Synthesis of MgO Nanoparticles and Its Enhanced Broad Spectrum Antimicrobial Activity Against Selected Bacteria and Fungus

Dr. Saba A. Mahdy
Applied Science Department, University of Technology/Baghdad.
Email:saba.hadi@yahoo.com

Received on:11/6/2015 & Accepted on:12/11/2015

ABSTRACT

MgO nanoparticles were prepared by using sol-gel technique, the MgO nanoparticle was pure and chemically homogeneous, the determination of physio-chemical characterization of MgO nanoparticles showed standard techniques.

MgO nanoparticles were used in study their ability to inhibit the growth of E. coli, pseudomonas aeruginosa, S. aureus, Candida albicans were found that MIC was studied by MTT assay in presence of different MgO nanoparticles concentrations for 24 hour, the results showed that 40 µg/ml of MgO nanoparticles was effective for E.coli and pseudomonas aeruginosa, 20 µg/ml and S. aureus while Candida albicans inhibited with 30 µg/ml.

The shelf-life of MgO determined by agar plate methods MgO nanoparticles showed highly effective against selective pathogens by preventing the growth of gram positive and negative bacteria to more than 40 days and 21 days for fungi and this could significantly enhance microbial safety and extend the shelf-life of foods.

Keywords: MgO nanoparticales, antimicrobial activity, shelf life activity

التأثير التثبيطي لمركب أوكسيد الماغنيسيوم النانوي في مجال المايكروبي

الخلاصة

تحضير مسحوق اوكسيد المغنسيوم الناوي تم بتقنية الصل- جل وقد امتازت المادة المحضرة بنقاوتها وتجانسها الكميائي وقد اعتمدت الفحوصات الفيزيوكميائية لتحديد مميزات المركب المحضر بهذه الطريقة كما تمت دراسة التاثير المثبط للنمو المايكروبي لمركب اوكسيد المغنسيوم النانوي ضد عدد من المسببات المايكروبية لتلف الاغذية والتسمم الغذائي باستخدام طريقة MTT وحددالتركيز المثبط الادنى بوجود تراكيز مختلفة من اوكسيد المغنسيوم النانوي واظهرت النتائج تثبيط بكتريا *E.coli* عند تركيز *40 µg/ml* و *S. aureus, Pseudomonas aeruginosa* بتركيز *20 µg/ml* و *Candida albicans* بتركيز 30 µg/ml كما تمت دراسة تاثير اوكسيد المغنسيوم النانوي على النمو المايكروبي مختبريا بطريقة الانتشار في الوسط الصلب عند استخدامها كمادة حافضة لزيادة فترة صلاحية المواد الغذائية وقد اوضحت النتائج قدرة اوكسيد المغنسيوم النانوي على منع النمو البكتيري لمدة تزيد عن 40 يوم ومنع النمو الفطري لمدة 21 يوم وقد سلطت هذه النتائج الضوء على امكانية استخدامه لتعزيز الامن الغذائي وتمديد فترة صلاحية الاغذية المعلبة.

INTRODUCTION

There is a mounting need and ever growing trend in the food industry to eliminate use of artificial ingredients, additives and antimicrobial agents that demands for minimally-processed and fresher foods, as well as for ready-to-eat food or the request for functional foods and nutraceuticals (Robertson *et al.,* 2004). In recent years, consumer demands for fresh,

minimally processed safe food, in addition to concern over the use of chemical preservatives in foods and the application of bio-preservatives has generated substantial interest in these compounds (Deegan *et al.,* 2006). The empirical use of microorganisms and/or their natural products for the preservation of foods has been a common practice down the history of mankind (Ross *et al.,* 1999).The elimination of food spoilage by the pathogenic organisms thus has become the focus of many researchers.

In order to effectively use antimicrobials in a food product, it can be delivered along with nanoparticles in a dose potent enough to kill the microorganism, but mild enough so as not to cause harm to the consumer, as of that there is a need to find new effective antimicrobials to ensure food safety and extend shelf-life. The MgO nanoparticles as one of metal nanoparticles are used a widespread in the consumer products which could be attributed to their potent antimicrobial activity against a wide range of pathogenic microorganisms (Fayaz *et al.,* 2010) In the current work MgO synthesize nanoparticles. The antimicrobial activity of MgO nanoparticles were also found out.

Sawai *et al.,* (2003) evaluated the antibacterial activity several metal oxide and found that MgO, CaO and ZnO exhibited strong antibacterial activities against *Staphylococcus aureus, E. coli* and fungi in culture media as well (Sawai and Yoshikawa 2004).

Metal oxide nanoparticles with sizes less than 100 nm promote significantly antimicrobial activities due to their features (e.g. small particle size, large surface area) that micro or macro-sized particles do not possess Nel et al. (2006).

Huang *et al.,* (2005) reported that increasing of antibacterial activity with reduce in particle size of the oxide ceramic. Stoimenov *et al.,* (2002) prepared nano-scale MgO powder, which exhibited bactericidal effect against bacterial vegetative cells and spores. However, there are few records on the antimicrobial activity of MgO nanoparticles in the literature bordering the use of MgO nanoparticles to kill or inhibit the growth of foodborne pathogens

The objective of this study was to investigate the antibacterial activities of MgO nanoparticles and its synergistic effect against foodborne pathogens (*E.coli*, *Pseudomonas aeruginosa*, *S. aureus* and *Candida albicans*) to expected knowledge to develop new antimicrobial strategies in food products technology.

Material and Methods

Chemicals

Muller Hinton media, Nutrient agar, Potato Dextrose Agar, Mg $(OH)_2$ and 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide) MTT dye was obtained from Hi-Media, mumbai, India.

Organisms

The microorganisms *Pseudomonas aeruginosa E.coli, Candida albicans, Staphylococcus aureus* isolated in microbiology labs of biotechnology division / university of technology-Baghdad and grown in Muller Hinton broth media aerobically at 37°C for 16–18 h. A 10 µl loop transfer was performed and the strains were grown at 37 °C for another 24 h to achieve a population of 1×10^9 CFU. Serial tenfold dilution were performed in sterile 0.1% peptone water and inoculated into Muller Hinton broth media so as to achieve target populations of 1×10^4 CFU and 1×10^8 CFU, respectively, all antimicrobial experiments were conducted in duplicate.

Synthesis and optimization of MgO nanoparticles

An MgO sample was prepared by refluxing 5.72 gm of Mg $(OH)_2$ with 4.5mL of water and 50 mL of ethanol. The hydrolysis of the magnesium alkoxide was accomplished by adding to the 0.60 mL of HCl before refluxing. The homogenous solution was maintained in reflux until the

gel is formed. Before characterization the sample was dried at 100°C for one hour and then calcined in air at 900°C for 13 hours. Thus the structure of the sample was optimized and found to be face centered cubic (Ansari et al, 2007). The reaction follows the equation given below:

$$Mg(OH)_4 + H_2O + C_2H_5OH \longrightarrow MgO + 3C_2H_5OH$$

Physio-chemical characterization of MgO nanoparticles

Surface plasmon resonance of the samples was studied with an UV-Vis Spectrophotometer at a resolution of 1 nm from 250 nm to 800 nm. Characterization of morphology of the nanoparticles was carried out using a SEM imaging and for determining the shape of nanoparticles XRD study was preformed the size, the infrared (FTIR) spectra of the crude extracts were obtained between 400 to 4000 cm-1 on a FT-IR Spectrophotometer. The symmetric, asymmetric stretching and the stretching frequencies were studied to determine the presence of functional groups in prepared sample (AbdulrahmaN, 2013; Rasheed, 2015).

Antimicrobial activity of MgO nanoparticles and MIC value by MTT (3- (4, 5 – Dimethylthiazol-2-yl)-2, 5-Diphenyltetrazolium Bromide) assay

The proliferation rates of microbial cells after treatment with MgO nanoparticles were determined by the colorimetric 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay To measure the activity of living cell by assessing the activity of the bacterial dehydrogenase enzymes. 95µl of the freshly prepared Muller Hinton Broth and the different concentration of MgO nanoparticles (10µg/ml, 20µg/ml to 100µg/ml) were added and the plates were kept in incubation at 37ºC for 24 hours. 5mg of MTT was weighed and dissolved in 1 ml of Double-distilled water and 10 µl of this preparation is added to each well (96-well Plate flat bottom) and kept for 4 hours incubation. The contents were collected and centrifuged at 8000 rpm for 15 minutes, and the pellets were dissolved with 100µl of Dimethyl sulphoxide. Then the contents were transferred to the appropriate well and read at 570 nm in the ELISA reader.(Zarai *et al.,* 2011) The percentage of viable cells were calculated using the following formula.

$$\% \text{ Viable Cells} = \frac{\text{Control O.D} - \text{Test O.D}}{\text{Control O.D}} \times 100$$

Determination the shelf life of MgO nanoparticles

MgO nanoparticles synthesized were tested using agar diffusion method for its potential antimicrobial activity and determine the shelf life of MgO nanoparticles against selective foodborn pathogens

Nutrient agar prepared and mixed with 10% of pasteurized apple juice was poured into petri dishes 24 hour old cultures were swabbed on the wells (10mm diameter) cut by a cork borer. Different concentration of MgO nanoparticles were loaded in to the respective wells. The plates were then incubated at 37 º C for 24 hrs. The inhibition diameter was measured at regular intervals up to 30 days.

To determine the anti-fungal activity, Potato Dextrose Agar (PDA) was poured after mixed with 10% of pasteurized apple juice into the petri dishes and 24 hour old cultures were swabbed on the wells (10 mm diameter) that were cut with a cork borer. Different concentrations of the metal nanoparticles and the protein were loaded in to the wells and one well was used as a control. The plates were then incubated at 37 ºC for 24 hrs. The diameter of the zone of inhibition was measured (Pissuwan et al., 2007).

RESULTS

Changing the color of the reaction to white precipitate was revealed the synthesis of MgO nanoparticles and UV-Visible spectrum was showed (Fig.1) an apparent broadening beak at 450 nm indicating the aggregation of MgO nanoparticles. X-ray diffraction refluxed nanocrystalline MgO in (Fig.2). The crystalline diameter estimated from Sherrer equation was approximately 27.38 nm. The information on the particle size was obtained from the full Width at Half Maximum (FWHM) of the diffracted beam using Debye–Sherrer formula.

$$D = \frac{0.9\lambda}{\beta \cos\theta}$$

Where D is the mean grain size, λ is the X-ray wavelength, β is the FWHM of diffraction peak and θ is the diffraction angle, these results compatible with SEM image of MgO sample (Fig.3), the image shows that all the particles are spherical in shape on a nanometer scale.

The FTIR spectrum of MgO nanoparticles in (Fig4) shows certain common absorption bands in the range of 3904-3628 cm^{-1} is a characteristic of hydroxyl υ (O-H) and υ (N-H) vibrational frequency which are interchangeable, the peak with maximum at 3429.2 cm^{-1} is assigned to the -OH stretch of water. The peak at 1439.4 cm^{-1} is due to the H2O bending. Peaks above 3600 cm^{-1} (Sharp intense beak) are assigned to three -OH groups on the surface of the material. The partly resolved broad peak just above 1000 cm^{-1} confirms the alkoxy group on the surface. Jianping *et al.,* (2007) concluded that the hydroxyl group was active in the reduction of metal ion.

Inhibition efficacy of MgO nanoparticles was tested against different pathogens by MTT assay which used to measure cytotoxicity (loss of viable cells) or cytostatic of potential medicinal agents and toxic materials (Xie et al. 2011).

The results of MIC showed that MgO nanoparticles inhibited growth *S. aureus* at a concentration of 20 µg/ml and 40 µg/ml on (*E.coli*), 20 µg/ml on *Pseudomonas aeruginosa*) and 30 µg/ml *Candida albicans*. (Table1).

The action of metal oxides against bacteria appears to be really close to the surface of the particle (Sawai *et al.,* 1995). Contact between MgO particles and bacterial cell is also an important factor in their activity (Sawai *et al.,* 2000). This is Because of major microbicidal effects of MgO alkalinity surface against bacteria. When the particle comes into contact with a bacterial cell at neutral or slightly acid pH, the active oxygen formed would increase the antimicrobial activity of the powder. A difference in the antimicrobial activity of MgO, CaO and ZnO comes from active oxygen species generated by the powder in solution (Sawai *et al.,* 2005).

Indeed, every bacterium responds unevenly to oxidative stress due to differences in the permeability of cell membranes. Some microbial strains succumb to damage of cell walls by O_2^- and greater sensitivity to H_2O_2, as is the case for *E. coli* (Zhang *et al.,* 2007)

A problem of the use of different metal oxides in a liquid medium, such as liquefied foods, as of use can change the visual appearance of the medium. Indeed, when the oxide powder, slightly soluble in solution, it is visible to the eye and tends to precipitate. A solution to this problem is using nanoparticle powder. A nanoparticle is a body having a size of about 100 nm or less (Heinlaan *et al.,* 2008).

The shelf-life studies experiments to measure the effectiveness of the MgO nanoparticles on different pathogens. The agar diffusion method was employed and the results are presented in (Table 2) which shows significant effect of the MgO nanoparticles on *S. aureus* with 30 mm inhibition zone, 20 mm on Gram negative bacteria *E.coli* and 22 mm *Pseudomonas aeruginosa* and 21 mm for *Candida albicans*.

No change was observed with regard to the inhibition zone during the period of 15 days for MgO nanoparticles). It was also observed (MgO nanoparticles) that no change took place for more

than 21 days except with *Candida albicans* for which the inhibition zone completely disappeared after 21 days. Shi et al. (2010) investigated the toxicity of many metal oxide nanoparticles against some yeasts and fungi strains. The results showed no effect to MgO nanoparticles on *C. albicans* and this may be to their cell wall and membrane structure and similar results demonstrated by Khan et al (2014).

The results of shelf-life studies with MgO nanoparticles as preservative agent revealed the ability of the MgO nanoparticles to damages cell walls (bactericidal and fungicidal activity) is directly related to the increase of active oxygen generated on the surface of particles of MgO nanoparticle, reducing the size of the particle. Moreover, the nanoparticle of oxides in solution enhances the possibility of interaction between the particle and the bacterial cell due to surface energy and its charge (zhang et al., 2007)

CONCLUSION

Which were used to synthesize MgO nanoparticles. The physio-chemical characterization of MgO nanoparticles showed that the particles which prepared from smaller. The MgO nanoparticles displayed good activity against all the indicator pathogens including both bacteria and fungus showing it potential broad spectrum antimicrobial activity and can be used as preservative which gave new hope to use MgO nanoparticles nanoparticles as a new generation of food preservative.

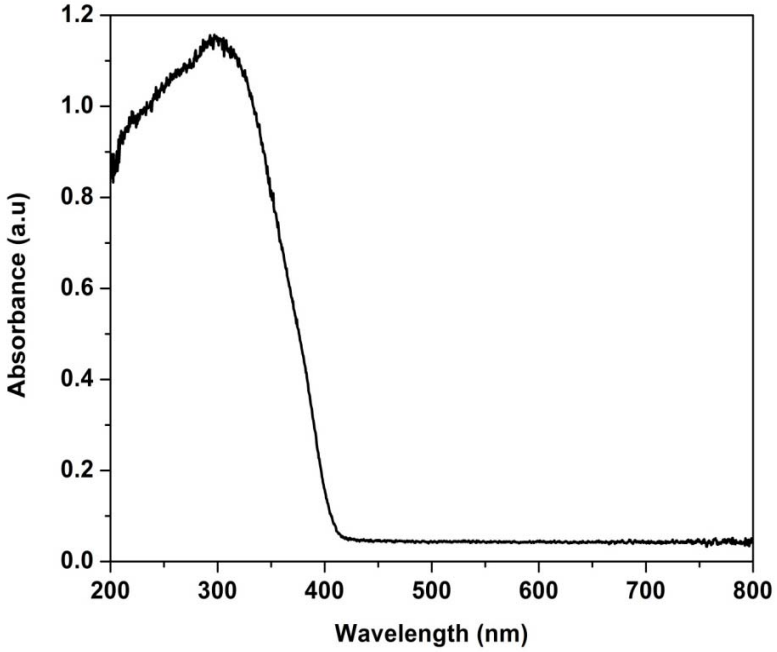
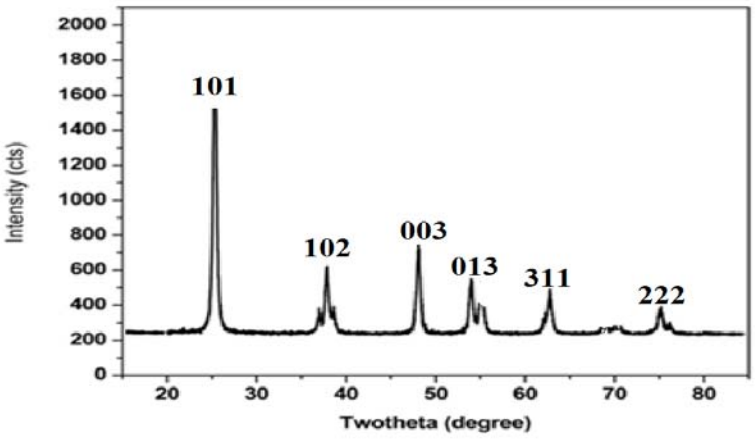
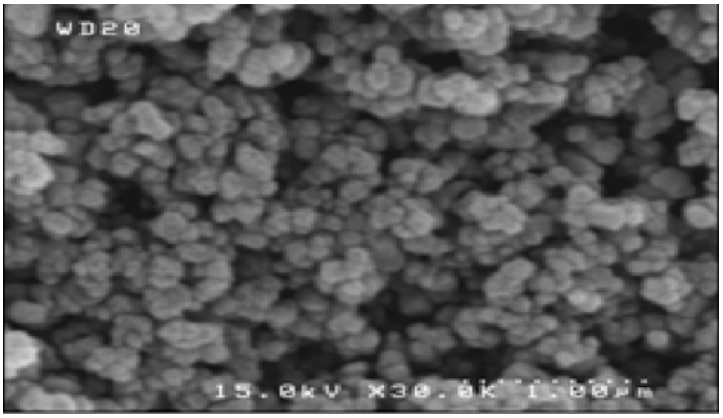


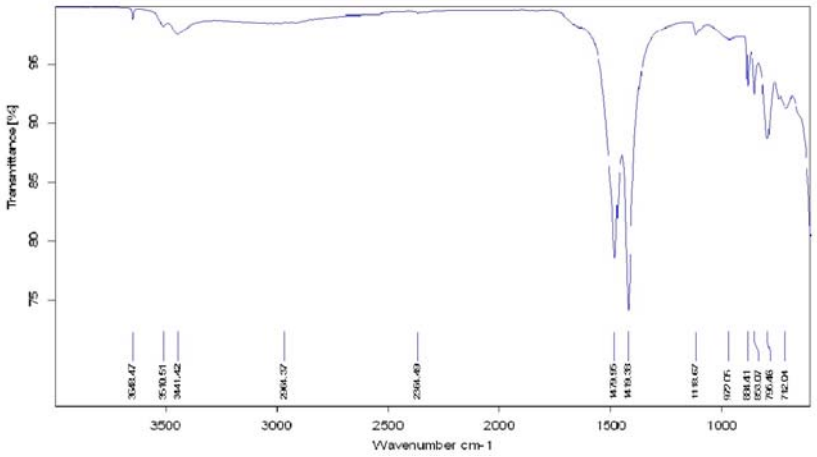
Figure.(1). UV–vis spectrum for MgO nanoparticles.



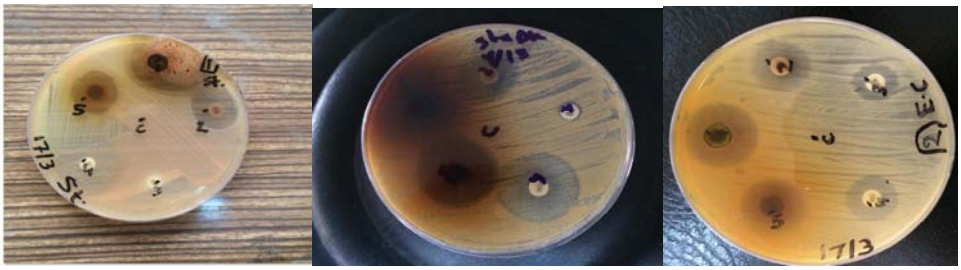
Figure(2). X-ray diffraction analysis of MgO samples.



Figure(3). SEM images of MgO nanoparticles.



Figure(4). FTIR spectrum of MgO nanoparticles.



A B C

Figure(5). Effect of MgO nanoparticles by agar diffusion method on: (A)Staph. Aureus(B) Pseudomonas(C)E.coli

Table.(1). MIC of MgO nanoparticles on selective pathogens by MTT assy

Concentrations of MgO NanoParticles.	E.coli	pseud. aeruginosa	Staph.aureus	Candida albicans
10	3.054	35.143	23.523	13.487
20	20.591	47.483	51.222	31.868
30	31.626	63.376	97.760	49.151
40	53.399	71.362	98.371	58.442
50	67.783	79.566	98.971	69.530
60	71.034	80.553	99.002	76.024
70	91.340	89.832	99.409	83.716
80	97.281	90.642	99.788	92.500
90	99.113	92.152	99.878	97.546
100	99.704	98.519	99.898	99.80

Table(2). Shown the effect of MgO nanoparticles on selective pathogens.

No	Concentrations of MgO Nanoparticles	E.coli mm	pseud. aeruginosa mm	Staph.aureus mm	Candida albicans mm
1	0.1	20	22	30	21
2	0.2	19	21	28	19
3	0.3	18.5	19.5	25	18
4	0.4	18	19	22	16.5
5	0.5	17	17	20	16

REFERENCES

[1].Abdulrahman K.Ali.(2013).One-Step Synthesis of Copper Oxide Nanoparticles Using Pulsed Laser Ablation in Water: Influence of the Laser Wavelengths on Optical Properties.*Eng.And Tech. Journal,Vol 31.Part (B), No.7.*

[2].Deegan L, P. D Cotter, C. Hill, and Ross R.P. (2006). Bacteriocins; biological tools for biopreservation and shelf life extension. Int. Diary J 16:1058-1071.

[3].Fayaz A, Balaji K, Girilal M, Ruchi Y, Kalaichelvan PT, Venketesan R. (2010). Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics: a study against gram-positive and gram-negative bacteria. Nanomedicine; 6:103–9.

[4].Heinlaan M, Ivask A, Blinova I. (2008). Toxicity of nanosized and bulk ZnO, CuO and TiO2 to bacteria Vibrio fischeri and crustaceans Daphnia magna and Thamnocephalus platyurus. Chemospher. 71: 1308–1316.

[5].Huang L, Li D, Lin Y, Evans DG, Duan, X. (2005). Influence of nano-MgO particle size on bactericidal action against.Bacillus subtilis var. niger. Chin Sci Bull 50(6):514–519.
[6].Jianping Xie,; Jim Yang Lee and Daniel I.C. Wang. (2007). Synthesis of Single-Crystalline Gold Nanoplates in Aqueous Solutions through Biomineralization by Serum Albumin Protein, J. Phys. Chem. C. 111 (28), 10226– 10232.
[7].Khan MF, Hameedullah M, Ansari AH, et al.(2014) Flowershaped ZnO nanoparticles synthesized by a novel approach at near-room temperatures with antibacterial and antifungal properties. Int J Nanomedicine. 9: 853- 64.
[8].Pissuwan, D., Valenzuela, S.M., Miller, C.M. and Cortie, M.B.(2007). A golden bullet? Selective targeting of Toxoplasma gondii tachyzoites using antibodyfunctionalized gold nanorods, Nano Letters 7: 3808– 3812.
[9].Qusay J. Rasheed. (2015). Synthesis and Optimization of Nisin-Silver Nanoparticles at Different Conditions. *Eng. And Tech. Journal,Vol 33.Part (A), No*.2.
[10].Robertson, A., Tirado, C., Lobstein, T., Jermini, M., Knai, C., Jensen, J.H., Ferro- Luzzi, A., James,W.P.T. (Eds.). (2004). Food and health in Europe: a new basis for action. WHO Regional Publications, European Series, vol. 96. Geneva.
[11].Ross, P. R.; Galvin, M.; McAuliffe, O.; Morgan, S. M.; Ryan, M. P.; Twomey, D. P.; Meaney, W. J. and Hill, C. (1999). Developing applications for Lactococcal bacteriocins. Antonie van Leeuwenhoek, 76, 337-346.
[12].Sawai J,Igarashi H, Hashimoto A.(1995). Evaluation of Growth Inhibitory Effect of Ceramics Powder Slurry on Bacteria by Conductance Method. Journal of Chemical Engineering of Japan.28:288-293.
[13].Sawai J, Kojima H, Igarashi H. (2000). Antibacterial characteristics of magnesium oxide powder. World Journal of Microbiology and Biotechnology. 16:187-194.
[14].Sawai J. (2003). Quantitative evaluation of antibacterial activities of metallic oxide powders (ZnO, MgO and CaO) by conductimetric assay. J Microbiol Methods 54:177–182.
[15].Sawai J, Yoshikawa T. (2004). Quantitative evaluation of antifungal activity of metallic oxide powders (MgO, CaO and ZnO) by an indirect conductimetric assay. J Appl Microbiol. 96:803–809.
[16].Sawai J, Himizu K and Yamamoto O. (2005). Kinetics of bacterial death by heated dolomite powder slurry. Soil Biology & Biochemistry. 37: 1484-1489.
[17].Lu-E Shi, Liangying Xing, Baochao Hou, Hongjuan Ge, Xiaofeng Guo and Zhenxing Tang(2010). Inorganic nano mental oxides used as anti-microorganism agents for pathogen control. current research technology and education topic in applied microbiology and microbial biotechnology.
[18].Stoimenov P. K, Klinger R. L, Marchin G. L, Klabunde K. J (2002). Metal oxide nanoparticles as bactericidal agents. Langmuir 18:6679–6686.
[19].Tony Jin and Yiping He.(2011). Antibacterial activities of magnesium oxide (MgO) nanoparticles against foodborne pathogens. J Nanopart Res 13:6877–6885. DOI 10.1007/s11051-011-0595-5.
[20].Xie Y, He Y, Irwin P, Jin T, Shi X (2011) Antibacterial activity and mechanism of zinc oxide nanoparticles against Campylobacter jejuni. Appl Environ Microbiol 77(7): 2325–2331.
[21].Zarai Z,Kadri A,Ben Chobba I.(2011). The in-vitro evaluation of antibacterial,antifungal and cytotoxic properties of Marrubium vulgare L. essential oil grown in Tunisia.Lipids Hlth Dis,10,161.
[22].Zhang L, Jiang Y, Ding Y. (2007). Investigation into the antibacterial behaviour of suspensions of ZnO nanoparticles (ZnOnanofluids). Journal of Nanoparticle Research. 93: 479–489.