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APPLICATION OF ZnO NANOPARTICLES IN DISINFECTION OF HOSPITAL WASTEWATER

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Abstract - *This work presents the disinfection property of ZnO NPs. The antimicrobial property of ZnO NPs, factors affecting ZnO, disinfection mechanisms and potential application of ZnO on hospital wastewater disinfection is discussed. The wastewater samples are collected from ETP of KLES Dr. Prabhakar Kore Hospital and Medical Research Center. The chemical and bacteriological parameters are tested using the standard techniques with varying dosages of ZnO. The bacteriological analysis is carried by multiple tube fermentation test to find the total coliform count, whose results are expressed as most probable number (MPN) index. The results indicate that the chemical and bacteriological parameters tested post disinfection with ZnO NPs are considerably reduced indicating ZnO NPs is a promising disinfectant. The chemical analysis of wastewater indicates considerable reduction in all the parameters as well as their values within the limits of CPCB Standards. Further, bacteriological tests indicated that ZnO NPs have significant bactericidal effect on Escherichia coli and Klebsilla species bacteria.*

Key Words: **Zinc oxide Nanoparticles, Escherichia coli, Klebsilla species, Hospital wastewater, antibacterial activity.**

1. INTRODUCTION

Water is essential factor for all forms of life. Around 71% of earth surface is covered by water. As the population is increasing, demand for water for various uses has been increased. Over a period of years, continued population growth and rapid industrialization have resulted in water scarcity. On the other hand, discharging greater concentration of untreated wastewater from various sources into water bodies is main reason for water pollution. Water resources are largely polluted by uncontrolled release untreated waste in to the water bodies. It is challenging to give resolution for the treatment and safe discharge of wastewater because it is concerned with environmental and other considerations [1].

2. WASTEWATER

Wastewater is an unnatural affected water due to human use. Wastewater may be organic or inorganic in nature depending on the source and characteristic of liquid waste in it. Organic wastewater includes the waste from hospitals, dairies, food factories, abattoirs. Inorganic wastewater includes water from industries, pesticide manufacturing units etc. Hospital wastewater encloses pathogens and hazardous compounds [2]. properties of wastewater are not constant and vary depending upon hours of the day, seasons of the year. Also, the physical and chemical characteristics of wastewater fluctuate depending on the source [3].

2.1 Sources of Hospital Wastewater

Hospitals are major consumer of water and generates significant amount of wastewater which comprises of pathogens, harmful bacteria, virus, metabolites. Main sources include wastewater from hospital wards, laboratories, water closet, bathrooms, outpatient's department, floor cleanings, kitchen waste and care units. Fig. 1 shows the sources of wastewater generated in hospital which enters the effluent treatment plant through sewer line.

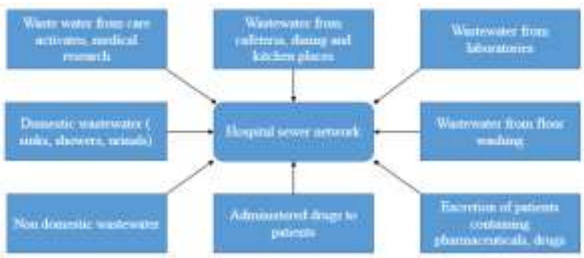

Fig -1: Flowchart of sources of wastewater generation in hospital

2.2 Pollutants in Hospital Wastewater

Pollutants in the form of heavy metals like mercury (Hg), silver(Ag), zinc(Zn) are found in hospital wastewater. Hospitals wastewater comprises chemicals, pharmaceuticals such as anesthetics, antibiotics, analgesic and anti-inflammatories in huge quantity. These end in wastewater through human excretion like urine and faece. Microbial contaminants mount on the sewer system by demanding high amount application of antibiotics. These chemicals cause antibiotic resistant to bacteria. Fig. 2 demonstrations different contaminants present in hospital wastewater [4].

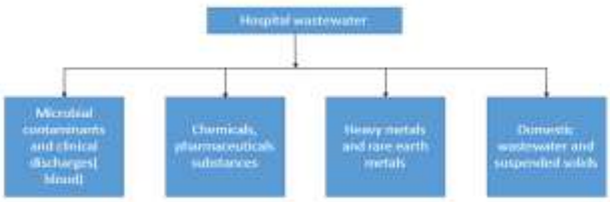

Fig -2: Pollutants in hospital wastewater

3. DISINFECTION

Disinfection is a method used to eliminate or immobilize pathogenic microorganisms. It is the last block of wastewater recovery process to shield ecosystem safety and social health. Disinfectants used must not only destroy microorganisms but it should also have a residual effect. Disinfection can be accomplished by means of physical (UV light, electronic radiation, heat, gamma rays) or chemical (chlorine, ozone, halogens, phenols) disinfectants. However, these methods have their limitations.

3.1 Zinc Oxide Nanoparticles

Zinc oxide nanoparticles (ZnO NPs) is considered as best disinfectant as it shows strong activity even in small magnitudes. ZnO is considered as good antibacterial agent due to its stability and is considered as harmless substance for humans and marine life. Zinc oxide have received significant consideration due to their matchless antibacterial activities toward several microorganisms that are generally existed in environment. Studies proven that the antibacterial activity of ZnO is very efficient and also said that the activity increases with increase in its concentration. ZnO NPs become toxic for some microorganisms by making them antiviral and antifungal agents.

3.2 Antimicrobial Activity of ZnO

The process by which microbial growth is depressed or destroyed is called antimicrobial activity. Among the various metal oxides ZnO has been found to have potent antimicrobial properties. Since ZnO NPs show strong UV absorption capacity and transparency to visible light, nanoparticles show very good antibacterial activities on a broad spectrum of bacteria. They are also used in paints, sunscreen lotions and coatings [5].

4. OBJECTIVES OF THE STUDY

The present study aims to examine the efficiency of ZnO nanomaterials towards disinfection of Hospital Wastewater so that quality of wastewater is enhanced to be able to discharged into water bodies or for farming lands. The objectives are:

1. To identify the suitability of ZnO NPs for hospital wastewater disinfection.
2. To obtain suitable ZnO NPs dosage for hospital wastewater.
3. Comparison of efficiency of ZnO NPs as disinfectant against chlorine used as disinfectant.

5. MATERIALS AND METHODS

Experiments were conducted in the Environmental Engineering Laboratory, Department of Civil Engineering, KLE Dr. M.S. Sheshgiri College of Engineering and Technology.

Samples were collected from effluent treatment plant of KLES Dr. Prabhakar Kore Hospital & M.R.C. The wastewater samples were collected in 1-liter glass bottles and brought to laboratory. Main chemical characteristics of the wastewater such as pH, Electrical Conductivity, Turbidity, DO, Biochemical Oxygen Demand, Chemical Oxygen Demand, and Total Dissolved Solids, Total Suspended Solid are determined before and after disinfection.

Most Probable Number is a technique used for the estimation of feasible concentration of microorganisms present in a sample by means of replicate liquid broth growth in ten fold dilutions. This technique is applied *to* confirm whether the water is safe or not in terms of microorganisms present in a sample. If water contains a small number of fecal coliform bacteria, then it shows that water may contains no disease causing bacteria, whereas the presence of huge numbers of fecal coliform bacteria designates that the water may contain disease causing organisms which makes the water hazardous for drinking [9].

Bacterial Culture is also done by preparing a plate which provides large surface for isolation and observation of colonies. Fermenting colonies that grew from positive tubes from MPN test were taken and were sub-cultured to MacConkey agar plate. The sample from fermentation tube was taken in sterile loop, streaking of sample was done on MacConkey agar plate. The bacterial culture was incubated at 37^0C for 24 hours. After incubation check for progress of microorganisms was done. The progression of microorganisms is characterized by a cloudy haze in the agar plate [10].

5.1 Nano Particle Size Analyzer

The SZ-100 measures ultrafine particles down to the nanometer range in extremely low as well as in very high sample concentrations. This recently advanced analyzer is used in the bio and nano technology (e.g. nano ceramics and nano metals). The antimicrobial activity of ZnO increases by decreasing the particle size. Smaller particle size has more mechanical damage on the cell membrane [11]. The average size of ZnO nanopaticle was found to be 845.8 nm as shown in Fig. 4.

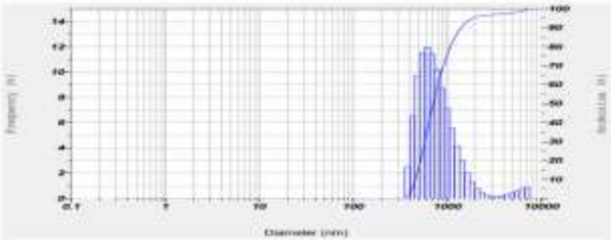

Fig -4: ZnO particle size

6. RESULTS AND DISCUSSIONS

The performance evaluation of ZnO nanoparticles on wastewater

The performance evaluation of ZnO nanoparticles on clarifier output wastewater for various parameters with their values are tabulated for each parameter and figures drawn for the results.

6.1 Electrical Conductivity

Electrical conductivity (EC) is a measurement ability of solution to carry an electrical current. In the present study electrical conductivity of wastewater collected from clarifier varied from 498.7 to 505.05 µs/cm. Electrical Conductivity of a zinc oxide treated wastewater ranges from 484.2 to 498.7 µs/cm. The values of Electrical Conductivity are given in Table 1. The average percentage reduction of electrical conductivity is 2.35%. ZnO may have agglomerated after being led into the wastewater by influencing the solution parameters such as ionic strength, dissolved organic carbon and thus causing decrease in conductivity .

Table -1: Electrical Conductivity at different stages of treatment

Day	Clarified wastewater (mg/L)	Treated wastewater with ZnO (mg/L)	% Reduction
Day 1	505.6	492.8	2.53
Day 2	498.7	484.2	2.91
Day 3	501.2	493.2	1.60
Average			**2.35**

6.2 pH

Samples with pH less than 7 are considered to be acidic and solutions with a pH greater than 7 are said to be basic. From this study, it was observed that pH of clarified wastewater was found in the range of 7.73 to 8.01. At the first collected clarified wastewater was treated with ZnO nanoparticle and allowed for 45 minutes for disinfection. pH of zinc oxide disinfected water was found to be 6.99 to 7.23. Fig. 5 shows pH values of treated and untreated wastewater. The mean reduction in pH value is found to be 9.75%. Similar results are reported by Shamsa et al. [12] who studied the effect of variation of pH by varying the exposure time. The slight decrease in pH of ZnO treated wastewater is due to varying the exposure time from 0 to 4 hours required for the degradation of pollutants.

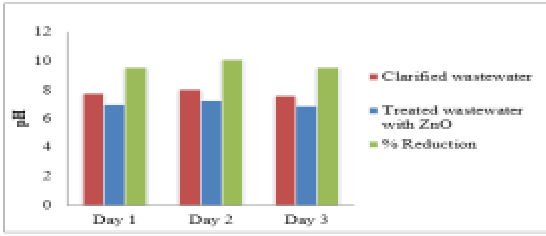

Fig -5: pH variations

6.3 Turbidity

In this present work, the turbidity of wastewater collected from clarifier was observed to be in the range of 18.6 to 20.6 NTU. After the disinfection of wastewater with ZnO, turbidity was reduced to range from 4 to 5.6 NTU. Table 2 shows the observed turbidity values of the study. The percentage decrease in turbidity due to ZnO disinfection is 76.07%.

Table -2: Turbidity at different stages of treatment

Day	Clarified wastewater (mg/L)	Treated wastewater with ZnO (mg/L)	% Reduction
Day 1	20.6	5.6	72.82
Day 2	18.2	4	78.02
Day 3	18.70	4.23	77.38
Average			**76.07**

5.4 DO

Dissolved oxygen is defined as the amount of oxygen dissolved in sample. DO is an amount of oxygen available to active aquatic organisms. Fig. 6 shows the Dissolved Oxygen at different stages of treatment. It was found that ZnO disinfection assisted in increasing the DO content of wastewater by 20.88%. The Dissolved oxygen increased with increase in exposure time with ZnO NPs up to 3 hours. [12].

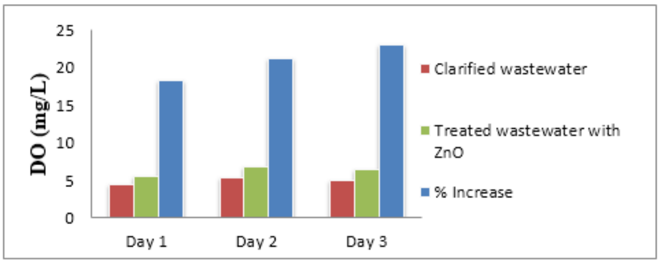

Fig -6: DO. Variations

6.5 Biochemical Oxygen Demand (BOD_3)

Biochemical Oxygen Demand is an amount of oxygen required by microorganisms to oxidize the organic matter present in one liter of wastewater. BOD of wastewater is used to designate the short time impact on the oxygen levels of the accepting water bodies. Present work shows, BOD_3 of clarified wastewater ranged from 30 to 35 mg/L, which after ZnO treatment is reduced considerably in the range of 12 to 13.8 mg/L. The obtained BOD_3 values are shown in Table 3. The percentage reduction in 3-days BOD after disinfection is 54.31%.

Liu et al. [14] reported that reduction level of ZnO NPs toxicity to the biological populations was solely due to soluble Zn^{2+} generated upon ZnO ENP dissolution.

Table -3: BOD_3 at different stages of treatment

Day	Clarified wastewater (mg/L)	Treated wastewater with ZnO (mg/L)	% Reduction
Day 1	35	17	51.43
Day 2	30	12	60
Day 3	33	16	51.51
Average			**54.31**

6.6 Chemical Oxygen Demand (COD)

Chemical oxygen demand is the amount of oxygen consumed in a wastewater during the decomposition of organic matter and in the oxidation of inorganic matter such as ammonia, nitrate. Present work showed the COD of wastewater collected from clarifier is in the range of 125 to 132 mg/L. The treatment considerably removed the COD of wastewater up to the range 90 to 95 mg/L. The reduction in COD due to ZnO effect is 28.92%. The values of COD obtained during the work are shown in Fig. 7.

There is a sharp decrease in COD reading when treated with ZnO whereas TiO_2 shows slight increase after 2 hours of treatment. An increase in exposure time results in decrease in COD [30]. ZnO agglomeration also have led to a drastic decrease in the COD. Notably the study reported wastewater exposed to high concentrations of ZnO NPs resulted in high COD removal [11].

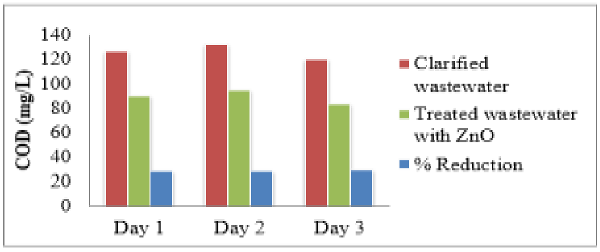

Fig -7: Chemical oxygen demand variations

6.7 Total Solids (TS)

Total solids are a combination of total suspended solids and dissolved solids in a given sample. It is correspondingly used to calculate a dry weight of sludge. The wastewater from clarified unit had considerable amount of total solids which varied from 300 to 352 mg/L. However, treated wastewater reduced the total solids from 172 to 200 mg/L. Total solids decreased with increase in exposure time of ZnO nanoparticles [12]. The values of total solids obtained during the work are shown in Table 4. The data shows that the percentage reduction in total solids is 43.71%.

Table -4: Total Solids at different stages of treatment

Day	Clarified wastewater (mg/L)	Treated wastewater with ZnO (mg/L)	% Reduction
Day 1	352	200	43.18
Day 2	300	172.5	42.5
Day 3	330	180	45.45
Average			**43.71**

6.8 Total dissolved solids (TDS)

Total dissolved solids defined as the combined dissolved content of organic and inorganic substances present in suspended form. The total dissolved solids of clarified wastewater changes from 180 to 200 mg/L. The treatment reduced the total dissolve solids from 130 to 136 mg/L. Fig. 8 shows the observed total dissolved solids values during study. ZnO disinfection is effective in reducing the total dissolve solids by 30.97%. Total dissolved solids decreased with increase in exposure time [12]. There was 23% drastic decrease in total dissolved solids for the first hour.

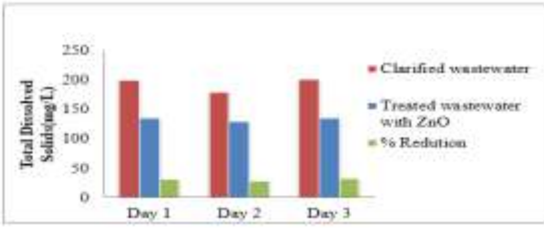

Fig -8: Total dissolved solids variations

6.9 Total suspended solids (TSS)

Total suspended solids are defined as the quantity of material suspended in a known volume of wastewater that is filterable in a filter. High TSS levels are associated with water pollution. It is important to consider the good type of filter to avoid TSS. Study observed that total suspended solids of clarified wastewater was found in the range of 120 to 150 mg/L. Total suspended solids decreased with increase in exposure time [12]. Treatment reduced the total suspended solids to 45-65 mg/L as shown in Table 5. The percentage reduction in TSS is 59.94%.

Table -5: Total Suspended Solids at different stages of treatment

Day	Clarified wastewater (mg/L)	Treated wastewater with ZnO (mg/L)	% Reduction
Day 1	150	65	56.67
Day 2	120	50	58.33
Day 3	128	45	64.84
Average			**59.94**

6.10 Most Probable Number

The reduction ratios of bacteria and growth curves of bacteria with and without the presence of different ZnO nanoparticle are investigated to estimate the disinfection activities of the ZnO nanoparticle. All the disinfection tests performed in the dark conditions. Bacterial count without disinfection was found to be 18 and 23.05 at 9am and 2pm respectively. Study observed that coliform count before the treatment of wastewater was found to be 25.5. With ZnO concentration of 10 g/L, 20 g/L, 30 g/L, 40 g/L, 42 g/L, 44 g/L 46g/L showed reduction in bacterial count 17.01, 13.84, 9.27, 6.85, 3.98,1.98 and zero respectively. As the concentration increased, the coliform count kept on decreasing. The reduction rate of the bacteria estimated is shown in Fig. 9 and Fig. 10.

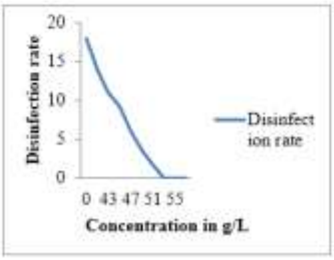

Fig. 9. Disinfection rate for different concentrations at 9am

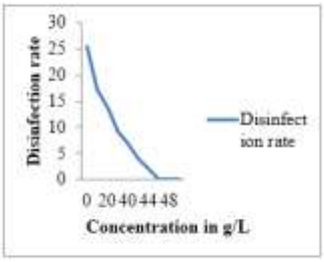

Fig. 10. Disinfection rate for different ZnO concentrations against at 2pm

6.11 Bacterial Culture

Fermenting colonies that grew from tubes positive for acid and gas production when sub-cultured to MacConkey agar, are identified as Escherichia coli (E. coli), Klebsiella. Growth of microorganisms are characterized by a cloudy haze in the media. Clarified wastewater yielded the colonies such as Escherichia coli and Kleblsiella species as shown in Fig. 11. Whereas the treated wastewater with ZnO concentration of 45g/L showed no growth of microorganisms proving the disinfection of ZnO is effective as shown in Fig.12. The culture test was done at Department of Microbiology, KLES Dr. Prabhakar Kore Hospital and M.R.C, Belagavi. Emelita et al. [5] reported the 90% death of E. coli bacteria at ZnO concentration of 20g/L for water. Adams et al. [7] obtained 90% growth reduction of B. subtilis and 48% growth reduction of E. coli at 10 ppm and 1000ppm ZnO respectively. According to Fatemeh Elmi et al. [8] ZnO NPs (20g/L) proved to have activities against E. coli and S. epidermidis for municipal wastewater.

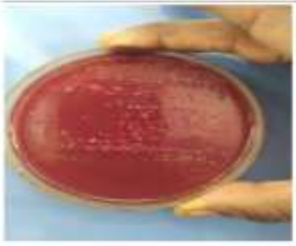

Fig. 11. Fermenting colonies of E.coli and Klebsiella sps on MacConkey agar

Fig. 12. Fermenting colonies with no yield on MacConkey agar

7. CONCLUSIONS

The following conclusions are drawn from the present experimental investigation:

1. It is found that the various parameters of hospital wastewater tested post disinfection with ZnO NPs are considerably reduced indicating ZnO NPs is a promising disinfectant.
2. The reduction in Electrical conductivity, pH, turbidity, BOD_3, COD, total solids, total dissolved solids and total suspended solids due to ZnO NPs disinfection is respectively 2.35 %, 9.75 %, 76.07%, 54.31 %, 28.92 %, 43.71 %, 30.97% and 59.94 %. However, the DO content is increased by 20.88 % after disinfection.
3. From the chemical analysis of wastewater, it is observed that all the parameters are within the limits of CPCB Standards.
4. The BOD_3, COD and TSS removal efficiency of ZnO NPs is good.
5. The coliform count that was 25.5 decreased with increasing ZnO concentration. The respective values of bacterial count with ZnO concentration of 10 g/L, 20 g/L, 30 g/L, 40 g/L, 42 g/L, 44 g/L 46g/L are 17.01, 13.84, 9.27, 6.85, 3.98,1.98 and zero. This indicates ZnO disinfection removed coliform completely with 100% efficiency.

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