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Bench-Scale and Pilot-Scale Photocatalytic Inactivation of Viruses with Titanium Dioxide Nanoparticles

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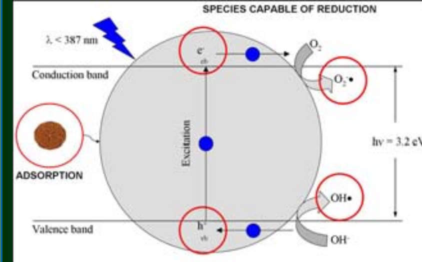
Motivation

Published studies regarding contaminants of emerging concern, particularly pharmaceuticals and endocrine disruptors, have led to increased public scrutiny of water quality. In addition, recent changes to water quality regulations, specifically the Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) and the Stage 2 Disinfectant and Disinfection Byproducts Rule (D/DBPR), have forced water utilities to consider implementation of advanced treatment technologies to remain in full regulatory compliance. Enhanced coagulation and granular activated carbon (GAC) are currently considered best available technologies for treating disinfection byproduct precursors and contaminants of emerging concern. However, enhanced coagulation and GAC are not always effective and have several limitations, including significant O&M costs. Utilities are now considering other alternatives, including advanced oxidation, to address their water quality issues. Due to its multi-barrier capabilities (oxidation, reduction, and adsorption), titanium dioxide (TiO_2) photocatalysis may become a viable water treatment technology. A tremendous amount of chemical research is available in the literature, but less is known regarding the potential for photocatalytic inactivation of viruses. With respect to both chemicals and microbes, the literature suggests that the energy requirements for photocatalysis may be cost-prohibitive, but these low efficiencies may be an artifact of inefficient reactor configurations. To address some of these issues, this study compares UV and photocatalytic inactivation with respect to four potential surrogate bacteriophages (MS2, PRD1, phi-X174, and fr), UV-resistant adenovirus (Ad4), and three enteroviruses (poliovirus 1, coxsackievirus B6, and echovirus 12). In addition, this study demonstrates the application of an integrated cell culture quantitative polymerase chain reaction (ICC-qPCR or ICC-qRTPCR) strategy for adenovirus and enterovirus disinfection studies.

Background

TITANIUM DIOXIDE PHOTOCATALYSIS



TITANIUM DIOXIDE

- Degussa P25 TiO_2 = 75% Anatase and 25% Rutile
- Individual particle size ≈ 25 nm
- Particle aggregation ≈ 500 nm
- Pure rutile TiO_2 was used as a turbidity control

BACTERIOPHAGES – DOUBLE AGAR LAYER

Double-stranded DNA	Single-stranded RNA	Single-stranded RNA	Single-stranded DNA
PRD1 (*Salmonella typhimurium*)	MS2 (*E. coli* 15597)	fr (*E. coli* 19853)	phi-X174 (*E. coli* 13706)
pH_{zpc} = 3.6	pH_{zpc} = 3.7	pH_{zpc} = 9.0	pH_{zpc} = 6.6

LIGHT SPECTRUM

The monochromatic output (254 nm) of low-pressure UV bulbs is considered the most effective wavelength for disinfection purposes. Black-light bulbs (350-400 nm), which have been used in similar photocatalysis studies, are capable of activating TiO_2, but they are not sufficient for disinfection in water and wastewater treatment.

HUMAN VIRUSES – ICC-qPCR or ICC-qRTPCR

Double-stranded DNA	Single-stranded RNA	Single-stranded RNA	Single-stranded RNA
Adenovirus 4 (PLC/PRF/5)	Coxsackievirus B6 (BGM)	Echovirus 12 (BGM)	Poliovirus 1 (BGM)

COLLIMATED BEAM DESIGN

15-Watt, Low-Pressure, 254-nm UV Bulb

Supports

Collimating Column

I_0 ≈ 0.15 mW/cm²
I_{AVE} ≈ 0.13 mW/cm²

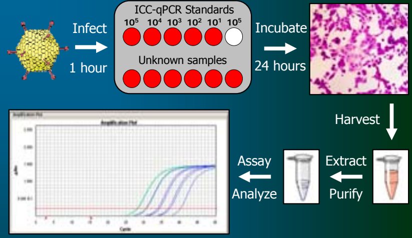
IL-1700 Radiometer

Correction Factors:
Petri = 0.890
Reflection = 0.975
Divergence = 0.984
Water = 0.995

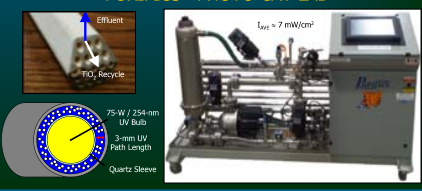
Intensity Calculation:
$I_{AVE} = I_0 \times PF \times RF \times DF \times WF$
Unit = mW/cm²

Dose Calculation:
$Dose = I_{AVE} \times Time$
Unit = mW-s/cm² = mJ/cm²

INTEGRATED CELL CULTURE qPCR (ICC-qPCR)



PURIFICS® PHOTO-CAT LAB®



Results

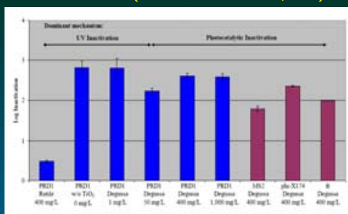
SYNERGISTIC INACTIVATION IN THE COLLIMATED BEAM

Virus	pH	TiO_2 dose (mg/L)	UV dose for 4-log inactivation (mJ/cm²)[a]	% Dose reduction based on UV baseline[b]
PRD1	7.0[c]	0	42	–
	7.0	1	41	2.4%
	6.0	1	34	19%
MS2	7.0	0	46	–
	6.0	1	39	15%
phi-X174	7.0	0	16	–
	7.0	1	15	6.3%
	6.0	1	16	0%
	7.0	1,000	181	N/A
fr	7.0	0	15	–
	8.0	1	15	0%

[a] Doses calculated using regression equation through triplicate points for four exposure times
[b] UV baseline denoted by a TiO_2 dose of 0 mg/L
[c] UV inactivation did not vary for each bacteriophage due to changes in pH (data not shown)

The collimated beam proved to be inefficient with high TiO_2 concentrations, but synergistic inactivation (1 mg/L of TiO_2) provided small reductions in the UV doses required for 4-log inactivation.

BACTERIOPHAGE INACTIVATION IN THE PHOTO-CAT (UV DOSE = 34 mJ/cm²)



The Photo-Cat achieved rapid viral inactivation—comparable to UV disinfection—even at high TiO_2 concentrations. High TiO_2 concentrations are more effective for chemical destruction.

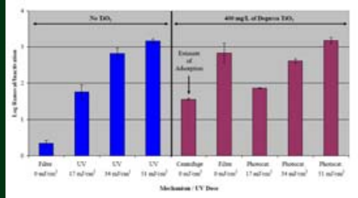
EFFECT OF PROTEIN CAPSID COMPOSITION ON PHOTOCATALYTIC INACTIVATION

Bacteriophage	PRD1		phi-X174		fr		MS2	
Spiking level (PFU/mL)	7.97×10^4		1.16×10^5		9.70×10^3		8.07×10^4	
Total number of amino acids[a]	303,040		42,336		23,400		23,400	
Alanine (A)	34,800	2.70[b]	2,952	1.50	2,880	1.73	2,520	1.19
Glycine (G)	19,100	1.48	3,192	1.63	1,620	0.97	1,620	0.76
Proline (P)	14,900	1.16	2,508	1.28	900	0.54	1,080	0.51
Level of inactivation in the Photo-Cat	2.61 logs[c]		2.36 logs		1.99 logs		1.80 logs	

[a] All amino acid values reported per viral particle.
[b] Hypothetical surface density reported in residues/nm².
[c] Pilot-scale photocatalytic inactivation: TiO_2 = 400 mg/L and energy input of 34 mJ/cm².

Hawkins and Davies (1998) and Jones et al. (2007) reported OH• specificity with alanine, glycine, and proline residues. The protein capsid compositions of the bacteriophages supported these theories.

INACTIVATION AND PHYSICAL REMOVAL OF PRD1 IN THE PHOTO-CAT

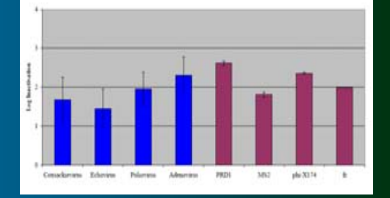


For similar energy inputs, UV and photocatalysis achieved similar levels of inactivation for PRD1. Due to a high level of adsorption, physical removal increased significantly after the addition of TiO_2.

INACTIVATION AND PHYSICAL REMOVAL OF ADENOVIRUS IN THE PHOTO-CAT

For each energy input, UV and photocatalysis achieved similar levels of adenovirus inactivation. Photocatalysis coupled with filtration exceeded the detection limit of the ICC-qPCR assay.

VIRAL INACTIVATION IN THE PHOTO-CAT (UV DOSE = 34 mJ/cm² AND TiO_2 = 400 mg/L)



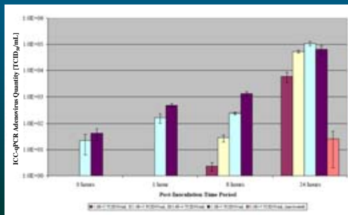
With a UV dose of 34 mJ/cm² (0.33 kWh/m³), photocatalysis generally achieved 2-log viral inactivation. The greater variability for the human viruses can be attributed to their more difficult infectivity assays.

COMPARISON OF ENERGY REQUIREMENTS FOR PHOTOCATALYSIS

Target	Electrical efficiency per log order reduction (kWh/m³-log): Based on lamp output	Electrical efficiency per log order reduction (kWh/m³-log): Based on energy use	Electrical efficiency based on treatment goals[e] (kWh/m³): Based on lamp output	Electrical efficiency based on treatment goals[e] (kWh/m³): Based on energy use
Bench-scale THMFP[a]	1.3	350	0.66	180
Bench-scale MS2[b]	11	720	44	2,900
Bench-scale phi-X174[c]	0.019	110	0.076	450
Pilot-scale Bacteriophages[d]	0.0072	0.17	0.029	0.66

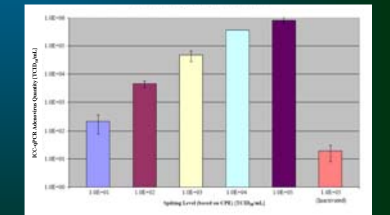
[a] Hand et al. (1995): 1 g/L Degussa P25 TiO_2, 1.8-L reactor volume, 450-W high-pressure mercury lamp, 0.91 W/L.
[b] Cho et al. (2005): 1 g/L Degussa P25 TiO_2, 50-mL reactor volume, 18-W black-light blue lamp, 7.9x10-6 einsteins/L-s.
[c] Current study: 1 g/L Degussa P25 TiO_2, 14-mL reactor volume, 15-W low-pressure mercury lamp, 0.13 mW/cm².
[d] Current study: 400 mg/L Degussa P25 TiO_2, 15-L reactor volume, 75-W low-pressure mercury lamp, 7 mW/cm².
[e] Treatment goals: 70% destruction of THMFP and 4-log viral inactivation. In Hand et al. (1995), 70% destruction of THMFP would be necessary to comply with the Stage 2 D/DBPR.

OPTIMIZATION OF ICC-qPCR FOR ADENOVIRUS



Cell monolayers were inoculated with different infectious spiking levels, and the viruses and cells were harvested at the specified time points. The 0-hr and heat-inactivated samples were performed as washing controls to characterize the detection limit of the assay. The 1-hr and 8-hr time points proved to be insufficient due to limited replication. The 24-hr time point provided sufficient replication to distinguish infectious and non-infectious viruses, but more consistency between spiking levels was necessary.

OPTIMIZED ICC-qPCR FOR ADENOVIRUS (24-HR INCUBATION)



This validation graph illustrates the optimized ICC-qPCR quantities for adenovirus, which can be used to create a standard curve based on the original spiking levels. The standard curve can then be used to determine the original number of infectious viruses in unknown disinfection samples. The enterovirus protocol differs in that the three viruses can be assayed simultaneously in the same wells. However, the qRTPCR assays must be completed individually using different primers and probes.

Summary

- In the collimated beam experiments, low levels of viral adsorption (data not shown) and an inefficient reactor configuration reduced the effectiveness of photocatalysis, particularly for high TiO_2 concentrations (100 – 1,000 mg/L).
- 1 mg/L of anatase TiO_2 produced a synergistic effect between UV inactivation and TiO_2 photocatalysis, which provided slight reductions in the UV doses required for 4-log inactivation. However, 1 mg/L of TiO_2 would likely have little impact on disinfection byproduct precursors and contaminants of emerging concern.
- The annular reactor configuration and increased adsorption in the Photo-Cat allowed for rapid viral inactivation (0.17 kWh/m³-log) even at high TiO_2 concentrations (400 – 1,000 mg/L). The Photo-Cat required significantly less energy than the bench-scale reactors in this study and the literature.
- The literature suggests hydroxyl radical specificity with alanine, glycine, and proline residues. Based on the level of inactivation in the Photo-Cat, there is a slight correlation between bacteriophage protein capsid composition and photocatalytic inactivation.
- UV disinfection is slightly more effective than TiO_2 photocatalysis for viral inactivation, but the potential for simultaneous inactivation and chemical destruction provides an additional benefit that is generally impractical with UV irradiation alone.
- ICC-qPCR is a promising strategy for the relative quantification of infectious viruses in disinfection studies. Washing out non-infectious viruses after a 1-hr infection period and harvesting the cells and viruses after 24-hr of incubation proved to be the optimal conditions for ICC-qPCR. ICC-qRTPCR allows for simultaneous assays of the three enteroviruses.

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