Assessment of *Escherichia coli* reactivation after photocatalytic water disinfection using flow cytometry: comparison with a culture-based method
Jasjeet Kaur, R. Karthikeyan and R. Smith

**ABSTRACT**

The photocatalytic process generates highly reactive oxidative species, such as hydroxyl radicals, which enable mineralization of cellular compounds. Microorganisms often tend to lose their culturability after disinfection, but could remain viable to proliferate under optimum conditions. Estimation of bacterial counts using culture-based methods pose limitations in differentiating viable, non-viable, and viable but non-culturable (VBNC) cells. Presence of viable and VBNC state cells in disinfected water could pose a potential health risk and accurate estimation of these cells through a molecular method is critical. Assessment of live/dead states of an indicator waterborne pathogen, *Escherichia coli* (ATCC®10798) after disinfection was conducted using flow cytometry. Photocatalysis was carried out under low pressure ultraviolet (LP UV) radiation alone and at four titanium dioxide (TiO₂) concentrations (1, 0.75, 0.5 and 0.1 g/L). During the repair period, flow cytometry showed 4–5 log₁₀ higher cell counts than the culture-based method. Photocatalysis using 0.1 g/L TiO₂ resulted in 50% cells with intact cell membrane during the repair period and lowered the repair rate of the ATCC®10798, *E. coli* after disinfection.

**Key words** | disinfection, flow cytometry, pathogens, water quality

**INTRODUCTION**

Water disinfection using ultraviolet (UV) rays is becoming a popular treatment option because of high inactivation rates with low doses and no carcinogenic by-product formation as compared with other disinfection methods (Lindenauer & Darby 1994; Boorman *et al.* 1999; Liu & Yang 2005; Galvez *et al.* 2007; Quek & Hu 2008; Shang *et al.* 2009; Hallmich & Gehr 2010). The most commonly used UV lamps are low pressure (LP) and medium pressure (MP) mercury lamps. LP UV lamps emit monochromatic UV radiation at 254 nm which is close to the germicidal wavelength of 260 nm, whereas MP UV lamps emit a broad spectrum of wavelengths ranging from 200 to 400 nm (Quek & Hu 2008). Upon absorption of high energy radiation, microbial DNA is altered via formation of pyrimidine dimers, namely: cis-syn cyclobutane pyrimidine dimers (CPD) and other photo-products which prevents DNA replication (Lindenauer & Darby 1994; Quek & Hu 2008; Shang *et al.* 2009; Hallmich & Gehr 2010).

However, microorganisms have evolved with repair mechanisms such as photoreactivation and dark repair, which are able to reverse the UV-induced damage to DNA (Lindenauer & Darby 1994; Sanz *et al.* 2007; Quek & Hu 2008; Shang *et al.* 2009; Hallmich & Gehr 2010). Photoreactivation involves photoreactivating light in the wavelength of 330–480 nm to activate photolyase enzyme, which enables splitting of the CPD back to its original monomerized state (Sanz *et al.* 2007; Shang *et al.* 2009). In general, dark repair involves nucleotide excision of damaged DNA which requires synchronization of dozens of proteins to repair and excise pyrimidine dimmers. Light independent repair of the damaged DNA is termed as dark repair mechanism. The reactivation of
microorganisms by either means affects the overall efficiency of the UV disinfection system (Sanz et al. 2007; Quek & Hu 2008; Shang et al. 2009).

In recent years, photocatalytic oxidation processes have emerged as cheap and effective means of water and wastewater disinfection (Liu & Yang 2003; Ibanez 2007; Theron et al. 2008; Shang et al. 2009; Chong et al. 2010). Advanced photocatalytic processes generate highly reactive species such as hydroxyl radicals in water, which enable mineralization of cellular organic compounds and inactivation of microorganisms (Dunlop et al. 2002; Liu & Yang 2003; Galvez et al. 2007; Ibanez 2007; Malato et al. 2007; Chong et al. 2010). Among the semiconductor catalysts, titanium dioxide (TiO₂) has gained a lot of interest, as it is the most active photocatalyst in the energy range of 300–390 nm (Dunlop et al. 2002; Galvez et al. 2007; Reddy et al. 2008; Chong et al. 2010). The energy range for excitation of TiO₂ falls in the UV radiation (200–400 nm), thus a combination of UV and TiO₂ may result in oxidation of cellular compounds and proteins through photocatalysis as well as DNA damage due to UV (Liu & Yang 2003; Shang et al. 2009; Chong et al. 2010). The reactivation kinetics of bacteria after photocatalysis should be studied to effectively determine the subsequent reactivation after disinfection.

Conventional culture-based methods have limitations in effective determination of membrane oxidation, cell state and cell viability. The culture-based method does not identify active, inactive, and dead bacteria (Berney et al. 2008). Also, it usually underestimates bacterial counts by at least 2 log₁₀ orders of magnitude and is unable to detect viable but non-culturatible (VBNC) state cells (Egli 2008; Hammes et al. 2008; Hammes & Egli 2010). The combination of flow cytometry with fluorescent stains has been applied to characterize bacterial cells based on membrane integrity, cell state, and conditions. A clear distinction between viable and non-viable cells, in contrast to the conventional plating method will be possible with flow cytometry (Berney et al. 2007, 2008; Hammes et al. 2008). Estimation of VBNC state cells in disinfected water would be helpful for assessing the disinfection efficacy, as some microorganisms lose their cultivability after stress conditions.

Thus, in this study, the effect of TiO₂-based photocatalysis on the reactivation potential of a non-pathogenic E. coli strain (Escherichia coli K-12 (ATCC®10798)) was evaluated. In general, E. coli is widely used as an indicator of fecal contamination. It was hypothesized that photocatalytic disinfection would decrease the subsequent photoreactivation and dark repair after disinfection. Also, higher titanium dioxide concentrations would decrease the rate of E. coli (ATCC®10798) cell repair. Assessment of flow cytometry along with nucleic acid stains to effectively enumerate live/dead states of E. coli during the reactivation period was studied. A membrane viability assay was utilized to assess the cell membrane permeability caused during disinfection treatment. Comparison with a culture-based method was also analyzed to assess the repair potential of bacteria after disinfection. Results from this laboratory study are presented in this manuscript.

MATERIALS AND METHODS

Titanium dioxide stock solution

Stock solution of titanium dioxide (10 g/L) was prepared by mixing 2 g of titanium dioxide powder (Degussa P25, Fisher Scientific, USA) in 200 mL nuclease-free deionized (DI) water. The stock solution was stored in a dark room at room temperature. Before each experiment, the stock solution was ultra-sonicated for 3 min to ensure homogenous suspension of titanium dioxide.

Bacterial strains and growth

Escherichia coli K-12 (ATCC®10798) strain was inoculated in 50 mL of Luria broth (Sigma Aldrich, USA) by transferring one loop from the culture stock and incubated at 37 °C for 22–24 h until the cell count reached 10^8 CFU/mL. The growth media was continuously mixed to ensure proper distribution of nutrients to the organisms. From the growth media, 20 mL culture was added to a 200 mL working volume in a 250 mL glass beaker to achieve 10⁻¹ dilution of the culture. All glassware, nuclease-free DI water and media used for bacterial growth were autoclaved at 121 °C for 20 min prior to each experiment.
Photoreactor setup

A UV-C LP germicidal lamp (Bryant energy, USA) with a wavelength of 254 nm was mounted horizontally in a polyvinyl chloride (PVC) box of dimension 81 × 61 × 51 cm (L × B × H). Three 250 mL Pyrex® beakers (7.62 cm in height and 6.35 cm in diameter) containing 200 mL water sample and magnetic stir bars, were placed on a magnetic stirrer under the LP UV lamp. The average UV irradiance reaching the irradiated sample was calculated using the Morowitz equation (Morowitz 1950; Hallmich & Gehr 2010):

\[
I_{avg} = I_0 \left( 1 - e^{-d \times \alpha} \right) \frac{d}{d \times \alpha}
\]

(1)

where, \(I_0\) is incident irradiance (mW/cm²); \(d\) is water depth under UV irradiation (\(d = 6.35\) cm); \(\alpha = \ln (1/T)\), \(T\) is transmittance at 254 nm with cell path length of 1 cm; \(I_{avg}\) is the average irradiance reaching the sample (mW/cm²). The average light intensity (irradiance) measured before sample exposure using a radiometer ranged from 220 to 245 μW/cm². The required UV dose was calculated using the following equation by adjusting the exposure time to achieve 5 log reductions for all the treatments (Hallmich & Gehr 2010).

UV dose (mW.s/cm²) = \(I_{avg}\) (mW/cm²) × time (s)

(2)

Batch disinfection study

All the experiments were conducted in triplicate at average pH 6.5 and 22 ± 2 °C to ensure their reproducibility. Photocatalysis and dark repair studies were conducted on different days. For each experiment, 200 mL of working volume was used with 0.34% (w/v) salinity. To the above mixture, a desired volume from TiO₂ stock solution and 20 mL of the bacterial culture was added. The bacterium was not washed before addition in order to mimic typical water and treated wastewater conditions, and to avoid any stress on bacteria due to centrifugation. The reactors were stirred at 400 rpm to ensure complete mixing. Photocatalytic disinfection was carried out at 1, 0.75, 0.5 and 0.1 g/L TiO₂ concentrations in suspensions subjected to LP UV irradiation. Disinfection without TiO₂ and exposed to UV-C irradiation was also studied. Two dark controls: one with 1 g/L TiO₂ and the other without TiO₂ were prepared to study the presence of inactivated or unexcited TiO₂ on bacteria for any lethal damage. After the required 5 log₁₀ reduction in cell counts, water samples were immediately exposed either to visible light with average intensity of 30,000 lux or covered with aluminum foil (dark conditions) for 4 h, respectively. The photocatalytic and dark repair were studied to determine the effect of TiO₂ in suppressing the repair after LP UV-TiO₂ disinfection. Regular time interval samples were taken and bacterial enumeration was done by the spread plate method and flow cytometry.

Bacteria enumeration

Culture-based method

*Escherichia coli* concentrations were determined by the spread plate method. For this method, appropriate dilutions were performed in DI and nuclease free water and 100 μL of the diluted sample was spread plated on the MacConkey agar plates. The plates were incubated for 24 h at 37 ± 1 °C. After incubation, *E. coli* colonies were counted and averaged for the triplicate plates and recorded as colony forming units (CFU/mL).

Flow-cytometric measurements

*Escherichia coli* cells with intact (live) and compromised cell membranes (dead) were also determined using a FACS Calibur (Becton Dickinson Immuno Cytometry Systems, San Jose, CA) flow cytometer, equipped with a 15 mW air-cooled argon laser, using CellQuest (Becton Dickinson) acquisition software. LIVE/DEAD Baclight® bacterial viability and counting kit (Invitrogen, USA) was used for differentiating the live and dead cells. For the flow cytometric measurement, 10 μL of the water sample from the reactors was taken in a 12 × 75 mm (H × D) BD Falcon tube (Fisher Scientific, USA). To this, components from the Baclight® kit were added. A final concentration of 30 and 5 μM for propidium iodide (PI) and STYO9, respectively, was used for the analysis. Green fluorescence from SYTO9 was collected through a 530/30 nm bandpass filter and red fluorescence from PI through a 670 nm longpass filter.

The instrument threshold was set on SYTO9 fluorescence. List mode data were acquired on a minimum of
10,000 viable cells defined by light scatter gates and SYTO9 staining. Data analysis was performed in FlowJo (version 8.8.7, Treestar, Inc., Ashland, OR). Bacterial cells were defined by sequential gates, first on their light scattering properties (forward versus side scatter) then on their SYTO9 staining patterns (SYTO9 versus side scatter).

Repair assessment

In order to assess the recovery of *E. coli* during photoreactivation and dark repair, two parameters, namely: percent log repair and repair rate were examined. To negate the variability in initial cell counts and log reduction after treatment, the following formula was used (Quek & Hu 2008):

\[
\% \text{log repair} = \frac{N_t - N_0}{N_{\text{initial}} - N_0} 
\]

(3)

where \(N_t\) is log CFU/mL of *E. coli* at time of exposure, \(t\), to photoreactivation/dark repair conditions; \(N_0\) is log CFU/mL of *E. coli* immediately after LP UV disinfection; and \(N_{\text{initial}}\) is the initial concentration of *E. coli* before LP UV irradiation (log CFU/mL). The rate of repair was calculated for the first hour of repair using the following equation (Quek & Hu 2008):

\[
\text{Rate of repair (log h}^{-1}) = \frac{N_1 - N_0}{t}
\]

(4)

where \(N_1\) is log CFU/mL of *E. coli* after 1 h of photoreactivation/dark repair and \(t\) is the time interval between two samples, here 1 h.

Statistical analysis

Response in terms of CFU/mL for the culture-based method and counts/mL for flow cytometry was recorded for each treatment and the data were analyzed using Design Expert®. The full factorial model was used to analyze the data from the culture-based and flow cytometry methods. A two factor model with different levels for each was selected in Design Expert®. Factor A was titanium dioxide concentration with five levels and factor B was time with five levels (photoreactivation and dark repair period). In total, nine data points were chosen for the statistical analysis. A Type I error value of 0.05 was selected for conducting analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Culturable *E. coli* cells after LP UV and LP UV-TiO₂ disinfection: culture-based method

The average initial concentration of *E. coli* (\(N_{\text{initial}}\)) was \(10^7\) CFU/mL and after LP UV-TiO₂ disinfection, the concentration was reduced to an average value of \(10^2\) CFU/mL. The LP UV-TiO₂ treatments had caused nearly 5 log₁₀ reductions for all the treatments. During the repair period, *E. coli* concentrations were increased to an average value of \(10^4\) CFU/mL (Figure 1). The photoreactivation and dark repair for all the treatments had caused nearly 2 log₁₀ increase in the *E. coli* counts. This resulted in a 3 log₁₀ net reduction after accounting for the repair period. So, in order to achieve 5 log₁₀ reduction, a disinfection treatment should be designed to achieve 7 log₁₀ reduction during the exposure time.

These results did not support the hypothesis that addition of TiO₂ would lower the repair after disinfection. The presence of TiO₂ did not drastically reduce the *E. coli* repair in comparison to LP UV (Figure 1). The hypothesis that a higher TiO₂ concentration would reduce the subsequent repair was also not supported. Data in Table 1 show that 0.1 g/L TiO₂ had the lowest repair rate of 0.87 and 0.95 log h⁻¹ during photoreactivation and dark repair period, respectively. The percent log repair in the presence of 0.1 g/L TiO₂ was the lowest among all other treatments during 4 h of repair (Figure 2). Photocatalysis at 0.1 g/L TiO₂ under LP UV resulted in 17.2% dark repair of cells with damaged DNA, whereas the remaining *E. coli* cells had their cellular compounds and membrane oxidized beyond repair. The percent repair during photoreactivation was slightly lower than the dark repair period for 0.1 g/L TiO₂ (Table 1). Photocatalysis might have caused oxidation of the photolyase enzyme which is responsible for repairing pyrimidine dimers back to their original state. This was achieved through the oxidative damage done to the cellular compounds and cell membrane, which had left cells in a condition beyond that which they could repair by photoreactivation (Dunlop et al. 2002; Benabbou et al. 2007; Shang et al. 2009; Chong et al.
It should be noted that Liu & Yang (2003) have found that higher TiO<sub>2</sub> concentrations resulted in a significant log reduction. Loss of cultivability was observed after disinfection under LP UV and photocatalysis, due to reduction in colony forming units on agar plates.

No reactivation in the dark was observed by Benabbou et al. (2007) after UV-C and photocatalysis using TiO<sub>2</sub> at varying concentrations. However, we had observed significant recovery of <i>E. coli</i> during photoreactivation and dark repair. It could be seen that <i>E. coli</i> cells were being repaired even after 2 h of dark repair (Figure 2(b)). This finding is not in agreement with a previous study (Quek & Hu 2008) in which dark repair was leveled off beyond 2 h of repair after reaching a maximum value. Although a gradual leveling off was seen for the photoreactivation period beyond 2 h which is in agreement with the previous study (Quek & Hu 2008). This shows that addition of TiO<sub>2</sub> had lowered the number of bacterial cells which could repair their damaged DNA and pyrimidine dimers under visible light conditions. The discrepancies in results could be attributed to differences in the <i>E. coli</i> strains used, variation in experimental geometry, and differences in UV intensities utilized for irradiation.

<i>Escherichia coli</i> with intact cell membrane after LP UV and LP UV-TiO<sub>2</sub> disinfection: flow cytometry

All water samples were analyzed by a flow cytometer to enumerate cells with or without cell membrane damage, as

Table 1 Culture-based assessment of <i>E. coli</i> (ATCC®10798) log reduction, percent log repair, and repair rate for different disinfection treatments for (a) photoreactivation and (b) dark repair

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Log reduction&lt;sup&gt;†&lt;/sup&gt;</th>
<th>% log repair&lt;sup&gt;†&lt;/sup&gt;</th>
<th>Repair rate (log h&lt;sup&gt;−1&lt;/sup&gt;)&lt;sup&gt;†&lt;/sup&gt;</th>
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<tr>
<td></td>
<td>a</td>
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<td>a</td>
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<tr>
<td>LP UV + 1 g/L TiO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>5.37 ± 0.15&lt;sup&gt;§&lt;/sup&gt;</td>
<td>5.27 ± 0.29&lt;sup&gt;§&lt;/sup&gt;</td>
<td>16.80 ± 0.39&lt;sup&gt;§&lt;/sup&gt; 20.28 ± 1.46&lt;sup&gt;§&lt;/sup&gt; 0.90 ± 0.01&lt;sup&gt;§&lt;/sup&gt; 1.07 ± 0.12&lt;sup&gt;§&lt;/sup&gt;</td>
</tr>
<tr>
<td>LP UV + 0.75 g/L TiO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>5.32 ± 0.07&lt;sup&gt;§&lt;/sup&gt;</td>
<td>5.50 ± 0.15&lt;sup&gt;§&lt;/sup&gt;</td>
<td>18.50 ± 1.04&lt;sup&gt;§&lt;/sup&gt; 23.42 ± 2.45&lt;sup&gt;§&lt;/sup&gt; 0.98 ± 0.04&lt;sup&gt;§&lt;/sup&gt; 1.29 ± 0.17&lt;sup&gt;§&lt;/sup&gt;</td>
</tr>
<tr>
<td>LP UV + 0.5 g/L TiO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>5.36 ± 0.02&lt;sup&gt;§&lt;/sup&gt;</td>
<td>5.28 ± 0.10&lt;sup&gt;§&lt;/sup&gt;</td>
<td>21.60 ± 1.66&lt;sup&gt;§&lt;/sup&gt; 22.97 ± 5.85&lt;sup&gt;§&lt;/sup&gt; 1.16 ± 0.09&lt;sup&gt;§&lt;/sup&gt; 1.21 ± 0.28&lt;sup&gt;§&lt;/sup&gt;</td>
</tr>
<tr>
<td>LP UV + 0.1 g/L TiO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>5.38 ± 0.13&lt;sup&gt;§&lt;/sup&gt;</td>
<td>5.56 ± 0.01&lt;sup&gt;§&lt;/sup&gt;</td>
<td>16.20 ± 0.52&lt;sup&gt;§&lt;/sup&gt; 17.16 ± 0.16&lt;sup&gt;§&lt;/sup&gt; 0.87 ± 0.03&lt;sup&gt;§&lt;/sup&gt; 0.95 ± 0.01&lt;sup&gt;§&lt;/sup&gt;</td>
</tr>
<tr>
<td>LP UV</td>
<td>5.29 ± 0.12&lt;sup&gt;§&lt;/sup&gt;</td>
<td>5.09 ± 0.38&lt;sup&gt;§&lt;/sup&gt;</td>
<td>17.50 ± 0.81&lt;sup&gt;§&lt;/sup&gt; 22.03 ± 9.75&lt;sup&gt;§&lt;/sup&gt; 0.92 ± 0.02&lt;sup&gt;§&lt;/sup&gt; 1.12 ± 0.56&lt;sup&gt;§&lt;/sup&gt;</td>
</tr>
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</table>

<sup>1</sup>Viable cell count log reduction during disinfection.

<sup>2</sup>Four hour repair period.

<sup>§</sup>Standard deviations of three experiments are presented.
assessed by their ability to exclude PI (Berney et al. 2007; Hammes et al. 2008; Khan et al. 2010; Remy et al. 2012). The Backlight® kit contains nucleic acid stains which compete for the same target location when both stains are present. SYTO9 binds to the nucleic acid of microorganisms irrespective of the cell membrane condition, whereas PI only enters cells with a damaged cell membrane and stains the nucleic acid. The standard side scatter plot generated for *E. coli* cells treated with 0.85% saline solution (intact cell membrane, live cells) and 70% isopropyl alcohol (compromised cell membrane, dead cells) is shown in Figure 3. The standard side scatter plot shows only two main regions, namely: live and dead region for intact cell membrane and compromised cell membrane, respectively.

The side scatter plot for all the treatments (Figure 4) shows three states of *E. coli* namely: intact cell membrane (live), compromised cell membrane (dead), and intermediate cells after 4 h of photoreactivation and dark repair. It is evident from flow cytometry analysis that LP UV-TiO₂ treatments had induced an intermediate state between *E. coli* with compromised (dead) and intact (live) cell membranes. The intermediate region might have resulted from the partial oxidation of cell membranes by reactive oxygen species, thereby leading to a state where SYTO9 and PI had equal competence for DNA (Figure 4). The detection of an intermediate state with flow cytometry using variable amounts of stains has also been reported in previous research (Berney et al. 2008; Khan et al. 2010).

*Escherichia coli* with intact cell membrane counts during photoreactivation and dark repair for all the treatments are shown in Figure 5. The initial *E. coli* intact cell membrane concentration was 10⁹ counts/mL and after disinfection, a minor log reduction in concentration was seen. It is in contrast to the CFU/mL obtained from the culture-based method, which showed a 5 log₁₀ reduction after disinfection. During the repair period, flow cytometry showed 4–5 log₁₀ higher counts than the culture-based method. Flow cytometry detection is irrespective of the culturability of the cells, and this finding is also supported by previous research (Egli 2008; Hammes et al. 2008; Khan et al. 2010). It could be seen that there was no extensive damage done to the cell membrane due to reactive oxygen...
species, such as hydroxyl radicals. The results are contrary to a previous study which had reported that cell membrane integrity was lost after UV-C- and TiO₂-based photocatalysis (Gogniat et al. 2006; Remy et al. 2012). They had utilized the LIVE/DEAD Baclight kit and assessed the membrane damage using a fluorescence microscope. Their results had indicated the cell membrane integrity was lost after exposure to UV-C irradiation and under photocatalysis. UV-C irradiation not only damages the DNA but also gets absorbed by membrane amino acids and lipids, which get oxidized, and results in cell membrane damage (Remy et al. 2012). However, in this study more than 80% cells had intact membranes for all the treatments, except for the treatment with TiO₂ at 0.1 g/L concentration, which had only 50% of cells with intact membranes. It could be seen...
that LP UV + 0.1 g/L TiO$_2$ treatment resulted in lower intact cell membrane counts in comparison with the remaining treatments. It is in agreement with the finding from the culture-based method in our study (Figure 1). However, the intact cell counts measured by membrane integrity were higher than culturable counts, implying the presence of VBNC state cells. However, Gogniat et al. (2006) found no VBNC state cells after TiO$_2$-based photocatalysis. It can be speculated that reduction seen in E. coli numbers could be due to DNA damage, some cell membrane damage, and possible reduction in metabolic activity.

While determining the water quality of highly sensitive distribution areas such as hospitals, nursing homes, healthcare facilities, and pharmaceutical and food manufacturing industries, culture-based methods will underestimate the concentration of pathogens, while flow cytometry will provide more realistic numbers (Egli 2008; Hammes et al. 2008; Khan et al. 2010). Furthermore, culture-based methods cannot detect VBNC cells. VBNC is that state of a bacterium which is related to the inability of the microorganism to recover metabolic activity and is considered to be reversible, as cells could be revived to a culturable state once they are exposed to favorable growth conditions. Cells retain their intact cytoplasmic membrane even when they have lost cultivability (Oliver 2010). Also, microorganisms might produce enterotoxins and remain virulent in the VBNC state (Khan et al. 2010; Oliver 2010). To avoid a potential health risk, further improvement in the water disinfection systems will be required to maintain an effective level of pathogen reduction.

**Effects of photocatalysis (LP UV-TiO$_2$) on repair of E. coli**

The E. coli concentrations measured in CFU/mL from culture-based, and cell counts/mL from flow cytometry, methods followed a normal distribution as determined from the normality plot of residual. The data also satisfied the conditions of randomness and equal variance as observed from residual vs. predicted and Box Cox plots, respectively (data not shown). The culturable (determined from culture-based method) and intact cell membrane counts (determined from flow cytometry) were significantly different for all the treatments ($p < 0.0001$, Table 2). The mean culturable E. coli cell counts were significantly affected by the titanium dioxide concentration. Cell counts during photoreactivation and dark repair showed a significant difference among five treatments (LP UV-TiO$_2$ and LP UV) for both culture-based and flow cytometry methods ($p < 0.0001$, Table 2).

Photoreactivated and dark repaired cell counts for LP UV were significantly higher ($p < 0.0072$, $p < 0.0001$, Figure 1) than LP UV + 1 g/L TiO$_2$ as determined by the culture-based method, but were not statistically different for flow cytometry methods ($p = 0.9118$, $p = 0.2165$, Figure 5). On the other hand, photoreactivated cells for LP UV + 0.1 g/L TiO$_2$ were significantly lower than LP UV treatment for both culture-based ($p < 0.0001$, Figure 1) and flow cytometry methods ($p = 0.0002$, $p < 0.0001$, Figure 2).

Thus, statistically it was evident that addition of a photocatalyst at 0.1 g/L along with LP UV had resulted in significant difference in cell counts and reduced the subsequent percentage repair after disinfection. It could be deduced that dark repair percentages could be significantly reduced by adding TiO$_2$ at 0.1 g/L to the UV disinfection systems, as compared with disinfection by LP UV alone. Previous studies have shown that photocatalysts play an important role in inactivation of pathogenic microorganisms present in water (Dunlop et al. 2002; Liu & Yang 2003; Shang et al. 2009). This study not only agrees with previous findings but also showed that a photocatalyst curbs the dark

**Table 2** | Two way ANOVA analysis on culture-based and flow cytometry data for (a) photoreactivation and (b) dark repair

<table>
<thead>
<tr>
<th>Source</th>
<th>Culture-based method</th>
<th>Flow cytometry method</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>F value $^a$</td>
<td>p-value $^a$</td>
</tr>
<tr>
<td>Model</td>
<td>819.61</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>A-TiO$_2$</td>
<td>20.34</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>B-Time (h)</td>
<td>1618.88</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

$^a$Photoreactivation.

$^b$Dark repair.
repair of E. coli after photocatalysis under LP UV. Modifications of the disinfection treatment should be done by accounting for repair rates of microorganisms in water. While considering the effectiveness of a water disinfection system, reactivation after disinfection should be evaluated. This study provides direct evidence that repair of E. coli is possible after disinfection.

Flow cytometry showed significantly higher cell counts than the culture-based method. The culture-based method had estimated only a fraction of the total bacterial cells present in water in contrast to detection using flow cytometry and nucleic acid dye stain. The number of viable pathogenic cells as enumerated by the widely used culture-based method will be less accurate in assessing the microbial quality of drinking water (Berney et al. 2007; Egli 2008; Hammes et al. 2008; Khan et al. 2010). Culture-based methods underestimate cell numbers due to: (1) cell injury, (2) inability to take up nutrients in the medium, and (3) other physiological factors which hamper culturability (Khan et al. 2010). Bacterial repair and regrowth is also affected by presence of inhibitors in selective media (Dunlop et al. 2002).

While assessing disinfection systems, total bacterial cell counts, rapid detection of pathogens, and microbial viability should be evaluated (Egli 2008). On average 60–90% microorganisms present in water are biochemically active and significantly higher than detected by cultured-based methods (Egli 2008; Hammes et al. 2008). Furthermore, intermediate states like cell membrane damage and cell injury are difficult to detect using culture-based methods. So, for bacterial enumeration there is a need for a fast, reliable and accurate method which could be utilized in optimizing a water disinfection system. The repair of bacteria should be considered for the time involved between the disinfection system and final end use points. It is very critical for selecting an appropriate retention time in water distribution systems after disinfection.

CONCLUSIONS

It can be concluded from this laboratory study that the photocatalyst had slightly lowered the percent log repair, and repair rate (log h⁻¹) of E. coli (ATCC®10798) strain during photoreactivation and dark repair after disinfection. Photocatalytic disinfection at 0.1 g/L TiO₂ resulted in better disinfection (log reduction) with a lower E. coli repair rate in comparison to LP UV alone. However, increasing the photocatalyst concentration did not significantly reduce E. coli repair after disinfection. Additionally, flow cytometry provided an accurate picture of the state of microorganisms by enabling fast and accurate counts of live and dead percentages of bacteria in water.

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