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Study of bacterial adhesion onto immobilized TiO_2: Effect on the photocatalytic activity for disinfection applications

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ABSTRACT

A study has been carried out to determine the influence of bacterial adhesion onto immobilized TiO_2 on the photocatalytic efficiency for bacteria inactivation. Two bacterial strains with differences in their membrane structure (*Escherichia coli* and *Enterococcus faecalis*) have been characterized in various suspensions for adhesion to the TiO_2 catalyst and surface charge. Non-meaningful differences have been observed regarding the adhesion properties between both bacteria. In contrast, the configuration of the catalyst and the composition of the suspension impacted the extent of bacterial adhesion. The solution affected the adhesion between bacteria and catalyst due to its influence on electrostatic forces between them. Under electrostatically favourable conditions, hydrophobicity is the primary mechanism of adhesion. Under unfavourable conditions aquatic chemistry governs the bacterial adhesion process. Organic matter in combination with divalent ions leads to the highest level of adhesion. This may be due to the presence of Ca^{2+} which can bridge between bacteria and catalyst. Additionally, Ca^{2+} can also bridge with organic matter, which can act as source of nutrients for bacteria. Despite the solution ionic strength being low, divalent cations can contribute to the compression of the electric double layer, enhancing cell-catalyst interactions and subsequent adhesion. The bacterial adhesion observed in wastewaters might be responsible for the fact photocatalytic bacterial inactivation efficiency was not as low as expected since the main role of ions and organic matter is to act as scavengers of hydroxyl radicals.

1. Introduction

Chlorine-based technologies have long been used as disinfection processes for drinking water supplies and also for the tertiary treatment of wastewater effluents. However, this technology is becoming of increasing concern due to recent studies about the formation of potentially harmful chloro-organic disinfection by-products (DBPs) with carcinogenic and mutagenic effects on mammals [1].

For that reason, new disinfection technologies are currently in development. Among them, the application of heterogeneous photocatalysis as an alternative for the inactivation of pathogenic microorganisms has attracted much attention in recent years. Since the early work of Matsunaga et al. in 1985 [2], many research groups have reported the successful killing of bacteria, viruses, algae, fungi or protozoa by semiconductor photocatalysis. Several research groups have studied in depth different aspects of the application of photocatalytic processes for the inactivation of microorganism such as catalyst loading, power irradiation, the influence of the water composition and bacteria cell wall structure, the use of solar radiation, and the efficiency of immobilized TiO_2 photoreactors [3,4]. Despite the limiting factor to effective photocatalytic inactivation being the proximity between microorganisms and the transient hydroxyl radicals produced by the catalyst, only a few studies have been focused on this interaction, mainly on the influence of the pH and the isoelectric point of the catalyst [4,5].

Bui et al. [6] pointed out that the differences in surface area, particle size and surface charge among different varieties of TiO_2 in powder affect the photocatalytic efficiency. However, according to Li and Logan [7], not only are the catalyst properties important, but also bacteria properties such as hydrophobicity and surface charge must be considered. It is important to note that the solution chemistry, not only the ionic strength of the suspension but also specific compounds, can also affect these properties. The presence of monovalent and divalent ions in water and even the kind of ion have led to differences in bacterial adhesion to solid surfaces due to both, electric double interactions and specific interactions or complexation [8,9]. In addition, the effect of organic matter on bacterial adhesion remains unclear as there is a lack of consensus in the literature as to its impact on cell attachment [10,11].

Properties such as cell type, solution chemistry, surface charge and hydrophobicity characteristics have been reported to affect adhesion [12]. It must be noted that electrostatic interactions are

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quite important in bacterial adhesion as a consequence of the charged groups being present on bacterial cell wall. In addition, since bacterial cell surface is highly depending on environmental changes, more complex interactions can appear such as the association or dissociation of charged groups or bacterial conformational changes leading to either complexation with certain compounds, making the process more difficult to understand [12,13].

Therefore, the aim of this work was to study the contribution of parameters known to affect bacteria–TiO_2 adhesion, such as bacteria cell structure and chemical composition of the solution. Additionally, the impact of the TiO_2 photocatalytic reactor configuration – and resulting bacteria–TiO_2 contact – was investigated using two different kinds of immobilized TiO_2 geometries [14]. And finally, the adhesion properties will be correlated between all of these various parameters with bacterial photocatalytic inactivation in different conditions.

2. Experimental

2.1. Bacterial selection and preparation

Escherichia coli K12 (ATCC 23631) strain was selected to carry out the photocatalytic experiments. The same strain together with *Enterococcus faecalis* (ATCC 11700) were selected and prepared to carry out the bacterial characterization and adhesion experiments. Fresh liquid cultures of both bacteria were prepared by inoculation in a Luria-Bertani (LB) growth medium and incubation under constant stirring on a rotary shaker at 37 °C for 24 h, until stationary growth phase.

For the photocatalytic experiments, bacterial cultures of $\sim 10^9$ CFU mL^{-1} were prepared. 5 mL of this culture was centrifuged and rinsed twice with sterile ultrapure water before diluting 1 mL of the resultant bacterial suspension to 1 L to prepare the reacting suspension, with an initial concentration of viable bacteria around 10^6 CFU mL^{-1}. More details of the procedures for preparing the cultures and the initial reaction suspension can be found elsewhere [14].

For the bacterial characterization, and adhesion experiments, the bacterial suspension was centrifuged at 3700 g under 4 °C for 15 min to separate bacteria from the growth medium. The medium was then decanted, and the pellet was resuspended in the test solution of choice. The centrifugation and rinsing steps with the chosen solution were repeated twice more to completely remove the growth medium to get a bacterial stock solution.

2.2. Solution chemistry

Several kinds of solution chemistry have been used to carry out the different experiments. Deionized water (DW), 0.01 M KCl, and simulated wastewaters (SWW) have been mainly used for carrying out the majority of experiments. Their values of ionic strength correspond to approximately 0, 0.01 and 5.72×10^{-5} M, respectively. Simulated wastewaters (SWW) consist of a mixture of salts (K_2HPO_4, NaCl, $CaCl_2$, and $MgSO_4$) and organic matter (meat peptone, beef extract and urea) diluted to a total organic carbon value of 15 mg L^{-1}, similar to effluents of a wastewater treatment plant [14].

2.3. Photocatalytic experiments

Photocatalytic experiments were carried out in an annular photoreactor of 188.5 cm^3 of irradiated volume (15 cm long, 3 cm inner diameter and 5 cm outer diameter) using two different catalytic systems: (i) a fixed-bed reactor with Degussa P25 TiO_2 immobilized onto 6 × 6 mm glass Raschig rings and (ii) a wall reactor with Degussa P25 TiO_2 immobilized onto the outer surface of the inner tube. More details of these photoreactor configurations, the immobilization procedure, and the optimization of the reactor system can be found elsewhere [14]. The system operates in a closed loop driven by a centrifugal pump with a reservoir tank; being the total working volume of 1 L. Illumination was performed by a Philips TL 6W black light lamp placed along the axis of the annular photoreactor. The UV-A incident photon flow, determined by ferrioxalate actinometry, was 2.8×10^{-6} Einstein s^{-1}, which corresponds to an irradiation flux in the inner tube of 64.5 W m^{-2}, with a maximum emission peak centred at 365 nm. More details about the reactor system can be found elsewhere [14].

E. coli K12 strain was used for the photocatalytic experiments. An initial bacterial concentration value of 10^6 CFU mL^{-1} was used. The quantification of viable bacterial concentration over the course of the experiment was carried out following a standard serial dilution procedure. Each decimal dilution was spotted 8 times on LB nutrient agar plates and incubated at 37 °C for 24 h before counting. Details of the procedures for the bacterial quantification can be found elsewhere [14]. Experiments were carried out in DW and SWW.

2.4. Bacterial and catalyst characterization

The electrophoretic mobility (EPM) of *E. coli* and *E. faecalis* were measured in DW, 0.01 M KCl and SWW. The EPM measurements were recorded with a ZetaPALS analyzer (Brookhaven Instruments Corporation, Holtsville, NY) according to procedures reported by Chen and Walker [13]. Electrophoretic mobilities were converted to zeta potentials (ZP) using the Smoluchowski equation [13]. Bacterial EPM was determined by diluting the previous bacterial stock solution to an optical density of ca. 0.2 measured at 546 nm (BioSpec-mini spectrophotometer, Shimadzu Corp.), corresponding to a bacterial concentration of ca. 2×10^8 CFU mL^{-1} for both strains. In addition, the hydrophobicity of bacteria was quantified by the microbial adhesion to hydrocarbons (MATH) test, which is a partitioning test for cells between the test solution and n-dodecane [13]. Samples were prepared by transferring 4 mL of the diluted bacterial stock solution to 3 test tubes and subsequently, adding 1 mL of n-dodecane. Hydrophobicity is reported as the percentage of total cells partitioned into the hydrocarbon. More details about the protocol can be found elsewhere [13].

Potentiometric titrations of bacteria were conducted to determine the relative acidity of the bacterial surfaces. For each measurement, 2 and 4 mL were taken from the bacterial stock solution for *E. coli* and *E. faecalis*, respectively (corresponding to concentration values of ca. 2×10^9 CFU mL^{-1}) and resuspended in the test solution of choice (DW, 0.01 M KCl, or SWW). Titrations were conducted in a sealed titration vessel with 50 mL of the test solution. After lowering the pH of the solution below 4 with 0.1 N HCl, a titrator (794 Basic Titrino, Metrohm, Switzerland) was used to carry out potentiometric measurements with 0.1 N NaOH in the presence of nitrogen gas purging and stirring. Acidity and the corresponding surface charge density were determined from the amount of NaOH consumed during a titration between pH 4 and 10. Data were processed by using FITEQL4 software. More details about the protocol can be found elsewhere [13].

Commercial Degussa P25 TiO_2 in powdered form was used at 0.1 g L^{-1}. Electrophoretic mobility (EPM) measurements of this catalyst were taken in DW, 0.01 M KCl and SWW. Measurements of pH were also recorded in DW, 0.01 M KCl and SWW. All of the suspensions showed pH values from 5 to 7.

2.5. Adhesion experiments

Adhesion experiments were carried out in the same fixed-bed and wall reactor used for carrying out the photocatalytic