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Comparative Study of Dye Degradation by Using Green Synthesized ZnO Nanoparticle and *Bacillus*

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Abstract: ***Comparative study of methylene blue degradation by using green synthesized zinc oxide nanoparticle and microorganism. Zinc oxide nanoparticle are synthesized by using Camellia sinensis plant extract. Comparative study shows that zinc oxide nanoparticle is more effective in degradation of methylene blue dye than microorganism. ZnO nanoparticle are capable to degrade methylene blue in brief time due to its extract capped zinc oxide nanoparticle. Using Bacillus species degradation of dye increase with increasing time.***
Keywords: ZnO, Bacillus species, Camellia sinensis, Nanoparticle

I. INTRODUCTION

Nanotechnology is science engineering and technology conducted at Nanoscale which is about 1 to 100 nm. The idea and concept behind nanoscience and nanotechnology started with talk entitled "The plenty of room at the bottum" By Richard fayman. The green synthesis of nanoparticle exhibit unique properties at nanoscale. Zinc oxide nanoparticle are synthesized by using green tea leaf plant extract Camellia sinensis or green tea .Further produced ZnO were alsocharacterized by UV visible, FTIR, and XRD.Zinc oxide nanoparticle belongs to class of metal oxides, which is characterized by photocatlytic and photo oxidizing activity Dyes are structural complex material which enter in the environment due to Various textile industry processes like dyeing and cloth completion process. Methylene blue is the one of the most important cationic dye which is now used in textile industry Nanoparticle are used as a photo catlyst in the presenceof uv to remove MB Dye. The focus on present work is to apply the accurate principles of green chemistry for the synthesis of zinc oxide nanoparticle by using green tea leaf. This work ZnO nanoparticle were prepared by biological method that means green synthesis. In this ZnO nanoparticle as precursors for the degradation of dyes. Comparison of dye degradation by using microorganism and nanoparticle.

Synthesize zinc oxide nanoparticle via biological method. To explore the degradation of dye using zinc oxide nanoparticle via photocatlytic reaction. Comparison of dye degradation by using nanoparticle and microorganism. Test the dye degradation in increasing time period.

II. MATERIALS AND METHODSCOLLECTION OF PLANT MATERIAL

Plant materials was collected from local areas in Palakkad, Kerala. Plant material was cleaned withdistilled water and dry at room temperature. The dried plant materials were crushed in to powder form.

A. Isolation of Organisms from soil.Sample Collection

Soil sample was collected from the nearby my native place .The soil sample forisolation of microbes were obtained from the 5 to 10 cm layer below the soil surface.

B. Isolation Method

Serial dilution of soil sample;

1) 10 g of soil sample was taken and add to 95 ml of distilled water. Shake the suspension well.
2) Before the soil settlers, remove 1 ml of the suspension with a sterile pipette and transferit to 9 ml distilled water.
3) Repeating the dilution step for 7 tubes containing 9 ml distilled water. This result inserial dilution 1st tube to 7th tube.

The diluted suspension was transferred into nutrient agar plates. The major bacteria present in the soil sample are selected and characterized by growing them on some selective media. The organisms was isolated on Mannitol salt agar media, MacConkey agar media and nutrient agarmedia. Then all the culture were maintained on the respective slant.

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C. Isolation on Selective Media

The identified colonies were then inoculated on to selective media such as Nutrient agar, Mannitol salt agar to study the colony characteristics.

Nutrient agar

Peptone	: 0.5
Beef extract	: 0.3 g
Sodium chloride	: 0.5 g
Agar.	: 2.0g
Distilled water	: 100 ml

Mac Conkey agar (pH 7.1)

Peptone.	: 1.7 g
Protease peptone.	: 0.3 g
Lactose.	: 1.0 g
Bile salt mixture	: 0.15 g
Sodium chloride.	: 0.5 g
Distilled water.	: 100 ml

One hundred ml of pseudomonas agar was prepared and sterilized at 121ºc for 15 minutes at 15 lbs pressure .Then it poured in to Petri plates and allowed to solidify. Then the organism were inoculated and incubate the Petri plates at 37ºc for 24 hours and observed the growth was observed.

D. Collection of Plant Material

Plant material was collected from local area in Palakkad Kerala. Plantmaterial was cleaned with distilled water and dry at room temperature. The driedplant material was crushed into powder form *Camellia sinensis*: green tea leaf **Bacterial isolates**
To isolate bacteria from soil. The *Bacillus Species* are isolated fromdifferent soil samples.
Isolation of microorganism Preparation of inoculum. Isolation on selective media.
Morphology and biochemical characterization of the isolates.

E. Preparations of Plant Extract.

The 10 g of dried leaves were heated in 100 ml deionised water with 80°c with thecontinuous stirring for 2 hours.

1) Then cooled at room temperature and filtered by using what's man filter paper no 40.
2) Centrifugation at 4000 rpm for 10 min and coloured pellets are settled at the bottom of thetube.

F. Green Synthesis of Zinc Oxide Nanoparticle.

Zinc nitrate solution of 50 ml was added to 5 ml of green tea leaf extract. The solution was stirred on magnetic stirrer at 120°c. The colour was observed from light brown to blackish brown.
The resultant solution was centrifuged for the minutes speed of50,000 rpm After discarding suspended zinc oxide nanoparticle were dried in watch glass Yellow coloured particle were collected for characterization. Characterization techniques of green synthesized zinc oxide NP

1) XRD
2) UV VISIBLE SPECTRA
3) FTIR

G. Dye

Methylene blue organic dye with greenish blue colour. Methylene blue does not undergo photo degradation without a photo catalyst. It is photo stable and would only degrade by photocatlysis.

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III. RESULT AND DISCUSSION

A. Microscopic Analysis

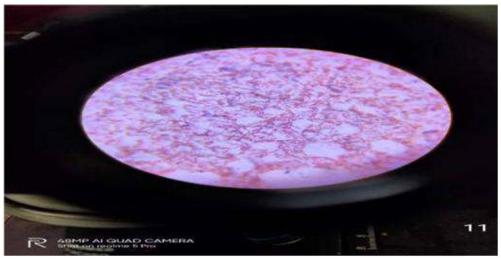
Table 1. Morphological classification of organisms

Sl No	*Organism*	*Selective media*				
		Nutrient agar	*Blood agar*	*MacConkeyagar*	*Mannitolsalt agar*	*EMB AGAR*
1	Sample 1	Circular colony ,rough opaque, fuzzy, white or slightly yellow withjagged edges	Non haemolytic	Large irregular plate colour colony	No growth	No growth
2	Sample 2	Large,opaque,irregular colony withdistinct earthly smell	Large, opaque hemolytic	Non lactose fermenting colonies	No growth	Irregular r colonies s
3	Sample 3	Large opaque, irregular colonywith distinct earthlysmell	Large opaque, hemolytic	Non lactose fermenting colonies	No growth	Irregular r colonies s
4	Sample 4	Circular colony,rough,opq ue,fuzzy ,white or slightly yellowcolour	Non hemolytic	Large irregular plate colour colony	No growth	No growth
5	Sample 5	Large opaque, irregular colony ,with distinctearthly	Large opaque hemolytic	Non lactose fermenting colonies	No growth	Irregular r colonies s

Table •1 morphological characterization of test organism

Sl No	Isolates	Morphology		Motility
		Gram staining	Spore staining	Hanging Drop
1	Sample 1	Gram positive rods	Non spore forming	Motile
2	Sample 2	Gram negative rods	Non spore forming	Motile
3	Sample 3	Gram negative rods	Non spore forming	Motile
4	Sample 4	Gram negative rods	Non spore forming	Motile
5	Sample 5	Gram positive rods	Non spore forming	Non Motile

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B. Isolation of Microorganism on Selective Media

After inoculation on to selective media organism was identified based on their colony morphology, the organism identified were *Bacillus.* The result have been tabulated in



Figure: *Bacillus species in Selective media*

C. Biochemical CharacterizationBacillus species

Isolated microorganism were further inoculated on to the various biochemical test media.

The result were tabulated in Table No 3

Table: 3 Biochemical test

Biochemical test	***Sample 1*** ***Sample 4***
Motility	+
Indole	_
MR	–
VP	+
Citrate	_
Urease	_



Figure: Biochemical test *Bacillus species*

D. Production of Zinc Oxide Nanoparticle

The production of zinc oxide nanoparticle by using dried green tea leaf. The pale yellow white colour of the zinc oxide nanoparticle arise due to capping action of biomolecule of green tea leaf extract on the surface of nanoparticle. When the mixture of salt zinc nitrate and green tea leaf was add and heat the colour change was appear .colour change indicate formation of zinc oxide nanoparticle.

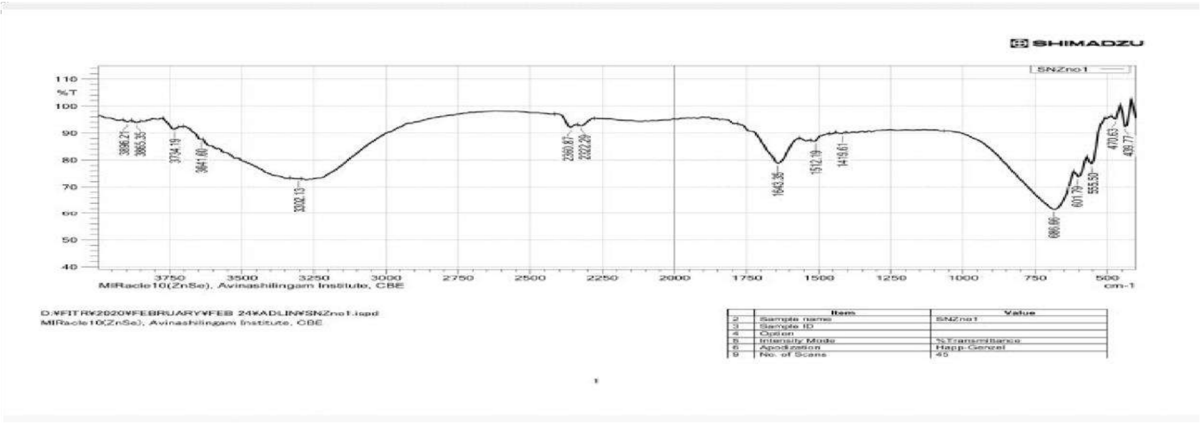
Dried green tea leaf powder Pale yellow colour ZnO NPs



IV. CHARACTERIZATION

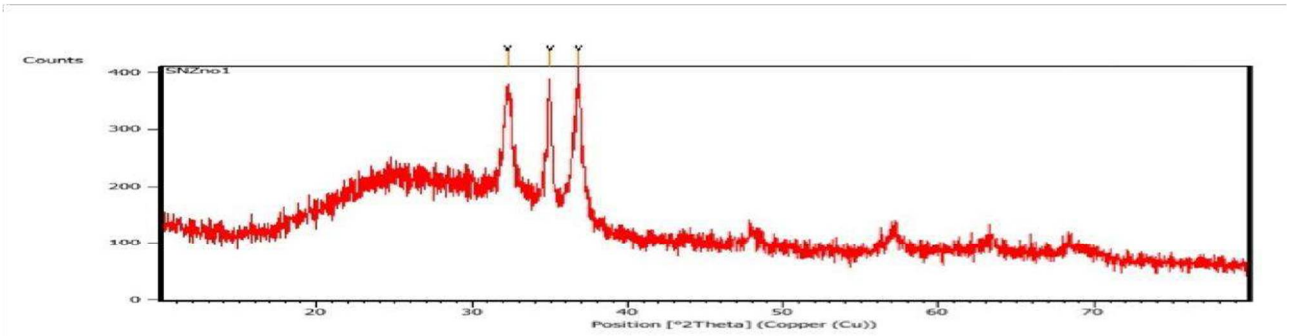
A. FTIR

The FTIR spectroscopy is the measurment of absorption of IR radiation by thesample plotted against the wavelength



B. XRD

The technique basically lets about the crystal density.Purity and size of the metal nanoparticle were examind by x-ray diffractometer



Peak List: (Bookmark 3)

Pos. [°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]
32.2275	176.88	0.4015	2.77770	76.47
34.9455	206.77	0.2676	2.56763	89.39
36.8250	231.30	0.4015	2.44079	100.00

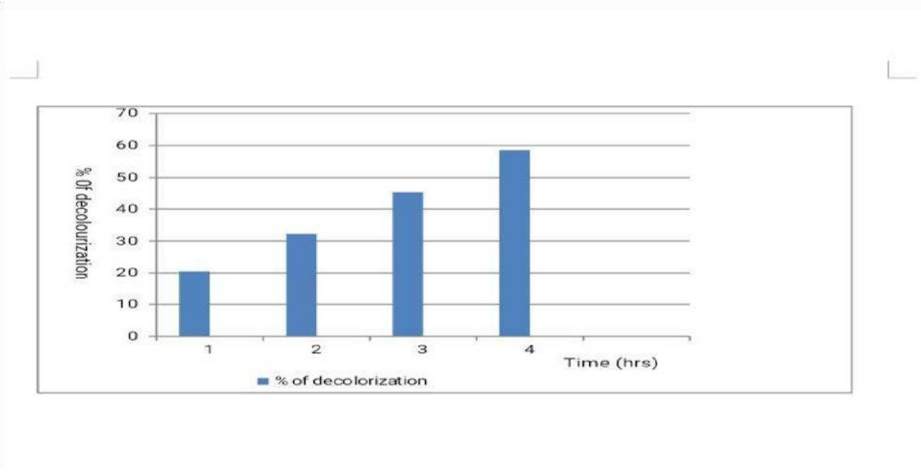
C. Methylene Blue Dye

Methylene blue is an organic dye with greenish blue color that is found in family of dyes known as phenothazine.it is used in the leather, paper, and textile industries for their product. Methylene blue does not undergo degradation by hydrolysis as well as photo degradation without a photo catalyst. The dye isphoto stable and would only degrade by photo catalysis.

D. Decolourization of Dye

Decolourization of dye by using microorganism. Effect of incubation time on decolourization of methylene blue. The course of decolorization of methylene blue dye bt bacillus species under the optimum conditions the persentage of decolourization its tabulated below. The result shows that decolourization rate ofmethylene blue by bacillus species increase with increasing time period

S.No	Time(hrs)	% of decolourization
1	1	20.50
2	2	32.23
3	3	45.25
4	4	58.56



E. Decolourization of Methylene blue by using ZnO Nanoparticle

Photo degradation of dye by using zinc oxide nanoparticle. The photo degradation of methylene blue observed at different time period. The absorbance OD MeB was decreased with increasing time period in presence of zinc oxide nanoparticle and uv light – visible spectra data of methylene blue treated with thephoto degradation reaction in presence of zinc oxide nanoparticle.

Reaction condition	Peck (nm)	Absorbance withZnO
Starting solution	663.73	0.5493
After 1 hr kept in sunlight	663.73	0.4532
After 2 hr kept in sunlight	663.73	0.3823
After 3hr kept in sunlight	663.73	0.2365
After 4 hr kept in sunlight	663.73	0.0740

V. CONCLUSION

Dye degradation process become main cost effective processes in now a days. Degradation of dye is very effective by using green synthesized nanoparticle. The biosynthesis of nanoparticle is very cost effective and ecofriendly using Bacillus species the degradation rate after 4 hour incubation is 58.69. In the case of ZnO nanoparticles degradation rate of starting solution is 0.5493 and after 4 hour incubation the rate become decreased. Combative study shows that zinc oxide nanoparticle e is more effective in degradation of. Methylene blue dye than microorganism.

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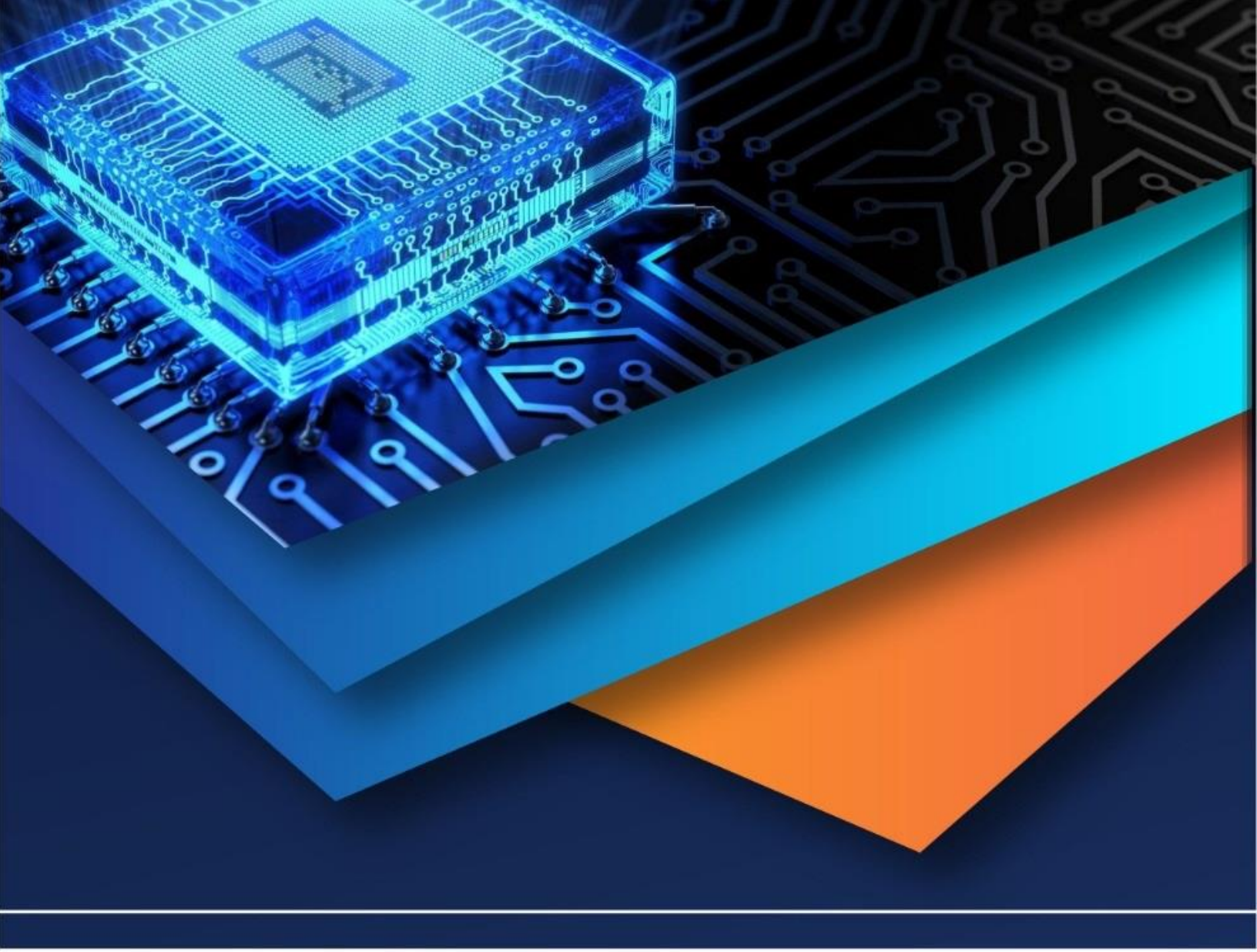
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