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# Antibacterial Effect of Magnesium Oxide Nanoparticle on Water Contaminated with *E.coli.*

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## ABSTRACT

Clean water that is free of toxic chemicals and pathogens is essential to human health. In countries such as India, 80% of the diseases are due to bacterial contamination of drinking water. The World Health Organization recommended that any water intended for drinking should contain fecal and total coliform counts of 0, in any 100 mL sample. When either of these groups of bacteria is encountered in a sample, immediate investigative action should be taken.The antibacterial potential of Magnesium oxide nanoparticle was detected by well diffusion method. Different concentrations of the nanoparticle were analysed by MIC and antibacterial effect. The MIC result reveals that MgO nanoparticle showed maximum inhibition at a concentration of 0.1 g/ml against *E.coli*. On comparing bactericidal activity it was observed that there was a continuous decrease in the number of colonies with the increase in concentration of MgO nanoparticle. Based on MIC results antibacterial activity of MgO nanoparticle was tested on water sample which was contaminated with *E.coli* culture. The growth of bacteria is completely inhibited when higher concentration (0.1 g /ml) of MgO nanoparticle was added in to 100 ml of water sample with 5ml of *E.coli* culture. Therefore from the above studies conducted, it can be inferred that the MgO nanoparticle has significant bactericidal activity, and overall may be more effective in the purification of water sample.

## INTRODUCTION

According to WHO, In the present world drinking water devoid of microorganisms is essential for primary health [1].Today a number of techniques are used for treatment of water i.e. chemical and physical agents.In industrial and municipal applications such as water and waste water plants, the three most widely used methods of liquid sterilization are ozone treatment, chlorine treatment, and UV irradiation[2]. Removal of microorganisms by chemical methods is the last step in the purification of water but they have a number of shortcomings. Such drawbacks include increasing microbiological adaptation to their destructive effects, Halogens such as chlorine (Cl) and bromine (Br) are well known and widely used as antibacterial agents, but the direct use of halogens as bactericides has many problems because of their high toxicity and vapour pressure in pure form[3].There is always a need to develop new apparatus and methods that will help perform these methods and processes more cost effectively than their traditional counterparts [4].

MgO nanoparticles and magnesium (Mg) nanoparticles are very effective biocides against Gram-positive and Gram-negative bacteria (*Escherichia coli* and *Bacillus megaterium*) and bacterial spores

(*Bacillus subtillus)* [5].Magnesium oxide nanoparticles are odorless, non-toxic and appear in a white powder form. They possess high hardness, high purity and a high melting point [6]. Nanomaterials reveal good result than other techniques used in water treatment because of its high surface area (surface/volume ratio). It is suggested that these may be used in future at large scale for water purification [7].

## MATERIAL AND METHODS

Commercial Magnesium oxide nanoparticle procured from IICT (Indian institute of chemical technology). *E.coli* culture, Nutrient broth, Nutrient agar, Eosin methelene blue agar (EMB), petridishes and test tubes.

### Antibacterial activity studies

Antibacterial activity of MgO nanoparticles was evaluated against the Gram-negative bacteria *Escherichia coli* by well diffusion method [8] . A loopfull of *E. coli* was grown overnight in nutrient Broth (NB) at 37$^o$ C for 24 h and after incubation 0.01ml of culture was inoculated in nutrient agar by spread plate technique and wells were made to add different concentrations of MgO nanoparticles (1, 2, 5 10, 20, 50 and 100 mg/ml).

The antibacterial activity of the MgO nanoparticle was evaluated by examining the zone of inhibition of bacterial growth and control was maintained by inoculating only *E.coli* culture without any nanoparticle and kept for incubation. And the antibacterial sensitivity is measured as zone of inhibition in mm in diameter [9].

### Minimum inhibitory concentration (MIC)

Various concentrations (40,60,80 and 100 mg/ml) of MgO nanoparticles were prepared and added into series of test tubes containing 5ml of sterilized nutrient broth and 0.1ml of *E.coli* culture and allowed to grow overnight at 37$^o$ c for 24h. Pathogen alone with nutrient broth was kept as control and they were examined for inhibition studies. MIC was the lowest concentration of the nanoparticle that did not permit any visible growth of bacteria. CFU was calculated by subculturing the above (MIC) serial dilutions after 24 h in nutrient agar petriplates using 0.01ml loop and incubated at 37$^o$ c for 24h [10].

### Effect of MgO nanoparticle on water sample

A loopful of *E. coli* was grown overnight in nutrient broth at 37$^o$C with shaking until late log phase. then 5ml of bacteria was transferred into 100 ml of water sample and then maintained as negative control. To determine the antibacterial activity of MgO nanoparticle in water sample, 0.1g/ml of nanoparticle was inoculated in 100 ml of water sample inoculated with 5 ml of *E.coli* culture [11].To assay for the viability of the bacteria with and without MgO nanoparticle 0.1ml of inoculum was spread from test tube before and after adding MgO nanoparticle onto the surface of nutrient agar plates and incubated at 37$^o$C for 24 h.

## RESULTS AND DISCUSSION

Antibacterial activity of Magnesium oxide nanoparticle against *E.coli* was tested by well diffusion method[8] In preliminary studies the MgO nanoparticle with concentrations of 1,2,5,and 10 mg/ml didn't show any antibacterial effect. And the plates with the concentration of 20 and 50 mg/ml had shown 2 and 10 mm zone of inhibition and it is represented in table 1. Then the concentration of MgO nanoparticle was increased to 0.1 g/ml which had shown 35 mm zone of inhibition on the growth of bacteria as seen in fig 1.

Various concentrations of MgO nanoparticle (40,60,80 and 100 mg/ml) were examined for inhibition studies to determine the minimum inhibitory concentration (MIC). No. of colonies was counted by subculturing the MIC serial dilutions and its effect on bacterial growth is shown in Figure 2 and table 2. In both negative control (only *E.coli* culture with out any nanoparticle) and 40mg/ml, mat growth was observed on agar plates. No. of colonies appeared in 60, 80 and 100mg/ml are 50, 10 and 2 respectively. On comparing it was observed that there was a continuous decrease in the number of colonies with the increase in concentration of MgO nanoparticle. The MIC of MgO nanoparticle from fig.1 appeared to be 100mg/ml against *E.coli.*[12].

MgO NPs have the advantage of being prepared from the readily available and economical precursors and solvents. So they have considerable potential as a solid bactericidal material under simple conditions. They have ability to prevent biofilm formation of common pathogens. MgO nanoparticles show biocidal activity by damaging the cellwall of bacteria or is commonly attributed to the production of reactive oxygen species (ROS). These nanoparticles have already proven their antibacterial effect against different organisms[13] .

MgO nanoparticles exhibit bacteriocidal activity which is highly dependent on the particle size and concentration. They are effective against both Gram positive and Gram negative organisms. These nanoparticles are harmless to mammalian cells and the environment. MgO NPs can be used in combination with other antibacterial substances which can be valuable[14]. In the area of water purification, MgO nanoparticles give promising results by removal of both chemical and biological substances[13].

**Figure 1: E.coli + MgO (100mg/ml) - Inhibition Observed**

**Figure 2: MIC Results of MgO nanoparticle against *E.coli***

**Figure 3: Effect of MgO nanoparticle on water sample plate 1 ( MgO + *E.coli* ) shows no growth & Plate 2 ( without MgO + E.coli )cultivated in NA media shows Mat growth**

**Table 1: Antibacterial activity of MgO nanoparticles in different concentrations Against *E.coli in mg/ml***

| Concentration Of MgO nanoparticle (mg/ml) | 1 | 2 | 5 | 10 | 20 | 50 | 100 |
|---|---|---|---|---|---|---|---|
| Zone of Inhibition (mm) in diameter | - | - | - | - | 2.0 | 10 | 35 |

**Table 2: MIC results of MgO nanoparticle against E.coli determined by viable count method**

| Concentration Of MgO nanoparticle (mg/ml) | Control (*E.coli* ) without nanoparticle | 40mg/ml | 60mg/ml | 80mg/ml | 100mg/ml |
|---|---|---|---|---|---|
| No. of colonies | Mat growth | Mat growth | 50 | 10 | 2 |

## CONCLUSION

Effect of MgO nanoparticle on water sample results revealed that the growth of bacteria is completely inhibited when higher concentration (0.1 g/ml) of MgO nanoparticle was added in to 100 ml of water sample with 5ml of E.coli culture. And full mat growth was observed in the plate without MgO nanoparticle which was maintained as control as seen in fig 3. Metallic sheen color colonies were observed when the cuture from negative control was inoculated in to EMB agar media. Nanomaterials reveal good result than other techniques used in water treatment because of its high surface area (surface/volume ratio)[15] .MgO nanoparticle can be used in future at large scale for water purification [11]. Research is underway to use advance nanotechnology in water purification for safe drinking. It is expected to play a crucial role in water purification [16] .

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