLES USING POTATO PEEL AND DEGRADATION OF TEXTILE MILL EFFLUENT BY PHOTOCATALYTIC ACTIVITY

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ABSTRACT

Textile industry is one of the oldest industries in India with over 1000 industry. A facile green recipe was developed to synthesize highly pure, safe and durable zinc oxide nanoparticles (ZnONps) using domestic waste-starch rich potato peel. The ZnONps were synthesized using zinc oxide powders and potato peel. The particle size and morphology of the synthesized nanoparticles is characterized by using UV VIS spectrophotometer, FTIR and SEM analysis. The effluents were treated with ZnONps and the photocatalytic degradation capability of the dyes significantly enhanced the great potential for wastewater treatment system.

KEYWORDS: zinc oxide nanoparticles, Effluent, Photocatalytic activity, Potato peel.

INTRODUCTION

Water is an essential compound for the survival and sustenance of life on the planet earth. The waste water or sewage water thrown out from industries is either used for irrigation purposes or it runs off to natural sources of water. If these effluents are not treated before their disposal they can be harmful for human consumption as well as for other uses too (Ahlawat and Kumar., 2009). One of the fast growing industries in India is textile industry, consuming large quantities of water and produces large volumes of wastewater during processing unit of textile manufacture. Continuous flow of textile industrial waste into river

and cultivated land now is one of the major global problems. Although efforts for reducing water pollution are very limited. The discharge of dye-containing effluents, directly released into the water channels is serious problem for human and animal (D Suteu *et al*., 2009).

Dyes can easily contaminate the water bodies through their discharge from the industries. Non-biodegradable nature of most of the dyes which result in perturbation in aquatic diversity by blocking the passage of sunlight through the water represents serious problems to the environment. It is reported that less than 1 ppm of dye content causes obvious water coloration (Al-Degs *et al*., 2000). There are more than 100,000 commercially available dyes and globally over $7x10^5$_tons of dye is produced in a year and 15% of which is lost during the dying process (Martinez-Huitle and Brillas, 2009).

Azo dyes are the largest and the most versatile class of dyes. They possess intense bright colors, in particular oranges, reds and yellows. In addition, azo dyes exhibit a variety of interesting biological activities. Medical importance of these compounds is well known for their antibiotic, antifungal and anti-HIV properties. On the other hand they bring a certain danger for health and environment because of cancero-and mutagenicity (Buazar *et al.*, 2016).

Nanotechnology is emerging as a rapidly growing field with its application in science and technology for the purpose of manufacturing new materials at the nanoscale level (MA. Albrecht *et al*., 2006).The use of plants for the synthesis of nanoparticles novel and provides a cost –effective and environmentally friendly alternative to chemical and physical synthesis.zinc oxide (ZnO) Nps is an important semiconductor with band-gap energy of 3.37 eV at room temperature and has a very large excitation binding energy of about 60 meV (Fernandes D *et al*., 2009).

The most important reason for choosing ZnO as photocatalyst is its ability to show photocatalytic efficiency in both UV and visible range of light i.e. direct sunlight can be used as light source.In nanoparticles synthesis chemicals used are toxic and lead to non-ecofriendly by products (Garima Singhal *et al,* 2010). Use of biological organisms such as micro organisms, plant extract or plant biomass could be an alternative to chemical and physical methods for the production an eco-friendly manner on of nanoparticles.

Potato skins are used in India to treat swollen gums and to heal burns. The tubers contain very small quantities of atropine alkaloids. One property of these alkaloids is the reduction of digestive secretions, including acids produced in the stomach. The leaves are antispasmodic. Photocatalysis is an advanced oxidation technique to use the catalytic activity of the semiconducting metal oxides. This technique produces OH , which has stronger oxidation power than ordinary oxidants normally used in the oxidation process, and decomposes the organic compounds into harmless compounds such as CO2, H2O or HCl (Lee SY *et al*., 2013).

MATERIALS AND METHODS

Collection of sample: Fresh potatoes were brought from local vegetable market from Eachanaari, Coimbatore district.

Sample preparation: The potatoes were washed thoroughly under running tap water. Then, the potatoes were peeled off with the help of a peeler. The peels were chopped into pieces with the knife. The peels were dried completely under sunlight.

Synthesis of zinc oxide nanoparticles: (Kannan *et al*., 2010)

20 ml of distilled water was taken in three beakers and marked as control, 0.5g zinc oxide and 1.0g zinc oxide. 1g of potato peel was weighed and put in each beaker containing distilled water and zinc oxide powder with 2 different concentrations. The mixture was mixed thoroughly using magnetic stirrer for about 1 hour. Then the beakers were incubated overnight at room temperature for the synthesis of zinc oxide nanoparticles.

Characterization of zinc oxide nanoparticles

UV –Vis spectroscopy: The bio reduction of zinc ions in the solution was monitored and sample of 2ml was withdrawn at different time intervals the presence of the confirmed by sharp peaks shown by the absorption spectrum of this solution and recorded by using UV-Vis spectrophotometer. A small of sample was taken in a quartz cuvette and observed for wavelength scanning between 200-700 nm using UV-Vis spectrophotometer (PHARAMASPEC UV-1700SERIES, SHI MADZU CORPORATION Ltd., JAPAN).

FTIR (Fourier transform infrared spectroscopy): The dried powder of Zinc oxide Nanoparticles was subjected to Fourier Transform Infrared Spectroscopy (FTIR) analysis. Two milligrams of the sample was mixed with 200mg KBr (FTIR grad) and pressed into a

pellet. The sample pellet was placed into the sample holder and FTIR spectra was recorded in the range 4000-450cm¯1 in FTIR spectroscopy at a resolution of 4cm¯1.

SCANNING ELECTRON MICROSCOPY (SEM)

The morphology of the Zinc oxide Nanoparticles was examined using Scanning electron microscope (SEM). The liquid sample was air dried to obtain the SEM analysis of zinc nanoparticles. Air dried sample were scanned on to Bruker AXS GmbH model D5005.

Collection of industrial effluents: Industrial effluent and textile mill effluents were collected in a plastic container from Coimbatore and Tirupur, respectively.

Isolation of microorganisms from the effluent: To isolate the microorganisms from the effluent, serial dilution was done. Then the sample were spread on nutrient agar and incubated at 37°C for 1-2 days for bacterial growth. After incubation, The single colonies were picked and repeatedly streaked on nutrient agar medium and potato dextrose agar.

Identification of isolates: Identification of the isolates were performed according to their morphological, cultural and biochemical characteristics by following Bergey's Mannual of Systematic Bacteriology (Kandler and Weiss, 1986). All the isolates were subjected to Gram staining and specific biochemical tests.

PRODUCTION OF BIOMASS

Nutrient agar was prepared and poured into 3 sterile petri- plates. The agar was allowed to solidify and 50µl, 100µl and 150µl of the effluent was spreaded using a sterile spreader (L-rod). The plates were incubated at 37ºC for 24 hours then pink colour colonies were isolated and screened for further studies.

Antimicrobial activity using zinc oxide nanoparticles

Antimicrobial activity of ZnONPs was tested against the microbe isolated from the effluent. Mueller Hinton agar was prepared and poured into 4 sterile petri-plates. The cultures from the nutrient broth were inoculated onto the plates.. Then, 4 wells were cutted.. Each wells were loaded with 20µl of control, 0.5g ZnO nanoparticles, 1.0g ZnO nanoparticles and an antibiotic. The plates were then incubated at 37ºC for 24 hours. After incubation, the zone of inhibition was measured.

Photocatalytic analysis: The photocatalytic experiments were conducted to investigate the photodegradation of the methylene blue and azo dyes by treating 10ml of the effluents with 7ml of control, 0.5g ZnO NP and 1.0g ZnO NPs, respectively and incubated under sunlight for certain time intervals. The concentration of the dyes were measured by UV-Vis spectrophotometer in the range of 200-700nm. The rate of degradation was studied in terms of changes of the absorption maximum at the absorption peak of 4000-450cm¯1.

MOLECULAR CHARACTERIZATION OF BACTERIA

Identification of the isolate by 16S rRNA gene sequencing

DNA extraction, polymerase chain reaction (PCR) and DNA sequencing Genomic DNA was extracted by using DNA purification kit (Genei, Bangalore, India). The PCR analysis was carried out with a volume of 20 mL of mixture in a DNA gradient thermocycler (Model no. P 818070424, Astec, Fukuoka, Japan). The procedure comprised 35 cycles at 92°C for 1 min, 45°C for 1 min and 72°C for 1 min. The primer used for amplification was 5' GCAAGTCGAGCGGACAGATGGGAGC 3' and 5' ACTCTCGTGGTGTGACGGGCGGTG 3'. The amplified PCR product was harvested from 1 % agarose gel and purified with gel extraction and PCR purification kit (Sigma-Aldrich, St. Louis, MO, USA). The purified product was sequenced with an automated sequencer (ABI PRISM 310, Applied Biosystems, Foster City,
CA, USA).

BLASTN analysis: The 16S rRNA sequence was then analyzed for similarities by using BLASTN tool (BLASTN, National Center for Biotechnology Information, Bethesda, MD, USA *www.ncbi.nlm.nih.gov:80/BLAST/*).

Phylogenetic tree analysis: The phylogenetic tree analysis was done by the Molecular Evolutionary Genetics Analysis MEGA 4.1 software and the tree was constructed by using the neighbor-joining method (Saitou and Nei, 1987).

RESULTS AND DISCUSSION

In the present study, green synthesis of zinc oxide nanoparticles from potato peel was performed and photocatalytic degradation of the industrial effluent and dye was carried out and to investigate experimentally the removal of dye from dying textile industrial waste water by photo sensitization process for its reuse in the same industry for domestic purpose or irrigation.

Effluent sample was subjected to serial dilution 10^{-4} to 10^{-7} and 10^{-6} dilutions was plated to facilitate the growth of bacteria . After appropriate incubations for bacterial growth, bacterial colony was observed. By frequent sub culturing, the single pure cultures was isolated and stored at 4°C for further studies. The morphology of the isolates was observed and genus was identified by biochemical tests as *Pseudomonas putida.*

The dyes can easily contaminate the water bodies and non- biodegradable in aquatic diversity by blocking the passage of sunlight into water causes serious problem in environment. More than one lakh commercially available dyes produced 15% over a year. This causes serious environmental problem to these water bodies (Martinez *et al*., 2009).

In the present study, the zinc oxide nanoparticle was synthesized within 24 hours of incubation using domestic waste-potato peel in a very less -expensive way.

Further SEM (Scanning electron microscopy) analysis confirmed the degradation of plastic and polymer by revealing the presence of porosity and fragility of the dye degraded surface compared to control. The SEM shows that the particles are uniform in shape and well dispersed with particle size below 150 nm . The nanoparticles were well distributed and it can be seen that relatively hexagonal shaped Zinc Oxide nanoparticles with diameters ranging from 30-150 nm were observed. These results illustrate the synthesis of Zinc Oxide nanoparticles through reduction of Zinc ions inside the starch-rich potato peel template.(PLATE-1).

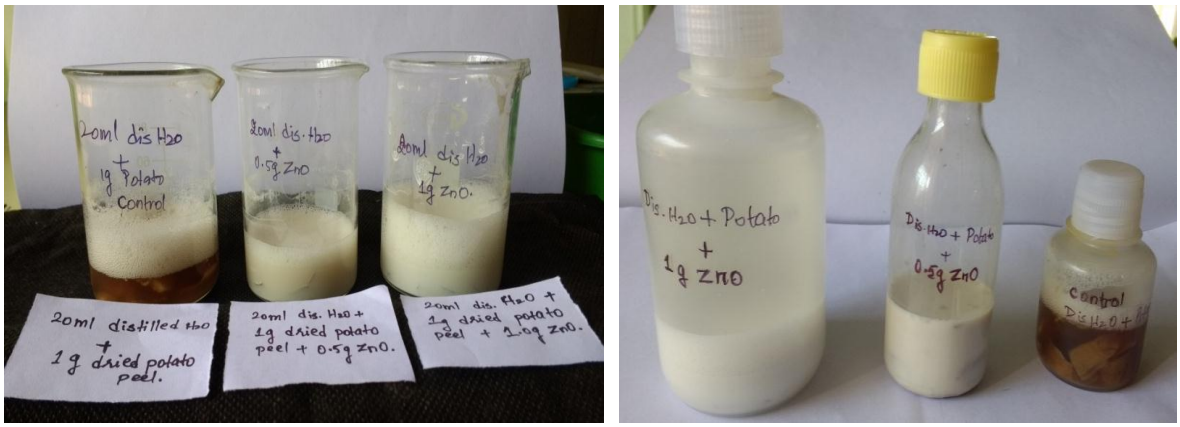

Plate-1.Synthesis of Zinc Oxide Nanoparticles from potato peel

The zinc oxide nanoparticles synthesized by isolates are irregular in size. SEM was done and the nanoparticle size was measured and found that the size 35-150nm. The synthesis of

zinc oxide nanoparticles was monitored by UV-Visible absorption spectra of the reaction mixture at a range of 200-400nm where an intense band was clearly detected at 430nm. This band was identified as a (Surface Plasmon resonance band) and describe to the excitation of the electron in the nanoparticles.

Photocatalytic activity: The UV spectral characteristics of the methylene blue and azo dyes after 24 hours and 72 hours are shown in (fig:3&4). The absorption peak gradually decreases as the concentration decreased. The decreasing of the absorption peak at 400-700nm indicates that the decomposition of organic compounds in the solution has taken place. To check the significance of the catalytic effect of Zinc Oxide nanoparticles, the dye was irradiated under sunlight without the presence of Zinc Oxide nanoparticles. The result showed that no degradation of the dye took place in the absence of Zinc Oxide nanoparticles after 24 and 72 hours of sunlight exposure. This follows from the fact that the active surface on the catalyst available for reaction is very crucial for the degradation to take place. As the catalyst concentration is increased, the reaction becomes faster and more feasible.(Banu *et al.*,2008).

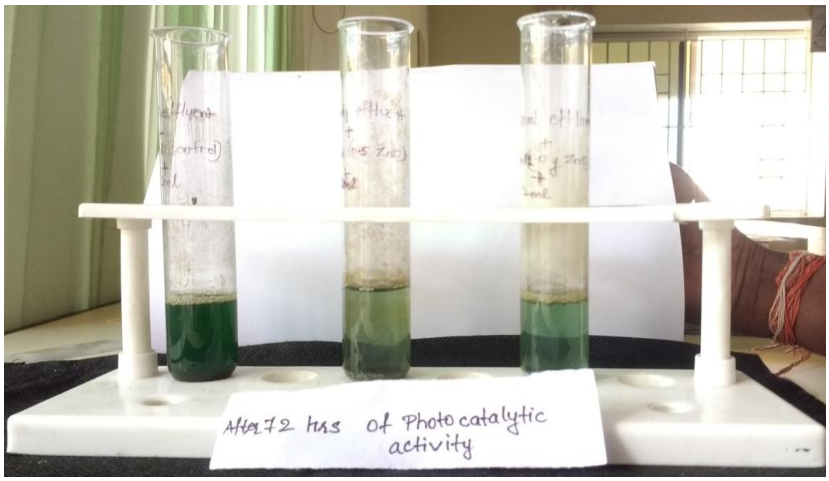

Plate-2. Photocatalytic treatment of textile mill effluent with zinc oxide nanoparticles for 72hours.

The preliminary screening of biodegradation capability was done by Fourier Transform Infra-Red (FTIR) Spectroscopy for surface changes (Sherly *et al.*, 2011). The changes in the spectra of the degraded samples confirmed the degradation occurred. In case of control, peak appeared at 1315.93 cm^{-1} but it disappeared after microbial treatment in case of test. The peak at 1768.72 cm^{-1} in control was reduced to 1760.23 cm^{-1} , thus indicating the breakdown of C=O bond. In case of test, the formation of new peaks in the region of 1417-827 cm^{-1} indicates the formation of the C-C due to the breakdown of C-H bonds. The FTIR spectra of

samples of Zinc Oxide particles are generally influenced by the particle size and morphology. The FTIR spectrum was taken using a FT-IR instrument operating at a resolution of 4000-800cm^{-1}in the percent transmittance mode. In addition to the absorption bands of the biomolecules used as reduction and stabilization (capping) agents, the absoption peak at 600cm^{-1} indicate the presence of Zinc Oxide nanoparticles. The spectral bands were interpreted for identification of functional moities of organic compounds adhering to zinc nanoparticles. The absorption of the isolates shows peaks at the presents of (Fig.1).

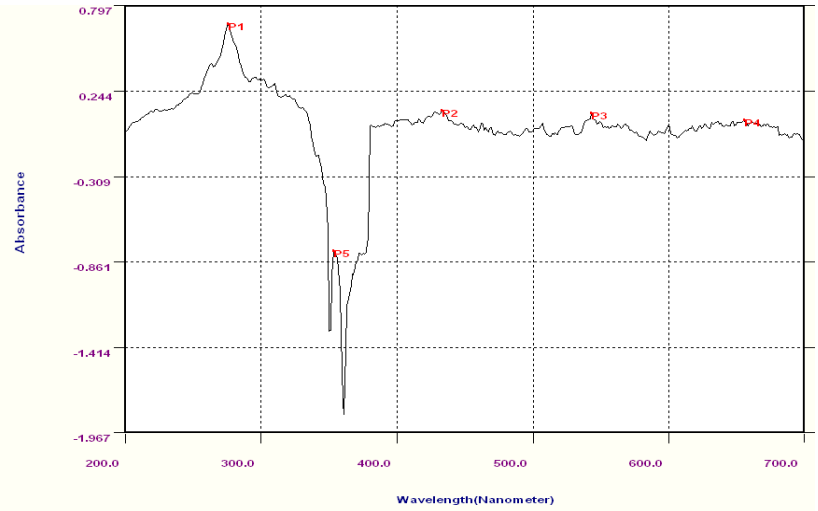

Fig.1.0g ZnO Nanoparticle treated after 72 hours of photocatalytic activity.

Antibacterial study: Antibacterial effect of nanoparticles was evaluated by well diffusion assay against bacteria *Pseudomonas putida* isolated from the effluent. Zone of inhibition was measured and tabulated (Table 1).

Zone formation (in cm) in four plates

Table: 1 Antimicrobial activity of Zinc Oxide Nanoparticles against *Pseudomonas putida.*

Sample	Plate 1	Plate 2	Plate 3	Plate 4
Control	0.8 cm	1.0cm	1.0cm	1.0cm
0.5g ZnO NPs	1.6cm	1.8cm	1.7cm	1.5cm
1.0g ZnO NPs	1.6cm	1.8cm	1.9cm	1.8cm
Antibiotic	1.0cm	1.0cm	1.0cm	1.0cm

A distinct PCR product of the 1500 bp size was produced when target DNA from isolated strain was used. From this analysis, it was determined that 25 cycles and 0.1ng of genomic DNA is required for successful amplification. Further, the amplicon was submitted for 16S rRNA sequencing after which BLAST and phylogenetic analysis was carried out.(Fig.2).

Fig-2 .Polymerase Chain Reaction (PCR)

>Pseudomonas putida strain

TGGTTTGCCAGTCAATTGCTCTAGGAATCTGCCTGGTAGCGGGGGACAACGTTTC
GAAAG
GAACGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGGACCTTCGGGCCTT
GCGCTA
TCAGATGAGCCTAGGTCGGATTAGCTTGTTGGTGAGGTAATGGCTCACCAAGGC
GACGAT
CCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAG
ACTCCT
ACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCC
ATGCCGC
GTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCA
GTAAGC
TAATACCTTGCTGTTTTGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGC
CAGCA
GCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCG
CGCGTA
GGTGGTTTGTTAAGTTGGATGTGAAAGCCCCGGGCTCAACCTGGGAACTGCATCC
AAAAC
TGGCAAGCTAGAGTACGGTAGAGGGTGGTGGAATCTCCTGTGTAGCGCTGAAAT
GCGTAG
ATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACT
GAGGTG

CGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAAC
GATGTC
AACTAGCCGTTGGAATCCTTGAGATTTTAGTGGCGCAGCTAACGCATTAAGTTGA
CCGTC
TGGGGAGTACGGCCGCAAGGTTAAAACTCACATGAATTGACGGGGGCCCGCACA
AGCGGT
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CAGAGA
ACTTTCCAGAGATGGATTGGTGCCTTCGGAAC

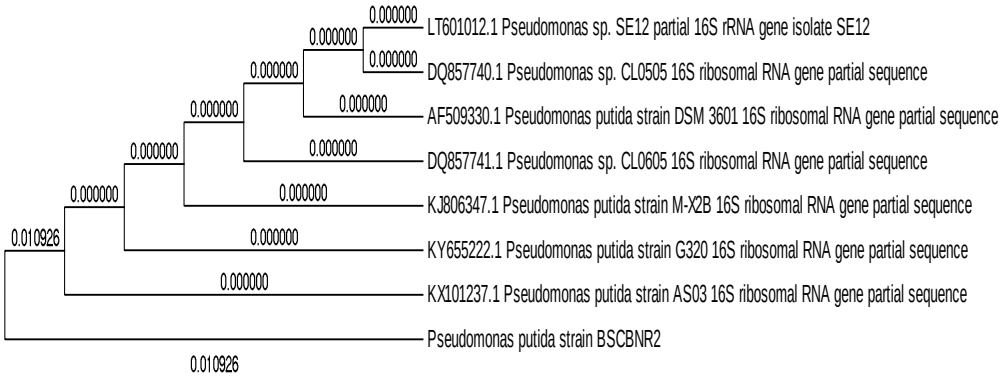

Plate 3.Gene sequencing

In this study synthesis of zinc oxide nanoparticle in an aqueous solution at a gentle temperature offers a green non toxic procedure which is attractive for biological and medically relative application. Our home made receipe will expended using extract from natural starch rich food sources such as Potato, rice, corn and other sources for eco friendly nanoparticle preparation.

CONCLUSION

In this study, synthesis of ZnO NPs in neutral aqueous solution offers a green and non-toxic procedure which is attractive for biological and medically related applications.Biosynthesis of nanaoparticles show a beautiful intersection between nanotechnology, biotechnology and physical chemistry. This technology had gained so much attention nowadays due to its growing demand of environmental friendly technology for material synthesis.Our domestic waste will extend using extracts from natural sources for eco-friendly nanoparticles preparation.

Furthermore, the ZnO nanocatalyst with high mineralization efficiency, high stability and recyclability could be the potential material for the wastewater treatment on an industrial scale.

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