Biosynthesis of Zinc Nanoparticles and their Applications

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***Abstract*—Biosynthesis of zinc oxide nanoparticles and its characterization was done and their applications were studied. In this study, zinc nanoparticles were rapidly synthesized from Zinc nitrate [Zn $(NO_3)_2$] solution using extract of *Hibiscus rosa-sinensis* leaves. The synthesised zinc oxide nanoparticles were characterized by different spectroscopic and analytical techniques such as SEM and FT-IR. The size of nanoparticles were from 40 to 60nm. The antimicrobial activity of the nanoparticles against *Escherichia coli, Staphylococcus aureus* was studied by using minimum inhibitory concentration method and bacterial growth was monitored by measurement of optical density (OD) of solution and estimation of colony forming units (CFU) on solid growth medium. In this report we have worked on the application of zinc oxide nanoparticles in photo-catalytic degradation of synthetic dyes.**

***Keywords*— Biosynthesis, Zinc oxide Nanoparticles, *Hibiscus rosa-sinensis*, Applications**

I. INTRODUCTION

Nanotechnology deals with particles less than 100nm size. Extensive research is going on for commercializing nano products throughout the world. The unique properties and utility of nanoparticles arise from a variety of attributes, including the similar size of nanoparticles and bio molecules such as proteins and polynucleic acids. Moreover, unlike their bulk counterparts, nanoparticles have reduced size associated with high surface/volume ratios that increase as the nanoparticles size decreases. As the particle size decreases to some extent, a large number of constituting atoms can be found around the surface of the particles, which makes the particles highly reactive with prominent physical properties [1].

The biosynthesis of nanoparticles utilizes naturally occurring reducing agents such as plant extracts, microorganisms, enzymes, which are simple and viable, an alternative to the complex and toxic chemicals. Biosynthesis of nanoparticles is cost effective, ecofriendly, nontoxic, and gives higher yield as compared to the chemical method which is highly expensive and emits hazardous by-products which can have some deleterious effects on the environment. The biological synthesis of nanomaterial can solve the environmental challenges like solar energy conservation, agricultural production, catalysis, electronic, optics, and biotechnological area. Among the different biological agents plants provide safe and beneficial way for the synthesis of metallic nanoparticles as it is easily available, there are possibilities for large scale production with the synthesis route being eco-friendly and the rate of production is faster. Plant extracts contain phytochemicals such as flavonoids, alkaloids and terpenoids etc which are mainly responsible for the reduction of ionic into bulk metallic nanoparticles formation and also help in the stabilization of these nanoparticles. These primary and secondary metabolites are constantly involved in the redox reaction to synthesize eco-friendly nanosized particles [2].

Zinc oxide, with its unique physical and chemical properties, such as high chemical stability, high electrochemical coupling coefficient, broad range of radiation absorption and high photo stability, is a multifunctional material[3]. During past few years, zinc oxide (ZnO) nanoparticles (NPs) have gained significant attention as it has been used in the field of dielectrics, photo catalysis, ceramics, piezoelectric film, piezoelectric sensor, solar cells, actuator, biosensors, bio-imaging drug delivery, water purification specifically arsenic removal, etc [4]. ZnO is non-toxic and self-cleansing. It is also compatible with skin, is anti-bacterial, dermatological and has UV radiation blocking property; hence it is used in cosmetics and many biomedical fields. Furthermore, ZnO NPs also act as good antimicrobial agent as they show antimicrobial activity against many pathogenic organisms like *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, etc [5]. ZnO NPs are less toxic and safe so they find increased applications in industries like food where they are used in packaging and processing of meat and vegetables. Recently, an awareness of general sanitation, contact disease transmission, and personal protection has led to the development of antimicrobial textiles. The use of ZnO NPs has been seen as a viable solution to stop infectious diseases due to the antimicrobial properties of these nanoparticles.

Wastewater released from textile industries contains excessive amount of dyes which are harmful and toxic. To treat such effluents, nanoparticles can be used to remove the dye. Mechanism implemented is photo-catalytic degradation. The dye gets adsorbed onto the nanoparticles and decolouration is observed. An investigation of solar light induced photo-catalytic decolourization of the synthetic reactive blue dye has been carried out in aqueous heterogeneous medium containing synthesized ZnO composite [6]-[7].

The plant *Hibiscus rosa-sinensis* belongs to family Malvaceae, and it is native from East Asia. The flower is used in hair care as a preparation. It can also be used as a pH indicator. When used, the flower turns acidic solutions to a dark pink or magenta colour and basic solutions to green. It is a very effective antimicrobial agent and may have some potential in cosmetic skin care.

II. MATERIALS AND METHODS

A. Preparation of extract

Plant leaves of *Hibiscus rosa-sinensis* were collected. All the leaves were washed thoroughly with water and kept in the sunlight to allow for drying. The dried leaves were made into a fine powder by grinding it. The powder was boiled in about 100-150ml of distilled water for 60 minutes. The pale yellow coloured extract was filtered using a filter paper and stored in the refrigerator for further experiments [8].

B. Synthesis of zinc oxide nanoparticles

50ml of the prepared *Hibiscus rosa-sinensis* extract is taken. It is boiled at temperature of 60-80°C and when the temperature reached 60^{o}C, 5grams of Zinc nitrate was added. This mixture was then boiled until the solution reduced to a deep yellow colour paste. The paste was collected in a ceramic crucible and dried in a furnace at 400°C for 2 hours. A light yellow or white crystalline powder is obtained. This is our , ZnO NPs from *Hibiscus rosa-sinensis* [8].

C. Characterization of Nanoparticles by SEM analysis and FT-IR spectroscopy

Surface Morphology of the Zinc oxide nanoparticles samples were analysed using SEM. From SEM analysis, the shape and sizes of the nanoparticles were identified. SEM Analysis was done using TESCAN Vega 3lmu, made in Czech Republic. Gold sputter coater was used. (Dried samples are sprayed on a carbon tape and plated with Gold). QUORUM instrument was employed for this coating process.

FTIR was recorded using Perkin Elmer Spectrum Two, with detector at 4000-400cm^{-1}. 20 scans per sample were taken. It helps in identifying the presence of functional groups. FTIR was conducted for the Zinc oxide nanoparticles obtained from *Hibiscus rosa-sinensis*.

D. Antibacterial activity of Zinc oxide nanoparticles

E. coli and *S. aureus* cultures were prepared by taking loop full of organisms into 5ml broth and incubating in shaker incubator overnight. The OD of the broth test tube was taken. Stock cultures were prepared. UV sterilized samples were placed 2mg each in test tubes containing autoclaved 4.8ml PBS. Dilution was calculated according to the OD so that load is $3x10^5$ CFU/ml and stock solution was prepared. 100µL of stock was added to all sample test tubes and control test tube. 100µL of culture was taken from control and serially diluted and plated as 0-hour control. The test tubes were incubated in shaker incubator at 37˚C for 1 hour. Incubated samples were serially diluted and plated as 1 hour and sample plates. The plates were incubated at 37˚C in incubator overnight. Colonies were counted and % reduction is [9]

E. Application in antibacterial textiles

3 conical flasks containing 50ml water were taken. Each containing 4mM, 6mM and 8mM concentration of zinc oxide nanoparticles synthesized from *Hibiscus rosa-sinensis*. Small round pieces (diameter 2.6cm) cotton fabric were cut out, autoclaved and put into the conical flasks. The flasks were kept in a shaker for 24 hours. Later, the cotton pieces had adsorbed nanoparticles on its surface. Meanwhile, nutrient media was prepared followed by streaking with the test organism (*E. coli*). The pieces with the adsorbed nanoparticles were kept on the media plates and incubated for 24 hours at 37°C. Clear zone of inhibition was observed around the fabric, thus indicating antibacterial activity of the fabric containing the adsorbed nanoparticles [5].

F. Application of Zinc oxide nanoparticles in degradation of Synthetic dye

Six conical flasks (5+ 1 control) containing 100ml water were taken. 0.1mg of Synthetic reactive blue dye was weighed and put into the 6 conical flasks. 1-5 mg of nanoparticles were then weighed and added to the 5 conical flasks. The initial absorbance of all the solutions was recorded at 440nm. The conical flasks were kept on a rotary shaker for 10-12 days, to allow for proper mixing of nanoparticles in the dye solutions. The mixing was left to proceed until a noticeable reduction in colour could be observed.

At regular intervals the optical density of the solutions were read at 440nm and the percentage of decolourization was calculated by using the following formula-

$$Removal(R\%) = \frac{(c_o - c_t)}{c_o} \times 100 \qquad (1)$$

where, c_o is the initial absorbance of the dye solution; c_t is the absorbance at time interval 't' [7].

III. RESULTS AND DISCUSSIONS

A. SEM Analysis

The size of the nanoparticles synthesized from various sources falls in the range of 40-70nm. The shape of these nanoparticles was spongy and rod like. SEM image has showed individual zinc particles as well as a number of aggregates.

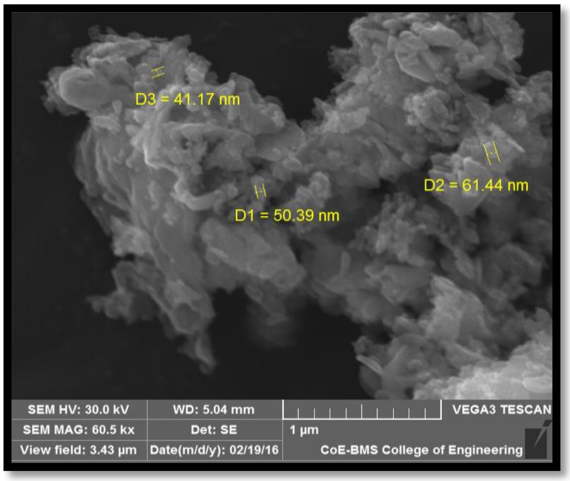

Figure 1: SEM images of Zinc oxide nanoparticles using *Hibiscus rosa-sinensis*

B. FTIR Analysis

FTIR Spectroscopy of ZnO NPs prepared from *Hibiscus rosa-sinensis* leaf extract was carried out to identify the possible biomolecules responsible for capping and efficient stabilization of the metal nanoparticles.

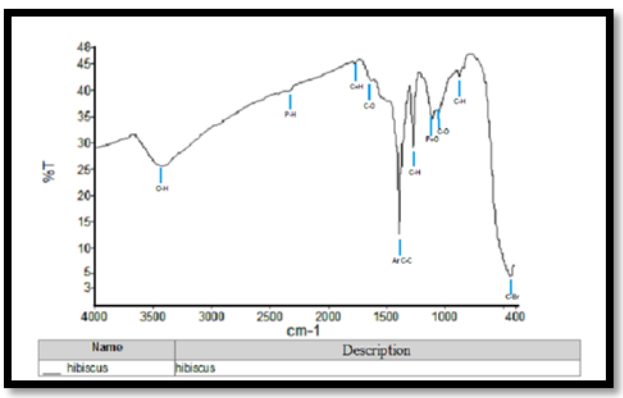

Figure 2: FTIR Spectroscopy of ZnO Nanoparticles from *Hibiscus rosa-sinensis* extract

C. Antibacterial activity

In order to obtain more accurate information of the antibacterial property of ZnO via a time dependent study, the broth inoculation method was carried out using two bacteria: *E. coli, S. aureus*. This method was used to determine the antibacterial properties of the synthesized nanoparticles by comparing the growth of the bacteria in the control tube (containing the broth and bacteria) and that in the test (containing the broth, bacteria and nanoparticles suspension). After one hour incubation the control and test were diluted and plated. The colonies were counted. Table 1 refers to the antibacterial activity of ZnO NPs.

Table 1: % antibacterial activity of ZnO NPs from *Hibiscus rosa sinensis* extract

Microorganism	% Reduction
E. coli	45%
S. aureus	48%

D. Application of Antibacterial textile

The formation of zones around the cotton cloth pieces proves that the nanoparticles were coated onto the cotton cloth disc. Amongst the 3 different concentrations used good results were seen for 6mg/ml and 8mg/ml concentrations of ZnO NPs from both the plant extracts (Figure 3).

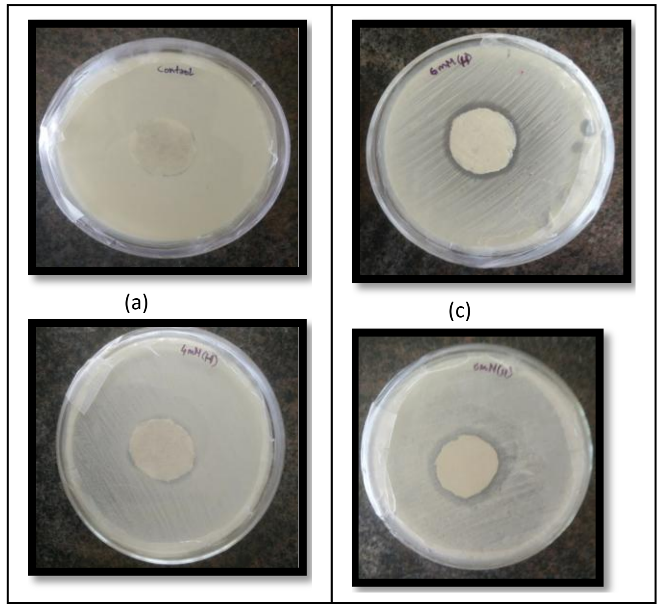

Figure 3: a) Control plate with no zone of inhibition, b) Plate with 4mg/ml of ZnO NPs, c) Plate with 6mg/ml of ZnO NPs, d) Plate with 8mg/ml of ZnO NPs.

E. Degradation of Synthetic dye by Zinc oxide nanoparticles

ZnO NPs synthesized from *Hibiscus rosa-sinensis* extract having concentration of 1-5mg/ml were put into aqueous solutions of synthetic reactive blue dye, the flask containing 5mg/ml of nanoparticles showed prominent degradation thereby proving the photo-catalytic degradation property of zinc oxide nanoparticles(Figure 4).

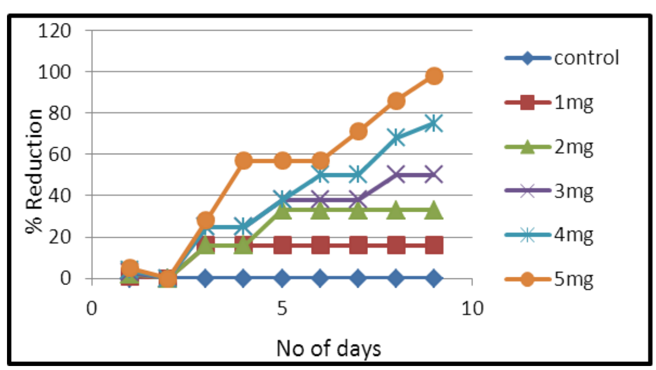

Figure 4: Graph of %reduction over the number of days for ZnO nanoparticles from *Hibiscus rosa-sinensis* extract.

IV. CONCLUSIONS

Biosynthesis of zinc oxide nanoparticles has many advantages over other methods. This method is both energy and cost effective with high yield at low concentration of plant extract. The size of the nanoparticles falls in the range of 40-70nm. The phytochemicals present on the surface of these nanoparticles keep them stabilized. The nanoparticles synthesized from this plant extract have an antibacterial activity of 40-50%. Even with minimum concentration of 5mg of nanoparticles, photo-catalytic degradation of synthetic dyes has been observed. There by showing that zinc oxide nanoparticles are useful wastewater treatment.

REFERENCES

[1]. Heiligtag F J and Niederberger M, (2013). The fascinating world of nanoparticle research, Materials Today, 16:262-27

[2]. Kuppusamy P, Yusoff M M, Maniam G P, and Govindan N, (2015). Biosynthesis of metallic nanoparticles using plant derivatives and their new avenues in pharmacological applications – An updated report, Saudi Pharmaceutical Journal,1-11.

[3]. Radzimska A K and Jesionowski Tl, (2014). Zinc Oxide—From Synthesis to Application: A Review, Materials, 7, 2833-2881.

[4]. Purkait P K, Roy J, Maitra S and Choudhuri M G, (2015). Green Synthesis of Zinc Oxide Nanoparticles – A Review, science voyage, 1(2):32-46.

[5]. Gunalan S, Sivaraj R, and Rajendran V, (2013). Green synthesized ZnO nanoparticles against bacterial and fungal pathogens, Progress in Natural Science: Materials International,22(6):693–700.

[6]. Baruah S, Pal and Dutta J, (2012). Nanostructured Zinc Oxide for Water Treatment, Nanoscience & Nanotechnology-Asia, 2:90-102.

[7]. Shilpa G , Yogendra K , Mahadevan K M and Madhusudhana N,(2015). Synthesis and Characterization of Zinc Oxide (ZnO) Composite Nanoparticles and its Efficiency in Photo catalytic degradation of Red HRBL dye in Aqueous Solution by Solar Radiation, International Journal of Environmental Science, 4(4): 188-194.

[8]. Devi R S and Gayathri R, (2014). Green Synthesis of Zinc Oxide Nanoparticles by using *Hibiscus rosa-sinensis*, International Journal of Current Engineering and Technology, 4(4):2445-2446.

[9]. Niranjan Bala, S. Saha, M. Chakraborty, M. Maiti,a S. Das, R. Basu and P. Nandy, (2015). Green synthesis of zinc oxide nanoparticles using *Hibiscus subdariffa* leaf extract: effect of temperature on synthesis, anti-bacterial activity and anti-diabetic activity, RSC Advances Issue 7.