Photocatalytic Killing of *Staphylococcus Aureaus* Bacteria by Using Naked and Coupled TiO_2 in Aqueous Solution.

Fatima Al-Zahra'a G.Gassim
College of Science for Girls
Babylon University

Abstract:-

Titanium dioxide (TiO_2) used as photocatalyst to sterilize an aqueous solution containing *staphylococcus aureus* bacteria under near UV-visible irradiation source in presence of oxygen. This research aimed to purify and disinfect water from *S.aureus* bacteria by using naked and coupled TiO_2 instead of using disinfectants that are used extensively in hospitals and other health care setting.

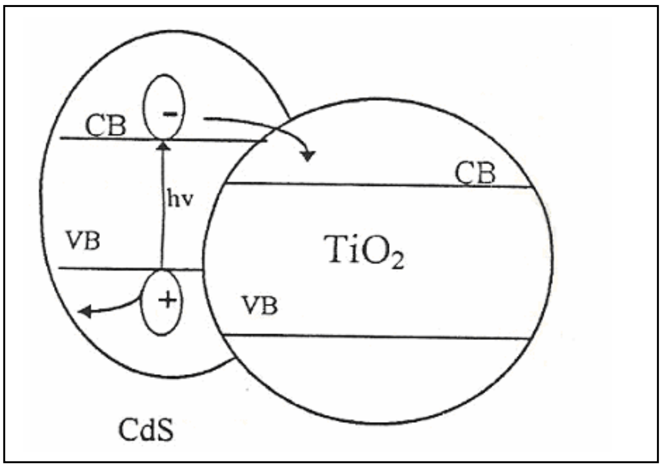
Primary experiments has been carried out to determined the optimum conditions which give high killing efficiency of *S.aureus* bacteria in aqueous solution. Survival ratio and rate constant of photocatalytic *S.aureus* photokilling were also determined under optimum conditions. These results indicate that 0.33 mg/ml TiO_2 concentration for photocatalytic S.aureus photokilling process. The results also indicate that the rate of photocatalytic killing was increased when TiO_2 was coupled with cadmium sulphate (CdS) instead of naked TiO_2 as photocatalyst, while the kinetic studies of this system reveal that the highest killing efficiency of *S.aureus* in aqueous solution was obtained in presence of CdS as photocatalyst. According to the experimental results the general photocatalytic reaction mechanism for this bacteria was suggested.

Introduction:-

Disinfectants are antimicrobial agents that are used in health care setting for a variety of topical and hard surface applications[(1)]. Compared to the widely used disinfectants applications of photocatalyst based untimicrobial disinfectant technologies are still in developmental stage[(2)].

Wast water from hospital ,food factories and contaminated sites. sometimes , virus and organic compound must be sterilized .One of the typical sterilization by illumination of UV light with the wave length of 254nm[(3)],which provides a high rate of sterilization at room temperature. Alternately, it is well known that the TiO_2 in anatase form is capable of oxidizing and decomposing various kinds compound[(4)].

TiO_2 is stable to photocorrosion and nontoxic and inexpensive. The band gap energy of TiO_2 is 3.2 ev and therefore absorbs in near UV- light ((λ<387 nm.)[(5)].Other semiconductors piratical such as CdS has band gap energy about 2.5 ev[(6)]and absorbs larger fractions of the solar spectrum than TiO_2 (about 496nm)but CdS does not reveal good results in water detoxification process. This is because CdS under go rapid photocorrosion. Coupled TiO_2/CdS semiconductors have more stability than CdS and absorbs larger wave length than naked TiO_2[(7)] . Coupled TiO_2/CdS semiconductors can be explained according to the following scheme.



Figure(1):- Illumination of coupled TiO_2/CdS .
Promotion of CdS an electron from V_B to the C_B and injected to the V_B of TiO_2 semiconductor[8]

.

The aim of this research is to study the effect of UV-visible light on the bacterial activity in presence of naked and coupled TiO_2/CdS semiconductors and using photocatalytic reactions for disinfecting water instead of chemical as antibiological.

2. Experimental:

2-1 Chemicals:-

Titanium dioxide was purchased from Degussa P-25 (mostly anatase BET 55m^2g^{-1}).Nutrient agar was supplied from HIMDIA. CdS ,ethanol and acetone were supplied by BDH.

2-2 Instruments :-

Low pressure mercury lamp (LPML) type OSRAM (160 W) was used as a source of irradiation after removing it's glass cover .Figure(2) shows the spectrum of irradiation lamp. photocell (35 cm^3) with quartiz window (2 cm^2) was used as reaction vessel . The temperature was adjusted by using regulator circulating thermostat (Desaga Frigostat) .Oxygen gas container was connected with flow meter (Rato) to control the rate of gas passing on the surface of aqueous solution. A magnetic stirrer (Abovolt) was used to keep the solution in homogenous suspension. TiO_2 partical was removed by using centrifuge (Hettich).

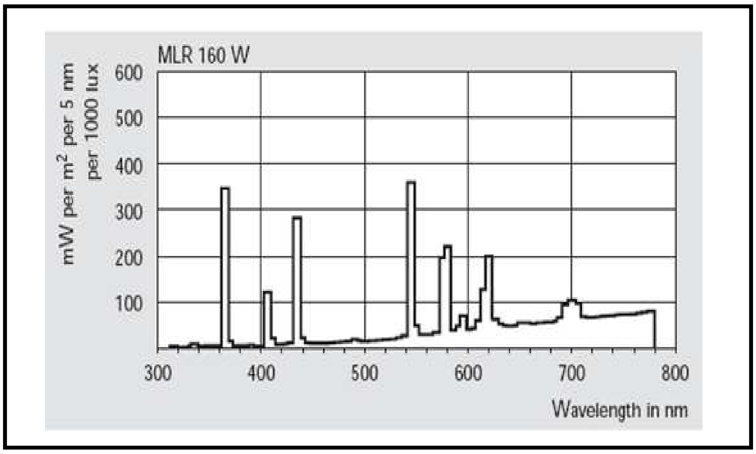


Figure (2):- The spectrum of irradiation OSRAM lamp.

2-3 Preparation of coupled TiO_2/CdS semiconductor system.

The coupled TiO_2/CdS semiconductors was prepared by precipitating CdS salt in a solution containing the same amount of dispersed TiO_2 and the product was washed with acetone, water and finally with absolute ethanol and dried at 110 ^{0}C.The sample containing CdS was dried at 400 C^o for one hour(9).

2-3 Photocatalysis Experiments :-

In all photocatalytic experiments , 30 cm^3 of aqueous solution of *S.aureus* cell suspensions was added to a known weight of TiO_2 particles in photocell quartz window and suspended by using a magmatic stirrer .The oxygen was passed on the surface of aqueous suspension at the rate 10 cm^3/min. The temperature was controlled at 25^{0}C by using circulating thermostat. The suspension was irradiated for 40 min.

Other experiments have been done by adding 0.33 mg/ml CdS and 0.33 mg/ml Coupled TiO_2/CdS semiconductors to *S.aureus* aqueous solution under dark and light conditions.

At each 10 min. samples of irradiated mixture were withdrawn by using a syringe with a long pliable needle .These were centrifuged at 1000 rpm for 10 min. to separate the semiconductors partials and the supernal liquid. For all experiments, 0.5cm^{3}of the suspension was immediately added to 20cm^3 nutrient agar media in a Petridish (9 cm-diameter) with trireplicates per each treatments. Petridishs were kept in the dark at 30 ^{0}C for 24 h. Colony forming units (CFU$_S$) of *S.aureus* were controlled.

The incident light intensity was measured by using Parcker and Hautchard method[10].This method consists of irradiated potassium ferrioxalate actinometry $K_3Fe(C_2O_4)_2.3H_2O$ for 3 nim. after passing nitrogen gas for 15 nim. at 25^{0}C. The average light intensity is 9.72 x 10^{-7} Einstein L^{-1}S^{-1}.

Results and Discussion :-

3-1 Primary experiments :-

Figure (3) shows that in the dark experiments the number of bacterial cell was increased with time due to once the bacteria have acclimatized to their new environment (such as aqueous solution) these bacteria will take part in the synthesis of the enzymes needed to utilize the available bacterial cell in which they start regular division by binary fission[11] .The second dark experiment shows that the presence of naked and coupled TiO_2 and in presence of CdS semiconductors does not effect on the bacterial activity because these semiconductors are biologically and chemically inert[12].Bacterial killing can be obtained by irradiation *S.aureaus* aqueous solution because a small amount of UV light emitted form irradiation lamp can be inert to the cell membrane of bacteria and damage it[13]. Figure

(3) shows that the best photocatalytic killing can be irradiated TiO_2 in presence of oxygen.

3-2.TiO_2 Concentration Optimization:-

As expected for photocatalytic reaction ,the rate of photocatalytic photokilling of *S.aureaus* has been increased with the amount of catalyst due to the complete absorption of incident light potentially absorbable by TiO_2[14] as shown in figure (4) .

Figure(5) shows that the maximum value of rate has been obtained at 0.33mg/ml TiO_2 concentration which is equal to 2.9x 10^{-2} CFUs/sec so that this concentration will be chosen to study the effect of CdS and coupled TiO_2/CdS semiconductors on the killing efficiency of *S.aureaus* in aqueous solution .Figure (4) shows that the killing efficiency was decreased by using TiO_2 more than 0.33 mg/ml of catalyst concentration because TiO_2 particles form inner filter which absorbs high portion of incident light as well as scatter part of light[15].

3-3 The effect of CdS and coupled TiO_2/CdS semiconductors :-

A series of experiments has been done to increase the photocatalytic killing efficiency of *S.aureaus* in aqueous solution .Figure (6) shows that the complete killing was achieved after 40 min of illumination in presence of 0.33mg/ml CdS concentration and all bacterial cells were killed .This observation can be explained that band gap of CdS equal (2.5ev)[6] it means that this semiconductor absorbs visible light and generate electrons and holes. Figure(7) shows the efficiency of photokilling of *S.aureaus* was increased by using 0.33 mg/ml coupled CdS/TiO_2 semiconductor compared with the same concentration of naked TiO_2 because CdS absorbs visible light and generate excited electrons and holes .Electrons

are injected in conduction band of TiO_2 and oxidizing radical species are produced[16]. These oxidizing agents were exhibited strong bacterial activity.

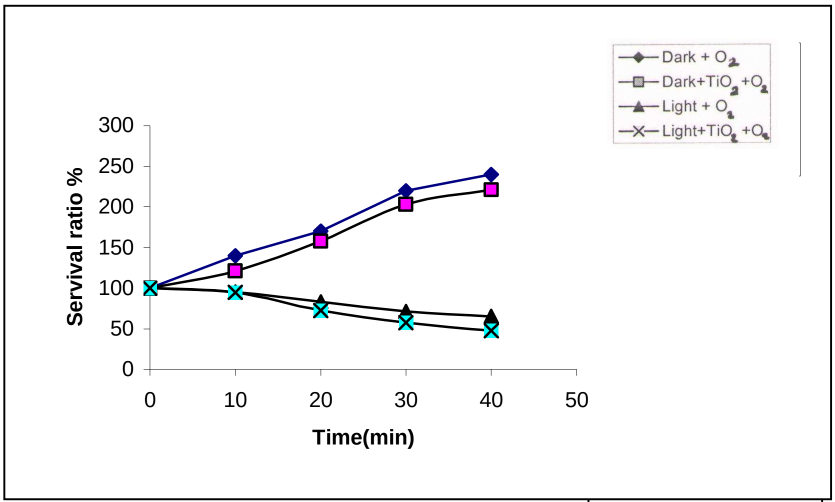
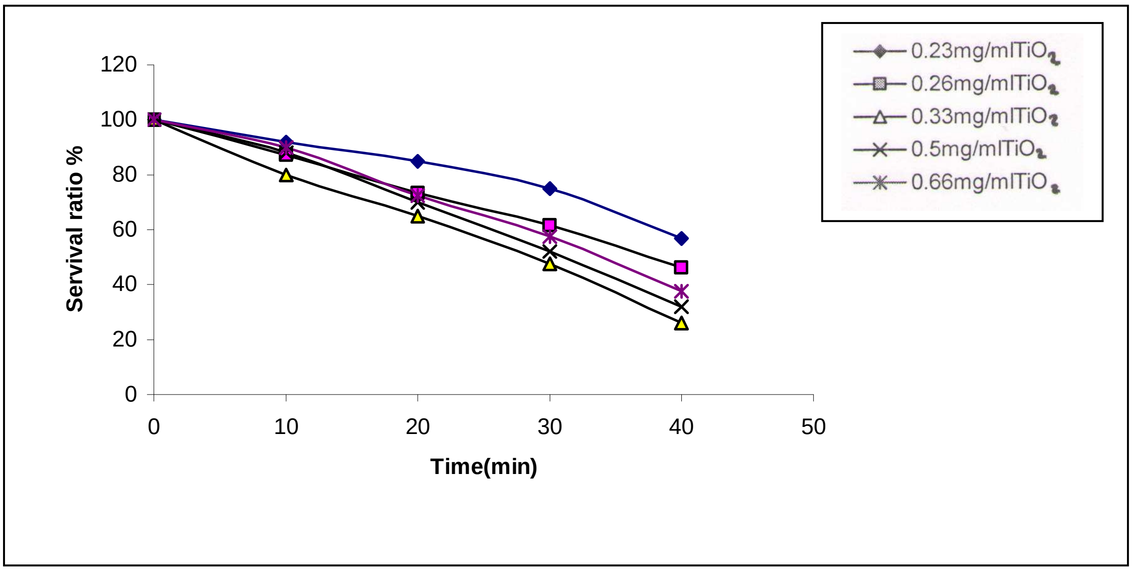
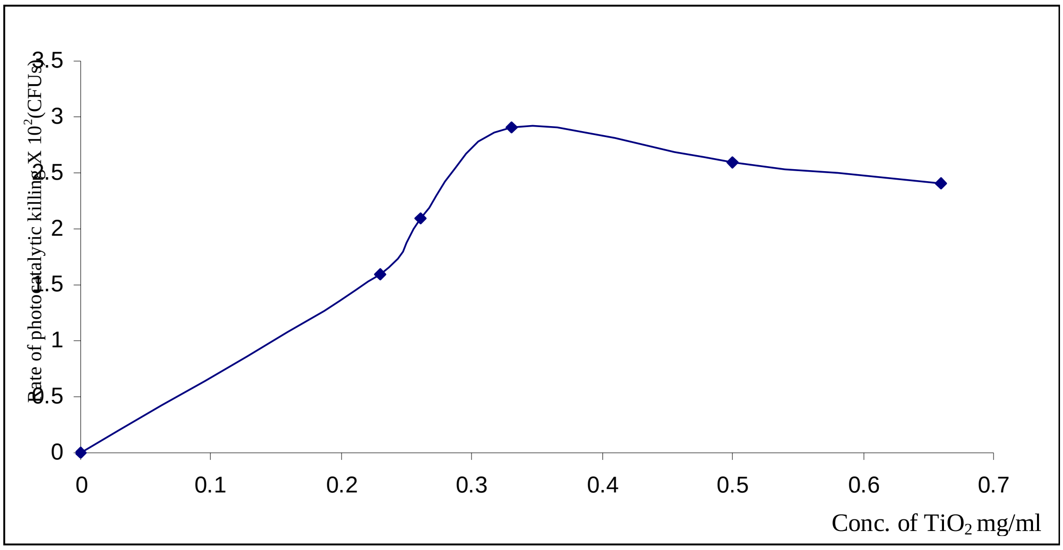


Figure (3):- Comparison of the survival ratio of *S.aureaus* in aqueous solution under various conditions at 25^0C.



Figure(4) :- survival ratio of *S.aureaus* in aqueous solution with different concentrations of TiO_2 at 25^0C.



Figure(5) :- The relationship between the rate of photocatalytic killing of *S.aureaus* in aqueous solution and concentration of TiO_2 at 25^0C.

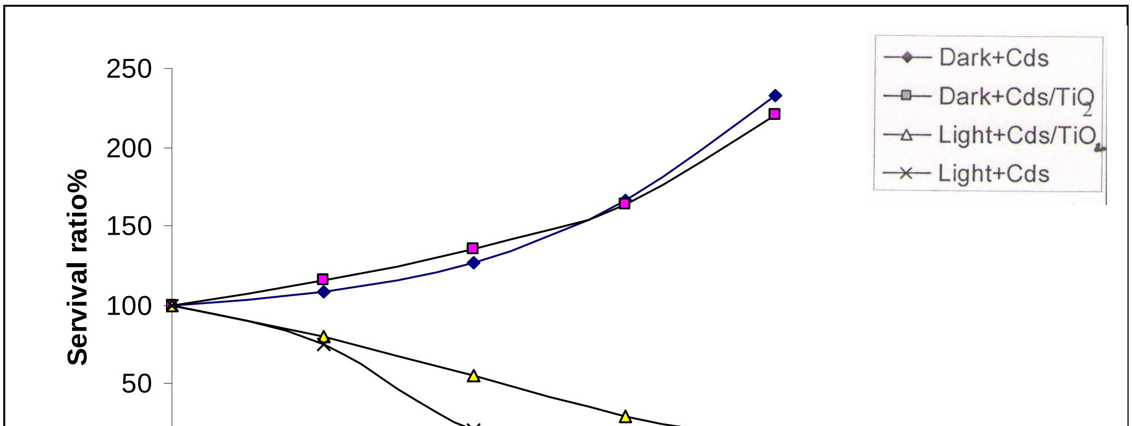


Figure (6):- Comparison of the survival ratio of *S.aureaus* in aqueous solution under various conditions at 25^0C.

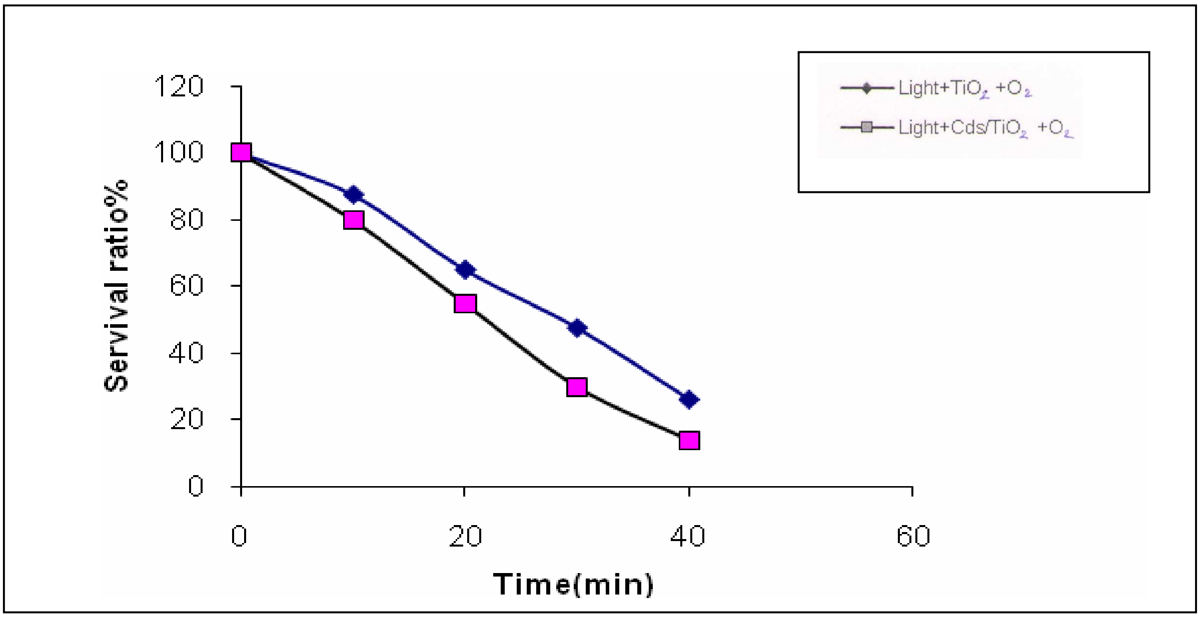


Figure (7):- Comparison of the survival ratio of *S.aureaus* in aqueous solution under various conditions at 25^{0}C.

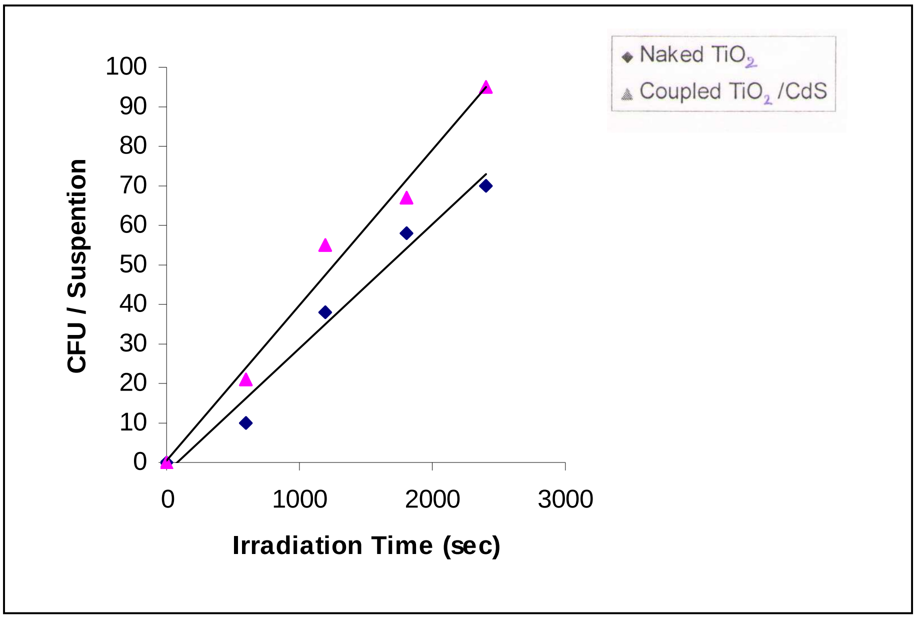


Figure (8):- The relationship between the rate of photocatalytic killing of *S.aureaus* in aqueous solution and concentration of TiO_2 at 25^{0}C.

4- Conclusions:-

The presence of catalyst , light and oxygen are very essential to generate (e_{cb}/h_{vb}) pairs.These pairs are very important to occurs any photocatalytic reaction in aqueous solution.

The rate of photocatalytic killing of bacteria in aqueous solution was reduced by using TiO_2 concentration more and less than 0.33mg/ml this mean that at this amount of catalyst there is complete absorption of the incident light by TiO_2 molecules .

When TiO_2 particles have been modified with CdS particles. CdS/TiO_2 system showed higher photocatalytic killing efficiency than the naked TiO_2 system because naked TiO_2 photocatalyst is effective only upon irradiation of UV-light ,while coupled CdS/TiO_2 photocatalyst have been identified to be active upon visible light so that TiO_2 /CdS system has been used as catalyst in water sterilization and purification.

5-The Suggested Mechanism of photocatalytic photokilling:-

When TiO_2 particle is illuminated with light (*hv*) of greater energy than of the band gab the photon energy excited valance band electron and generates pair of electrons and holes (electron-vacancy in valence band) that diffused and trapped on near TiO_2 surface[17].These excited electrons have strong redusing and oxidizing activity and reacted with atmospheric oxygen and water to yield reactive oxygen species such as hydroxyl radicals (ÓH) super oxygen (O_2^-) hydrogen dioxide (H_2O_2)[18] according to the following equations:-

$$TiO_2 \xrightarrow{hv} TiO_{2(h-e)exc.} \qquad \ldots\ldots\ldots\ldots(1)$$

$$\ldots\ldots\ldots\ldots(2)$$

$$(h-e) \rightarrow h_{vb}^{+} + e_{cb}^{-}$$

$$\ldots\ldots\ldots\ldots(3)$$

$$\ldots\ldots\ldots\ldots(4)$$

$e^{-} + O_2 \rightarrow O_2^{-}$

$O_2^{-} + H^{+} \rightarrow HO_2^{\cdot}$

. . . .(5) $\bar{O}H + h_{vb}^{+} \rightarrow \dot{O}H$

$2HO_2^{\cdot} \rightarrow H_2O_2 + O_2$ . . ..(6)

TiO_2 photocatalyst is effective only up on irradiation of UV – light and levels that would also induce serious damage to human cells[19].This greatly restricts the potential application coupled TiO_2/CdS photocatalyst have been identified to be active upon visible –light it is also believed that coupled TiO_2/CdS particles have large surface area are efficient for decomposition of pollutants (such as organic compound and bacteria)[20].

When CdS absorbs visible light and generate electrons in conduction band and positive holes in valance band excited electrons are injected in conduction band of TiO_2 as shown in figure (1)

The radicals (ÓH) and (O_2^{-}) **are extremely reactive upon contact with organic compounds. Complete oxidation of organic compound and bacterial cells to carbone dioxide could be achieved.**

reactive oxygen species such as hydroxyl radicals (ÓH) (O_2^{-})**and (H_2O_2) generated on the light irradiated TiO_2 surfaces were shown to operate in concert to attack polyunsaturated phospholipids in bacteria[18] .**
TiO_2 has shown a pronounced activity in the adsorption of basic L-amino acids such as L-lysine and L-arginine in an aqueous solution[21].TiO_2 is also capable of absorbing and in activating various bacteriocin . It has been demonstrated that the nitrogen moiety in various amino acids are converted

predominantly into NH_3 up on exposure to TiO_2 illuminated with UV-light[(22)].

6- References:-

1- C.Kampf and A.Kramer,Clin. Microbiol.Rev.,2004.17,863.

2- L.Frazer, Environmental Health perspectives,2001,109,4.

3- .Y.Kim,K.H.Park,T.G.Jeoung,S.J.Kim and S.Y.Cho,The Anual meeting Sanfrancisco CA.,2006.

4- D.Y.Gaswami,D.M.Trivedi and S.S.Block ,J.Sol. Energy,1997,119,92.

5- O.Lengrini,EOliveros and A.M.Broun,Chem.Rev., 1993,39,671.

6- A.Henglein, J.Phys. Chem., Newyork,1982,86,2291.

7- H.A.Habeeb,MSC., Thesis, Babylon University ,1998.

8- R.Vogel,P.Hoyer and H.Weller, J.Phys.Cem., 1994,98,3183.

9- P.C.Maness, S.Smolinski, D.M.Blake, Z.H.Wolfrum and W.A.Jacoby, Appl. Environ. Microbiol,1999,65,9,4094.

10- T.E.Parker and B.G.Henrich.,Anal.chem.,1955,27,1986.

11- S.Hogg, Essential Microbiology , 1st Edition,England, 2005,P102.

12- N.Ehrhrdt and G.Petrick, Marine Chem.,1984,15,47.

13- S.W.Radi, MSC.,Thesis,Salah AL-Den University,Irag,1989.

14- F.H.Hussein and H.A.Habeeb,AL-Qadisia,2000,1,5.

15- A.G.Attia,and F.H.Hussein ,National J. Chem.,2001,2,230.

16- A.C.Diener and F.M.Ausubel, Dominant Arabidopsis Disease, 2005,171(1),305.

17- J.Wang et al , Electronic journals ,2006,17,4561.

18-P.C.Maness et al , Appl.Environ. Microboil,1999,65,4094.

19- W.A. ,P.C. Maness ,E.J.Wolfram,, D.M.Blake and J.A.Fennel ,Environ Sci. Technol,1998,32,2650.

20- C.L.Cheng et.al ,Journal of Biomedical Science,2009, 16(1),7.

21- M.S.Wong et.al, Appl. Environ Microbiol,2006,72,6111.

22- Q.Li , R.Xie,E.Mintz,Environ. Sci Technol., 2007,41,5050.