Application of the photocatalytic reaction of TiO\textsubscript{2} to disinfection and the killing of \textit{Escherichia coli} bacteria

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Abstract

Photocatalytic disinfection reaction under UV- visible light in presence of oxygen and Titanium dioxide (TiO\textsubscript{2}) in aqueous solution has been investigated. This method is effective for killing \textit{Escherichia coli} bacteria in water. TiO\textsubscript{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 \textit{Ecoli} bacteria.

Primary experiments have been done to determined the optimum conditions which lead to high killing efficiency of \textit{Ecoli} bacteria in aqueous solution. The results showed that the bacterial effect of TiO\textsubscript{2} under UV-visible light irradiation on \textit{Ecoli} bacterial suspension was much higher than without using TiO\textsubscript{2}. The photocatalytic reactions were carried out with various TiO\textsubscript{2} concentrations. The highest photocatalytic \textit{Ecoli} photokilling rate was obtained at 0.5 mg/ml TiO\textsubscript{2} concentration, which is equal to $3.25 \times 10^{-2}$ CFUs/sec. This rate was increased to $5.8 \times 10^{-2}$ CFUs/sec when 10 ppm of hydrogen peroxide was add to \textit{Ecoli} aqueous solution under UV-visible light in presence of oxygen and ideal concentration of TiO\textsubscript{2} (0.5mg/ml). The mechanism of TiO\textsubscript{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\textsubscript{2} كعامل مساعد والأوكسجين، واستخدام الأشعة فوق البنفسجية والطيف المرئي، وقد أظهرت هذه الطريقة فعالية عالية في قتل البكتريا \textit{Ecoli} الموجودة في الماء، حيث أظهرت هذه الدراسة أن تأثير شبه الموصل TiO\textsubscript{2} على فعالية البكتيريا \textit{Ecoli} تجاه البكتيريا البكتيرية. يهدف هذا البحث إلى تصميم مفاعل ضوئي جديده وتطبيقه في تعقيم المياه من هذه البكتيريا.

تم إجراء عدة تجارب أولية لتحقيق الظروف المثلى والتي تؤدي إلى أعلى كفاءة في قتل البكتيريا \textit{Ecoli} في المحالولة المائية، وقد أوضح النتائج أن تأثير تشعيب Alv. TiO\textsubscript{2} بالأشعة فوق البنفسجية والضوء المرئي على فعالية البكتيريا أكبر بكثير من عند التشعيب دون استخدام TiO\textsubscript{2} كعامل مساعد. كما تم إجراء سلسلة من
التفاعلات الضوئية المحفزة بوجود تركيز مختلفة من TiO_2 وقد تم الحصول على أعلى سرعة قتل ضوئي عند تركيز 0.5mg/cm^1 وتركيز TiO_2 من 0.5x10^{-2} CFUs/sec. وكانت هذه السرعة مساوية إلى 3.25 CFUs/sec عند تركيز TiO_2 من 0.5mg/cm^1. وقد ازدادت هذه السرعة إلى 5.8x10^{-2} CFUs/sec عند إضافة 10 ppm من بروكسيد الهيدروجين إلى المحلول المائي لإستخدام الأشعة فوق البنفسجية والطيف المرئي بوجود الأوكسجين والتركيز المثالي للعامل المساعد E-coli ليكترس. تم اقتراح ميكانيكا تشيع 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 a long 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 suitable semiconductor for photocatalysts reaction because it is stable to photo and chemical corrosion, nontoxic and inexpensive. The band gap 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 (hv) of greater energy than of the band gap, 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.
Matsunaga and coworkers\(^{(8)}\) reported that microbial cell in water could be killed by contact with TiO\(_2\) catalyst upon illumination with near UV light. They reported that oxidizing the cell membrane and losing its semi permeability, the intracellular Co-enzyme (CoA) is photooxidized and this cause decrease in respiratory activity which ultimately led to cell death.

Sunado and coworkers\(^{(9)}\) measured the destruction of endotoxin from E-coli. The endotoxin of Gramnegative bacteria. Its toxicity resides mainly on the lipid fraction i.e. lipid A, while the sugar moiety acts as antigenic determinate. The endotoxin 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 55m\(^2\)g\(^{-1}\)). Nutrient agar was supplied from HIMEDIA. H\(_2\)O\(_2\) 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 wavelength of this lamp range between 306-750 nm. Photocell (35 cm\(^2\)) with quartz 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\(_2\) particl was removed by using centrifuge (Hettich).
Photocatalysis Experiments

The instruments used in this work was previously described 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\(^0\)C by using circulating thermostat. The suspension was irradiated for 40 min.

Other experiments have been done by adding 10 ppm of H\(_2\)O\(_2\) 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 min. to separate the semiconductors partials and the supernal liquid. For all experiments, 0.5 cm\(^3\) of the suspension was immediately added to 20 cm\(^3\) nutrient agar media in a Petridish (9 cm-diameter) with triplicates per each treatments. Petridishes were kept in the dark at 30 \(^0\)C for 24 h. Colony forming units (CFUs) 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\(_3\)Fe(C\(_2\)O\(_4\))\(_2\).3H\(_2\)O for 3 min. after passing nitrogen gas for 15 min. at 25\(^0\)C. 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 conduction 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 O2. The best result has been obtained at 0.5 mg/ml TiO2 concentration.

Figure(4) shows that the rate of photokilling increases with increasing of TiO2 loading. The maximum rate value has been produced at 0.5mg/ml TiO2 concentration which is equal to 3.25 x 10^2 CFU/sec. However above 0.5 mg/ml TiO2 concentration it showed a negative deviation as TiO2 semiconductor increases, while using low TiO2 concentrations (0.23-0.33) mg/ml produced direct proportional between TiO2 concentration and the rate of E-coli photokilling. This observation can be explained as follow, at low concentration the number of TiO2 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 TiO2 particles, but when using TiO2 concentration more than 0.5 mg/ml TiO2 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 H2O2 on the killing efficiency of E-coli bacteria in aqueous solution. Two dark experiments have been done in absence and presence H2O2 and passing O2. Figure(5) shows that the number of bacterial cell was increased with time in the absence of H2O2 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 H2O2 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 H2O2 E-coli aqueous solution with UV-visible light in presence of O2. 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 TiO2 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 H2O2 and 0.5 mg/ml TiO2 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 :-

\[ \text{H}_2\text{O}_2 + e^{-}_{cb} \rightarrow \text{OH} + \text{OH} \]

Both hydrogen peroxides whose added to E-coli aqueous solution and generated on irradiated TiO2 surface can inhibite the electron – hole recombination process and hence prolong the life time of the photokilling of electron-hole pair on TiO2, 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° C.

Figure (3) :- survival ratio of *E-coli* in aqueous solution with different concentrations of TiO₂ at 25° C.

Figure (4) :- The relationship between the rate of photocatalytic killing of *E-coli* in aqueous solution and concentration of TiO₂ at 25° C.
Figure (5): - Comparison of the survival ratio of *E. coli* in aqueous solution under various conditions at 25° C.

Figure (6): - Comparison of the survival ratio of *E. coli* in aqueous solution between absence and presence H$_2$O$_2$ at 25° C.

**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$^{(15)}$:

\[ TiO_2 \xrightarrow{hv} TiO_2(\text{h}^-\text{e}^+) \]

\[ (h^- - e) \rightarrow h^*_\text{vb} + e^-_{\text{cb}} \]

The excited electrons react with the dissolved atmospheric oxygen to yield super oxide (O$_2^-$)

\[ e^- + O_2 \rightarrow O_2^- \]

\[ O_2^- + H^+ \rightarrow HO_2^- \]

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

\[ \dot{OH} + h^*_\text{vb} \rightarrow OH \]

Hydroxyl radical contact with each other to yield the hydrogen peroxide$^{(16)}$

\[ 2HO_2^- \rightarrow H_2O_2 + O_2 \]

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 \(^{(18)}\).

\[ \text{Fe}^{+2} + \text{H}_2\text{O}_2 \rightarrow \cdot \text{OH} + \cdot \text{OH} + \text{Fe}^{+3} \]

\( \cdot \text{OH}, \cdot \text{O}_2 and \text{H}_2\text{O}_2 \) have been proposed to attack poly unsaturated phospholipids in bacterial cell membrane and causes a break down of the cell membrane structure 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 \(^{(20)}\).

References

