Developing Anti-Fungal and Anti-Bacterial Mortar Using Zinc Oxide Nanorods

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Abstract Fungal and bacterial growth are two factors primarily responsible for the degradation and breakdown of the surfaces of buildings and structures. Fungi, which cause molds in buildings, develop rapidly in humid places like Assam and can cause deadly diseases such as Histoplasmosis. Remedial measures for these molds are expensive and cumbersome. Bacteria can result in Sick Building Syndrome (SBS), which causes acute health problems in people living in these buildings. Bacteria mostly grow in the damp walls of hospitals and cause acute diseases in people, which is a worldwide problem in humid countries. Nanotechnology can be utilized to solve these problems to a great extent, providing relief to the world's population. Tests with Zinc Oxide (ZnO) Nanorods grown on prepared mortar samples using hydrothermal techniques revealed dense growth of Nanorods deep into the pores of the mortar samples on scanning electron micrographs. This anti-fungal and anti-bacterial study was done on the prepared Ordinary Portland cement mortar sample with fungus Aspergillus Niger and bacteria Escherichia coli (gram-negative) and microorganisms samples collected from local hospital buildings.

Keywords Mortar, Zinc Oxide, Nano Rods, Aspergillus Niger, Escherichia Coli

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Introduction

Nanotechnology has made significant advancements in almost all aspects of technology, including the construction industry in civil engineering. It has provided many benefits and possibilities to the construction sector. The use of nanotechnology has helped to produce stronger and more durable materials

than conventional ones, like concrete, which occupies nearly 70% of the materials by volume in construction. Researchers have incorporated Nano-sized particles into concrete using appropriate methods in suitable proportions to improve compressive and flexural strengths at an early age, along with enhancing the pore structure of concrete. Nano-sized materials absorb less water and require less cement content than conventional concrete. Extensive studies have been conducted on the use of nanotechnology in civil engineering, and its possible effects (Redlich *et al.,* 1997). Research has shown that traditional construction materials, such as concrete and steel, can perform better when nanotechnology is applied. The use of metal/metal oxide nanoparticles and engineered nanoparticles, such as carbon nanotubes and carbon nanofibers, has resulted in achieving remarkable improvements in concrete strength, durability, sustainability, and environmentally responsive anti-corrosion coatings formed using Nanoencapsulation techniques, showing promise in laboratory settings (Yu and Crump 1998). Advancements in the development of novel materials and technologies have been observed in the field of nanotechnology and the world's construction industry. The obvious advantage of applying nanotechnology is to promote the use of more efficient nanomaterials to make building structures and infrastructures "smarter" (Pelczar *et al.,* 2001). A review has also been conducted on the knowledge of nanomaterials and nanotechnology used by the construction industry. The areas covered include the nanoscale analysis of Portland cement hydration products, the use of nanoparticles to increase the strength and durability of cementitious composites, the photocatalytic capacity of nanomaterials, and the risks of nontoxicity (Ananthnarayan and Paniker 2009). Fungal growth, bacterial growth, VOCs (volatile organic compounds), and formaldehyde are primarily responsible for the degradation of structures. Remedial measures are being taken using nanotechnology. These problems must be addressed appropriately; otherwise, they have a strong probability of resulting in Sick Building Syndrome (SBS). VOCs and formaldehyde, which originate from building and finishing materials and consumer products, can cause adverse health effects to building occupants and may contribute to symptoms of SBS when emitted continuously over prolonged periods. The demand for low-emission VOC products has increased. Studies have been conducted on the emission of VOCs from polymeric building materials, the level of emissions in the indoor environment, and the requirements for testing the materials (Crook and Burton 2010). Exposure to a large number of mold spores is attributed to symptoms of asthma, rhinitis, or bronchitis.

Some methods have been described that were used to investigate exposure to indoor mold contamination. Strategies for remediating mold-contaminated buildings were discussed, taking examples of the after-effects of flooding in the UK in 2007 and Hurricane Katrina in the USA in 2005 (Harold Zeliger, 2011). It has been observed that mucous membrane (nose, throat, and eye) irritation, skin symptoms, for example, itching, and sensory irritation (primarily odor) are some health issues that can occur due to SBS. SBS has its presence in office structures, warehouses, and homes. The association of SBS has also been observed with airborne biological and chemical components, including bioaerosols, volatile organic compounds released from building materials and furnishings, personal use products (e.g., perfumes), and environmental tobacco smoke (Torgal and Jalali, 2011). The sensation of dry mucous membranes, red skin, headaches, mental fatigue, nausea, and dizziness have also been observed to have a direct link with SBS. The World Health Organization has recognized SBS as a group of symptoms that occur due to exposure to building variables like indoor air quality, lighting, noise, and psychological factors (Monica *et al.,* 2013). Studies related to SBS suggested that on-site assessment had proved to be very useful. The patient and the building were both involved in the treatment. Initiation of works like reduction of sources of environmental contamination and ventilation improvements were highly recommended (Srikanth *et al.,* 2013). Researchers have compared the biological resistance of green and conventional building materials (BMs) before and after nano-metal treatment. They have also explored the best Nano-metals to improve the fungal growth resistance of building materials. It was observed that Nano-zinc was probably the most favorable Nano-metal for wood and wood composite materials. Green materials were less resistant to fungal attack relative to their conventional counterparts treated by Nano-metals. A few building materials

were selected with specific proportions, and it was seen that all test Nano-metals failed to provide complete protection against fungal growth. However, it was concluded that the higher the Nano-metal concentration was, the longer the lag period until growth began, and fewer fungi grew on the materials (Huang *et al.,* 2013). Researchers have tried to establish an advantageous position in the field of nanotechnology. In this matter, a special mention should be given to China as it is the second-largest producer of research papers on nanotechnology. The first place belongs to the United States of America. It has been observed that a lot of emphasis has been given to the incorporation of nanotechnology in the construction sector (Pacheco-Torgal, 2019). Recent studies have focused on antimicrobial surfaces with functional material coatings, such as cationic polymers, metal coatings, and antifouling micro-/nanostructures. These studies provide insights into the development of virus-inactivating surfaces, which could be particularly useful in controlling the currently confronted pandemic coronavirus disease 2019 (COVID-19). Additional recent studies have been conducted on the beneficial role of zinc oxide nanoparticles in improving the performance of cement composites. Zinc oxide (ZnO) nanoparticles have unique optic, antimicrobial, and photocatalytic properties. These ultra-fine nanoparticles have a filler effect and have been found to impact the hydration reaction in the cement matrix, as well as impart photocatalytic properties in the cement structures. The impact of ZnO nanoparticles on setting time, microstructure, and strength has also been studied in detail in recent years. Studies reveal that with an optimized dosage of these nanoparticles, mortars and concrete with increased antimicrobial properties can be prepared, which provides an innovative pathway in the construction industry to build self-cleaning, durable, and eco-friendly structures.

Importance and Objectives

Experimental results show the possibility and prospect of using such treated and smart building materials or anti-fungal/anti-bacterial materials for the construction of structures. This will be particularly attractive for structures in humid areas, hospitals, etc., as it would be helpful and effective in increasing the longevity and performance of the structures.

Our study aims to find a unique preparation technique for the ZnO nano-rods-based mortar sample, which has not been done previously in any related study. Moreover, we will ascertain the performance of the prepared sample against specific microorganisms, which has not been done in any previous research.

Methodology

Materials and Methods

Bacterial growth is one of the factors primarily responsible for the degradation and breakdown of building surfaces. Hence, smart building materials are increasingly being used in the construction of structures nowadays. Tests with Zinc Oxide (ZnO) Nanorods grown on prepared mortar samples using the hydrothermal technique revealed a dense growth of Nanorods deep into the pores of the mortar samples in Scanning Electron Micrographs. These Nanorods exhibit anti-bacterial properties.

The steps adopted are described below:

(i) Firstly, samples of mortar (Figure 13.1) were prepared using cement and sand in a proportion of 1:2 and with a 0.45 water-cement ratio. 50 grams of cement and 100 grams of sand were used and cast using Le-Chatelier's apparatus. They were then cut into three equal pieces with the help of a hacksaw blade.

(ii) For the synthesis of ZnO Nanorods in the prepared samples, the seeding process was initiated. The samples were dried at 120°C for 15 minutes. In 250ml of distilled water, 1mM of Zinc Acetate

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(C4H6O4Zn) was added and stirred for 10 minutes at normal temperature. (Figure 13.2)

(iii) The heated samples were dipped for 1-2 minutes in the solution. Then they were dried at 120°C. This process was repeated 3 times. The samples were then annealed at 250°C for four hours.

After the seeding process, the Nanorod growth process was initiated. For this, equimolar solutions of Zinc Nitrate and Hexamine were prepared, where 10 mM Zinc Nitrate (ZnNO3) was added to 300 ml distilled water, and 10mM Hexamine or Hexamethylenetetramine (C6H12N4) was added to 300 ml of distilled water. Equimolar solutions of Zinc Nitrate and Hexamine were added to the mortar samples and heated for 5 hours in the oven at 250°C. After this, the Nanorods synthesized mortar samples were again heated at 250°C on a hot plate for 1 hour. Similarly, the same process has been followed for the growth of Nanorods for 30 hours.

Figure 13.1. Mortar sample

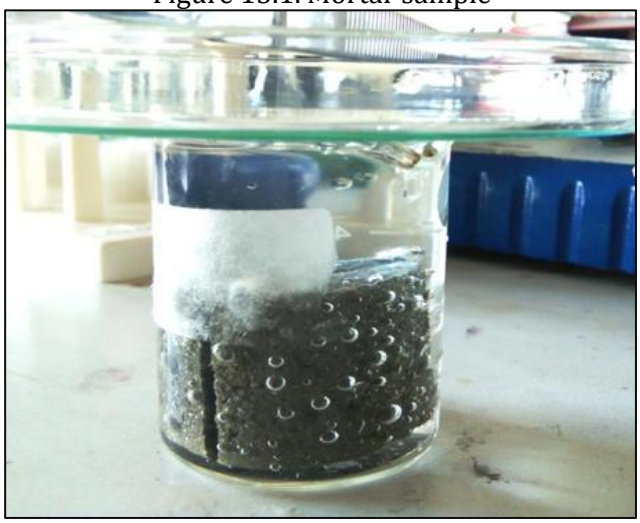

Figure 13.2. Immersed sample in 10 m Zinc Acetate Solution

Anti-Bacterial Test

The antibacterial test is carried out using two types of bacteria: Escherichia coli, commonly known as E.coli, and Streptococcus pneumoniae. E.coli and Streptococcus pneumoniae are the most common bacterial species and have therefore been selected for the experiment.

E.coli is a gram-negative bacteria commonly found in the lower intestine of warm-blooded organisms.

They can cause food poisoning in their hosts and are expelled into the environment within fecal matter. They do not form spores (Rao *et al.,* 2014). Streptococcus pneumoniae is a gram-positive bacteria that resides colonizing in the respiratory tract, sinuses, and nasal cavity. It spreads from person to person via contact with the respiratory tract. It can also cause neonatal infections and is the main cause of pneumonia and meningitis in children and elders. Pneumonia is the most common and serious disease, which shows symptoms such as fever and chills, cough, rapid breathing, difficulty breathing, and chest pain. It affects the lungs. Pneumococcal meningitis is an infection that affects the brain and spinal cord and shows symptoms of a stiff neck, fever, headache, confusion, and photophobia. Sepsis caused by an overwhelming response to infection leads to tissue damage, organ failure, and even death. The symptoms of sepsis are confusion, shortness of breath, elevated heart rate, pain or discomfort, over-perspiration, fever, shivering, or feeling cold (Mendes and Teixeira, 2014).

E.Coli Treatment

Two 200 mL Luria Bertani Broth samples (one for the control sample and one for the Nano-synthesized sample) were prepared. The control and Nanorods-synthesized samples were immersed in the L.B. Broth samples in the beakers and sterilized using an autoclave process. In the incubation process, equal amounts of bacteria were added to the conical flasks after cooling down and then placed in a shaker for 24 to 48 hours (Figures 13.3 and 13.4). Afterward, the optical density (O.D.) values were measured using a colorimeter.

Before treatment, the samples were kept in the Luria Bertani broth samples and E. Coli bacteria was added to both beakers. After incubation for 48 hours, the sample with the Nano-synthesized sample is less turbid than the one with the non-treated sample. (Figure 13.5 and Figure 13.6). Results are shown in Figures 13.7 and 13.8.

Anti-Fungal Test

A series of experiments are performed successively for this test taking Aspergillus Niger fungus and Potato Dextrose Broth (PDB) as the media. A.Niger is the most common species and causes a disease called black mold on certain fruits and vegetables and acts as a common contaminant of

Figure 13.3. Incubation process

Figure 13.4. E.coli treatment

Figure 13.5. After incubation

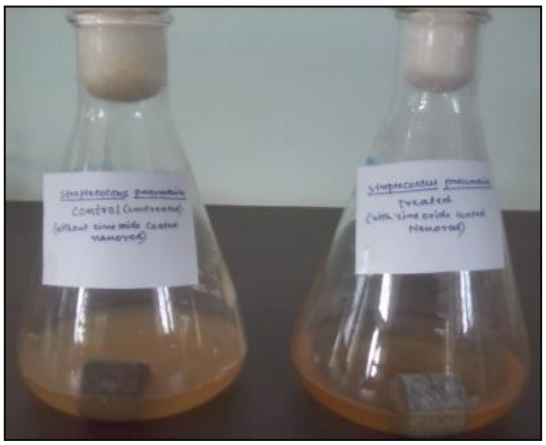

Figure 13.6. Streptococcus treatment

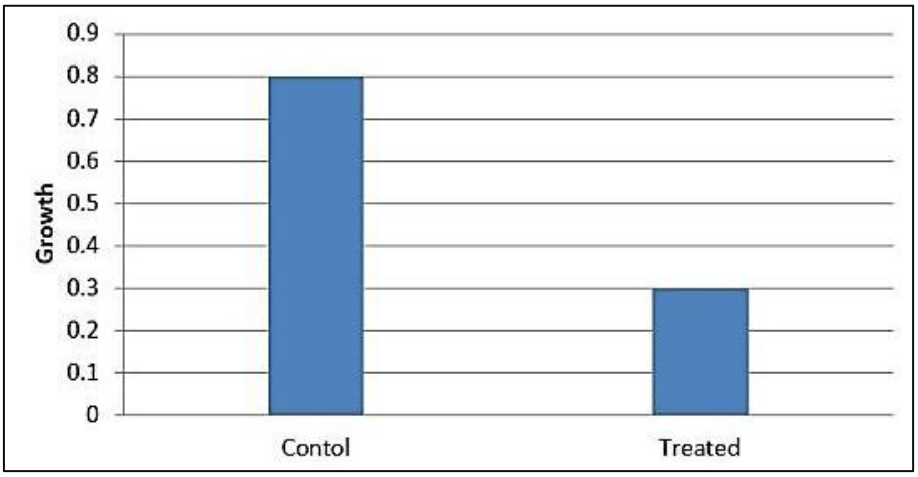

Figure 13.7. O.D. Results for E.Coli

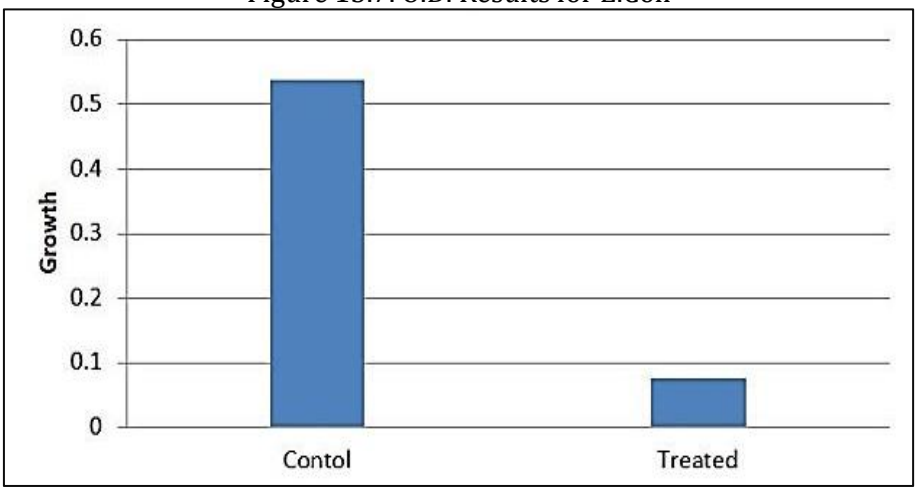

Figure 13.8. O.D. Results for S. pneumoniae

food. This fungus is pre-dominant in the walls of damped buildings and can easily affect the occupants. The ZnO nanorods were synthesized at 10mM concentration for 15 hours initially. The following are the steps taken for performing the test:

(i) 100ml of PDB samples are made in triplets (i.e, 3 treated samples, 3 untreated, and 3 control.) The media is prepped on 9 conical flasks. (Figure 13.9)

(ii) All the samples are autoclaved for 30min @121^{0}C to sterilize the samples.

(iii) The grown fungus on the agar plates is taken into 'laminar air flow' and is then transferred to a flacon tube and 10ml is weighted. Next, 1ml of liquid is poured (containing fungus) into 9 conical flasks 3 treated samples, 3 untreated, and 3 controlled. (Figure 13.10)

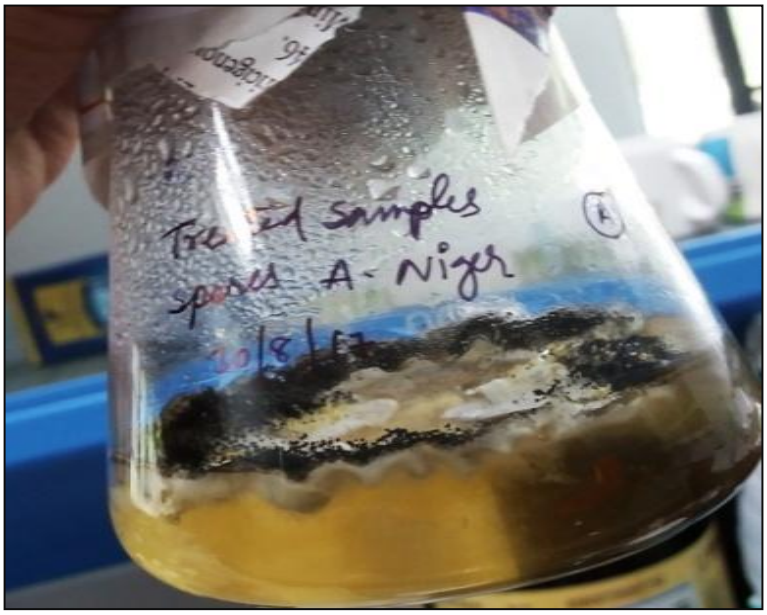

Figure 13.9. Fungal growth over a treated sample in PDB

Figure 13.10. Fungal growth over an untreated sample in PD

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For spore counting, the aspergillus niger mycelia were extracted by using forceps and suspended in 14ml of autoclaved distilled water in a falcon tube. The solution is vertexed, mixed, and filtered using filter paper and 100µl of the filtrate was taken in a sterile Eppendorf tube and 10µl of lactophenol cotton blue was added to the Eppendorf. It is then observed under a hemocytometer by compound microscope. The spores are then mixed in a 2ml Eppendorf tube. From there 213µl solution is poured into each conical flask by using a micropipette.

Satisfactory results were not found on the first attempt. It has been seen that the treated as well as the untreated samples settled to the bottom of the conical flask due to gravity and the since the fungal growth is reliable even in anti-gravitational directional, it remained floating at the top of the conical flask, and the nanorods samples couldn't resist the growth of fungus.

Similarly, the successive experiment was carried out in agar triplets (Figure 13.11 to 13.13). This time the mortar samples were first placed and then sterilized agar solution was poured into them. It was then allowed to get thickened and solidified. 12.7ml of spore solution was prepared for 9 agar plates. The samples are then incubated for three days.

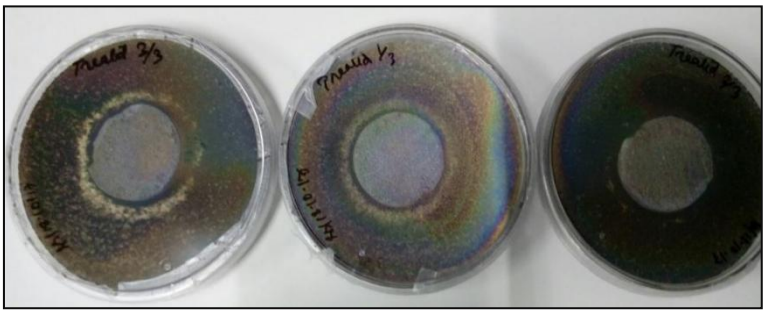

Figure 13.11. After the experiment treated samples in PDA

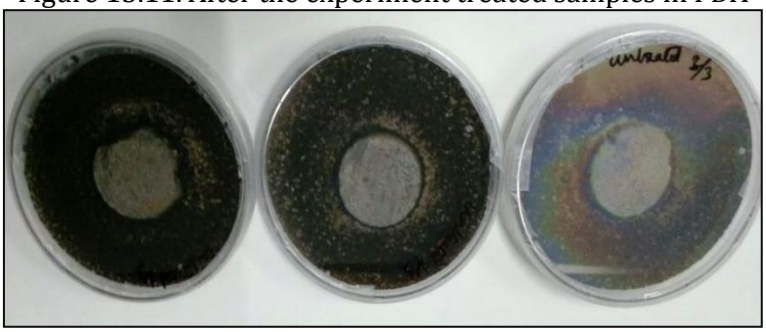

Figure 13.12: After the experiment untreated samples in PDA

Figure 13.13. Controlled samples

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The expected results were not very positive. The Aspergillus niger interaction has repeatedly come negative with a minimum concentration of fungal inoculum in the PDA medium. This one might be a very aggressive strain. Further, fungal experiments were carried out with fungal samples collected from local hospital buildings in North Lakhimpur, Assam. A total of 26 microbial colonies were obtained on PDA plates from three hospital buildings. Samples were collected by taking wall samples from these hospital buildings. The respective colonies were named in numbers. Two colonies were picked up. Experiments based upon their rapid growth pattern, fast sporulation, surface texture, and color. The colonies taken were colony numbers 18 and 26 (Figures 13.14 and 13.15).

Nanorods were grown on mortar samples strictly for 20 hours, changing the solution at 5-hour intervals. The experiment was carried out in triplicates with nano-treated and untreated samples in PDA plates. Since this experiment is focused on fungal growth and resistance, streptomycin antibiotic is used for bacterial treatments. Streptomycin is used mainly to kill any bacterial contamination in the fungal experiments to be performed so that only fungi can survive. It is a substance that exhibits antibiotic activity against gram-positive and gram-negative bacteria. It is water soluble and is effective against bacterial growth.

Figure 13.14. Colony number 18

Figure 13.15. Colony number 26

The spore counting for the respective colonies was done under a compound microscope using a hemocytometer. The spore counting for colony 18 was found to be 7.9×105 spores /ml and spore concentration was taken for 104 spores /ml (Figure 13.16). However, no spores for colony 26 have been

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seen when observed under a microscope (Figure 13.17). Since it has a glistening surface texture the spores are absent. So, an optimal amount of the sample was taken for test experiments. For the antibacterial streptomycin, a stock solution of 100mg/ml was prepared in autoclaved distilled water weighing 100mg of streptomycin in 1ml of water and filter sterilizing the solution. For performing the experiments the required working concentration was taken from the preserved stock solution.

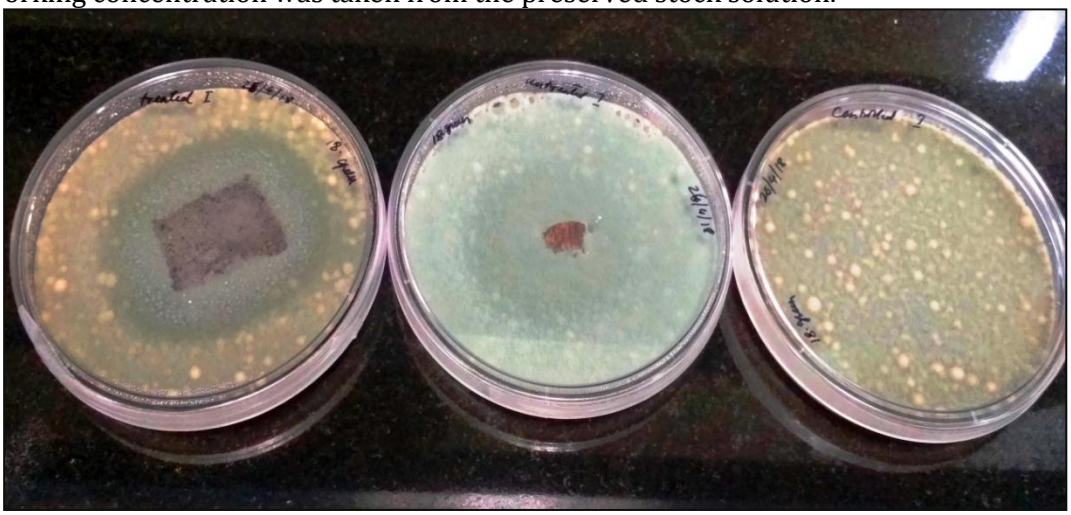

Figure 13.16. Experimental results with colony 18

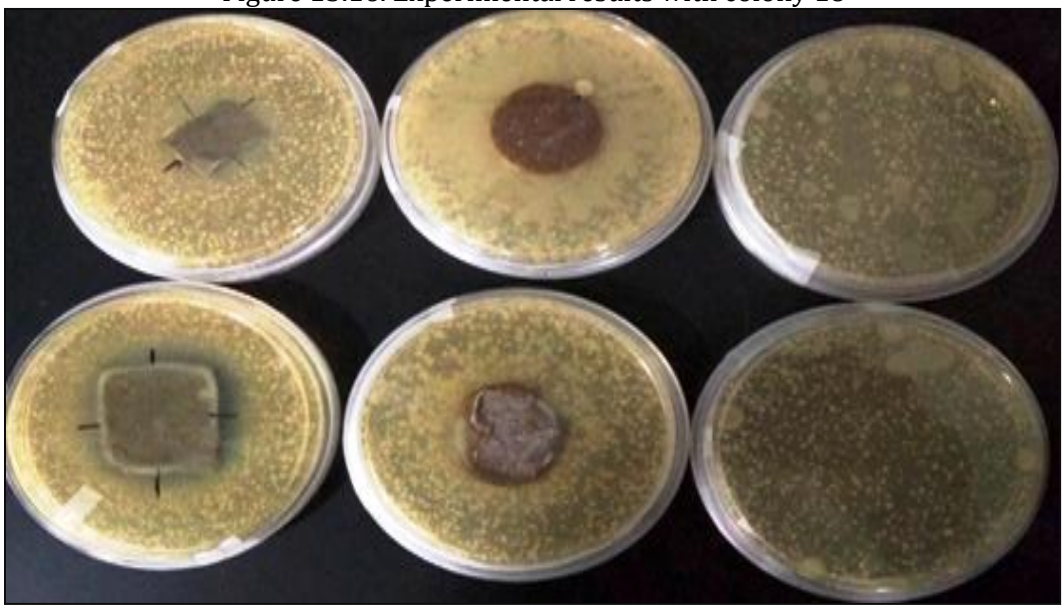

Figure 13.17. Experimental results with colony 26

Interpretation and Analysis

In the bacterial analysis, the growth of E. Coli and S. Pneumoniae has been inhibited by the Zinc Oxide nanorods coated samples. The optical density (OD) value obtained, in the case of E. Coli is 0.299 and 0.077 in the case of S. pneumoniae when observed under wavelength 600nm. The decreasing value of OD indicates lower microbial growth, indicating inhibiting their growth.

It was seen from experiments that out of the two microbial species taken for observation, Colony

number 18 showed a clear zone of teal green around the mortar sample but did not show a clear zone of inhibition. The nanorods have likely to reduce the sporulation around the treated mortar sample. The untreated sample as shown above has been engulfed by the species. The complete zone of minimum sporulation is 5.7 in the circumferential area. Colony number 26 showed a zone of inhibition of area 0.45cm when measured from the edge of the sample.

The samples previously taken for experiments were examined with Scanning Electron Microscopy (SEM) and it was observed that the growth of zinc nanorods was not uniform and was not straight enough to directly act upon the microbial action (Figures 13.18 and 13.19). There might be two possible reasons. One, the time required for growth was not strictly followed as a lapse of half an hour came into intervention. Secondly, the required chemical composition might be quite low for the rods to grow up to certain nanometers, and maybe their resisting capacity was weaker. It was also reported that organic matter was present in the samples in large amounts.

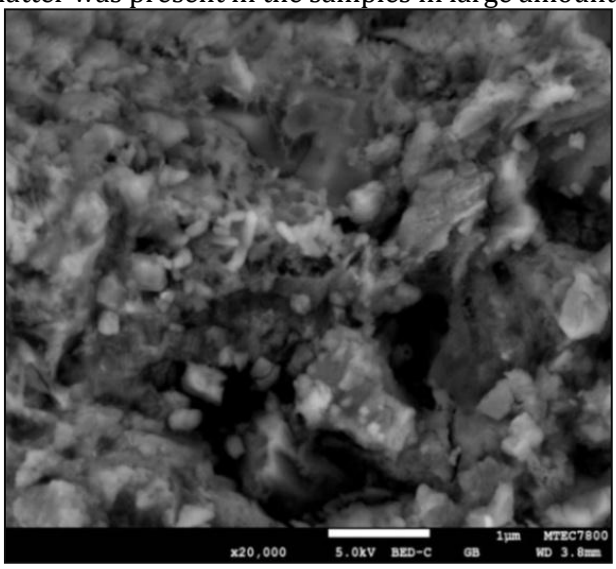

Figure 13.18. SEM image at 20,000x

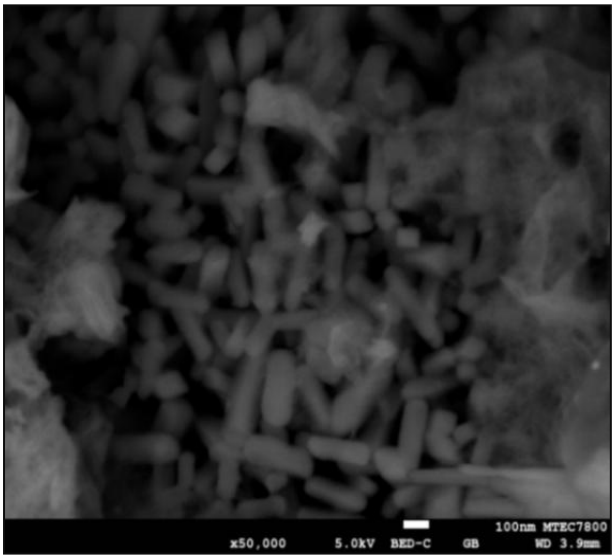

Figure 13.19. Nanorods at 50,000x

Figure 13.20. Mortar samples with reduced samples

The sample sizes and thickness has been reduced lately for the next experiments and this time the nanorods were synthesized at 20mM for 20 hours straight strictly maintaining the time variation and changing the solution every 5-hour interval (Figure 13.20). The rods were grown at 900C with the same chemicals used earlier, Zinc Nitrate and Hexamine solutions.

Limitations

The primary limitation of the work lies in the cost of preparation of the mortar sample with ZnO Nanorods grown on them. In this study, a small volume of samples was prepared. Whereas for large-scale production of the same sample proper research must be done in order to finally arrive at a manufacturing procedure which will also have to be cost-effective. Moreover, Nanotechnology is a comparatively costly area which ultimately leads to an increase in the price of the final product.

Conclusions

The test for anti-bacterial experiments was done with E.coli and Streptococcus pneumoniae. Experiments showed that the mortar samples with ZnO Nanorods possess anti-bacterial properties. This is evident from the decrease in the O.D. value of the bacterial cells in the Luria Bertani (L.B.) medium with time. The ZnO Nanorods embedded samples showed 63% and 85% anti-bacterial activity on E. coli and Streptococcus pneumoniae respectively. Mortar structures embedded with ZnO Nanorods offer promise as a possible anti-bacterial building material.

The test for anti-fungal properties of ZnO Nanorods was performed with fungal species which grow commonly on walls of buildings. The treated mortar samples were tested with Aspergillus Niger initially in triplicates. The test with A. niger was not successful. The test was performed on a conical flask, and it was observed that the mortar samples settled at the bottom of the conical flask due to gravity and the fungal biomass remained afloat at the top of the broth media. From Scanning Electron Microscope (SEM) images it was observed that too much of organic deposits were present on the surface of the mortar samples. The growths of Nanorods were nonuniform and were not in perpendicular alignment.

The fungal species collected from hospital buildings of North Lakhimpur district, Assam, were cultured. Out of the 26 colonies obtained, two colonies tested against the treated mortar samples using an anti-bacterial streptomycin solution to terminate the growth of bacterial species if in case present in the colonies, it was observed that colony number 18 showed a zone of minimum sporulation, insisting that Nanorods have reduced the rate of sporulation near the treated sample area and 26 showed zone of inhibition of area 0.45cm as measured from edges.

Acknowledgment

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Conflict of Interest Statement

The author declares that there is no conflict of interest.

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