STUDY OF BACTERIAL ADHESION ONTO IMMOBILIZED TiO₂: 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 (*E. coli* and *E. 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.

KEYWORDS: photocatalysis, disinfection, immobilized TiO_2, *E. coli*, *E. faecalis*, adhesion.

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 & 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 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 37ºC for 24 h, until stationary growth phase.

For the photocatalytic experiments, bacterial cultures of ~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 4ºC 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 0, 0.01 and 5.72 x 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 6x6 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 optimisation 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].

Escherichia 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 37ºC 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 Walker et al. [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 x 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 x 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 inactivation experiments, at a flow rate of 0.025 L min^{-1}. The total void volume of each reactor, which is the volume not occupied by the catalyst or the glass and therefore available for being occupied by the liquid, is 0.121 and 0.189 L for the fixed-bed and wall reactors, respectively. The resulting residence time of each reactor corresponds to 5 and 7.5 min, respectively.

Bacterial stock suspensions were prepared as described previously. 2 and 9 mL were taken from the bacterial stock solution for *E. coli* and *E. faecalis*, respectively to carry out the experiments, corresponding to an initial *E. coli* and *E. faecalis* concentration of ca. 10^8 CFU mL^{-1}. Firstly, a bacteria-free suspension whose chemical composition corresponded to that chosen for the test was injected in the reactor for the equilibration of the system. Then, the bacterial suspension was pumped through the reactor for an equivalent of 6 times the residence time. This suspension was passed through the reactor six times the void volume of the reactor to guarantee that saturation of the adhesion sites in the reactor is achieved. Afterwards the influent was switched to a bacteria-free suspension of identical chemical composition, leading to a drop-off in the effluent concentration of bacteria until it eventually approached zero. During the whole experiment, the outlet of the reactor was connected to a spectrophotometer where bacterial breakthrough curves are obtained by measuring the absorbance at a wavelength of 546 nm, a signal correlated with the concentration of bacteria in the suspension [15]. The concentration of bacteria leaving the reactor (normalized by the injected concentration) was plotted against the number of void-reactor volumes eluted through the system to obtain bacterial breakthrough curves [15]. The delay in the elution of bacteria in comparison with a tracer compound that shows null adhesion in the studied system was tested. That result can be then compared with the adhesion of bacteria under the same tested conditions. Potassium nitrate was used as inert tracer (0.01 M KNO$_3$). It was fed into the photoreactor and the absorbance values of the outlet were measured at 248 nm [15]. Experiments were carried out in DW, 0.01 M KCl and SWW for both bacteria.

3. RESULTS AND DISCUSSION

3.1. Photocatalytic bacterial inactivation experiments.

Figure 1 shows the photocatalytic *E. coli* inactivation in a fixed-bed and wall reactor for DW and SWW. As expected [14], a longer irradiation time is required to reach a complete bacterial inactivation for simulated wastewaters (SWW) as compared to DW due to the presence of scavengers of hydroxyl radicals such as organic matter and ions. However, the irradiation time needed to reach the detection limit must be increased only in 20 min as compared to that corresponding to DW in a fixed-bed reactor. In contrast, the irradiation time needed to reach the detection limit must be increased in ca 120 min for the wall reactor. One of the mechanisms responsible for these differences in time of treatment depending on both, composition of water and catalyst configuration, may be bacterial adhesion to the catalyst. Some results correlating bacteria-catalyst adhesion with photocatalytic activity have been reported [7, 16]. Actually, Gogniat et al. [16] pointed out the importance of the chemical composition of water which directly affected to bacteria adhesion onto the catalyst, leading to photocatalytic differences in bacterial inactivation efficiency.
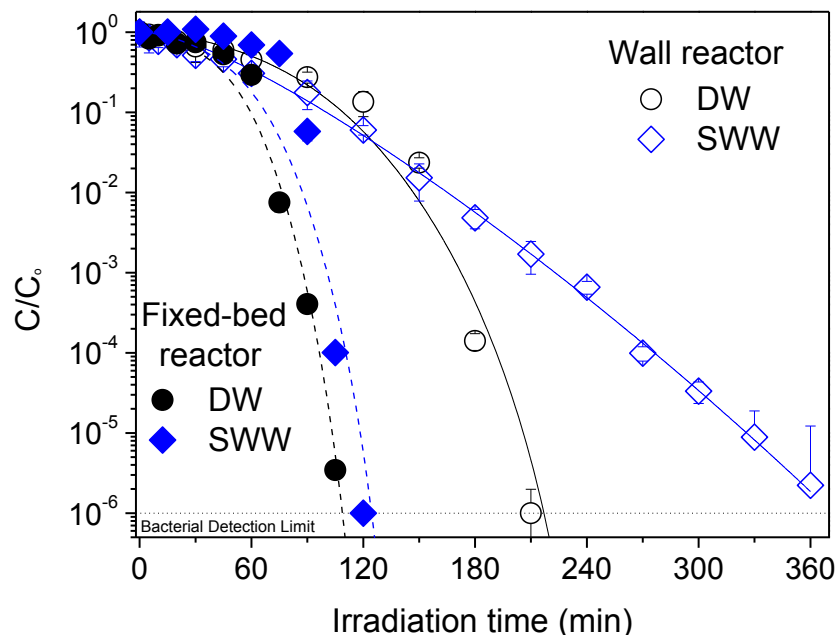

Figure 1. Photocatalytic *E. coli* inactivation for deionized water (DW) and simulated wastewaters (SWW) in a fixed-bed and wall reactor. Solid lines correspond to the fitting with the kinetic model detailed in a previous work [17]. Error bars calculated from eight independent bacterial counts.

Other studies [18, 19] have also reported the influence of cell wall differences on photocatalytic bacterial inactivation. Other groups [15, 20, 21] have also pointed out the influence of the bacterial cell wall characteristics on bacterial adhesion. Therefore, if cell wall differences can affect adhesion, they can also have influence on photocatalytic bacterial inactivation.

Consequently, the contribution of adhesion to the photocatalytic bacterial inactivation process is analyzed below. Previously, a bacterial and catalyst characterization mainly based of surface charge present on the cell wall and on TiO$_2$ depending on the solution chemistry was carried out. A bacterial characterization and adhesion experiments were also carried out to compare Gram negative with Gram positive bacteria.

3.2. Bacterial and catalyst characterization.

Table 1 reports the surface charge characterization of *E. coli*, *E. faecalis* and colloidal TiO$_2$. Electrophoretic mobility (EPM) measurements of *E. coli* in DW, 0.01 M KCl and SWW suspensions, indicating bacterial velocity under an applied electric field, were used to calculate zeta potential (ZP). Tose values indicate the electrical potential or charge at the bacterial shear plane which is useful surrogate for the surface potential [20] and are shown in Table 1. Negative values of ZP are obtained for *E. coli* in all these three suspensions which indicate that bacteria are negatively charged under the conditions present in the solutions used for the photocatalytic and adhesion experiments. Additionally, similar absolute values of ZP are observed for *E. coli* in these three suspensions, indicating a similar bacterial surface charge. As expected, higher pH values than 4 in the test suspensions lead to a negative charge surrounding *E. coli* since the isoelectric point of bacterial cell walls is between 2 and 4 [20]. Despite Konhauser [20] suggested that magnitude of negativity or charge on bacterial surface is expected to rise when increasing pH, the SWW having pH of ca. 6.5 as compared to that of 5.5 for DW and KCl solutions does not give to rise to a significantly different magnitude of the ZP. Actually, this data agrees with those reported by Kim et al. [21], who remarked the buffering capacity of bacteria. Similar values of ZP have been reported by other groups for KCl (ionic strength = 0.01 M) [15] and in the presence of organic matter (500 mg L^{-1} of dissolved organic matter, DOC) [22].

Table 1. Bacterial and catalyst characterization as function of solution chemistry. Deposition rate coefficients (k_d) obtained after bacterial adhesion experiments for the fixed-bed reactor in different solution chemistry conditions.

Bacteria/ Catalyst	Suspension	pH	[a]Ionic Strength (M)	[b]k_d x 10^{-4} (s^{-1})	[c]MATH (%)	[d]ZP (mV)	[e]Surface Charge Density ($\mu C\ cm^{-2}$)	[f]Acidity (meq/10^8 cell) x 10^{-5}
E. coli	DW	5.59	0	4.2 ± 2.7	37.7 ± 8.65	-56.93 ± 0.89	216 ± 29.5	1.06 ± 0.07
E. coli	KCl	5.56	0.01	5.2 ± 0.3	68.1 ± 1.61	-52.12 ± 3.13	225 ± 0.20	1.14 ± 0.20
E. coli	[g]SWW	6.47	5.72 x 10^{-5}	7.7 ± 1.6	38.1 ± 9.91	-53.50 ± 0.82	211 ± 43.8	1.06 ± 0.03
E. faecalis	DW	5.59	0	3.5 ± 2.0	48.1 ± 15.1	-49.43 ± 3.19	249 ± 34.7	1.21 ± 0.09
E. faecalis	KCl	5.56	0.01	6.0 ± 1.1	73.7 ± 23.2	-35.33 ± 6.02	179 ± 24.3	0.88 ± 9 x 10^{-16}
E. faecalis	SWW	6.47	5.72 x 10^{-5}	6.5 ± 3.2	42.4 ± 8.43	-49.64 ± 0.96	162 ± 11.8	0.95 ± 0.05
TiO$_2$	DW	5.59	0	–	–	30.91 ± 4.23	–	–
TiO$_2$	KCl	5.56	0.01	–	–	26.11 ± 2.83	–	–
TiO$_2$	SWW	6.47	5.72 x 10^{-5}	–	–	-30.26 ± 3.43	–	–

[a]Ionic strength given by the concentration and valence of the ions present in the solution. [b]k_d, deposition rate coefficient, quantification of bacterial adhesion to the catalyst. [c] The microbial adhesion to hydrocarbons (MATH) test is a measurement of cell hydrophobicity determined from the fraction of cell partitioned into n-dodecane versus an electrolyte. [d]ZP, Zeta Potential indicates the surface charge and magnitude of the bacteria or the catalyst. [e]Surface charge densities and [f]acidities of bacteria were determined from potentiometric titration from pH 4 to 10. [g]SWW, simulated wastewaters which consist of a mixture of ions and organic matter (meat peptone, beef extract and urea) (TOC =15 mg L^{-1}). –, not available.

E. coli surface shows a hydrophilic behaviour in DW and SWW as expected for pH values higher than 4, similar to that shown by Kim et al [21]. Unexpectedly, a higher hydrophobicity value is observed in KCl despite bacteria possessing similar values of ZP in DI, SWW and KCl, indicating a negatively charge surface as well as similar surface charge densities and acidities. This experimental fact agrees with Khemakhem et al. [23], who explained high hydrophobicities due to the presence of cations which can attach to negatively charged amino acids present in bacterial proteins, neutralizing their surface charge and increasing hydrophobicity. Therefore, hydrophilic bacteria surfaces (negatively charged in this case) are likely to interact with oppositely charged compounds and surfaces in DW and SWW; whereas a hydrophobic bacterial behaviour will lead to interactions with neutral surfaces in KCl.

No significant differences between suspensions were observed for either the surface charge density or acidity, both parameters calculated from potentiometric titration data. It agrees with the fact that functional groups in cell surfaces are hardly affected by solution chemistry [20].

On the one hand, no notable differences between both bacteria are observed in surface charge density and acidity data either, despite the magnitude of surface charge has also been reported to depend on composition of cell walls of several kinds of bacteria, such as *E. coli* [12, 20]. On the other hand, despite having different cell wall structure, no differences are supposed to appear in isoelectric behaviour of Gram-negative (*E. coli*) and Gram-positive (*E. faecalis*) [20]. Concerning the acidity and surface charge density, although values of *E. faecalis* in KCl and SWW seem to be slightly lower, no differences are observed in either sign or absolute values of ZP and hydrophobicity, despite the fact that other authors have reported differences for the kind of bacteria and even strain [12, 15, 20, 21].

Since the bacterial surface is negatively charged under most physiological conditions (pH = 5 - 7) as well as the test solutions in this study, the surface charge of the photocatalytic substrates must also be taken into account. ZP values of TiO_2 (Table 1) indicate sensitivity of the charge to the chemical composition of each suspension. As expected, the TiO_2 surface is positively charged for deionized water and KCl, due to the isoelectric point of 5.6 [5]. The less negative ZP observed in KCl as compared to DW may be attributed to the presence of

ions. The presence of K^+ and Cl^- can act as a shield of the charge on the surface making it appear less charged that would happen in a lower ionic strength solution as compared to DW. Such decrease of ZP for TiO_2 with ionic strength has also been reported in other studies [24]. In contrast, the ionic content and organic matter presence in simulated wastewaters (SWW) leads to a higher pH (above the isoelectric point) responsible for a shift in TiO_2 surface charge from positive to negative. It is also supported by negative values of ZP due to both, an increase in pH and a plausible sorption of organic matter as reported in another study [25].

Based on the values of ZP, in SWW the negatively charged bacteria and TiO_2 surface will undergo electrostatic repulsion when approaching each other. This should result in less interaction and subsequent adhesion. In contrast, a higher attachment between bacteria and TiO_2 is expected in DW and KCl suspensions as bacteria experience electrostatic attraction to the catalyst due to oppositely charged surfaces. Theoretically, the highest attachment between bacteria-catalyst would be expected for DW, followed by KCl due to the ion presence, responsible for the neutralization of the electric double layer on the bacterial and catalyst surfaces.

3.3. Adhesion experiments.

Adhesion experiments were carried out in DW, SWW and 0.01 M KCl to establish a relationship among bacterial characterization, catalyst properties and adhesion results. The breakthrough concentrations (C/C_o) for *E. coli* in the fixed-bed reactor are shown in Figure 2. These experiments allow the calculation of the amount of bacteria retained on the solid surface available in the fixed-bed reactor corresponding to the solid-liquid interface showed by the titania-coated Raschig rings that filled the entire annular space. The concentration of cells breaking through the column can be considered as a measure of the relative adhesion. The concentration of bacteria leaving the reactor (normalized by the injected concentration) is plotted versus the number of void-reactor volumes of liquid that have passed through the system (or pore volumes) in the three test suspensions. After 6 void-reactor volumes the bacterial suspension is no longer injected and is replaced with a bacterial free solution in a continuous way, leading to a drop-off in the effluent concentration of bacteria until it eventually approaches zero.
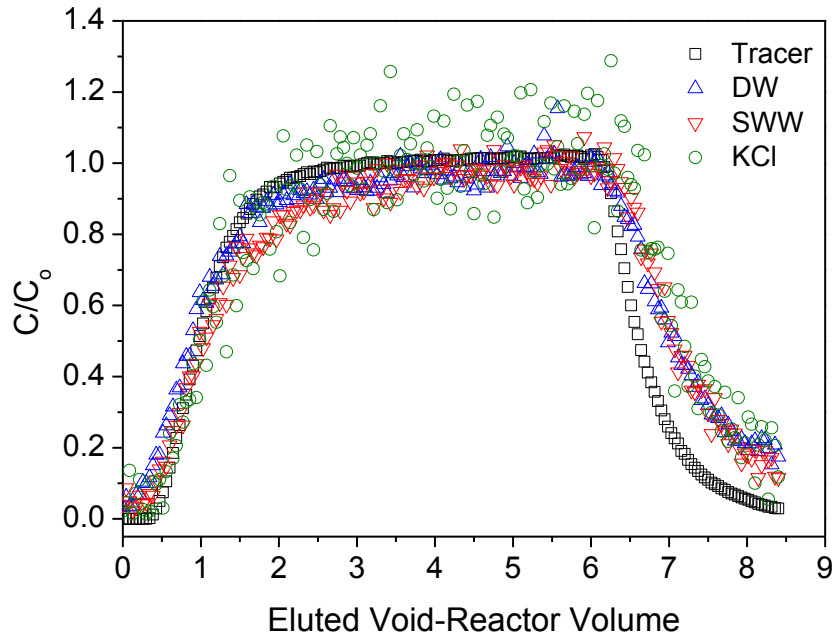
Figure 2. Breakthrough curve for *E. coli* for DW, SWW and 0.01 M KCl in a fixed-bed reactor. Experiments have been repeated in triplicate for each suspension. Error bars are not shown for clarity purposes.

After approximately 1 void-reactor volume, the injected bacteria appear in the effluent. To quantitatively compare the adhesion kinetics of the three different suspensions, the deposition rate coefficient ($k_d \times 10^{-4}$, s^{-1}) was determined between 1.8 and 2 reactor (or pore) volumes assuming clean-bed conditions according to the following expression [15]:

$$k_d = -\frac{U}{f \cdot L} \ln \frac{C}{C_o}$$
(1)

where C/C_o is the normalized breakthrough concentration, U is the (superficial) fluid velocity, f is the reactor porosity, and L is the length of the reactor. The resulting rate coefficients ($k_d \times 10^{-4}$, s^{-1}) for *E. coli* and *E. faecalis* for the fixed-bed reactor in deionized water (DW), simulated wastewaters (SWW) and KCl are reported in Table 1. It must be noted that the values of the deposition rate coefficients have not been normalized for surface charge or mass, since the comparison must be done in terms of the absolute values that can be achieved with each system. Both bacteria resulted in almost similar deposition rate coefficients from the adhesion experiments, as expected since negligible differences in their surface charge were observed.

It must also be noticed that the differences in solution chemistry seem to influence bacterial adhesion according to the variation in the k_d values. Although the highest bacterial deposition

rate coefficient was expected for DW and KCl due to the electrokinetic characterization of the surfaces (which suggested attractive forces existing between bacteria and catalyst), other interactions must also be considered according to the highest k_d observed in SWW for both strains of bacteria.

The addition of KCl has been reported to increase bacterial adhesion towards substrates as a consequence of the increase in solution ionic strength, which favours the compression of the electric double layer [8, 11-13, 15, 20]. However, the increase of solution ionic strength only seems to enhance adhesion process under unfavourable electrostatic conditions, when separation distance between bacteria and surfaces is governed by electrostatic repulsion forces [26, 27]. Therefore, taking into account the similar absolute values of ZP for bacteria in DW and KCl, it suggests that no effect on electrical charge on bacterial surface is observed due to increasing ionic strength solution. DLVO [20, 25] profiles are based on the combination of van der Waals attraction and the electrostatic repulsive forces in the electric double layer to predict bacterial attachment. Those profiles predicted a higher bacterial interaction for DW as compared to KCl (data not shown). It confirms that although the presence of KCl increases the ionic strength of the suspension, it does not seem to enhance the attachment of the bacteria to the substrate. Thus, the higher hydrophobic behaviour of bacteria surface in KCl as compared to DW is the key point in this case to understand the mechanisms which may lead to a higher bacterial adhesion to TiO_2 in KCl as compared to DW. On the one hand, larger attractive forces occur between bacteria and TiO_2 in KCl as confirmed by deposition rate coefficients and DLVO profiles. Therefore, repulsion forces between bacteria with a significant hydrophobic behaviour in KCl and positive charges of the catalyst surface can be dismissed. On the other hand, TiO_2 has been reported to have a hydrophobic behaviour [7], which can favour the interaction with bacteria possessing a high hydrophobic character, as it is the case in KCl solution. The experimental results here described suggest that the hydrophobic interactions are important even at these low ionic strength conditions where the electrostatic interactions should prevail. The importance of the hydrophobic interactions over the electrostatic forces was previously stated by Portinga et al. [12] but at high ionic strength conditions, where electrostatic interactions are favoured.

Despite repulsive electrostatic conditions that theoretically should exist between the bacteria and catalyst, there was substantially more deposition of cells on the catalyst in SWW. The contribution of the organic matter in the SWW is a subject of debate in the bacterial adhesion

literature. Several studies have reported the negative effect of anionic organic matter in bacterial adhesion since it can increase repulsion between bacteria and substrates by providing additional negative charges to the bacteria by adsorption onto bacterial cell walls, leading to a thicker electric double layer [11, 12, 20, 28, 29]. In this case, bacteria should show a higher absolute value of the ZP in SWW in comparison with the values of ZP in the other suspensions to confirm that the presence of organic matter increases repulsion between bacteria and the substrate; but this was not the case for the bacteria we investigated in this study (Table 1). In contrast, other authors propose that organic matter enhances the bacterial attachment as it alters the bacterial surface [26], may be used as carbon source for survival purposes by microorganisms [10] and increases hydrophobicity [11] and surface roughness [7, 20, 30]. In addition, organic matter can act as a polyelectrolyte, affecting adhesion by steric interactions. According to Portinga et al. [12], smaller polyelectrolytes may act as bridges between bacteria and substrates which would otherwise repel each other.

When SWW is used as medium, repulsion forces exist between bacteria and catalyst (Table 1), and therefore, the effect of ionic strength may gain importance. Although the ionic strength of SWW is low (5.72×10^{-5} M), there are divalent cations such as Ca^{2+} and Mg^{2+}, which have a greater effect on compressing electric double layer [20, 22]. Moreover, divalent cations also lead to the formation of cationic bridges between specific negatively charged bacterial functional groups and surfaces [8, 13, 31]. Therefore, those cations might neutralize the repulsion between bacteria and TiO_2 by reducing electric double layer. Other studies have also reported that a low ionic strength in presence of organic matter increases adhesion due to the reduction in electric double layer repulsion between bacteria and the surface [30]. It has also been identified that cations play a role in the neutralization of negatively charged organic matter [11, 32]. In addition to the increase of pH above the isoelectric point of TiO_2, the negative values of ZP observed for the catalyst in SWW (Table 1) might also be consequence of the adsorption of organic matter onto the catalyst as stated by Shim et al [22]. Other studies [33] have also pointed out that adsorption of organic matter on TiO_2 in the presence of Ca^{2+} is favoured due to cation bridging, as carboxyl groups of organic matter can bind with TiO_2 via this cation. In such scenario, bacteria may be more disposed to attach to the catalyst for survival purposes [10]. Moreover, if the surface of the catalyst becomes more negatively charged due to organic matter adsorption, cations could easily be attracted and further act as bridges between the catalyst and bacteria. Figure 3 shows schematically how this bacteria-TiO_2 interaction seems to occur in presence of organic matter.
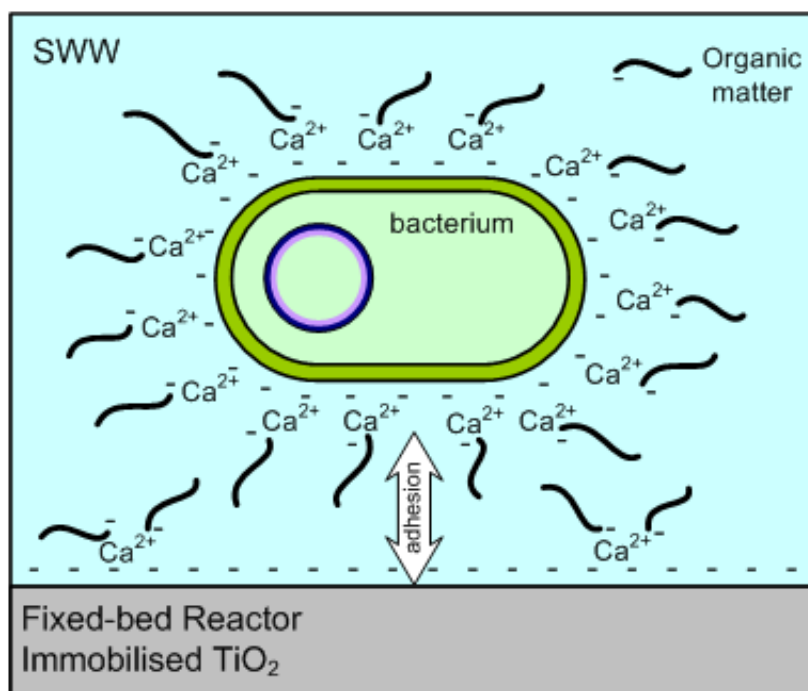
Figure 3. Schematic representation of the suggested mechanism of bacteria-TiO$_2$ interaction in SWW for a fixed-bed reactor.

Similar bacterial adhesion experiments were carried out in a wall reactor in deionized water and simulated wastewaters (SWW) to compare *E. coli* adhesion as function of the reactor configuration. The surface available in the wall reactor for the retention of the bacteria corresponds to the walls of the annular space, mainly the outer surface of the inner tube where titania is deposited. *E. coli* deposition rate coefficient ($k_d \times 10^{-4}$, s^{-1}) values obtained are 3.4 and 2.4 s^{-1} in deionized water and SWW, respectively for the wall reactor, being the difference between both values inside the experimental error. Under these same solution conditions, the deposition rate coefficients for the fixed-bed reactor corresponds to 4.2 ± 2.7 and 7.7 ± 1.6 s^{-1} for DW and SWW respectively. Notably, less bacterial attachment to the catalyst is observed in the wall reactor as compared to the fixed-bed reactor, which is probably a consequence of the lower TiO$_2$ surface area in the former. In addition, it has been reported [34] that bacteria are under mechanical stress in the fixed-bed reactor due to the impact with the Raschig rings present in this reactor. In these conditions, extracellular polymeric substances (EPS) could appear for protecting the bacteria under stressful conditions. It must be noticed that these secreted substances might also contribute to the bacterial adhesion onto the catalyst, in agreement with results reported by others [35].

3.4. Adhesion significance in a photocatalytic bacterial inactivation process.

These results suggest that the enhancement of adhesion observed for wastewater effluents (SWW) in comparison with the observed for DW for the fixed-bed reactor might favour the interaction between bacteria and catalyst counterbalancing the presence of other species capable to react with hydroxyl radicals.

Since the presence of such species in water acting as scavengers was quite significant for photocatalytic inactivation experiments, increasing the irradiation time (as shown in Figure 1), equivalent photocatalytic bacterial inactivation experiments in a fixed-bed reactor at 10^3 CFU mL^{-1} bacterial initial concentration were carried out at a lower flow rate (0.250 L min^{-1}). A complete bacterial inactivation was achieved faster in DW compared to that in SWW (data not shown). Since the flow rate used was more similar to that corresponding to adhesion experiments, a much clear correlation between adhesion and irradiation time required to reach the detection limit would have been expected. However, chemical composition of water affects the bacterial adhesion to the catalyst although the role of substances in water as scavengers maintains a significant role.

Concerning the differences in catalyst configuration, adhesion in SWW for the wall reactor was not as high as compared to the fixed-bed reactor. In addition, photocatalytic inactivation also required a higher irradiation time to reach the bacterial detection limit for the wall reactor compared to the fixed-bed reactor (as shown in Figure 1). Therefore, the lower adhesion obtained for the wall reactor in comparison to the fixed-bed reactor may be one of the reasons for the lower photocatalytic activity registered for the wall reactor. This fact seems to support the clear role that adhesion may have in photocatalytic inactivation as function of catalyst configuration. Of course, the amount of catalyst is different depending on the reactor considered, being higher in the fixed-bed configuration than in the wall reactor. However, the activity must not be compared in specific terms of area or catalyst loading, but rather in terms of the maximum activity that can be achieved with each optimized system. For instance, a catalyst or reactor configuration with a very low loading of TiO_2 will be probably very active in specific terms, but that does not mean that increasing ten times the catalyst loading will lead to a ten-fold increase in the activity. A discussion of this point can be found in a previous paper about photocatalytic oxidation of organic molecules [36]. In the case of

inactivation of bacteria this extrapolation will be even more erroneous, as bacteria-catalyst interaction, mechanical stress and other microbiological aspects play a significant role. Previous work on wall reactor reported negligible difference between the irradiation times required for reaching the detection limit for *E. coli* and *E. faecalis* in DW or SWW [37]. These trends agree with the current bacterial characterization and adhesion experiments, since no appreciable differences between both bacteria have been observed from both points of view, bacterial characterization and adhesion.

However, further investigations must be done since the surface charge and properties of the TiO_2 may change under UV irradiation, and the net charge on the TiO_2 will also depend on the kinetics of electron and hole transfer during photocatalysis and these phenomena may affect the interaction between the bacteria and the TiO_2 surface [38].

4. CONCLUSIONS

E. coli (Gram negative) and *E. faecalis* (Gram positive) do not seem to show appreciable differences in measured surface characteristics such as hydrophobicity and surface charge. Consequently, there was no noticeable difference in the extent of attachment to the catalyst. Rather, the configuration of the photochemical reactor (and hence surface area of the catalyst) and the aquatic chemistry played the most significant role in identifying the extent of cell adhesion and subsequent disinfection.

The influence of water composition on bacterial-TiO_2 interactions was substantial as it impacted subsequent electrostatic and hydrophobic interactions between the bacteria and catalyst. Under electrostatically favourable conditions in which bacteria and TiO_2 showed opposite surface charge, such as for DW and KCl suspensions, the increase in ionic strength given by the presence of KCl does not seem to be the main mechanism responsible for the enhanced bacterial attachment observed in KCl as compared to DW. But the bacterial hydrophobic behaviour shown in KCl seems to play a determinant role in that case. In contrast, under unfavourable conditions, the solution chemistry played a more substantial role, particularly when considering the presence of organic matter and ion content (SWW), which lead to substantially more attachment to the catalyst in the fixed-bed reactor.

As photocatalytic bacterial inactivation is a promising technology for tertiary treatment of wastewaters (such as SWW), optimization of bacteria-catalyst interactions and subsequent disinfection process is a critical issue.

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