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Comparative study of homogeneous and heterogeneous photo-oxidative treatment on bacterial cell via multianalytical techniques

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ABSTRACT

The present article demonstrates the comparative efficiency of homogeneous and heterogeneous photocatalyst on cell membrane destruction using electron microscopy and spectroscopy techniques to understand the mechanism and evolve an effective water disinfection technique. The antibacterial activity of a heterogeneous photocatalyst nano-TiO_2 was compared with a homogeneous neutral Photo-Fenton reagent ($Fe^{2+/3+}$, H_2O_2) against *Salmonella typhimurium*, a gram negative water borne bacterium. To confirm the extent of both types of photooxidation treatment on bacterial cell, production of MDA, release of RNA, DNA and protein were carefully studied and analyzed. Based on the above experimental results, heterogeneous photocatalyst shows better performance than homogeneous neutral Photo-Fenton system and the difference is attributed to the mode of disinfection in the two systems. The damage of cell membrane and release of inner cell components is higher in heterogeneous system in contrast to the neutral Photo-Fenton system which causes disinfection through both cell membrane and internal cell damage. The entrapped RNA/DNA within the cell up to 80% in Fe^{2+} treated bacteria substantiates destruction via internal cell damage.

1. Introduction

Harmful microorganisms are a serious problem in drinking water. Hence, there is an immediate need for an appropriate disinfection technique. *Salmonella typhimurium* is a typhoid fever causing Gram negative, rod-shaped bacterium and the salmonella infections in humans are divided into two categories – *Salmonella typhi* (*S. typhi*) and *Salmonella paratyphi* (*S. paratyphi*). The disease is transmitted through the ingestion of infected foods and contaminated water. Recently, the Photo-Fenton (PF) ($Fe^{2+/3+}/H_2O_2/h\gamma$) system under sunlight, at physiological pH was reported [1,2] to be an effective option for bactericidal activity. The antibacterial activity of Photo-Fenton, $Fe^{3+}/h\gamma$ was associated with adsorption of Fe^{3+} ions on the bacterial cell membrane, together with formation of iron-bacteria exciplexes, thus, leading to oxidation of cell membrane, in contrast to $Fe^{2+}/h\gamma$, which causes diffusion of Fe^{2+} and by intracellular dark Fenton reactions, leads to generation of $^{\bullet}OH$ radicals resulting in direct damage of DNA. Photo-Fenton was demonstrated to be highly efficient not only for the acceleration of solar disinfection of water, but also for elimination of natural organic matter [3–9]. But since, decades, titanium dioxide has been extensively used for surface coating as a self-cleaning and self-disinfecting material, as these materials have many attractive features, such as optical properties, low cost, high photocatalytic activity, chemical stability and non-toxicity [10,11]. Titanium dioxide in the anatase crystalline form behaves as a classical semiconductor. Illumination of TiO_2 in water with light of $\leq$400 nm generates electrons in the conduction band (e^-_{cb}) and positive "holes" in the valence band (h^+_{vb}) [12]. This potent redox power characteristically results in the lysis of bacteria and other organic substances [13–16]. Spuhler et al. [1] in their extensive study in 2010 claimed that solar Photo-Fenton system at low concentration of reagent and at near neutral pH proved efficient for both bacterial inactivation and mineralization of model organic matter. Although, for the last couple of years there are few papers reporting the effect of Photo-Fenton and TiO_2 (Degussa P25) individually on organic degradation/antibacterial activity, a comparative study of their efficiency on a single pathogen to evaluate the best disinfection system and its mechanism is reported for the first time to the best of our knowledge. Also a highly efficient, single phase of anatase and nano-titania with a high surface area, which is uniformly sized and regularly shaped, has been experimented to validate the true comparative capability of nano-TiO_2 photocatalyst over Photo-Fenton reagents in causing disinfection of water.

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2. Experimental materials and methods

2.1. Material

$TiCl_4$ (99.5% Loba Chemie) was used as a titanium source for the preparation of TiO_2. The pure culture of *S. typhimurium* (ATCC14028) was obtained from Defense Food Research Laboratory, Mysore, India. Other chemicals, namely, sulphuric acid (98%, 36 N), sodium hydroxide (97%), hydrogen peroxide (30%), ferric chloride (96%), ferrous sulphate heptahydrate (99%) and ammonium hydroxide (30%), were of Merck brand. The photocatalyst TiO_2 was prepared as per our earlier reported protocol [17].

2.2. Preparation of bacterial culture

The photocatalytic performance of the synthesized TiO_2 was evaluated on *S. typhi* ATCC14028, prepared by picking a loop full of exponential growth phase of pure culture colonies into 500 mL of brain–heart infusion (BHI) broth and incubated at 37° C for 15 h. The density of the bacterial suspension was adjusted approximately equivalent to 10^7 CFU/mL. The bacterial cells were collected in an exponential phase by centrifugation and the bacterial pellet was washed three times with double distilled water. Finally the bacterial pellet was resuspended in double distilled water. The bacterial suspension ($\sim 10^7$ CFU/mL) was placed in the dark at 25 °C under stirring for 30 mins to allow the bacteria to acclimatize to the new matrix, also to allow die-off of the most stress sensitive species and in all to stabilize the bacterial population.

2.3. Photoreactions

Experiments were conducted in six of the reactor vessels having an exposure area of 176.62 cm^2 into which the sunlight was directly focused. The concentration of photocatalysts used was as follows: 0.4 g/L TiO_2 as heterogeneous catalyst, 0.06 g/L of ferrous sulphate heptahydrate and ferric chloride as Fe^{2+} and Fe^{3+} PF sources respectively, with 0.01 g/L of H_2O_2. The photocatalytic reactions were conducted on intense sunny days in the month of March between 11 AM and 2 PM in Bangalore city (13°00.57′N and 77°34.15′E) which is about 1800 km from New Delhi, the capital of India. The average solar intensity was around 900 W/m^2 and no steps were taken to maintain the intensity of sunlight during the reaction. The reaction mixture was stirred continuously with a magnetic stirrer in atmospheric oxygen during photoreaction. The samples were collected every 15 mins for various analysis such as standard plate count method (SPC), dye exclusion, lipid peroxidation, protein, RNA/DNA estimation and TEM/SEM analysis. The protocols used for the above estimations are as mentioned in our earlier papers [18,19]. The above tests were conducted in triplicate giving fairly good reproducibility. The quantitative detection of hydroxyl radicals produced by various photocatalysts is estimated by using coumarin as a probe molecule [20], which was converted to a highly fluorescent 7-hydroxy coumarin (7HC) when reacting with OH radicals.

3. Results and discussion

3.1. Comparative bacterial inactivation mechanism under near-neutral Photo-Fenton (homogeneous) and TiO_2 (heterogeneous) photocatalysis

The synthesized photocatalyst used for the present study has been characterized by XRD, EDS, SEM, TEM, BET and spectral study in depth and is as reported in our earlier paper [17]. Fig. 1 depicts the extent of bacterial inactivation by cultivability measurements, where cultivability is N/N_o (N is the number of colonies obtained after an irradiation time period t and N_o is the number of colonies obtained before any irradiation). The heterogeneous catalysis causes complete inactivation within 120 mins of reaction. It is observed that all the reagents cause rapid decrease in the initial 25 mins of irradiation due to quick action of the catalyst and 80% of inactivation occurs within that period. The remaining 20% takes another 95 mins for completion. The difference in the efficiency of the photocatalysts (either heterogeneous or homogeneous) with regard to loss of cultivability appears marginal in Fig. 1. This may be because, both the internal damage due to diffusion (by homogenous catalysis) and cell damage due to oxidation (by heterogeneous catalysis) leads to ultimate loss of cultivability and hence the marginal difference.

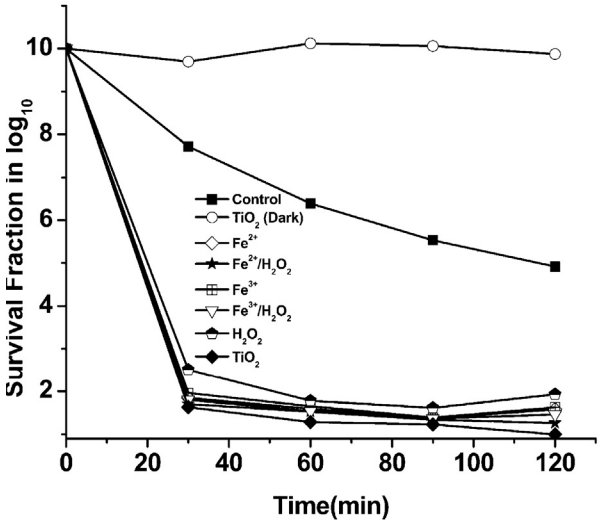

Fig. 1. Extent of photoinactivation of *S. typhimurium*.

Fig. 2 represents the accumulation of malondialdehyde (MDA), one of the most abundant aldehyde forms originating from peroxidation of bacterial lipid membranes. The heterogeneous catalyst is almost on par with other catalysts up to 60 mins beyond which there is a tremendous increase in MDA content. The high efficiency of the TiO_2 photocatalyst to generate ROS and cause membrane oxidation is in agreement with the high rate of lipid peroxidation occurring by hydroxyl radicals. The final MDA concentration reaches 99.323 nmol/mL on treatment with the TiO_2 photocatalyst, being much very higher than 17.478 nmol/mL obtained without the photocatalyst. The order of MDA concentration obtained with different compositions of Photo-Fenton reagent is 55.251 nmol/mL > 49.064 nmol/mL > 40.187 nmol/mL > 32.154 nmol/mL for Fe^{3+}/H_2O_2, Fe^{3+}, Fe^{2+}/H_2O_2 and Fe^{2+} respectively.

The same has been substantiated by hydroxyl radical scavenging investigation as shown in Fig. 2a. The spectrum denotes the hydroxyl radicals produced during a photocatalytic reaction highlighting the ability to generate both $^{\bullet}OH$ and $O_2^{\bullet -}$ radicals proportionately with time. The plot indicates the generation of hydroxyl radicals to be highly linear with time for the heterogeneous catalyst unlike the PF reagents, and this is clearly indicative of the enhanced inactivation (membrane oxidation and membrane permeability) occurring with TiO_2, which in fact evidences to be highly efficient in producing hydroxyl radicals. The phenomenon points to a classical mechanism of the light induced promotion of valence band electron to conduction band which yields valence band holes and produce hydroxyl radicals at the particle surface. The $^{\bullet}OH$ radicals (along with other ROS such as $O_2^{\bullet -}$ and H_2O_2 generated on the illuminated TiO_2 surface) can attack polyunsaturated components in *S. typhi*, leading to cell membrane breakage.