Application of the photocatalytic reaction of TiO_2 to disinfection and the killing of *Escherichia coli* bacteria

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Abstract

Photocatalytic disinfection reaction under UV- visible light in presence of oxygen and Titanium dioxide (TiO_2) in aqueous solution has been investigated .This method is effective for killing *Escherichia -coli* bacteria in water. TiO_2 semiconductor exhibits strong bacterial activity .The aim of this research is to design a new photobioreactor and its application to sterilize the water from *E-coli* bacteria .

Primary experiments have been done to determined the optimum conditions which lead to high killing efficiency of *E-coli* bacteria in aqueous solution . The results showed that the bacterial effect of TiO_2 under UV-visible light irradiation on *E-coli* bacterial suspension was much higher than without using TiO_2 .The photocatalytic reactions were carried out with various TiO_2 concentrations. The highest photocatalytic *E-coli* photokilling rate was obtained at 0.5 mg/ml TiO_2 concentration ,which is equal to 3.25×10^{-2} CFUs /sec .This rate was increased to 5.8×10^{-2} CFUs /sec when 10 ppm of hydrogen peroxide was add to *E-coli* aqueous solution under UV- visible light in presence of oxygen and ideal concentration of TiO_2 (0.5mg/ml). The mechanism of TiO_2 illumination to produce oxidizing species and the effect of these species on the bacterial activity in aqueous solution has been suggested.

الخلاصة

تضمن البحث الحالي دراسة التفاعل الضوئي المحفز لتعقيم المياه بوجود TiO_2 كعامل مساعد والأوكسجين وباستخدام الأشعة فوق البنفسجية والطيف المرئي ، وقد أظهرت هذه الطريقة فعالية عالية في قتل بكتريا *E-coli* الموجودة في الماء ، حيث اظهر شبه الموصل TiO_2 تأثير عالي تجاه الفعالية البكتيرية . يهدف هذا البحث إلى تصميم مفاعل حيوي ضوئي جديد وتطبيقه في تعقيم المياه من هذه البكتريا.

تم إجراء عدة تجارب أولية لتحديد الظروف المثلى والتي تؤدي إلى أعلى كفاءة في قتل بكتريا *E-coli* في المحلول المائي .وقد أوضحت النتائج أن تأثير تشعيع ألـــ TiO_2 بالأشعة الفوق البنفسجية والضوء المرئي على فعالية البكتيريا اكبر بكثير منه عند التشعيع دون استخدام TiO_2 كعامل مساعد. كما تم إجراء

سلسلة من التفاعلات الضوئية المحفزة بوجود تراكيز مختلفة من TiO_2 وقد تم الحصول على أعلى سرعة قتل ضوئي عند تركيز $0.5mg/cm^{-1}$ من TiO_2 وكانت هذه السرعة مساوية إلى $3.25\ x10^{-2}$ CFUs/sec. . وقد ازدادت هذه السرعة الى $5.8\ x10^{-2}$ CFUs /sec عند اضافة 10 ppm من بيروكسيد الهيدروجين الى المحلول المائي لبكتريا *E-coli* باستخدام الاشعة الفوق البنفسجية والطيف المرئي بوجود الاوكسجين والتركيز المثالي للعامل المساعد (0.5 mg/ml). تم اقتراح ميكانيكية تشعيع TiO_2 لتكوين أجزاء مؤكسدة ودراسة تأثير تلك الأجزاء على الفعالية البكتيرية في المحلول المائي.

Introduction

A wide variety of active chemical agents exhibit bactericidal activities some of the most widely used, including alcohols, iodine and chlorine have been employed for along time in disinfection and preservation [(1)]. Compared to these widely used disinfectants, application of photocatalyst based antimicrobial disinfectant technologies rare still in the development stage[(2)].

Photocatalytic oxidation of a very wide range of organic compounds has been observed .Therefore, it is not surprising that cellular molecules, such as carbohydrates, lipids, proteins and nucleic acids can be damaged and subsequently lead to cell death[(3)] . TiO_2 has shown a pronounced activity in the adsorption of L-amino acids such as L-lysine and L-arginine in aqueous solution [(4)].

TiO_2 is the most best suitable semiconductor for photocatalysts reaction because it is stable to photo and chemical corrosion, nontoxic and inexpensive. The band gab energy of TiO_2 in the anatase crystal is 3.2 ev and therefore absorbs in near UV light (λ<387 nm.)[(5)].When TiO_2 particle is illuminated with light ($h\nu$) of greater energy than of the band gab, an electron is promoted from the valance band (vb) to the conduction band (cb) leaving a positive hole in the valance band[(6)].After separation, the electron and hole pair may recombine generating heat or can be separated to produce electron donor sites (reducing sites) and electron acceptor sites (oxidizing sites)[(7)] as illustrated in the following figure.

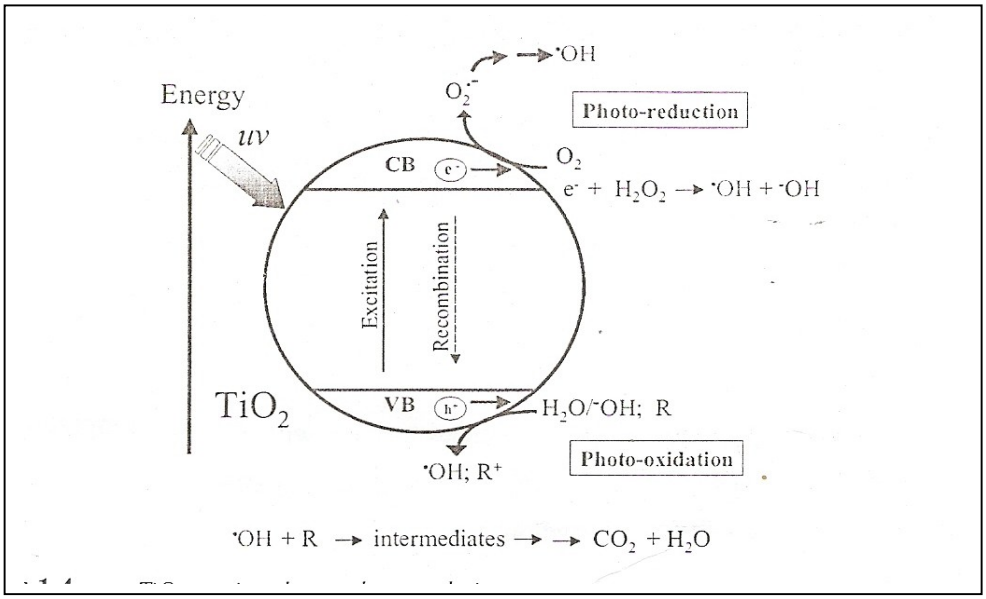


Figure (1):- TiO_2 –semiconductor photocatalysis process.

Matsunaga and coworkers[8] reported that microbial cell in water could be killed by contact with TiO_2 catalyst upon illuminat5ion with near UV light. They reported that oxidizing the cell membrane and losing its semi permeability ,the intra cellular Co-enzyme (CoA) is photooxidized and this cause decrease in respiratory activity which ultimately led to cell death.

Sunado and coworkers[9] are measured the destruction of endotoxin from E-coli. The endotoxine of Gramnegative bacteria . It's toxicity resides mainly on the lipid fraction i.e. lipid A ,while the sugar moiety acts as antigenic determinate . The endotoxn is an integral part of the bacteria cell envelope and is released only when the intact cellular structure is destroyed.

The aim of this study is to investigate the effect of UV- visible light on the antibacterial activity in presence of TiO_2, and using photocatalytic reactions for sterilizing water instead of chemical as antibiological.

Experimental

Chemicals

Titanium dioxide was purchased from Degussa P25 (mostly anatase BET $55 m^2 g^{-1}$).Nutrient agar was supplied from HIMDIA. H_2O_2 was supplied from BDH company at 30%.

Instruments

Low pressure mercury lamp (LPML) type Emaky (160 W) was used as a source of irradiation . The wave length of this lamp rang between 306-750 nm. . 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).

Photocatalysis Experiments

The instruments used in this work was previously descrided in details[10]. In all photocatalytic experiments , 30 cm^3 of aqueous solution of *E-coli* 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 25^0C by using circulating thermostat. The suspension was irradiated for 40 min.

Other experiments have been done by adding 10 ppm of H_2O_2 to *E-coli* aqueous solution in absence and presence TiO_2 catalyst 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 5 nim. to separate the semiconductors partials and the supernal liquid. For all experiments, 0.5$cm^3$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 *E-coli* were controlled.

The incident light intensity was measured by using Parcker and Hautchard method[11].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 25^0C. The average light intensity is 6.2 x 10^{-8} Einstein $L^{-1}S^{-1}$.

Results and Discussion

Determination of optimum conditions for photocatalytic reactions

A number of primary experiments have been done to determine the optimum conductions which lead to high killing efficiency . Figure (2) shows that, in the first three experiments the number of bacterial cell was increased with time because 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 .This leads to the exponential increase in the number of cells with time in aqueous solution[12].The results shows that the presence of TiO_2 semiconductor without using light dose not affect the bacterial activity because TiO_2 is biologically and chemically inert [13].the survival ratio of *E-coli* bacteria under UV-visible light is higher than that of irradiated of TiO_2 because the light lamp energy is not enough for killing bacterial cell , so that the optimum condition for bacterial killing can obtained by irradiating TiO_2 in presence of O_2 . This means that the presence of light,$O_2$and TiO_2 catalyst was very essential for photocatalytic reaction.

The effect of catalyst concentration

A series of experiments has been accomplished including irradiation of aqueous suspension of *E-coli* bacteria with different TiO_2 concentrations ranging between (0.23-0.66)mg/ml in presence of O_2 at 25 ^{0}C. Figure (3) shows a comparison of survival ratio of *E-coli* in aqueous solution under different TiO_2 concentrations in presence

of O_2 . The best result has been obtained at 0.5 mg/ml TiO_2 concentration.

Figure(4)shows that the rate of photokilling increases with increasing of TiO_2 loading. The maximum rate value has been produced at 0.5mg/ml TiO_2 concentration which is equal to 3.25 $x10^{-2}CFU_S$/sec. However above 0.5 mg/ml TiO_2 concentration it showed a negative deviation as TiO_2 semiconductor increases, while using low TiO_2 concentrations (0.23-0.33) mg/ml produced direct proportional between TiO_2 concentration and the rate of *E-coli* photokilling. This observation can be explained as follow, at low concentration the number of TiO_2 particles are few as compared with number of incident photons and according to the second law of photochemistry each atom or molecule can absorb one photon only .So that the rate of *E-coli* photokilling is increased with increasing TiO_2 particles, but when using TiO_2 concentration more than 0.5 mg/ml TiO_2 concentration (0.66 mg/ml). This particles form inner filter[14]which absorbs high portion of the incident light as well as the excess number of scattering another part of it, which lead to reduce the rate of photocatalytic reaction.

The effect of hydrogen peroxide

A number of experiments has been carried out including the effect of adding H_2O_2 on the killing efficiency of *E-coli* bacteria in aqueous solution . Two dark experiments have been done in absence and presence H_2O_2 and passing O_2 . Figure(5) shows that the number of bacterial cell was increased with time in the absence of H_2O_2 and light because these bacterial cells are able to start division by binary fission , but the number of bacterial cell was increased by adding 10 ppm of H_2O_2 under dark condition . This effect can be explain that the hydrogen peroxide was disrupted of bacterial cell membrane and cause of decreases in respiratory activities that led to cell death[8].

The killing efficiency of *E-coli* bacteria was increased by irradiated H_2O_2 *E-coli* aqueous solution with UV-visible light in presence of O_2. Figure (6) shows a comparison of survival ratio of *E-coli* in aqueous solution with and out hydrogen peroxide in presence of 0.5 mg/ml TiO_2 concentration as a function of irradiation time . The highest *E-coli* photokilling rate was obtained when irradiated *E-coli* aqueous solution after adding 10 ppm H_2O_2 and 0.5 mg/ml TiO_2 concentration ,which is equal to 5.8 x 10^{-2} CFUs/sec.

This effect was explained by Matsunaga and coworkers[8]for the photokilling of *E-coli* in water .Hydrogen peroxide can be react with conduction band electrons to produce the more damaging hydroxyl radicals as the following equation :-

$$H_2O_2 + e^-_{cb} \longrightarrow \bar{O}H + ?H$$

Both hydrogen peroxides whose added to *E-coli* aqueous solution and generated on irradiated TiO_2 surface can inhibite the electron – hole recombimation process and hence prolong the life time of the photokilling of electron-hole pair on TiO_2 , so that the efficiency of photocatalytic killing was increased

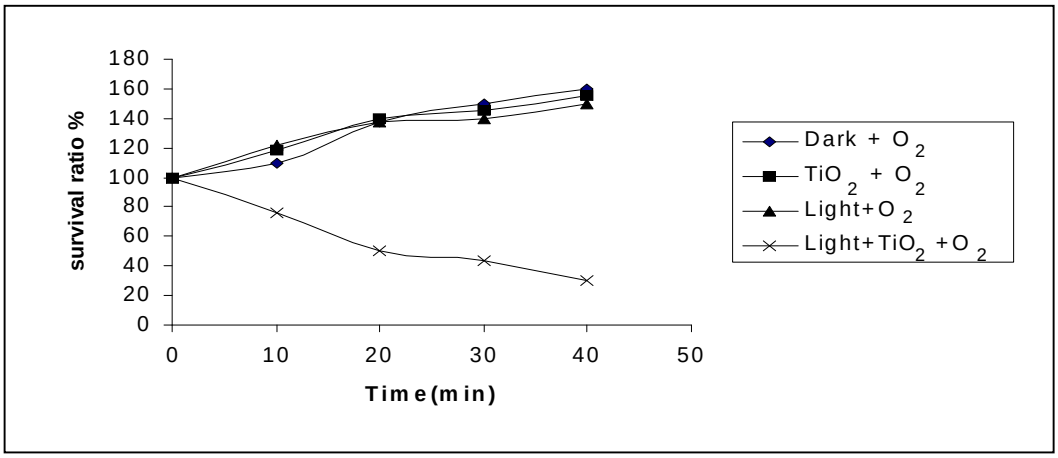
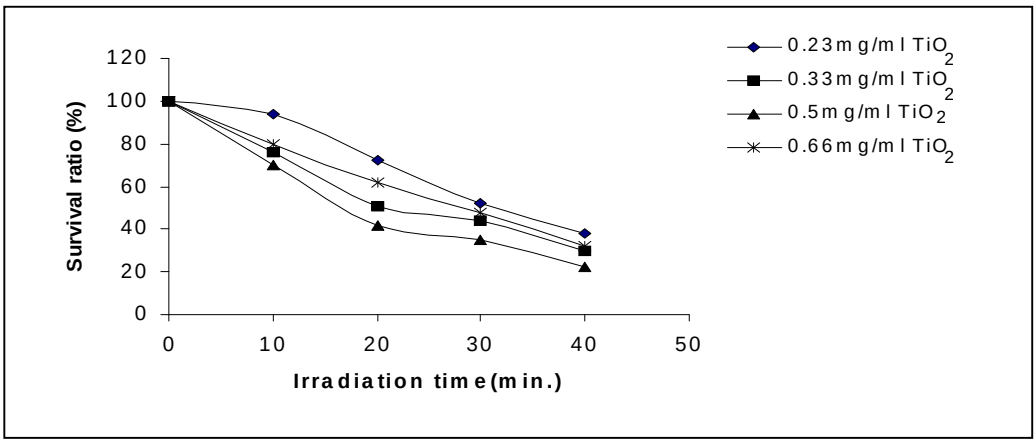
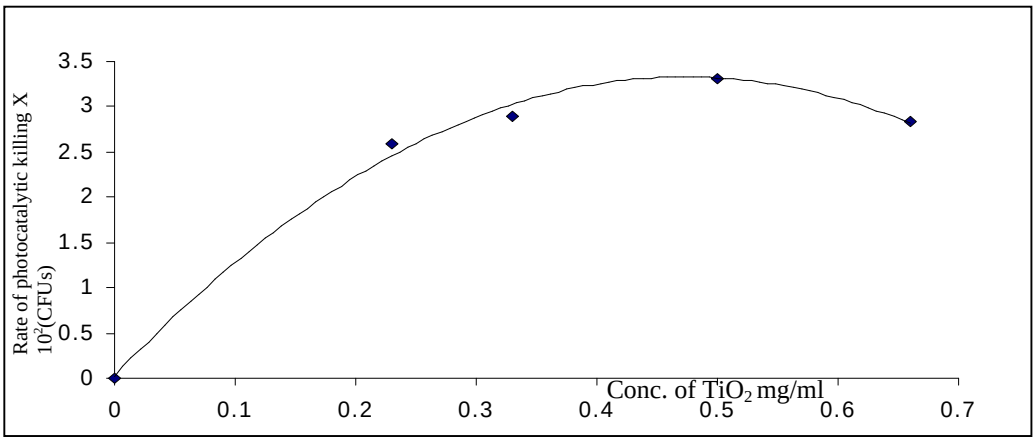


Figure (2):- Comparison of the survival ratio of *E-coli* in aqueous solution under various conditions at 25^0C.



Figure(3) :- survival ratio of *E-coli* in aqueous solution with different concentrations of TiO_2 at 25^0C.



Figure(4) :- The relationship between the rate of photocatalytic killing of *E-coli* in aqueous solution and concentration of TiO_2 at 25^0C.

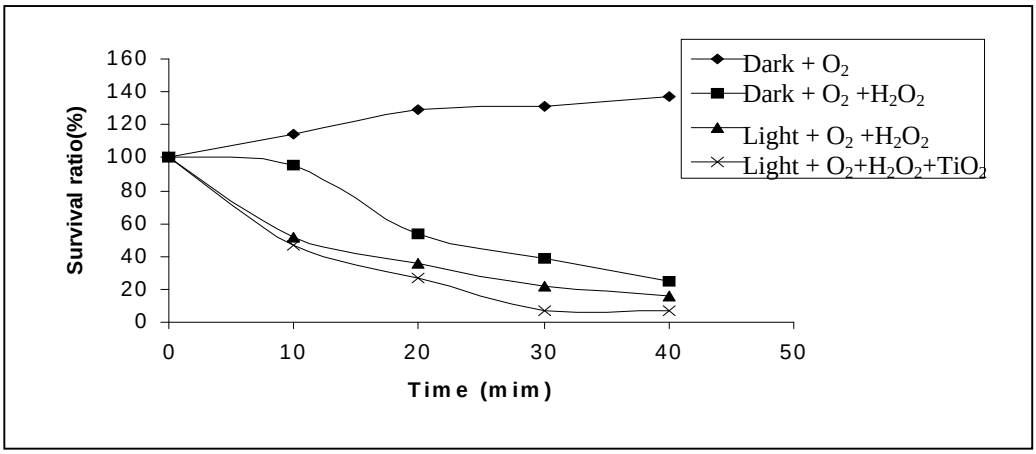


Figure (5):- Comparison of the survival ratio of *E-coli* in aqueous solution under various conditions at 25^0C.

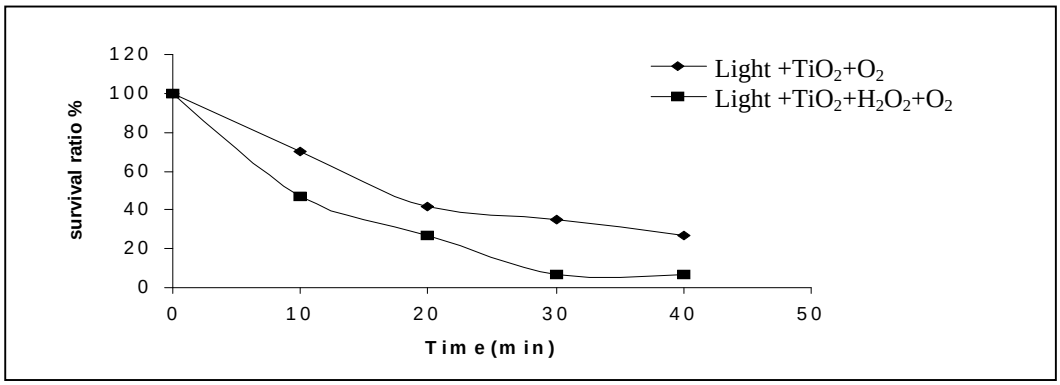


Figure (6):- Comparison of the survival ratio of *E-coli* in aqueous solution between absence and presence H_2O_2 at 25^0C.

The suggested mechanism of photocatalysis.

When the suspension of TiO_2 irradiated with light , the photon energy excited valance electrons and generated pair of an electron in conduction band and a positive hole in the valance band[15] :-

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

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

The excited electrons react with the dissolved atmospheric oxygen to yield super oxide $(O_2^{\cdot -})$

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

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

Photoholes are trapped by hydroxide groups of water to produce hydroxyl radicals.

$$\bar{O}H + h^+_{vb} \rightarrow \dot{O}H$$

Hydroxyl radical contact with each other to yield the hydrogen peroxide[16]

$$2HO_2^{\cdot} \rightarrow H_2O_2 + O_2$$

When bacterial cell contact with TiO_2 surface they may be direct photoelectron or hole transfer to the organism or one of it components . The authors concluded that a direct contact between cells and semiconductor is a

prerequisite for cell killing . The thick wall of bacteria spores is impermeable to most damaging agent[17].

Hydroxyl radicals generated by TiO_2 irradiation are highly reactive and therefore have a short half lived. Super oxide ions are more long half lived however due to the negative charge. Both super oxide and hydroxyl radical can not penetrate the cell membrane[18], while hydrogen peroxide can enter the cell and interact with ferrous ion in the periplasmic space or inside the cell, either as iron clusters or iron storage protein (such as ferritin) to produce the more damaging hydroxyl radical and this type of reaction is called Fenton reaction[18].

$$Fe^{+2} + H_2O_2 \rightarrow \dot{O}H + \bar{O}H + Fe^{+3}$$

$\dot{O}H, \bar{O}_2$ *and* H_2O_2 have been proposed to attack poly unsaturated phospholipids in bacterial cell membrane and causes a break down of the cell membrane stracture and therefore its associated to cell death, also the reactive oxidizing species can disturb cell membrane lipoprotein and nucleic acids, which place cell in state of oxidative stress and eventually leads to cell death.So that TiO_2 particles can exert oxidative action directly on all the essential components in the cytoplasm and oxidative the cell membrane[20] .

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