Structural and antibacterial studies of rice straw based ZnO nanocomposite

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Abstract. The present study is focused on the structural and antibacterial studies of rice straw based ZnO nanocomposite. For comparison, counterparts were also synthesized. Nanoparticles of ZnO were synthesized through combustion method and co-precipitation method. Structural characterizations of all the synthesized samples were carried out using XRD. Antibacterial studies of the synthesized samples were carried out. The present study investigates the antimicrobial activity of the synthesized samples as antibacterial agents. The selected microbes for the present study are gram-positive bacteria: Bacillus cereus (B. cereus), the gram-negative bacteria: Escherichia coli (E. coli).

1. Introduction
Natural fiber has many advantages like biodegradability, flammability, and non-toxicity [1, 2]. The source of natural fiber are stalks, leaves, and seeds, for instance, kenaf, sisal, flax, wheat straw and rice straw [3, 4]. In most countries, straw is an abundant cellulosic by-product from the production of crops such as wheat, corn, soybean, and rice. The utilization of easily available biodegradable farming residues like rice straw, rice husks and corn Stover can be used to rationalize the cost effect. Rice straw is best known to be a potential source of energy and also a value-added by-product [5]. Due to the availability of natural/bio-fibers from the renewable resources, the use of bio-composites has been found to be expanding in the recent years. Nanocomposite materials formed by metal nanoparticles that have been appropriately incorporated into the polymer matrix were experimentally found to be very significant due to their diversity in electrical, catalytic and optical properties. These diversities have potential applications in the fields of electronic, photonic, catalysis and bioengineering [6]. Synthesis of nanocomposites is an expanding trend of nanoscience. In order to exploit the full potential of the technological applications of the nanomaterials, the scientists were guided towards the conventional polymers as one component of the nanocomposite. The control of the proliferation of microbes in nature is one of the most important concepts for the survival of human beings, animals and plants. Antimicrobial operators are of incredible significance in businesses, for example, water cleansing, materials, bundling, development, medication and sustenance bundling [7,8]. As of late, nonstop endeavors have been made to create polymer-based nanocomposites with antimicrobial capacity. The polymeric frameworks are considered as great host materials for metal and metal oxide nanoparticles. They give extra characteristics, for example, processability, solvency and warm
solidness to the frameworks shaped[9]. Several efforts have been made for the fabrication of polymeric material with potential antimicrobial activity [10]. Moreover, the nanocomposites prepared using inorganic metal oxide nanoparticles and organic polymers can find better utilization due to the enhanced antimicrobial activity. All these open the possibility of the formulation of a new generation of bactericidal materials. In the present work, we are focussed on the comparison of structural and antibacterial properties of the nanocomposite of ZnO formed with rice straw.

2. Experimental

AR review synthetics got from Merck were utilized for the arrangement of rice straw based ZnO nanocomposite. ZnO nanoparticles were set up by the co-precipitation method in presence of capping agent and also by combustion method.

Metal oxide ZnO was prepared as follows. Zinc nitrate dihydrate and sodium hydroxide were used as beginning materials. The citrus extract was utilized as the stabilizer. Aqueous solutions of 25ml 0.1 M zinc nitrate, and 50ml 1 M of sodium hydroxide were slowly mixed drop wise into a beaker containing aqueous solution of 25 ml 0.02 M citric acid and stirred well using a magnetic stirrer for two hours. The stabilizer was used to prevent growth and agglomeration of the particles. The molecule measure was driven by the test parameters, for example, the convergence of the reactants, rate of blending, pH, thickness of the arrangements and so forth. The stabilizers used for controlling the precipitation reaction should be easily and completely removable from the sample so as to avoid any possible contamination of the samples. After two hours of continuous stirring, the precipitate formed was in hydroxide form. The hydroxide precipitate formed was washed several times in distilled water to free it from ions and other impurities. The wet accelerate got was dried at room temperature and completely grounded utilizing an agate engine to get the hydroxide antecedent as fine powder. The powder so obtained was annealed at 600°C for 3 hours in a muffle furnace to acquire the particular metal oxide nanoparticles.

Nanoparticles of zinc oxide were prepared by combustion method using analytical grade chemicals. Zinc nitrate dehydrates and urea was used as starting materials. Urea was used as fuel. 5 g zinc nitrate was taken to which urea and glycol were added mixed to get a clear solution. pH of the solution was made neutral by controlling the amount of urea and glycol. The solution was then heated. The chemical reactions between the reactants produce exothermic energy and as a resulting flame is produced and fine powders of the nanoparticles were formed. The powder so obtained was annealed at 600°C for 3 hours in a muffle furnace to acquire the particular metal oxide nanoparticles.

After legitimate cleaning and drying process rice straw waste was utilized. For the treatment of rice straw waste, basic NaOH solution and methanol were used. Small pieces of 5 g rice straw was washed using distilled water. NaOH solution was prepared by measuring NaOH equal to the weight of 10% of rice straw (.5g) and making it up to 50ml by dissolving NaOH in distilled water. Liquor to fiber ratio was taken as 10:1, ie, 50g of methanol for 5g of rice straw. Liquor and NaOH solution were added to the rice straw and were cooked on a heater for 20 minutes until the solution reduced to some amount on open air. It cool for some time and the pulps was further applied for acid pulping, where nitric acid (69% concentration) diluted (2.5 ml of acid and making it up to 25 ml using distilled water) was used in the pulp for heating until it reduces. It was cooled for some time. Again 25 ml of concentrated acid was added. After reduction and cooling process, another 25 ml of acid was added and heated. The pulp turned to particles. After cooling distilled water was added to it and stirred for 15 minutes on a magnetic stirrer. After some time the powder settled. Water was decanted. The process was repeated thrice and finally, washing was done using 50 ml of methanol. The solution was filtered using filter paper and dried at room temperature. Dried rice straw powder was collected and used for analysis. In case of nanocomposite based on rice straw annealed ZnO prepared from combustion and co-precipitation methods added at the time of pulp formation, separately.
Table 1. Sample notations used in the present work.

| Sample                                                                 | Notations |
|-----------------------------------------------------------------------|-----------|
| ZnO nanoparticles prepared from co-precipitation annealed at 600°C     | ZS        |
| ZnO nanoparticles prepared from combustion method annealed at 600°C    | ZCS       |
| Rice straw                                                            | RS        |
| Rice straw based zinc oxide nanocomposites                            | RSZS      |

3. Results and discussions

Metal oxide prepared using co-precipitation method in the present study was obtained in the form of hydroxide. In the case of combustion method, black powder was obtained. Both annealed samples of zinc oxide are obtained as white powders. Rice straw and rice straw based nanocomposites were obtained as pale yellow.

3.1. XRD Analysis

3.1.1. XRD analysis of ZS and ZCS

XRD studies were done by XPert-PRO model powder diffractometer (PAN analytical, Netherlands) employing Cu- Kα radiation (λ = 1.54060 Å) operating at 40kV, 30mA. Figure 1 shows XRD of ZS and ZCS. The interplanar spacing (d_hkl values), 2θ values and relative intensity values of zinc oxide (ZS and ZCS prepared in the present work) corresponding to the observed diffraction peaks were compared with the standard values of zinc oxide as reported by JCPDS-International Centre for Diffraction Data which also matched with JCPDS-ICDD pattern number pattern number #79-0205 of zinc oxide. Both ZS and ZCS matched with the standard ZnO hexagonal system with a primitive lattice of ZnO (JCPDS: #79-0205). For ZS, the diffraction peaks at 2θ values 31.71°, 34.39°, 36.27°, 47.49°, 56.61°, 62.68°, 66.29°, 67.99°, 69.1°, 72.75° and 77.12° corresponds to the crystal planes of (100), (002), (101), (102), (110), (013), (200), (112), (201), (004) and (202) respectively with no characteristic peaks corresponding to the impurities, which further confirms the formation of pure stable ZnO phase.

The average crystallite size of ZS and ZCS calculated from the line broadening of the XRD pattern, utilizing FWHM values of three noteworthy tops in the XRD spectrum making use of Scherrer equation are shown in Tables 2 and Table 3. The Scherrer equation is

\[ t = \frac{K\lambda}{\beta \cos \theta_{hkl}} \]

Here t is the average crystallite size normal to the reflecting planes, K is the shape factor which lies between 0.95 and 1.15 depending on the shape of the grains (K = 1 for spherical crystallites), λ is the wavelength of X-rays used, β_hkl is the full width at half maximum of the diffraction line in radians and θ_hkl is the Bragg angle corresponding to the diffraction line arising from reflections from the planes designated by Miller indices (h k l).

Table 2. Crystallite size calculation of ZCS using Scherrer equation.
### Table 3. Crystallite size calculation of ZS using Scherrer equation.

| $2\theta$ | $\theta$ | $\cos \theta$ | FWHM | $D$ (nm) |
|-----------|----------|----------------|-------|----------|
| 31.71     | 15.86    | 0.9619         | 0.3945| 20.94    |
| 34.39     | 17.19    | 0.9553         | 0.3603| 23.08    |
| 36.27     | 18.14    | 0.9504         | 0.4308| 19.40    |

Average Crystallite size = 21.14 nm

### 3.1.2. XRD analysis of RS

The XRD pattern of RS was found to match well with the XRD patterns in literature [7]. The XRD of RS showed the presence of a few crystalline peaks in the diffraction pattern having $2\theta$ values...
between 15° and 30° and broad peaks between 30° and 50°. Figure 2 shows the XRD diffraction pattern of RS.

Figure 2 XRD Spectrum of RS

3.XRD analysis of RSZS and RSZCS

Figure 3 shows the XRD diffraction pattern of RSZS and RSZCS respectively. The variation of XRD of RSZS and RSZCS when compared with RS confirms the formation of nanocomposites. Since doping level of metal oxide was low no sharp peaks of metal oxide was obtained in case of nanocomposites.

Figure 3 XRD Spectrum of RSZS and RSZCS

3.2. Antibacterial Studies
There is a pressing demand in the present world to develop new antimicrobial agents and to find out novel strategies due to the emerging infectious diseases and the development of drug resistance in the pathogenic bacteria and fungi. The present study investigates the antimicrobial activity of the synthesized samples as antibacterial agents. The selected microbes for the present study are the gram positive bacteria: Bacillus cereus (B. cereus), the gram negative bacteria: Escherichia coli (E. coli). These test organisms were collected from Institute of Microbial Technology, Microbial Type Culture Collection Centre (IMTECH), Chandigarh. The strains were maintained on their respective medium in slants at 2-8°C.

The antibacterial activities of the prepared samples in the laboratory had been calculated using the modified Kirby-Bauer disc diffusion method. The pure cultures of the given organisms were subcultured in the Müller-Hinton broth at a temperature range of 35°C ± 2°C on an orbital shaking incubator at 160 rpm. For the microbial growth, a large lawn of culture was prepared by spreading the 100 μL freshly prepared culture having 106 colony-forming units (CFU)/mL of each testing organism on nutrient agar plates by the assistance of a sterile glass-rod spreader. Plates were left standing for 10 minutes to allow the culture to get absorbed into the plates. Thereafter, 8 mm wells were punched into the nutrient agar plates to test the nanomaterial antimicrobial activity. Wells had been sealed with one drop of molten agar (0.8% agar) to avoid leakage of nanomaterials from the bottom of the wells. Using a micropipette, 100 μL (50 μg) of the prepared sample of nanoparticle suspension was poured onto each of the wells on all plates. After an overnight incubation at a temperature range of 35°C±2°C, the various levels of the zone of inhibition were measured around the disc in units of millimeter. The Solvent blank was used as negative control and Antibiotic tetracycline was used as a positive control.

The antibacterial activities of all the five samples were studied in detail. The antimicrobial activity of the nanoparticles with which is in contact with the microorganisms is generally known to be a function of the surface area. Since, the reactions take place at the surface of a chemical or material, smaller the size, higher is the surface to volume ratio i.e., in case of larger surface area, better interaction with the microbes is seen. Figure 4 shows the comparison of antibacterial activity of ZS, ZCS, RS, RSZS and RSZCS. Table 4 shows this comparison. Antimicrobial effects of ZnO nanoparticles can be attributed to several mechanisms: ROS (reactive oxygen species) generation results in induction of oxidative stress, due to accumulation of nanoparticles in the bacterial membrane, membrane disorganization occurs and also their cellular internalization, antimicrobial activity may also be due to the release of metal ions and their binding to the membrane of microorganisms.
The destruction of the outer membrane of bacteria by the generated superoxide anion radicals (•O₂⁻), which acts as the reactive species, results in antibacterial activity. At the catalyst's surface, the reactive species such as •OH and •O₂⁻ are generated. Hence the high surface area is very beneficial for degradation of bacteria. The activity was found to be highest in case of gram negative bacteria. All the five samples showed very low activity for B.cereus. The variation in the sensitivity or resistance to both gram positive and gram negative bacteria populations may be due to the differences in the cell structure, physiology, metabolism or degree of contact of organisms with nanoparticles.

4. Conclusions
In the present work we are focussed on the comparison of structural and antibacterial properties of nanocomposite of ZnO formed with rice straw. ZnO nanoparticles were prepared using co-precipitation and combustion methods. Structural characterizations of all the five synthesized samples were carried out using XRD. Comparison of XRD’s of metal oxide with JCPDS confirmed that the formed metal oxide was ZnO. Crystallite sizes were calculated using Scherrer equation. Crystallite sizes calculated for ZCS was 18.54 nm and that of ZS 19.33 nm. The variation of XRD of RSZS and RSZCS when compared with XRD of RS confirms the formation of nanocomposites. Since doping level of metal oxide was low no sharp peaks of metal oxide was obtained in case of nanocomposites. The present study investigates the antimicrobial activity of the synthesized samples as antibacterial agents. The selected microbes for the present study are the gram positive bacteria: Bacillus cereus (B. cereus), the gram negative bacteria: Escherichia coli (E. coli). The activity was found to be highest in case of gram negative bacteria. Antibacterial activity of RS can be attributed to the activity of organic groups which are present in the biopolymer chain. The presence of polyfunctional groups is supposed to be a perfect recipe for making an antibacterial agent. In addition to this, the presence of metal oxide in the biopolymer chain might have enhanced the antibacterial activity of the nanocomposite. All the five samples showed very low activity for B.cereus. The variation in the sensitivity or resistance to both gram positive and gram negative bacteria populations may be due to the differences in the cell structure, physiology, metabolism or degree of contact of organisms with nanoparticles.

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