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Influence of Bacterial, Environmental and Physical Factors in Design of Photocatalytic Reactors for Water Disinfection

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Abstract

The design of photocatalytic reactors for use in bacterial disinfection studies is often based on defined laboratory conditions, which are not a true representation of the harsh and ever-changing environment that bacteria encounter in nature. In this study four parameters (growth phase, biofilm production, pH and irradiation source) subject to continuous flux in nature, which could affect the efficacy of photocatalytic disinfection studies, were examined and their importance in process design was considered. The results produced a number of key findings which should be taken into consideration when designing photocatalytic reactors for biological processes. Using *Escherichia coli* as a model organism, studies of effects of pH and bacterial growth phase showed that cells in the stationary phase and at a pH of 8 were more resistant to photocatalytic breakdown. Only at a pH of 4 and while in the logarithmic growth phase, was complete photocatalytic destruction achieved. This process was further enhanced by replacing six 8 W black lamps with a single high-power UV-LED operated at 1.05 W. The impact of virulence was investigated by comparing photocatalytic destruction of a biofilm producing and non-producing strain of *Staphylococcus epidermidis*. The results indicated that there were no differences in susceptibility to disinfection suggesting that the capacity alone to express a virulence factor may not generate greater resistance to photocatalytic destruction.

Keywords

Photocatalysis, stationary and logarithmic bacteria growth phase, biofilm production, pH, photonic efficiency, photocatalytic reactor design parameters, spinning disc reactor.

1. Introduction

Semiconductor photocatalysis is a technology which has been studied for over four decades and is very effective in pathogen destruction [1]. Photocatalysis has also been used as a means of eliminating both pathogens and minerals from water and solid surfaces as well as for air treatment, water splitting and unlocking sources of renewable fuels [2–9]. When photocatalysts such as titanium dioxide (TiO_2) are illuminated with UV light, powerful oxidising agents capable of destroying bacteria are generated [10–12]. As many studies have shown [10, 11, 13, 14] the primary target for photocatalytic destruction of bacteria is hydroxyl (OH) radical attack on the cell wall. In the majority of aerobic water based systems, a superoxide radical ($\text{O}_2^{\cdot-}$) can also be generated from O_2 acting as an electron acceptor at the conduction band. While the superoxide radical is also capable of forming OH radicals via continued O_2 reduction, it is largely believed that the OH ion is the main source of OH radicals that primarily damage the cell wall [1]. It follows therefore that the more complex the cell wall the more resistant microorganisms will be to photocatalytic degradation [15, 16]. When exposed to stressful conditions in the natural environment, however, the cell wall of bacteria may undergo changes to enable the organism to withstand these conditions. Under such conditions e.g. dehydration, acidic environment or lack of nutrients, many bacteria revert to the stationary phase of growth since it is during this stage that many of the cells survival mechanisms become activated; the most significant of which, in terms of photocatalytic destruction, is the rebuilding and strengthening of the bacterial cell wall. Cells in the stationary phase have been shown to be many orders of magnitude more resistant to antimicrobials and many have the ability to survive under extremely adverse environmental conditions [17]. In this study bacterial growth phase was examined as a determinant of bacterial susceptibility to photocatalytic disinfection by comparing photocatalytic destruction rates of *Escherichia coli* K12 grown in stationary phase and logarithmic phase of growth and to ascertain more particularly if the known changes that occur in cell wall structure during the stationary phase of growth, have any noticeable impact on photocatalytic disinfection rates.

Another process parameter to be considered in bacterial disinfection studies is the influence of pH. The pH of many natural water samples is likely to be different to that used in laboratory conditions and is also likely to be subject to fluctuation. Therefore,

any photocatalytic reactor must be designed to cope with such variations. Most organisms are neutrophils i.e. they grow optimally between pH 5.5 -8.5 and while many can still grow outside of this pH range the changes in pH may affect how they respond to external stressors. Rincon and Pulgarin [18] have previously shown that low pH helped to enhance the photocatalytic disinfection of *E. coli*. The present study, however, has examined susceptibility of *E. coli* K12 to photocatalytic disinfection at both pH 4 and pH 8 since many natural water samples tend to be slightly more alkaline and this increase in pH may be more representative of natural water conditions.

The question of whether or not more virulent bacterial strains demonstrate greater resistance to photocatalytic destruction is of particular interest when setting parameters for disinfection processes. Virulence factors of microorganisms confer upon them the ability to invade a host, cause disease within that host and evade the host's immune system. Examples of virulence factors include toxin production, expression of fimbriae and/or flagella and biofilm production. Virulence factors, however, may not always be expressed or may only be expressed under certain conditions. This study consequently studied whether or not the capacity of an organism to express a virulence factor, in this case biofilm production, conferred upon it greater resistance to photocatalytic destruction. Biofilms are groups or communities of microorganisms which produce an extracellular polymeric glue-like substance which helps them anchor themselves to each other and to solid surfaces like water pipes or medical devices. They are notoriously much more difficult to destroy than bacteria in planktonic form and represent a unique challenge in water disinfection studies [19]. The ability to form a biofilm is a virulence mechanism possessed by some but not all bacteria and even those capable of biofilm production will often exist in planktonic form. This study examined whether or not the ability of an organism to form a biofilm confers, upon it, greater resistance to photocatalytic destruction by comparing the photocatalytic destruction of a biofilm producing strain of *S. epidermidis* in planktonic form with that of a non-biofilm producing strain.

The plethora of variables that need to be considered when setting process parameters for photocatalytic disinfection studies is considerable and represents a serious challenge in transferring such studies from the laboratory to the natural environment. Many problems relate to scale up issues such as selection of the most effective method for deployment of the photocatalyst, ensuring effective irradiation of the

photocatalyst with the appropriate light sources and particularly the minimisation of mass transfer limitations within the reactor configuration [20, 21]. The role that bacterial factors play in this process, however, should clearly not be overlooked. We have previously reported that bacteria which are serially passaged show greater resistance to photocatalytic destruction than those which are not [22]; these findings serve to highlight the fact that in the natural environment bacteria may undergo many changes not encountered in the laboratory which may alter their susceptibility to photocatalytic destruction. This paper seeks to build upon previous work [22] and investigate the impact of three key factors on photocatalytic disinfection; bacterial growth phase, pH and irradiation source. Moreover, this study helps to build a platform to further investigate crucial parameters with a view towards deploying photocatalytic technology in real life scenarios.

2. Materials and methods

2.1 Bacterial strains and culture conditions

The bacterial strains used in this study were *Escherichia coli* K12 (NCTC 10538) and *Staphylococcus epidermidis* strain 1457 [23]. Frozen stock cultures (-80 °C) of bacteria were sub-cultured into nutrient agar and subsequently transferred to nutrient broth. Broth cultures were incubated statically at 37 °C overnight to produce stationary phase cultures. To produce logarithmic phase cultures, bacteria were cultured overnight in nutrient broth as described above and the following morning 1 ml of the overnight culture was inoculated into a fresh 100 ml of nutrient broth and incubated at 37°C in an orbital incubator set at 100 rpm for a further 3 hours. To prepare bacteria (in both growth phases) for photocatalytic disinfection experiments, cells were centrifuged at 3,500 rpm for 10 minutes then washed twice in 10 ml of sterile distilled water before being re-suspended in a final 10 ml of distilled water. The optical density (O.D.) of the washed bacterial solutions was adjusted to 0.5 at $\lambda = 600$ nm which was equivalent to a starting concentration of 10^6 cfu/ml. All experiments were conducted at room temperature (18-20°C). The illumination sources did not increase the temperature of the surrounding area enough to influence the results.

2.2 Assessment of the photocatalytic disinfection of *E. coli* K12 in stationary and logarithmic phases of growth

Reaction vessels consisted of three sterile 250 ml beakers containing 100 ml of sterile distilled water with or without (UV only control) 1 g/L Degussa (Evonik) P25 TiO₂. All three reaction vessels were inoculated with 1 ml of the appropriate washed bacterial culture. UV illuminated beakers were placed at a distance of 10 cm from a 6 x 8 W UVA lamp (spectral output 311 – 415 nm peaking at 368 nm; Philips TL 8W/08 F8 T5/BLB) which was housed within a UV protective light box and were irradiated from the side. To accurately monitor the spectral characteristics of the lamps and the photonic efficiency of the system, the photon flux was determined using the potassium ferrioxalate actinometrical method [41,42]. The photon flux was determined by replacing the photocatalytic solution with actinometry solution. The photonic efficiency was then determined based on the calculated photon flux along with Equation 1.

$$\text{Photonic efficiency} = \frac{\text{Reaction rate (mole min}^{-1}\text{)}}{\text{Photon flux (Einsteins L}^{-1}\text{min}^{-1}\text{)}} \quad \text{Equation 1}$$

Using this method, the photon flux of the 48 W lamp was determined to be 6.8×10^{-5} Einstein's min⁻¹. The other beaker was placed in the dark (dark control) for the duration of the experiment. Magnetic stirrers were employed to prevent settling within each reaction vessel. Samples (1 ml) were taken from all three reaction mixtures at time zero and then every half hour for a period of 2 hours. Each sample was diluted tenfold in sterile distilled water and plated onto nutrient agar. Agar plates were incubated at 37°C overnight and viable counts were performed the following day using the Miles and Misra method [24].

2.3 Comparison of photocatalytic disinfection of a biofilm and a non-biofilm producing strain of *S. epidermidis* and assessment of biofilm destruction

Photocatalytic disinfection of biofilm and non-biofilm producing strains of *S. epidermidis*, grown to stationary phase, was performed as described in section 2.1. For assessment of biofilm destruction, *S. epidermidis* biofilms were grown in 1/20 strength TSB medium in 24-well plates (Nunc surface plate) using the method

described by Solomon *et al.* [25] and biofilm growth was assessed using a crystal violet binding assay. For photocatalytic disinfection experiments, the media was carefully removed from the surface of each biofilm by gently pipetting. This was then replaced with either 400 ml of TiO₂ (1 g/L in sterile distilled water) or with 400 ml of sterile water only (UV control). Plates were placed under the UV lamp at a distance of 10 cm and illuminated for 2 hours. At times 0, 30, 60 and 90 minutes, groups of wells were covered with aluminium foil to prevent further UV exposure. Dark controls were achieved by covering wells containing the TiO₂ suspension with aluminium foil for the duration of the experiment. At the end of the illumination period cell viability was assessed using the resazurin assay as previously described [22]

2.4 Assessment of the effect of pH on photocatalytic disinfection on *E. coli* K12

Photocatalytic disinfection experiments were performed as described in section 2.1, using *E. coli* K12 grown to stationary phase, except that this time the pH of each reaction mixture was maintained at 4 or 8 for the duration of the illumination period by the addition of either 1 M HCl or 1 M NaOH.

2.5 Photocatalytic disinfection of *E. coli* K12 using a Light Emitting Diode (LED)

Photocatalytic disinfection experiments using stationary phase *E. coli* K12 were repeated as described in section 2.1 using an energy efficient 1.05 W 365 nm UV LED array. The UV LED array was positioned 4 cm above the reaction vessels with a viewing angle of 70 ° to allow for the best coverage of the reaction mixture. As described in the previous section, the photon flux of the LED was determined using actinometry and was found to be 5.59×10^{-8} Einstein's min⁻¹.

3. Results and discussion

3.1 Assessment of photocatalytic disinfection of *E. coli* K12 in stationary and logarithmic phases of growth

The impact of bacterial growth phase on photocatalytic activity was found to be significant, as shown in Figure 1. Cells in the logarithmic phase were seen to be

completely removed within a 90 min irradiation period. In contrast to this however, cells in the stationary phase showed higher resistance to photocatalytic oxidation and only achieved 99.9 % disinfection following 2 hrs of irradiation. These findings agree with those of Rincón and Pulgarin [16] who also showed that *E. coli* grown in stationary phase was more resistant to photocatalytic destruction than logarithmic phase cultures. They suggested that the *rpoS* regulator, which activates the expression of the genes required for survival under stress and starvation conditions such as those experienced in the stationary phase of growth, was responsible for this resistance. Photocatalytic destruction of bacteria has been shown to take place via cell wall destruction [10, 11, 13, 14] and a correlation between complexity of the cell wall structure and microbial resistance to photocatalytic destruction has been reported [15, 16]. Results from this study, however, suggest that in addition to the known structural differences in cell wall structure among different microorganisms, the changes to cell wall structure which take place during the stationary phase of growth, to strengthen and protect it from mechanical damage, are also important in determining microbial susceptibility to photocatalytic disinfection. These findings have important implications for the design of disinfection parameters in photocatalytic reactors and show that residence times must be enhanced where possible to ensure sufficient destruction of all bacteria present, regardless of the growth phase.

Figure 1.

3.2 Comparison of photocatalytic disinfection of a biofilm and a non-biofilm producing strain of *S. epidermidis* and assessment of biofilm destruction

Biofilm production is a virulence mechanism which enables bacteria to grow in sessile communities and confers upon them resistance to many antimicrobial agents. In their natural environment bacteria more often form biofilms rather than grow as single planktonic cells. In this study we wanted to investigate whether or not the ability to form a biofilm conferred upon bacteria greater resistance to photocatalytic disinfection when in planktonic form. The *S. epidermidis* strain capable of biofilm production, however, did not appear to be more difficult to destroy by conventional photocatalysis when it was not grown under conditions to promote biofilm production, Figure 2.

Figure 2.

To further investigate the results shown in figure 2, the photocatalytic destruction of the actual biofilm was also assessed. Results from the resazurin viability assay (Figure 3) show that *S. epidermidis* biofilms were destroyed relatively quickly (within 30 minutes treatment time). Dark controls remained viable at the end of the treatment time although some cell death was observed in the UV only control group. Biofilms are notoriously difficult to destroy using conventional water treatment methods, yet few studies have been carried out assessing the efficacy of photocatalysis to destroy biofilms in water [22, 26,27]. We previously reported on the successful photocatalytic destruction of a biofilm from a gram negative organism (*E. coli*). The results from this study, further support the use of photocatalysis as a reliable method for biofilm destruction in both gram positive and gram negative bacteria.

Figure 3.

3.3 Assessment of the effect of pH on photocatalytic destruction of *E. coli*

The impact of pH on any photocatalytic system is crucial, however understanding its role in a biological based reaction can present a challenge. Figure 4 shows that when photocatalytic disinfection experiments were performed at pH 8 no bacterial destruction was observed. Control experiments were not affected by the increase in pH indicating that pH changes alone were responsible for the observed effects. Previous studies looking at the effect of gradually reducing the pH during treatment time, on photocatalytic destruction of *E. coli* K12 [18] showed a greater loss of bacteria occurred when compared to controls. Authors suggested that photocatalytic disinfection was due to the interaction between pH modification, direct action of light and attack of photogenerated oxidative species. In this study we did not observe any adverse effects of maintaining the pH value at 4 for the duration of the disinfection period. We have however shown that maintaining a constant pH value of 8, for the

duration of the illumination period resulted in no bacterial destruction taking place. The observed effect of no bacterial destruction under these conditions is likely to be due to changes in the electrostatic charge of TiO_2 which is known to be affected by changes in pH [28] rather than any direct pH effects on the bacteria. At neutral pH TiO_2 has a point of zero charge but as the pH is increased the surface of the catalyst becomes increasingly negatively charged which repels the already negatively charged bacteria resulting in reduced or no bacterial destruction. Schwegmann *et al.* [28] found that photocatalytic disinfection rates depended on the electrostatic interaction between cells and TiO_2 . Furthermore, catalyst particle size, surface charge, and band edge positions of TiO_2 have been shown to be strongly influenced by pH [29]. The pH of many freshwater sources is around 6-8 and the pH of seawater is around 8.1 therefore pH could potentially be a limiting factor in enabling efficient photocatalytic disinfection to take place. These findings have important implications in the design of photocatalytic reactors for use in water disinfection studies and suggest that water samples may require buffering to ensure efficient functioning of the catalyst.

Figure 4.

3.4 Photocatalytic disinfection of *E. coli* using an energy efficient LED light source

The reported use of UV LED's to improve the photocatalytic process has been steadily increasing in the literature due to a number of key advantages in relation to energy efficiency; minimised photon wastage due to specific peak wavelengths, reduced power consumption and the ability to be incorporated into larger arrays [30–32]. In order to improve energy efficiency in this study, the 48 W UV lamp was replaced with a 1.05 W 365 nm UV LED array. The photocatalytic disinfection experiments carried out using the 48 W lamp and the UV LEDs showed a similar level of bacterial disinfection (Fig. 5). The photon flux and photonic efficiency of the two different light sources were calculated. The results shown in Table 1 also highlight the key advantages of deploying LEDs over high power lamps that often provide a wider spectral output. Interestingly, the photon flux of the 48 W lamp was higher than that of LEDs, despite being positioned further from the reaction solution and being required to pass through a borosilicate beaker to reach the TiO_2 surface. The photonic

efficiencies however, showed that the LEDs, per photon, were capable of achieving increased disinfection over that of the 48 W lamp. This was likely due to the number of ‘wasted photons’ delivered by the 48 W lamp, which while contributing to the photon flux may not be capable of promoting electron excitation in TiO₂. As already stated, the spectral output of LEDs provides specific peak wavelengths which, when matched with the electronic structure of catalysts, can significantly reduce photon wastage.

Table 1

Figure 5.

When designing a photocatalytic reactor, one of the fundamental design features is the light source, as photon delivery, penetration and distribution is vital to achieving electron excitation. The illumination system which was employed in most of the experiments in this study was a 48 W UV lamp which peaks at 370 nm. While this lamp has enabled high levels of bacterial disinfection, the implementation of the high-powered UV LEDs has enhanced the process further. UV LEDs are more easily incorporated into reactor designs, they use less energy and allow for a much more controlled reaction. By using UV LEDs, the photons are delivered in a narrow spectral range, meaning they reach the photocatalyst more efficiently, so the quantity of electrons being promoted from the valence band to the conduction band can be better controlled. A consequence which is often seen with high powered lamps in photocatalytic reactions is the high volume of electrons being promoted to the conduction band, which results in an increased rate of recombination [33, 34]. This has a negative impact on the efficiency of the reaction, as so much power is being fed into the reaction as light, but only a small amount of photocatalytic activity can take place with most light energy being wasted as heat in recombination. High-power lamps also accelerate reactions so that the subtle differences in mechanisms are not seen; whereas lower power lights will slow the kinetics and may show these differences.

4. Considerations for future reactor development

Addressing, and simulating, the vast range of real-world parameters that can impact on photocatalytic bacterial disinfection in a laboratory is a significant challenge. Despite this, it is crucial these parameters are considered in view of reactor designs

moving forward. The results shown in this study, highlight two key parameters which should be incorporated into designs; catalyst-target interactions at the surface along with irradiation delivery and distribution. It is also worth noting, that these parameters should not be considered as independent of one another as adjusting them will have significant impact on the overall system.

The impact of pH and bacterial growth phase in this study, clearly indicated the importance of surface chemistry and increased contact time between cells and the photocatalyst in bacterial disinfection. When the growth phase of bacterial cells is not controlled, it becomes paramount that sufficient interaction is achieved to ensure complete bacterial destruction. Maintaining a mildly acidic pH will also enhance this by creating an electrostatic attraction between the negatively charged bacteria and positively charge TiO_2 surface. The deployment of a suspended photocatalytic system is advantageous in view of these parameters as TiO_2 suspensions can provide a pH range of 4-6, while maintaining a high surface area to facilitate surface bound reactions. A major limitation to suspended systems, however, is scale and downstream processing requirements, which is further highlighted when dealing with nanoparticles and any form of water treatment. An additional limitation is photon masking and shading as a result of using highly loaded suspensions.

Based on the above observations, catalyst platform and deployment clearly becomes a key parameter. The development of an immobilised catalyst reactor can alleviate a number of the issues around downstream processing and photon delivery. A fixed catalyst surface requires little to no filtration, while being fully irradiated with no significant impact from shading or masking. The limitations to such a system, however have been well documented in bacterial disinfection studies and includes mass transport limitations and reduced surface interactions [20].

A spinning disc reactor may offer a possible solution to this problem. The continuous movement of the coated disc may present greater opportunities for interactions between catalyst and bacteria to take place, while maintaining excellent photon penetration. Spinning discs have been used previously for biodiesel production and condensation polymerisation, [35,36]. They have also been used for the successful photocatalytic degradation of constituents from wastewater e.g. phenols, dyes and pharmaceuticals [37–40] but there have been no reports, to date, of their use in bacterial disinfection studies. The development of a spinning disc reactor for

photocatalytic bacterial disinfection is currently the focus of on-going work with a view towards addressing the parameters discussed above.

5. Conclusion

This study highlights several important factors which must be considered when designing parameters for photocatalytic disinfection studies. The first of these is the phase of growth that target bacteria are in. Results from this study have shown that cells in the stationary phase of growth are considerably more resistant to photocatalytic destruction than cells in the logarithmic growth phase. This is likely to be due to the strengthening of the cell wall which takes place during stationary phase to protect bacteria from external stressors. Since bacteria in the natural environment may be in different growth phases; the design of photocatalytic disinfection reactors must be such that they can effectively kill all bacteria regardless of growth phase. Some studies have shown differences in disinfection times between gram negative and gram positive bacteria [15, 16] however, in this study no differences in disinfection times were observed for both *E. coli* and *S. epidermidis*.

Studies assessing whether or not the capacity of an organism to express a virulence factor, in this case biofilm production, conferred upon it greater resistance to photocatalytic destruction, proved negative. Results showed that no differences in disinfection rates were observed for a biofilm producing strain of *Staphylococcus epidermidis* compared with that of a non-biofilm producing strain, when grown in planktonic conditions. Results did however show that *S. epidermidis* biofilms could be successfully destroyed by photocatalysis.

This study also showed the importance of pH control during photocatalytic disinfection studies. Raising the pH to 8 prevented any bacterial destruction from taking place. This is thought to be due to lack of effective combination of catalyst and target bacteria due to changes in the electrostatic charge of TiO₂. This is particularly significant since the pH of many natural water sources is around 8. Therefore, to be effective a photocatalytic reactor would need to have an in-built pH measurement and buffering system.

Taking into consideration the bacterial, environmental and physical parameters shown to be of importance in reactor design, a spinning disc reactor was fabricated with an emphasis on greater light penetration, increased mass transfer and photonic efficiency to promote OH radical formation. Control of pH can also easily be incorporated in this unit with readily available process control modules. Initial tests showed it to be photocatalytically active; this reactor will be used in future bacterial disinfection studies.

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Captions for Figures and Table.

Figure 1 Photocatalytic destruction of *E. coli* in log and stationary phase at pH 4. ●

:TiO₂ + UV log, ◆ : UV Only log, ■ : TiO₂ Only log, ● : TiO₂ + UV stat, ◆ : UV Only stat, ■ :TiO₂ Only stat.

Figure 2 Photocatalytic destruction of biofilm producing and non biofilm producing strains of *S. epidermidis* in stationary phase at natural pH. ● :TiO₂ + UV biofilm, ◆ :

UV Only biofilm, ■ : TiO₂ Only biofilm, ● :TiO₂ + UV non biofilm, ◆ :UV Only non biofilm, ■:TiO₂ Only non biofilm.

Figure 3 Photocatalytic destruction of *S. epidermidis* biofilms. (Blue: dead cells, Pink: live cells)

Figure 4 Photocatalytic destruction of *E. coli* in stationary phase at pH 4 and pH 8.●:

TiO₂ + UV pH 4, ◆ : UV Only pH 4, ■ : TiO₂ Only pH 4, ● :TiO₂ + UV pH 8, ◆ : UV Only pH 8, ■ :TiO₂ Only pH 8.

Figure 5 Photocatalytic destruction of *E. coli* in stationary phase at pH 4 with 1.05 W

UV LEDs and 48 W lamp ●: TiO₂ + UV 48 W, ◆ : UV Only 48 W, ■ : TiO₂ Only 48 W, ● :TiO₂ + UV LED, ◆ : UV Only LED, ■ :TiO₂ Only LED.

Table 1 Photon flux and photonic efficiency comparison between 48 W lamp and 1.05 W LED

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Table 2

Illumination Source	Photon flux (Einstein's/min)	Photonic efficiency (CFU/Photon)	83.62 % increase in efficiency
48 W Lamp	1.06×10^{-7}	1.22×10^{11}	
1.05 W LED	5.59×10^{-8}	7.45×10^{11}	