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Disinfection of *E. coli* by the Ag-TiO_2/UV system: lipidperoxidation

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Abstract

The photocatalytic disinfection of *Escherichia coli*, a pathogenic micro-organism was investigated. An aqueous suspension of the micro-organism (1×10^8 cfu/ml) and neat TiO_2 or silver-loaded TiO_2 were irradiated with a high pressure mercury lamp for various time periods at neutral pH (7.0). Silver loading (1%, w/w) dramatically reduced the illumination time for complete degradation (less than 1–2 min) and minimum catalyst concentration was determined as 0.1 mg/ml. Possible killing mechanism and intermediate products were also determined. Occurrence of lipidperoxidation which produces malondialdehyde (MDA) was proposed but the method employed was so effective that all micro-organisms and intermediate products were further degraded to harmless products.

Keywords: *Escherichia coli*; Photocatalytic degradation; Silver loading; TiO_2/UV; Lipidperoxidation

1. Introduction

Water supply resources consist of a variety of physical, chemical and biological constituents. Particularly, reclamation of wastewater is more and more widely practised since the limitations of the fresh water supply. Therefore, disinfection is mostly applied before usage of the fresh and reclamated wastewater.

Chlorine has been widely used for the treatment of water disinfection throughout the world. The well-known interaction of chlorine with organic materials (humic substances) that produces by-products called 'chloroorganic compounds' with trihalomethanes (THMS) which are presumed to be carcinogenic chemicals led to the employment of new alternative methods that gain great interest in recent years [1,2].

Advanced oxidation technologies (AOTs) have been investigated with the aim of developing methods to pure water and several results have been reported using TiO_2 powder as photocatalyst for varying waste materials [3,4]. Applications of TiO_2 assisted photodegradation of *E. coli* have appeared in recent literature [5–10]. For instance, it has been showed that *E. coli* (10^3 cells/ml) was totally inactivated in 60 min using BLF lamp (light intensity) in the presence of TiO_2 (1 mg/ml) [5]. In another experiment, *E. coli*-deposited TiO_2 was oxidised by the air to produce CO_2 in the presence of UV light and the mass balance was then determined [7]. Bactericidal activity of TiO_2/UV reaction and its killing mechanism was also reported by Maness and co-workers and the lipidperoxidation reaction was the first evidence proposed as the underlying mechanism of the death of *E. coli*.

A wealth of information has demonstrated the efficacy of the biological disinfectitious actions of TiO_2 as photocatalyst. The aim of our study is to offer a modified TiO_2 catalyst system for the complete disinfection of *E. coli* within a shorter illumination time.

Since the effectiveness of metal loading on TiO_2 has been discussed and the results have been reported before [4], an application of silver-loaded TiO_2 was evaluated as catalyst for *E. coli* system. Recently, engineering developments of aqueous phase solar photocatalytic detoxification and disinfection was reviewed by Goswami [11] and the application of this technology was discussed in details. Especially, the development of TiO_2 immobilised photoreactor systems are gained great interest which leads to physical and chemical modification of TiO_2 to improve the catalyst performance.

It is well-known that, Ag ions have bacteriostatic/bactericidal effects and as declared by the EES, the WHO and the UESEPA, silver does not cause adverse health effects setting a secondary minimum concentration level (MCL) as 90 ppb [12]. Therefore, loading TiO_2 with silver was thought to increase effectiveness of this catalyst system in terms of its effectiveness with its economical importance, since the catalyst could be used several times after regeneration. The degradation products and intermediates

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of Ag-TiO2/UV system was investigated as well as its disinfection activity.

When irradiated, TiO_2 particles are in direct contact with or close to micro-organisms, the oxidative specie hydroxyl radicals that are produced by the irradiation of the catalyst, will attack to the microbial surface. Unsaturated fatty acids (polyunsaturated phospholipids) are the primary target of hydroxyl radicals and the other reactive oxygen species such as, superoxide radical anions (O_2^-) or H_2O_2 [6,8].

Oxidative deterioration of lipids leads the formation of malondialdehyde (MDA) during the last stage of the breakdown of endoperoxidases which are formed during intramolecular rearrangements in the structure of unsaturated fatty acids. It is highly reactive substance that can cause deterioration of molecules, such as proteins, nucleic acid bases [13]. Its mutagenic effects on the bacteria and carcinogenic effects on the animals have been reported [2,13]. Therefore, photodegradation mechanism of Ag-TiO2/UV system for micro-organisms should be well-defined and either degradation products or intermediates should be determined comprehensively.

The effectiveness of Ag-TiO2/UV and neat TiO_2/UV systems were compared and the optimal dose of Ag-TiO_2 for given cell concentration were determined. Formation and degradation of MDA with the same catalytic system was also carried out and degradation pathways were then proposed.

2. Materials and methods

2.1. Silver loading

Titaniumdioxide (TiO_2) (anatase form 99.9%) was supplied by Aldrich and Ag loading procedure was carried the following steps given below.

First, to a TiO_2 slurry, made by adding 9.2 ml of a 0.1 M solution of $AgNO_3$ to 10 g of TiO_2 was added to ca. 10 ml of a 1.0% (w/v) solution of Na_2CO_3. The suspension was dried and allowed to be baked for 6 h at 400°C. Then, the catalyst system was finely ground and kept in a bottle to avoid moisture. Requisite amounts of TiO_2 or Ag-TiO_2 were weighed and placed into Pyrex reaction vessel containing *E. coli* suspension with a condenser. The catalyst was added to the sterile water prior to the reaction.

2.2. Culture of micro-organisms

E. coli K-12 strain ATCC 25922 was grown aerobically in 100 ml of brain–heart infusion at 37°C for 18 h and the micro-organisms were cultured at 25°C. Immediately, 100 ml aqueous samples containing 1×10^8 cfu/ml initial *E. coli* concentration were prepared and subjected to treatment

2.3. Photocatalytic reaction procedure

The amounts of the catalyst were varied between 0.1 and 1.0 mg/ml. The illumination was carried out using a low pressure mercury lamp (300 W, 294 nm, light intensity ca. 5.8 W/m^2 near the 254 nm wavelength) located 10 cm from the reaction vessel. The system was covered with aluminium foil and the temperature of the reaction mixture was kept between 40 and 85°C. At regular intervals, 1 cm^3 of aliquots of irradiated sample were withdrawn and kept in dark. Analysis were in triplicates and control runs were also carried out under the same conditions.

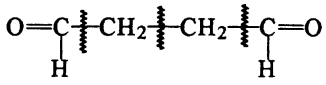
The number of viable cells in the suspensions of all TiO_2 or Ag-TiO_2-light treatments and control runs were determined by plating 30–100 μl aliquots of withdrawn samples onto blood agar plates. The plates were incubated at 30°C for 24 h and the numbers of colonies were counted.

2.4. Determination of lipidperoxidation

Lipidperoxidation was determined by monitoring the formation of MDA. Quantification of MDA was done following the methods described before [8,14]. The method is actually based the formation of pink pigment which has an absorption maximum in acidic solution at 532 nm. The pink pigment is formed from the reaction of MDA with thiobarbituric acid (TBA). A volume of 1 ml of photocatalyst-cell slurry was mixed with 2.0 ml of 10% (w/v) trichloroacetic acid (TCA) and the solid and the precipitated proteins were completely removed via centrifugation at 4000–10,000 rpm. Then aqueous supernatants were obtained and 3 ml of a freshly prepared 0.67% (w/v) TBA solution was added. The samples were placed in a boiling water bath for 10 min and cooled, and its absorbency was recorded at 532 nm using a Unicam UV2 spectrophotometer. MDA values were given as nanomoles of MDA per mg (dry weight (DW)) of cells.

2.5. Photocatalytic degradation of MDA

Photocatalytic degradation of MDA itself was also carried out to determine the degradation pathways, reaction mechanism and possible degradation products. As reported before, MDA is a lipidperoxidation product being formed from the oxidation of *E. coli* membrane phosphatidylethanolamine [8]. As emphasised in the same literature, MDA concentration had been reduced nearly 88% within 30 min illumination, but the degradation products have not been discussed. As a dialdehyde, MDA has two aldehyde groups and degradation products are expected to be ring cleavage products such as monoaldehydes (formaldehyde, acetaldehyde), monoketons (acetone), carboxylic acids (formic and/or acetic acid) or complete mineralisation products like CO_2 and H_2O.



As a result of lipidperoxidation, the formation of four major lipid metabolites (malondialdehyde, formaldehyde, acetaldehyde and acetone) was reported by Bagchi et al.

[15]. Therefore, the formation of these possible metabolites were also investigated. A series of samples consisting of 10.0 nmol/ml of MDA, formaldehyde (FA), acetaldehyde (ACT), and acetone (ACON) were prepared and 2,4-dinitrophenylhydrazine (DNPH) was used as the derivative agent for the identification of the metabolites on GC–MS, using helium as the carrier gas. A volume of 1 ml of DNPH (saturated solution in water) was made to react with 1 ml of MDA, FA, ACT and ACON solutions, individually, and a mixture of them at room temperature. The precipitated hydrozones were extracted into 1 ml of pentane for GC–MS analysis.

A Shimadzu GC–MS QP5000 system with a 30 m × 0.32 mm i.d. SE 54 capillary column (30 m) was used with an ionisation energy of 70 eV electron ionisation mode.

2.6. Determination of silver ions

Aliquots of 50 ml of 30 min illuminated sample containing only the Ag-loaded TiO_2 were collected and filtered in 0.45 μm membrane. After filtration, the solution was acidified with HNO_3 to pH 2.0 and the soluble silver was measured using a Unicam FAAS.

3. Results and discussion

3.1. Effects of Ag-loaded catalyst concentration

The effect of illuminated catalyst was investigated using the catalyst system TiO_2 or 1% (w/w) Ag-loaded TiO_2 (Table 1). In a previous work [8], the 70% of initial *E. coli* (1×10^8 cfu/ml) was destroyed after 30 min illumination using a 40 W near-UV light. We observed 100% cell damage in 15 min illumination period with TiO_2/UV.

As can be seen from Table 1, silver loading was so effective that Ag-loaded titania killed all *E. coli* cells just after addition. Killing possibly occurred due to bacteriostatic effect of silver. However, the illumination was carried out and the MDA levels were determined as a function of illumination. Although silver loading was so effective for the killing of *E. coli*, the formation of toxic product MDA did not significantly differ for both systems. The formation of MDA and its further degradation profile is shown in Fig. 1 for both catalyst systems.

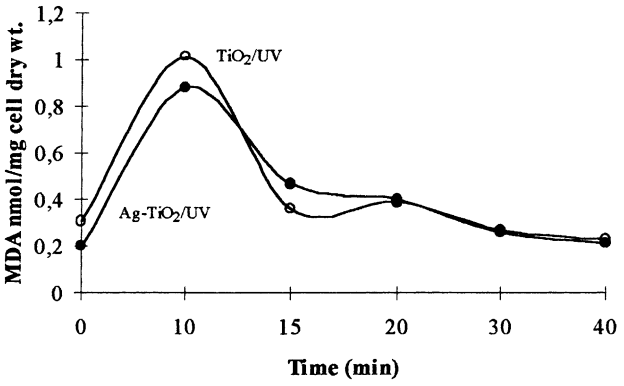


Fig. 1. MDA formation during the photocatalytic degradation of *E. coli* using neat TiO_2 and 1% Ag-TiO_2 (1 mg/ml).

Therefore, the influence of the amount of Ag-TiO_2 was investigated since the using concentrations greater than the required might cause the shading of the cells by the particles and preventing the light penetration. Catalyst amount was changed between 0.1 and 1.0 mg/ml for constant cell concentration (1×10^8 cfu/ml) to determine the optimal dose and findings were given in Table 2.

As can be seen from Table 2, although less Ag-TiO_2 catalyst used the system is still effective for *E. coli* (0.5–0.1 mg/ml) and a significant improvement was observed for further degradation of MDA. Since the strong adsorption of MDA on Ag-TiO_2 solid surface, MDA was not observed before illumination, then adsorbed MDA should be released by increasing temperature after 10 min (approximately 50°C) illumination then released MDA was further decomposed. However, using 0.5 mg/ml of 1% Ag-TiO_2 catalyst seems to be quite effective but when the dissolved silver ions, which is released to water from catalyst surface, is considered both 1 mg/ml and 0.5 mg/released Ag^+ ions more than MCL, 500 and 250 ppb, respectively. Therefore, using 0.1 mg/ml of 1% Ag-TiO_2 catalyst provide the best conditions with this system releasing only 50 ppb Ag and destroying all *E. coli* and MDA in 15 min illumination period.

Table 1
Inactivation of *E. coli* (1×10^8 cfu/ml) upon irradiation[a]

TiO_2/UV			Ag-TiO_2/UV	
Time (min)	Viable cell concentration (cfu/ml)	MDA (nmol/mg cell DW)	Viable cell concentration (cfu/ml)	MDA (nmol/mg cell DW)
0	1×10^7	0.31 ± 0.02	NVC	0.2 ± 0.03
10	1×10^6	1.01 ± 0.07	NVC	0.88 ± 0.07
15	0.5×10^6	0.36 ± 0.02	NVC	0.47 ± 0.09
20	NVC	0.39 ± 0.03	NVC	0.40 ± 0.06
30	NVC	0.27 ± 0.04	NVC	0.26 ± 0.05
40	NVC	0.23 ± 0.09	NVC	0.21 ± 0.04

[a] TiO_2, 1.0 mg/ml; Ag-TiO_2, 1.0 mg/ml (1%, w/w); NVC, no viable cell.

Table 2
Inactivation of *E. coli* (1×10^8 cfu/ml) upon irradiation[a]

Illumination period	1% (w/w) Ag-TiO_2 concentration (mg/ml)	Viable cell concentration (cfu/ml)	MDA concentration (nmol/mg cell DW)
0	1	NVC	Not observed
	0.5	NVC	Not observed
	0.1	NVC	Not observed
10	1	NVC	0.88 ± 0.05
	0.5	NVC	0.52 ± 0.07
	0.1	1.5×10^3	0.41 ± 0.09
15	1	NVC	0.47 ± 0.08
	0.5	NVC	Not observed
	0.1	NVC	Not observed

[a] Ag-TiO_2, 1% (w/w); NVC, no viable cell.

3.2. MDA degradation

It is known that once the reactive oxygen specie is formed it will degrade all organic compounds in the reaction media. Lipidperoxidation product MDA is also a target of oxidative degradation. Photocatalytic oxidative degradation of MDA (2.5 nmo1/ml) is carried out under the same condition using MDA–TBA method with neat TiO_2 and 1% Ag-TiO_2 (0.1 mg/ml). Residual MDA concentration upon illumination period is given in Fig. 2.

Kinetic model which is common in photocatalytic processes was determined as pseudo-first-order and rate constants were determined.

$$-\frac{dC}{dt} = kC$$

At maximum intensity of the light source, the initial rate constants of *E. coli* inactivation were 0.0691 for TiO_2 and 0.357 for Ag-TiO_2.

Although, the initial MDA solution gave a clear MDA-hydrozone precipitate the aliquots withdrawn at different time intervals did not give the corresponding hydrozones. However, the reaction mixture is extracted into pentane and the GC–MS carried out but corresponding hydrozones were far below the detection limits and only observed product was carbon dioxide.

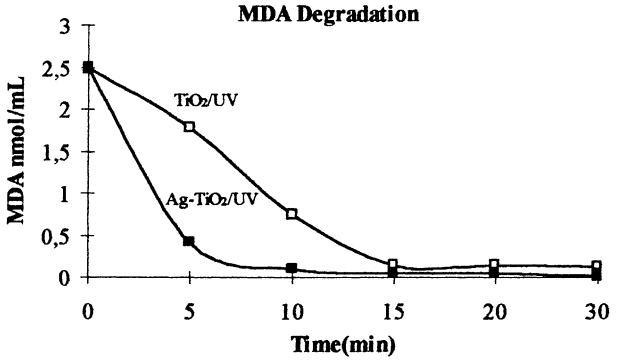


Fig. 2. Photocatalytic degradation of MDA using neat TiO_2 and 1% Ag-TiO_2 (0.1 mg/ml). Mean values for 5 min: 1.77 ± 0.09 and 0.42 ± 0.11, respectively, pooled standard deviation $= 0.10$, $t_{exp} = 16.46$ and $t_{exp} > t_{cri}$ (for $N = 4$; $P = 05$).

Consequently, it was concluded that the proposed method degraded all micro-organisms to MDA via lipidperoxidation and further degradation of MDA led to harmless products. Silver loading (1% w/w, 0.1 mg/ml) dramatically reduced the reaction time for complete degradation of *E. coli*. when the photocatalytic inactivation of micro-organisms in a photoreactor is considered, immobilised Ag-loaded TiO_2 layer will be more effective than the neat TiO_2 layers [10] and this will provide more economical and effective inactivation system. Silver loading possibly increased the reaction rate and presence of silver ions enhanced the reaction rate by trapping the conducting band electrons besides reducing the band gap energy of titanium dioxide.

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