Effect of water compounds on photo-disinfection efficacy of TiO₂ NP-embedded cellulose acetate film in natural water

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ABSTRACT

Photocatalysis disinfection has great potential for irrigation water disinfection to improve fresh produce safety. Titanium dioxide (TiO₂) nanoparticle (NP)-embedded cellulose acetate (CA) film has shown effectiveness against *Escherichia coli* (*E. coli*) O157:H7 in water. The current study evaluated the effect of natural water compounds on the photo-disinfection efficacy of TiO₂ NP-embedded CA film. Humic acid, calcium carbonate (CaCO₃), and kaolin clay solutions were prepared at four concentrations, respectively. When concentration increased from 0 to 20 ml/L, inactivation of *E. coli* O157:H7 in humic acid, CaCO₃, and kaolin clay solutions decreased from 6 log to 5, 4, and 2 log CFU/ml, respectively after 3 h treatment. Turbidity, UVT-254, water hardness, total suspended solids (TSS), and total organic carbon (TOC) of the solutions were measured. UVT-254 and turbidity had the highest correlation with the inhibition effect of water compounds on photo-disinfection efficacy. A prediction equation was developed with UVT-254 and water hardness as independent variables to predict photo-disinfection efficacy in natural water. *E. coli* O157:H7 decreased by 1 and 2.5 log CFU/ml in unfiltered and filtered natural creek water samples after treatment. The results from this study showed promise in the use of TiO₂ NP-embedded CA film to inactivate pathogens in natural water.

Key words | *E. coli* O157:H7, natural water compound, photocatalysis, TiO₂ nanoparticle, water disinfection

HIGHLIGHTS

- Natural water compounds affect TiO₂ photo-disinfection efficacy in different degrees.
- UV transmittance and water hardness are important water quality parameters that can affect TiO₂ photo-disinfection efficacy.
- Prediction equation was developed to predict TiO₂ photo-disinfection efficacy in natural water.
- Filtration is an effective pre-treatment method for improving TiO₂ photo-disinfection in natural water.

INTRODUCTION

Agriculture consumes 70% of the freshwater used worldwide, and the number increases to 95% in developing countries (FAO 2008). As a crucial part of agricultural water, irrigation water is one of the main pathways by which pathogenic microorganisms can reach fresh produce (FDA 2015). Irrigating produce to be eaten raw with contaminated water may increase the risk of foodborne illness (Chalmers et al. 2000; Gu et al. 2019; Rodrigues et al. 2020). Evidence of produce contamination through contaminated irrigation water has been found in epidemiological investigations (Steele &
Irrigation water has been implicated in several *E. coli* O157:H7 outbreaks (Ackers et al. 1998; CDC 1999; Iwu & Okoh 2019). Several studies have demonstrated that pathogens can transfer from contaminated irrigation water to soil and persist in the soil for a long period (Islam et al. 2004, 2005; Jokinen et al. 2019; Kabir et al. 2020). Moreover, the occurrence of the internationalization of pathogens in plants through contaminated irrigation water has been reported (Erickson et al. 2010; Erickson et al. 2019). Improving irrigation water safety can effectively reduce the risk of fresh produce contamination.

Chlorination is the most commonly used disinfection method, however, disinfection byproducts (DBPs) that are potentially carcinogenic may be produced when chlorine reacts with natural organic matter (Marugán et al. 2008). Treatment methods such as UV and ozone are effective and have been demonstrated for drinking water treatment (Solomon et al. 2002; Loeb et al. 2012). However, these methods are costly and may not be suitable for irrigation water disinfection. The development and implementation of alternative disinfection methods are therefore needed.

Photocatalysis disinfection inactivates microorganisms by reactive oxygen species (ROS) generated when a semiconductor photocatalyst is activated by light in the presence of water and oxygen. Photocatalysis disinfection is an economic and environmentally friendly process and might be an alternative water disinfection method for irrigation water (Dasgupta et al. 2017). Titanium dioxide (TiO$_2$) has been considered the most suitable photocatalyst for solar photocatalytic reactors for water treatment. However, when applied in natural water, various organic and inorganic compounds may detrimentally affect photocatalysis disinfection of water. The effect of these water compounds on photocatalysis disinfection needs to be understood before this technology can be practically applied. Humic substances constitute the major fraction of natural organic matter (NOM) in natural water and 90% of total dissolved organic carbon (DOC) in surface water may come from humic acid (Alkan et al. 2007). It has been reported that humic acid can inhibit the photocatalysis process by acting as an ·OH scavenger (Garbin et al. 2007; Cheng et al. 2008). Calcium carbonate (CaCO$_3$) occurs in rocks, soil, and natural water world-wide. The presence of CaCO$_3$ affects water hardness and alkalinity, and it is the predominant component of scales deposited from natural water (Wang et al. 2009). The effect of CaCO$_3$ on photocatalysis inactivation is rarely reported. Kaolin clay is one of the common inorganic particles in natural water, and it contributes to turbidity in water and might be the worst particle for shielding (Liu & Zhang 2006). The effect of kaolin clay on photocatalysis disinfection in water has not been reported. Also, there is a lack of information on the comparison of the inhibition effect of different water compounds on photocatalysis disinfection.

The overall purpose of this study was to determine the effect of natural water compounds on the photo-disinfection efficacy of TiO$_2$ NP-embedded CA films. Specific objectives include:

(i) to determine the photo-disinfection efficacy of TiO$_2$ NP-embedded CA film in water containing humic acid, CaCO$_3$, and kaolin clay;

(ii) to evaluate the correlation between water quality parameters and the photo-disinfection efficacy of TiO$_2$ NP-embedded CA film in water;

(iii) to develop a prediction equation for predicting the disinfection efficacy of TiO$_2$ NP-embedded CA film in natural water.
MATERIALS AND METHODS

Preparation of TiO₂ NPs-embedded film

Aeroxide® P25 TiO₂ nanoparticles (anatase–rutilite), acetone, cellulose acetate (average Mn ~30,000 by GPC), and triethyl citrate (TEC) (>99%) were purchased from Sigma-Aldrich (St Louis, MO, USA). TiO₂ NPs-embedded CA film was prepared using a solution casting method as described in Xie & Hung (2018). An optimum TiO₂ NPs concentration at 0.82 mg/cm² on the film to achieve the highest bactericidal effect had been determined in a previous study (Xie & Hung 2020 submitted). To fabricate the film, 4 g of CA and 0.4 g of TiO₂ were dissolved and suspended in 20 ml of acetone at room temperature (24 °C), separately. TEC (1.2 g) was added to TiO₂ NPs suspension as plasticizer. An ultra-sonication bath (Model FS60, Fisher Scientific, Waltham, MA, USA) was used to assist the suspension of TiO₂ NPs. After sonication, TiO₂-solvent suspension was added into the CA solution gradually using a pipette with stirring, and the solution was continuously stirred for 2 h. Five millilitres of the mixed solution was added into each glass Petri dish (88 mm in diameter, Corning®, Sigma-Aldrich (St Louis, MO, USA)) and allowed to dry with Petri dish lid covering in a fume hood at 24 °C overnight, and then stored in a vacuum desiccator for 24 h. The thickness of the prepared film was 53.5–54.7 μm.

Preparation of bacterial strains and inoculum

A five-strain cocktail of nalidixic-acid-adapted E. coli O157:H7 strains (E009 (beef), EO932 (cattle), O157-1 (beef), O157-4 (human), O157-5 (human)) was used in this study. The strains were stored at −70 °C in tryptic soy broth (Becton Dickson and Company, Sparks, MD, USA; TSB) containing 20% glycerol. To resuscitate the bacteria, each strain was streaked on Sorbitol MacConkey agar (Hardy Diagnostics, Santa Maria, CA, USA) supplemented with 50 μg/ml nalidixic acid (Sigma-Aldrich, St Louis, MO, USA; SMACNA) and incubated at 37 °C for 24 h.

One isolated colony of each strain was then transferred into 10 ml of tryptic soy broth supplemented with 50 μg/ml nalidixic acid (TSBNA) and incubated overnight. After incubation, bacterial suspension was centrifuged at 4,000 × g for 12 min, and the supernatant was decanted and the cell pellet was resuspended in 9 ml of sterilized phosphate-buffered saline (Acros Organics, NJ, USA; PBS, pH 7.2). The inoculum was prepared by mixing 9 ml of each strain to obtain a five-strain cocktail with a concentration of about 9 log CFU/ml. The inoculum was further adjusted to a concentration of about 8 log CFU/ml by making ten-fold dilution.

Preparation of water samples

CaCO₃ was purchased from Fisher Scientific (Waltham, MA, USA). Kaolin clay and humic acid were purchased from Sigma-Aldrich (St Louis, MO, USA). Stock solutions of CaCO₃, kaolin clay and humic acid were prepared at 200 mg/L each and stored at 4 °C until use. Water samples containing various levels of CaCO₃, kaolin clay, or humic acid were prepared using the stock solutions before the experiment. The pH of these water samples was measured as about 6.5 ± 0.4. The natural water sample was obtained from a creek in Griffin, Georgia (33.2545488, −84.3114407).

Photo-disinfection of E. coli O157:H7 in water

Photo-disinfection of E. coli O157:H7 in water was performed as described in Xie & Hung (2019). In brief, the photocatalytic inactivation of E. coli O157:H7 in water was carried out in 150 ml glass beakers. TiO₂ NP-embedded CA films (4.5 cm in diameter) were adhered to the bottom of the beakers using ethylene-vinyl acetate (EVA, AdTech™, adhesive technologies, Hampton, NH, USA). The beakers were sterilized with UV-C light for 30 min in a bio-safety cabinet (Class II Type A/B3, Nuaire, Plymouth, MN, USA) before the experiment. The beakers were then placed on a platform shaker (Model Classic 10, New Brunswick Scientific Co., Inc., Edison, NJ, USA) inside a photocatalytic disinfection chamber (Figure 1). Four 40 W UV-A lamps (American DJ®, Model UV Panel HP™, LL-UV P40, Los Angeles, CA, USA) were fitted on the inside top of the photocatalytic disinfection chamber. The shaker was set at a speed of 100 rpm during the photo-disinfection experiment. Water samples were inoculated right before the
photo-disinfection experiment. In this study, 30 ml of water sample was inoculated with 300 μl inoculum. The experiments were carried out at a light intensity of 1 mW/cm².

**Sampling and bacterial enumeration**

Water samples were taken hourly for three hours and 1 ml of sample was taken each time. Serial dilutions were made in PBS and appropriate dilutions were plated on SMACNA agar and incubated at 37 °C for 24 h. Colonies were counted and recorded as log CFU/ml.

**Water quality parameter measurement**

Water quality parameters including turbidity, UV transmittance at 254 nm (UVT-254), total organic carbon (TOC), total suspended solids (TSS), and total hardness of all the lab-prepared water samples and natural water samples were measured. Turbidity, TOC, and TSS were measured following Hach methods 8237, 10129, and 8006, respectively, using a DR/90 colorimeter (HACH, Loveland, CO, USA). UVT-254, which measures the percentage of light that passes through a water sample at 254 nm, was measured using a UV-vis spectrophotometer (Orion™ AquaMate 8000, Thermo Fisher Scientific, Waltham, MA, USA). Total hardness was determined following USEPA method 8226.

**Statistical analysis**

Experiments were replicated at least twice. Duplicate measurements were made on each sample. Pearson correlation analysis and partial correlation analysis on the water quality parameters and bacterial reduction were conducted using JMP 14 (SAS Institute, Cary, NC, USA). The regression equation was developed by least squares regression also using JMP 14. All the tests were performed at a significance level of 0.05.

**RESULTS AND DISCUSSION**

**Effect of water compound on photo-disinfection efficacy of TiO₂ embedded CA film**

Figure 2 shows the results of the photo-disinfection of *E. coli* O157:H7 using TiO₂ NP-embedded CA film in DI water and lab-prepared water samples with different natural water compounds under UV-A light illumination. In DI water, *E. coli* O157:H7 population was reduced by about 5.8 log after 3 h of photo-disinfection treatment. Increasing humic acid concentration from 2 to 20 mg/L reduced bacterial reduction from 3.9 to 0.8 log CFU/ml. Increasing CaCO₃ concentration from 2 to 20 mg/L reduced bacterial reduction from 4.3 to 1.9 log CFU/ml. In comparison with humic acid and CaCO₃, kaolin clay had the least inhibition effect on photo-disinfection. When adding 2 mg/L of kaolin clay in water, 5 log reductions were achieved after 3 h of photo-disinfection treatment. When the concentration of kaolin clay was increased to 20 mg/L, more than 4 log reductions could still be achieved after 3 h of treatment.

Inactivation of bacteria using TiO₂ immobilized systems has been demonstrated in published studies. Xiong & Hu (2013) evaluated the inactivation of *E. coli* using a UVA/
LED system with a crystallizing dish coated with TiO₂. They reported that at a light intensity of 6 mW/cm², it took 145 min for 3 log inactivations of *E. coli* in 30 ml of inoculated distilled water. Rodrigues *et al.* (2007) evaluated the inactivation of *E. coli* in synthetic water and natural water using a glass reactor with immobilized TiO₂ (catalyst). They found 100% bacterial reduction in synthetic water, and 80% bacterial reduction in natural water after 1 h treatment under solar light illumination. Due to the variation in factors such as experimental setting, TiO₂ immobilized system, light intensity, and water volume among different studies, the treatment efficiency may not be compared directly.

The disinfection kinetics in the current study follows a non-linear trend with a ‘shoulder’. The ‘shoulder’ can be explained by the cumulative-damage nature of photo-disinfection treatment on the cytoplasmic membrane rather than an instantly lethal effect (Gyürék & Finch 1998). The disinfection kinetics for CaCO₃ and humic acid tend to have a longer ‘shoulder’ period. This indicates that the bacterial inactivation process was subject to interference by these two water compounds. Many empirical kinetic models have been reported in the literature for the interpretation of disinfection data. The following models have been summarized as the most well-known disinfection models (Marugán *et al.* 2008; Chong *et al.* 2010; Yemmireddy & Hung 2015a).

The Chick–Watson (C-W) model (Chick 1908; Watson 1908), which is the most commonly employed disinfection model in photo-disinfection:

\[
\log \frac{C}{C_0} = -kt
\]

where \(C/C₀\) is the reduction in bacterial concentration, \(k\) is the disinfection rate constant, and \(t\) is the treatment time.

The delayed Chick–Watson model (Cho *et al.* 2004) was developed to accommodate any initial lag time \(t₀\):

\[
\log \frac{C}{C_0} = \begin{cases} 
0 & \text{for } t \leq t₀ \\
-k(t - t₀) & \text{for } t > t₀ 
\end{cases}
\]

The modiﬁed Chick–Watson model (Cho *et al.* 2003) was to fit either an initial shoulder or a tail at the end of the reaction:

\[
\log \frac{C}{C_0} = k₁[1 - \exp(-k₂t)]
\]

The Homs model (Hom 1972) was reported for predicting a curvilinear or non-linear function:

\[
\log \frac{C}{C₀} = -ht^h
\]

where \(h\) is the second parameter. If \(h = 1\), the Homs model becomes a linear Chick–Watson equation and, if \(h > 1\), this model can fit a line with a tail.

The modified Homs model (Cho *et al.* 2003), which expands the applicability of the Homs model for the fitting of disinfection data with an initial shoulder, log-linear reduction, and prolonged tailing behaviors:

\[
\log \frac{C}{C₀} = k₁[1 - \exp(-k₂t)^h₁]
\]

The data generated in the current study were fitted using these empirical models and RMSE (root mean square error) was calculated using the following equation:

\[
RMSE = \sqrt{\frac{\sum_{n=1}^{N} (\tilde{y}_n - y_n)^2}{N}}
\]

where \(N\) is the number of observations, \(y_n\) is the observed value, and \(\tilde{y}_n\) is the predicted value. The results in Table 1 show that the delayed Chick–Watson model provided a better fit with the smallest RMSE for all water compound solutions tested. The three water compounds inhibited the photo-disinfection of Ca film following the order of: humic acid > CaCO₃ > kaolin clay.

**Effect of kaolin clay on water property and disinfection efficacy**

The effects of kaolin clay on water quality parameters and bacterial reduction are shown in Table 2. Kaolin clay did not have a strong inhibition effect on photo-disinfection in the current study compared with humic acid and CaCO₃.
Results of water property analysis showed that increasing kaolin clay concentration in water did not significantly change TOC and hardness. UVT-254 slightly decreased when kaolin clay concentration increased. However, kaolin clay concentration significantly affected the total suspended solids (TSS) and turbidity. It has been reported that kaolin clay might be the worst particle for shielding light (Liu & Zhang 2006) and hence may affect photo-disinfection efficacy. However, the current study showed that when the turbidity and TSS of kaolin clay solution were increased to 24 FAU and 24 mg/L, respectively, more than 4.2 log bacterial reductions were achieved. This indicates that turbidity and TSS might not be significant parameters affecting photo-disinfection efficacy.

Effect of CaCO3 on water properties and disinfection efficacy

Results in Table 2 show that increasing CaCO3 concentration did not affect TOC and TSS concentration in water. Increasing CaCO3 slightly increased UVT-254 and turbidity. Water hardness significantly increased when increasing CaCO3 concentration. The hardness of the CaCO3 solution increased to 22 mg/L, bacterial reduction decreased to about 1.9 log CFU/ml. Similar findings regarding the effect of CaCO3 on photo-disinfection or photo-degradation have been reported in other published reports. Cohen-Yaniv et al. (2008) studied the inactivation of Flavobacterium and E. coli in water by a continuous stirred
tank reactor (CSTR) fed with suspended or glass immobilized TiO$_2$. They found water hardness increase reduced photocatalytic inactivation efficiency, and they suggested the treatment of water chemically before disinfection. Sreethawong et al. (2014) studied the degradation of Congo Red (CR) azo dye using nano-Ag/sol-gel TiO$_2$-In$_2$O$_3$ mixed oxide mesoporous-assembled nanocrystals. Their results showed that the water hardness reduced the photocatalytic CR dye degradation activity. In natural water, CaCO$_3$ is the major compound that causes an increase in water hardness (Sreethawong et al. 2014). Increased water hardness can cause a variety of problems such as reduced efficiency of chlorine treatment and decreased life of plumbing and appliances (Pangloli & Hung 2013). Results from the current study suggest that water hardness might be a significant parameter affecting photo-disinfection efficacy.

**Effect of humic acid on water properties and disinfection efficacy**

Results in Table 2 show that increasing humic acid concentration significantly increased TOC, turbidity, and TSS in water, whereas the UVT-254 reading decreased significantly. When humic acid concentration increased to 20 mg/L, turbidity increased to 37 FAU, TSS increased to 27 mg/L, TOC increased to 5.8 mg/L, while UVT-254 decreased to 40.35%. At this humic acid concentration, bacterial TOC increased to 5.8 mg/L, while UVT-254 decreased to 40.35%. At this humic acid concentration, bacterial reduction decreased significantly to 0.899, TOC and UVT-254 have a correlation coefficient of 0.884, turbidity and UVT-254 have a correlation coefficient of –0.884, turbidity and UVT-254 have a correlation coefficient of –0.881, and UVT-254 and TSS have a correlation coefficient of –0.872. These suggest collinearity exists increased from 0 to 2.0 mg/L, disinfection efficacy reduced from 5 log to about 2 log after 1 h treatment. According to these studies, the main reason that humic acid inhibited photo-disinfection efficacy was due to the consumption of hydroxyl radicals produced by TiO$_2$ photocatalysis.

Humic acid is a major fraction of natural organic matter (NOM) in natural water (Wang & Hsieh 2001). The C = C double bonds and C – O double bonds in humic acid chemical structure can absorb UV light at 254 nm and prevent light at this wavelength transmitting through water (Cheng et al. 2018), and hence caused low UVT-254 readings for humic acid solutions in the current study (Table 2). UVT-254 measures the transmittance of UV light at 254 nm through the water. It has been reported that the efficiency of UV disinfection will be affected by the turbidity of the target water and the resultant transmittance of UV light through the water (Cantwell & Hofmann 2011), while the current study showed that for photocatalytic disinfection, the effect of turbidity and UVT-254 on disinfection efficiency varied among the different compounds studied. For example, kaolin clay caused a significant increase in turbidity, but it did not have as much effect on bacterial reduction as other compounds such as humic acid.

**Prediction equation development**

As discussed above, various water compounds such as humic acid and CaCO$_3$ have strong inhibition effects on the TiO$_2$ NP-embedded CA film photo-disinfection effect. However, in a practical situation, it is unrealistic to monitor the level of all compounds in water. Identifying common water quality parameters that can be used as water quality indicators for photo-disinfection is a more efficient solution.

To select the proper water quality parameters that can be used for predicting TiO$_2$ NP-embedded CA film disinfection efficacy, the Pearson correlation of all the water quality parameters reported in Table 2 was first performed and the results are presented in Table 3. It shows that several variables are strongly correlated with another variable. For example, turbidity and TSS have a correlation coefficient of 0.899, TOC and UVT-254 have a correlation coefficient of –0.884, turbidity and UVT-254 have a correlation coefficient of –0.881, and UVT-254 and TSS have a correlation coefficient of –0.872. These suggest collinearity exists...
among these variables and using all the variables for model development would cause an inaccurate predictor contribution to the model. Therefore, only one variable between highly correlated pairs should be considered for regression development.

To determine which variable should be excluded from model development, a partial correlation between the predictor variables and the response variable was performed and the results are shown in Table 4. Pearson correlations reported in Table 3 demonstrated the linear correlation between two variables without controlling other variables in the model, while partial correlation measures the correlation between two variables with the effect of other controlling variables removed. Results show that turbidity, TOC, and TSS have Pearson correlation coefficients of \(-0.617\), \(-0.702\), and \(-0.433\) with bacterial reduction, respectively. However, these three variables have much lower partial correlation coefficients of \(0.161\), \(0.272\), and \(0.097\) with bacterial reduction, respectively, after controlling other confounding variables in the model. As a result, UVT-254 and total hardness with high partial correlation coefficients with bacterial reduction (Table 4) are further considered for regression analysis. This conclusion further supports the observations reported above that changes in total hardness and UVT-254, respectively can explain the changes in bacterial reduction.

In addition, for practical applications, some water properties can be affected by more than one chemical compound. For example, UVT-254 and TOC can all be used to indicate organic matter in water. However, TOC is a more time-consuming measurement compared with UVT-254. It is also desirable to select the parameters that are easy to measure. In the current study, UVT-254 and water hardness are the two parameters that are easy to measure and can explain the changes in bacterial reduction. A linear prediction equation is then developed using the least squares method and listed in Equation (7) with the adjusted \(R^2\) and root mean square error of 0.78 and 0.74, respectively:

\[
\text{Bacterial reduction} = -2.150 + 0.075 \times \text{UVT} - 254 - 0.142 \times \text{Hardness} \tag{7}
\]

### Verification of prediction equation using laboratory-prepared water samples and natural water

In order to test whether the prediction equation can accurately predict bacterial reduction in a complex environment that contains different water compounds, four different simulated water samples were prepared with different combinations of the three water compounds based on the formula shown in Table 5. Table 6 shows the observed and predicted bacterial reductions using TiO\(_2\) NP-embedded CA film in these simulated water samples.

| Turbidity | UVT-254 | Total organic carbon | Total hardness | Total suspended solids | Bacterial reduction |
|-----------|---------|----------------------|----------------|-----------------------|--------------------|
| Turbidity | 1.000   | \(-0.881\)           | 0.674          | 0.027                 | \(-0.617\)         |
| UVT-254   | 1.000   | \(-0.884\)           | 0.179          | 0.079                 | 0.685              |
| Total organic carbon | 1.000 | \(-0.161\)          | 0.672          | 0.672                 | \(-0.702\)         |
| Total hardness | 1.000 | \(-0.284\)             | 0.097          | 0.097                 | \(-0.432\)         |

**Table 4** | Partial correlations between water quality parameters and bacterial reduction

| Turbidity | UVT-254 | Total organic carbon | Total hardness | Total suspended solids |
|-----------|---------|----------------------|----------------|-----------------------|
| Bacterial reduction | 0.161 | 0.461 | \(-0.272\) | \(-0.726\) | 0.097 |
The highest bacterial reduction was detected in sample 4 (2.1 log CFU/ml), followed by sample 2, sample 1, and sample 3 with reductions of 1.8, 1.6, and 1.5 log CFU/ml, respectively. Water property analysis shows that sample 3 had the lowest UVT-254 of 61.62%. The UVT-254 of sample 1, 4, and 2 was 73.3%, 79.47%, and 81.85%, respectively. Sample 2 had the highest hardness of 20 mg/L. The hardness of sample 1 was 14 mg/L and the hardness of both samples 3 and 4 was 6 mg/L. The prediction results show that half of the samples have a prediction percentage error of less than 15%, however, the other half have a prediction percentage error of more than 30%. The prediction equation developed in the preceding section was based on the disinfection experiment carried out in individual water compounds. The prediction equation might be improved by studying the interaction effects between different water compounds during treatment.

Table 7 shows that in natural creek water, bacteria can be reduced by 1 log CFU/ml after 3 h treatment using TiO2 NP-embedded CA film. After filtration using a 0.45 μm filter, 1.4 log reductions were achieved. After filtration using a 0.2 μm filter, bacterial reductions increased to 2.5 log CFU/ml. As shown in Table 7, filtration also improved water quality. The hardness, turbidity, TSS, and TOC all decreased after filtration, and UVT-254 increased with filtration. Filtration can remove insoluble particles that cause the increase of these parameters and can therefore improve water quality (O'Melia 1985). Bacterial reductions were also calculated using Equation (7). The predicted bacterial reduction was \(-0.9\) CFU/ml reduction for the water sample without filtration, and 0.9 and 1.1 CFU/ml reductions for samples filtered with 0.2 and 0.45 μm filters, respectively. The total hardness of the unfiltered water sample was over the ranges of the hardness of water samples used for the development of the prediction equation, which may affect the prediction accuracy. More types of natural water and more water property information are needed to further improve the prediction equation.

Nevertheless, the current study has demonstrated that using TiO2 NP-embedded CA film has the potential to inactivate pathogens in natural water. Filtration has also been found as a simple and effective method to remove water compounds that may affect bacterial inactivation during the photo-disinfection process.

**CONCLUSIONS**

The results of this study showed that natural water compounds such as humic acid, CaCO3, and kaolin clay can all affect the photo-disinfection efficacy of TiO2 NP-embedded CA film. Humic acid had the highest inhibition effect on photo-disinfection, followed by CaCO3 and kaolin clay. The effect of different water quality parameters on TiO2 NP-embedded CA film indicates that different water compounds affect photo-disinfection efficacy through different mechanisms. It was found that UVT-254 and turbidity can be used as indicators for predicting the effect of natural water compounds on bacterial inactivation. Further studies are needed to develop a comprehensive model that can accurately predict the photo-disinfection efficacy of TiO2 NP-embedded CA film in natural water.
water compounds on photo-disinfection efficacy. A predictive equation was developed using UVT-254 and turbidity as independent variables. Photo-disinfection using TiO₂ NP-embedded CA film reduced *E. coli* O157:H7 by 1 log CFU/ml in the inoculated natural creek water sample. After filtration using a 0.2 µm filter, about 2.5 log CFU/ml reductions were achieved with the same treatment. Hence, filtration can be used as an effective pre-treatment for photo-disinfection by TiO₂ NP-embedded CA film.

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**DATA AVAILABILITY STATEMENT**

All relevant data are included in the paper or its Supplementary Information.

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| Filter (µm) | Hardness (mg/L) | Turbidity (FAU) | Total suspended solids (mg/L) | Total organic carbon (mg/L) | UVT-254 (%) | Bacterial reduction at 3 h (log CFU/ml) | Prediction percentage error (%) |
|-----------|----------------|----------------|-------------------------------|-----------------------------|-------------|---------------------------------|-------------------------------|
| 0.2       | 20 ± 1         | 3.5 ± 2.1      | 0                             | 4.9 ± 1.6                   | 85.75 ± 2.25| 2.5 ± 0.4                      | 1.4 ± 0.2                     | --44                          |
| 0.45      | 24 ± 1         | 7 ± 2.8        | 1.5 ± 0.7                     | 5.9 ± 1.6                   | 80.71 ± 0.95| 1.4 ± 0.1                      | 0.5 ± 0.1                     | --64                          |
| w/o       | 32 ± 1         | 10.5 ± 2.1     | 3 ± 1.4                      | 8.7 ± 3.3                   | 77.82 ± 1.90| 1.0 ± 0.3                      | -0.9 ± 0.1                    | --190                         |

*Prediction percentage error (%) = (Predicted bacterial reduction − Observed bacterial reduction) / Observed bacterial reduction × 100.
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