

Photo Catalytic Inactivation of *Escherichia coli* Using Titanium (IV) Oxide-Tungsten (VI) Oxide Nanoparticles Composite

Mbugua S.N.^a, Oyoo W.S.^{b*}, Njuguna K.G.^a

^a Department of Chemistry, P.O. BOX 30197, Nairobi

^b P.O. Box 42792-00100, Nairobi

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Abbreviations:

CFU: Colony Forming Units, USEPA: United States Environmental Protection Agency, XRF: X-ray Florescence, XRD: X-ray Diffraction, UNEP: United Nations Environmental Programme, UV/Vis: Ultra Violet/Visible, BLB: Black Light Blue, nm: Nanometer, g: Gram, ml: Milliter, L: Litre, MCL : Maximum Contaminant Level, MCLG: Maximum Contaminant Level Goal, AOP: Advanced Oxidation Processes, CB: Conduction Band, USAID: United States Agency for international Development, ANCAP: Africa Network for Chemical Analysis of Pesticides, SPE: Solid Phase Extraction

Corresponding Author:

Oyoo W.S.

Professor

Email: wandigas@uonbi.ac.ke

Njuguna K.G.

Professor

Abstract

This work focused on synthesis of Titanium (IV) Oxide and Tungsten (VI) Oxide composite and testing the composite as a photocatalyst in

deactivation of *Escherichia coli* in water. Modified wet chemistry method was used and the synthesized nanoparticles calcined at 575° C, taken through X-Ray Fluorescence and X-Ray Diffraction. The result showed a particle size diameter of 18.99nm. The nanoparticles photocatalytic inactivation efficacy of *Escherichia coli* in water was tested. 3M Petrifilms from 3M Microbiology Products, U.S.A., was used for *Escherichia coli* colony forming units' counts. ATUV 8W G8 T5 lamp from PHILIPS emitting between 350-600nm was used as energy source. The catalyst reduced *Escherichia coli* count by log 3.415 at an optimum catalyst amount of 0.75 g/L at pH 7.3 using the Chick-Watson model for disinfection kinetics. This work proved that photocatalysis is a promising technology in water purification with possible and practical opportunities existing especially for small-scale point-of-use water purification units where potable water could be treated for disinfection of pathogens or trace priority pollutants remaining in water distribution network after conventional treatment methods. This work proved that nano particles can provide solutions in treatment of drinking water especially for poor communities living in the tropics.

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1. Introduction

African water resources contain high levels of microbiological pathogens that include bacteria, viruses and protozoa as well as chemical contaminants like pesticides, heavy metals, and persistent organic pollutants amongst others (Masakazu and Masato, 2001). *Escherichia coli* and related bacteria constitute about 0.1% of gut flora, and fecal-oral transmission is the major route through which pathogenic strains of the bacterium cause disease. Cells are able to survive outside the

body for a limited amount of time, which makes them ideal indicator organisms to test environmental samples for fecal contamination (Feng et al., 2002).

The USEPA gives Maximum Contaminant Level Goal (MCLG) for Total Coliforms (including fecal coliform and *Escherichia coli*) as zero and a Maximum Contaminant Level (MCL) as 5%. This means that for water systems doing sampling, no

more than 5.0% water samples should test positive for total coliform in a month, and for water systems that collect fewer than 40 routine samples per month, no more than one sample should test positive for total coliform per month (USEPA 2010). Water treatment processes like filtration and adsorption are widely used both in homes and industries. However, these purification processes only transfer the removed pollutants from one matrix (water) into another (filter or adsorbent). This necessitates further remediation of the adsorbent or filter for subsequent use. The trapped pollutants especially microorganisms may in some cases accumulate posing secondary pollution challenges where they can be re-introduced into the drinking water.

A conclusive water treatment method is therefore crucial in total elimination of organic and inorganic pollutants in drinking water without the danger of inter-matrix transfer of the pollutants. Other drawbacks in water purification is the use of costly energy in processes like reverse osmosis and electro dialysis. Chemical treatment methods like oxidation technologies utilizing chlorination and ozonolysis have long been used for the disinfection of water. However, several concerns have emerged regarding these treatments, mainly costs, and for chlorine, formation of potentially harmful disinfection by-products such as trihalomethanes and haloacetic acids upon reaction with the naturally occurring organic matter and halide ions (Grieken et al., 2009).

2. Review of literature

Heterogeneous photo catalysis has emerged as an alternative process to advanced oxidation processes (AOP) for bacteria inactivation and oxidation of organic pollutants (Gumy et al., 2006 and Sobana et al., 2008). The process utilizes semiconductor materials and a UV energy source. Pin-Ching et al., (1999) carried out experiments on inactivation of *Escherichia coli* on Titanium (IV) Oxide photo catalysts and concluded that TiO₂ photo catalysis promoted peroxidation of the polyunsaturated phospholipid component of the lipid membrane initially and induced major disorder in the *Escherichia coli* cell membrane. Subsequently, essential functions that rely on intact cell membrane architecture, such as respiratory activity, were lost, and cell death was inevitable. Coupling TiO₂ with tungsten trioxide (WO₃) has been widely studied to improve the photocatalytic performances of TiO₂, since WO₃ can serve as an electron accepting species (Ke et al., 2008). As a basic function, WO₃ has a suitable conduction band potential to allow the transfer of photo-generated electrons from TiO₂ facilitating effective charge separation. Photo excited electrons are transferred

from conduction band (CB) of TiO₂ to CB of WO₃ and therefore are separated from photo induced holes (Kwon et al., 2000). Additionally, formation of a monolayer of WO_x species on TiO₂ can significantly increase the surface acidity as WO₃ is 15 times more acidic than TiO₂ (Kwon et al., 2000). Because of this increased acidity, TiO₂/WO₃ composites can absorb more hydroxyl groups and simultaneously more organic reactants on its surface. Thus, significant improvement of the photocatalytic activity has been achieved in comparison with the pure TiO₂ photocatalysts (Kwon et al., 2000).

2.1 3M Petrifilms

3M Petrifilm *Escherichia coli* Count Plate is a reliable, sample-ready medium system for enumerating *Escherichia coli* and other coliforms. The Plates contain violet red bile nutrients, a cold water soluble gelling agent, a glucuronidase indicator to identify *Escherichia coli*, and a tetrazolium indicator to enhance the visualization of other gram negative coliform (non-*Escherichia coli*) bacteria. Coliforms ferment the lactose in the medium to produce gas. This gas is trapped around the coliform colony and allows the differentiation of coliform bacteria from other gram negative bacteria. In addition, glucuronidate, produced by most *Escherichia coli* will react with the glucuronidase indicator in the medium to produce a blue precipitate around the colony allowing visual identification of *E. coli*. Plates are incubated in a horizontal position with the clear side up at an incubation temperature range of 37-38° C for 24 + 2 hr, and examined for coliform and *Escherichia coli* growth. Various enumeration experiments have shown very little or no variance between counts obtained through Petrifilm and standard agar counts (Watterworth and Schraft, 2005).

3. Materials and Methods

3.1 *Escherichia coli* samples

Experiments were carried out with the water samples collected from the Nairobi dam next to Kibera slums South of Nairobi, six kilometers from the Department of Chemistry where analysis was done. Samples were transported in two-liter amber bottles and stored at 4° C prior to analysis. All experiments were performed within three days of sample collection at the natural pH 7.3 of the samples without any pH adjustment. The water contained 2,600 CFU/ml *Escherichia coli*. A portion of the water solution (10 ml) was diluted by a factor of 10 which gave a count of 260CFU/ml after 24hr incubation.

3.2 Preparation of the photocatalyst composite

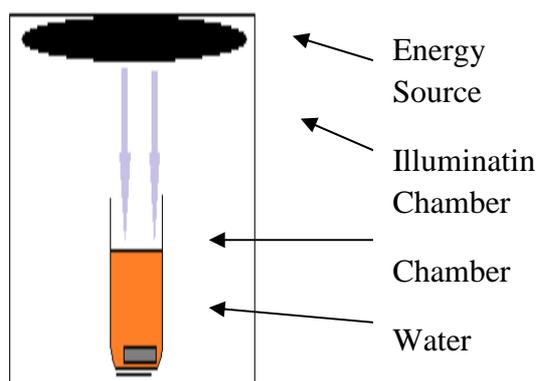
Solid powder precursors of TiO₂ and WO₃ (the British Drug Houses Limited of 99.4% and 99.5%

purity respectively) were utilized and hydrolysis was carried out in acidified isopropanol (J.T.Baker Inc.). The addition of nitric acid together with 12-hour stirring followed by drying and calcinations at 575° C for 2 hours yielded the desired product. The nanoparticles were characterized using a Philips Minipal2 XRF instrument (Shimadzu) and an XRD 600 analyzer (Shimadzu), at two theta (2θ) angles between 0°-80°. The mean crystallite size was determined from the broadening of the most intense line at Full Width Half Maxima (FWHM), based on the Debye-Scherrer equation (Habibi et al., 2010). Our results showed a particle size diameter of 18.99 nm.

3.3 Photo catalytic tests

Tests using 50 ml of the water samples and 0.75 g/L of the photocatalyst were carried out in triplicates and 1ml aliquots sampled every 15 minutes for a total illumination time of 75 minutes. A TUV 8W G8 T5 lamp from PHILIPS was used as energy source. The intensity reaching the solution was 0.3520 watts (350–560 nm) as measured using an optical power meter Radiometer (USB2000 Spectrometer from Ocean Optics). The test sample was positioned approximately 3 cm from the BLB lamp. The sample was continuously stirred using a magnetic stirrer (400 rpm). Control tests without the photocatalyst were carried out to establish inactivation by photo catalysis only. Dark control experiments were also carried out to exclude UV light. This was done by tightly covering the reaction vessel with aluminium foil to exclude all light. All experiments were performed in triplicates. The set-up was as shown in Figure 1:

Figure 1: Illumination set-up for the Photocatalysis Experiments.



A portion of 1ml of raw water and 1 ml of each of the samples subjected to photo catalysis was cultured and incubated for 24 hours at 37.2° C and the colony forming units counted. Using 3M Petrifilm *E. coli* count plates, the data was recorded as Colony Forming Units (CFU)/ml versus illumination time (minutes) for analysis.

Results on photocatalytic inactivation with time were recorded using 3M Petrifilms *Escherichia coli* test kits, where colony forming units' counts versus illumination time were recorded for different variables of photocatalyst and pH. Consequently, the residual fraction, percentage *Escherichia coli* removal efficiency, log reduction of *Escherichia coli* and the variation of the rate constant versus time were analyzed.

4. Results

4.1 Effect of catalyst load versus efficiency of *Escherichia coli* removal from water

A trend of the residual fraction with time in minutes using different photocatalyst amounts was generated as shown in Figure 2 below. Photocatalytic action is evident as the highest residual fractions were obtained during photolysis and dark reactions. It can be seen that photolysis gradually reduced the residual fraction consistently, while during the dark reaction, an increase of the residual fraction was observed at 75 minutes. This can be explained that the reduction during dark reaction was as a result of adsorption of the *Escherichia coli* onto the surface of the TiO₂-WO₃ photocatalyst. With time, the *Escherichia coli* got desorbed back into the solution thereby raising the number of *Escherichia coli* detected after 75 minutes.

The photokilling effectiveness of the catalyst increased with catalyst amount up to 0.75 g/L optimum amount. Increasing the photocatalyst amount resulted to no further increase in photokilling effectiveness. A plot of degradation efficiency versus amount of catalyst was plotted which showed that 0.75 g/L catalyst amount as the optimum. Percent removal efficiency rises with photocatalyst amount upto 0.75 g/L and then decreases at a photocatalyst amount of 1.0 g/L. Thus, 0.75 g/L photocatalyst amount was taken as the optimum amount at these experimental conditions and other experiments were conducted at this catalyst amount. The effectiveness of photocatalytic degradation of *Escherichia coli* with the amount of catalyst is as shown in Figure 4 below.

4.2 Effect of pH on *Escherichia coli* photokilling efficiency

Next, the effect of pH on photokilling efficacy was investigated. The data on the residual fraction with time at different pH values is as shown in Figure 4 below, starting with 2600 CFU/ml count of *E. coli* in the test water. It was found that varying pH did not significantly vary the *Escherichia coli* inactivation rate after 15 minutes.

Figure 2: Residual Fraction of *E. coli* over time at different catalyst amounts

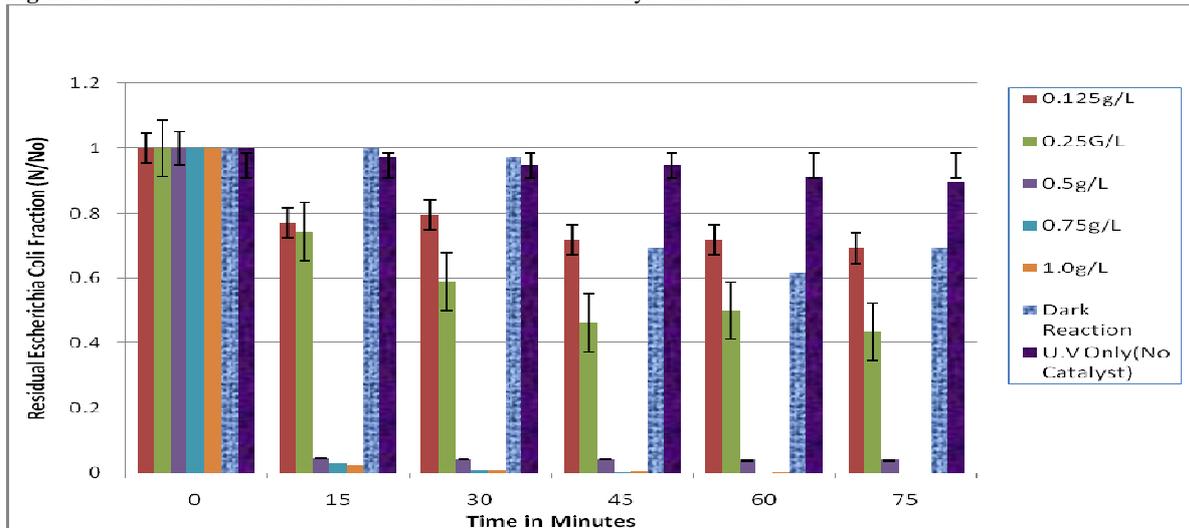


Figure 3: Effect of Catalyst Concentration on *Escherichia coli* removal, N= CFU/ml Irradiation. Time = 75 min, pH=7.3

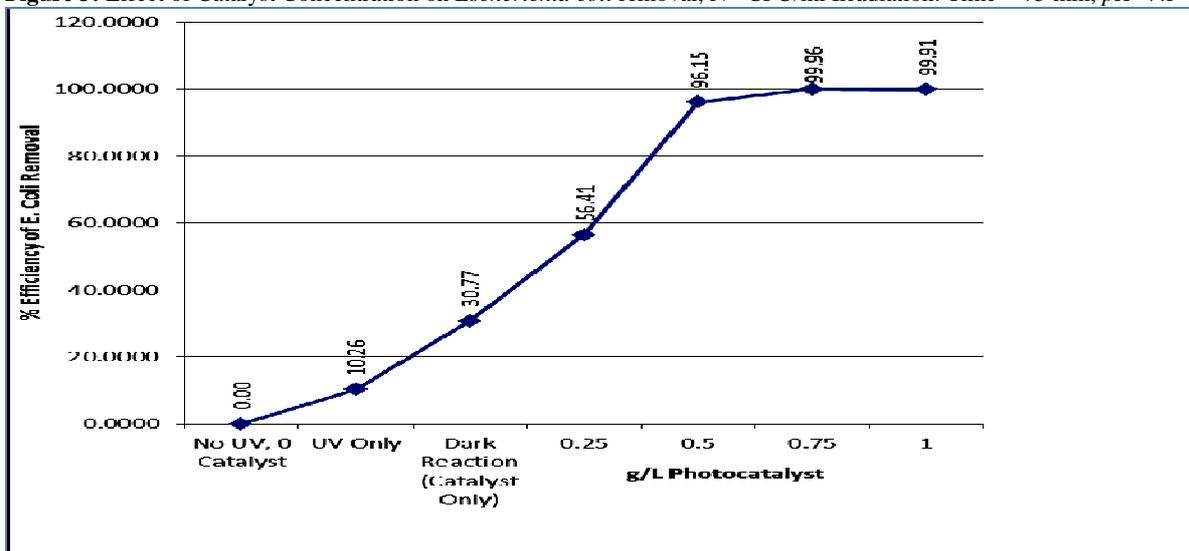


Figure 4: Effect of PH on Photokilling of *E. coli* 0.75 g/L Catalyst

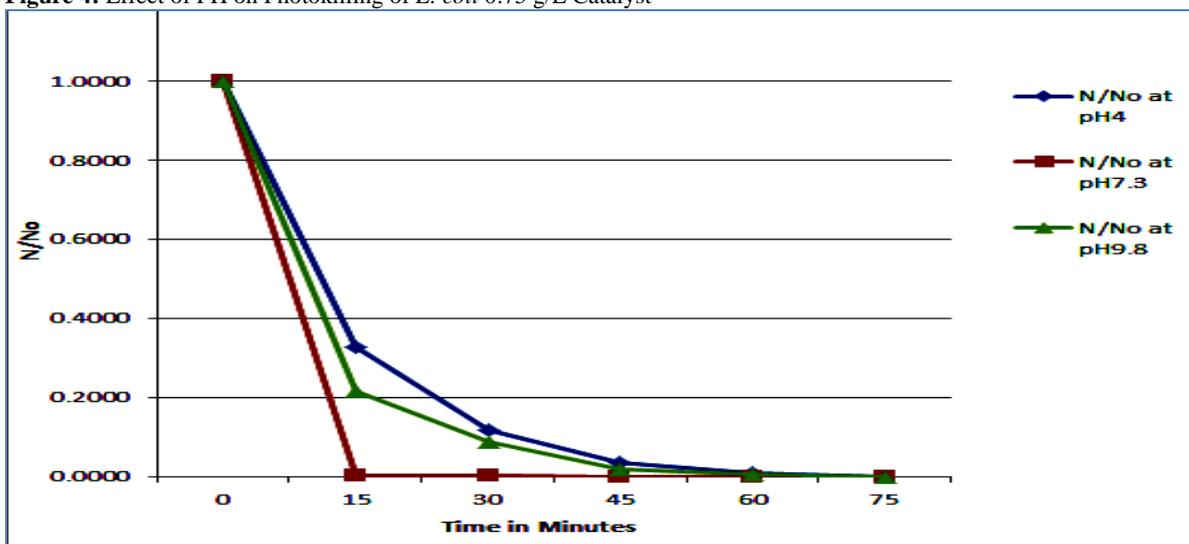


Figure 5: Log Inactivation of *Escherichia coli* with Time, pH 7.3

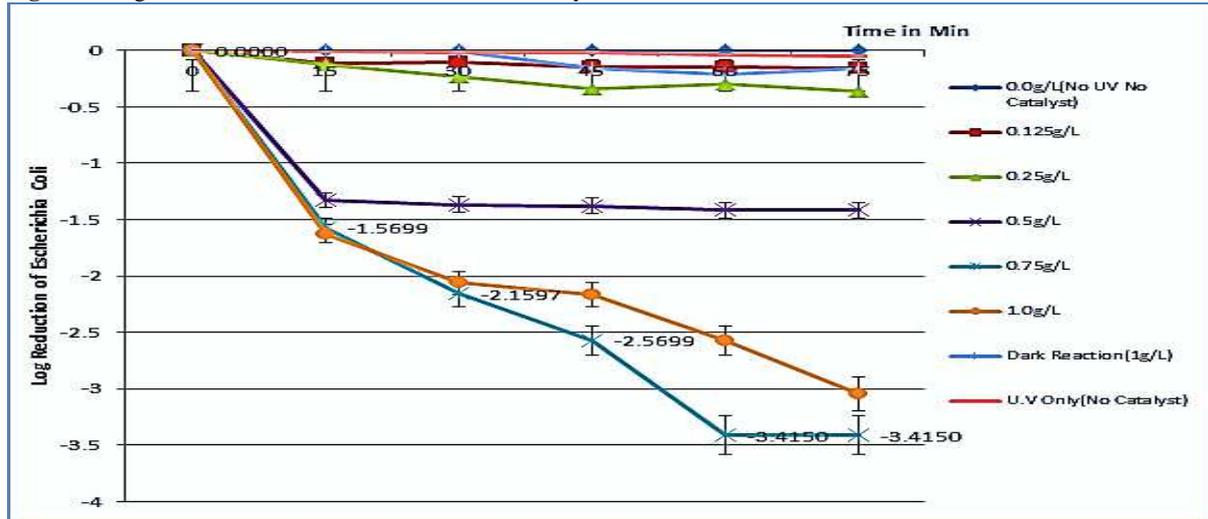
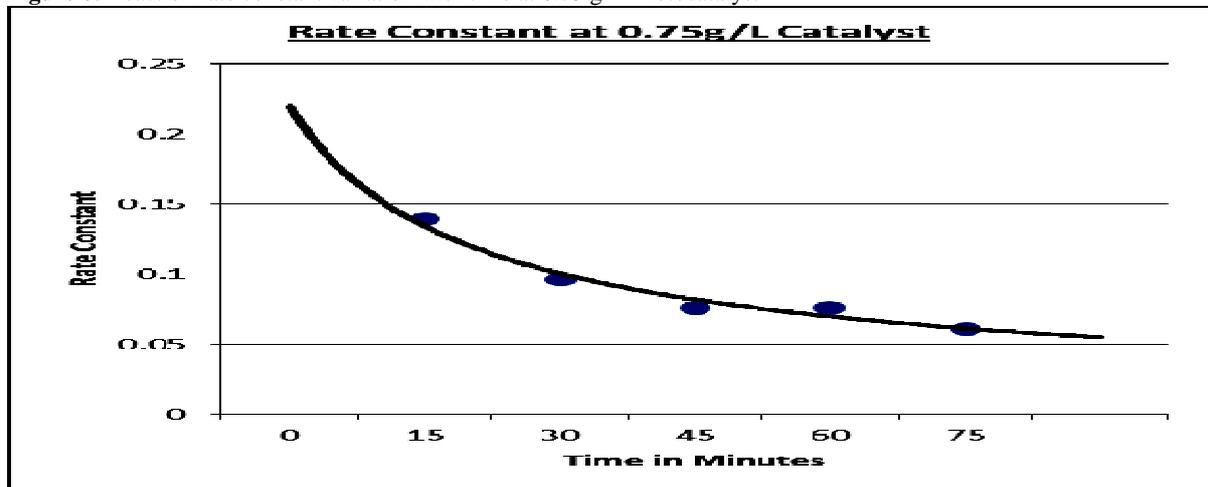


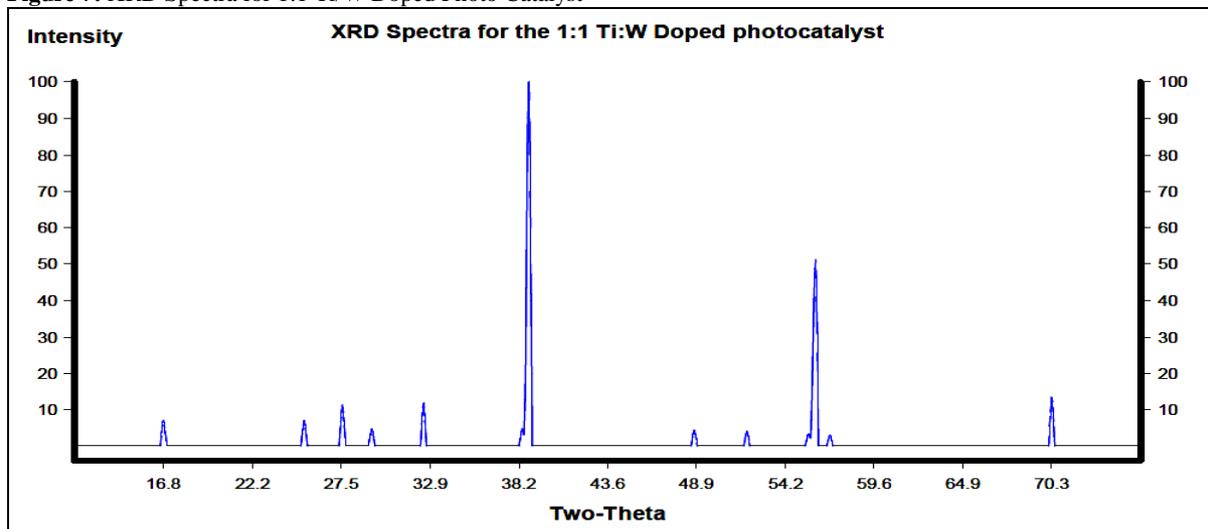
Figure 6: Reaction rate constant variation with time at 0.75 g/L Photocatalyst



Using the equation for log inactivation, the rate constant for the photokilling was determined as follows:
 $\log N/N_0 = -kCT$, Where k = the rate constant, C = the amount of catalyst, 0.75 g/L, T = the illumination time

The reaction rate constant at different time intervals was determined for the 0.75 g/L photocatalyst amount. Figure 6 shows the plot obtained.

Figure 7: XRD Spectra for 1:1 Ti/W Doped Photo Catalyst



The particle size for the TiO₂-WO₃ compound was calculated using the rearranged Scherer equation and using the peak broadening of the base peak [Habibi et al., 2010] as follows:

$$L = K\lambda / (B\cos\theta)$$

Where B at 2θ is the Full Width at Half Maxima (FWHM) of the base peak (I/I₁=100), in radians, calculated as 0.007652.

K is the Scherer constant, =0.89

λ is the wavelength of the Cu-ka x-rays used = 1.54060Å

L is the particle size (diameter) in nanometers θ is the angle in degrees, 19.31915 (2θ=38.6383) for the base peak, cos θ = 0.9437 Substituting these figures into the Scherer equation yielded a particle size of 18.99 nm~ 19nm.

It can be seen that pH 7.3 was best for the photokilling of the *Escherichia coli*. Efficiency was lowest at pH 4 but followed almost a similar trend for all pH from 45 minutes of illumination.

These agreed with the findings by Gummy and co-workers (2004), who found that the photolytic inactivation of *Escherichia coli* was independent of initial pH between 4.0 and 9.0 (Gummy et al., 2004).

Heyde and Portalier (1990) explained that the negligible *Escherichia coli* reaction to acid conditions was due to the presence of an acid tolerance response in the bacterium itself, which secreted the acid-induced proteins for bacterial acid-shock protection (Heyde et al., 1990). Our results of Log residual fraction Versus Time in minutes showed a peak log reduction of 3.415 corresponding to 99.9615% *E. coli* removal at 0.75 g/L catalyst loading as shown in Figure 6 below.

It can be seen that the rate constant is highest within the first 15 minutes, decreasing gradually as time progresses. This could partly be explained that with time, there was fouling of the photocatalyst by suspended solid content of the test water which blocked the surface active sites and therefore reducing the photocatalytic rate. Further work with pure water spiked with pure strains of *Escherichia coli* is therefore recommended to fully assess the potential of catalyst fouling from the suspended solid content of the environmental samples.

6. Discussion

The XRD data in Figure 7 below was used to determine the particle size of the synthesized TiO₂-WO₃ compound using the Debye-Scherrer equation as calculated below.

From the results obtained, it was found that the composite at 1:1 ratio gave the best results. This can be explained by the fact that since the excited electrons from the valence band of titanium (IV) oxide are transferred to the conduction band of tungsten (VI) oxide to avoid charge recombination, the best performance can only be obtained in the presence of equal donor and acceptor particles. This would allow for capturing of all electrons generated from the valence band of titanium (IV) oxide once they migrate to the surface of the TiO₂ by WO₃, thus preventing charge recombination,

and effectively improving the quantum yields of the composite.

Results on *Escherichia coli* and total coliforms indicated a negligible *Escherichia coli* reduction rate variation to acid conditions. According to Hyde, (1990), this is due to the presence of an acid tolerance response in the bacterium itself, which secretes acid-induced proteins for bacterial acid-shock protection [Heyde et al., 1990].

Conclusion

Although a lot has been done on photocatalytic decomposition of pollutants in water, this work went further to explore the potential of simple composite synthesized using locally available and abundant resource in Kenya (TiO₂). The composite was synthesized using fairly mild conditions and later tested for water purification applications using *Escherichia coli* in natural water samples collected from the environment as opposed to lab cultured strains. The experiments proved that the titanium (IV) oxide- Tungsten (VI) oxide composite can be effectively used in the removal of microbial pollutants from water. The results indicated a negligible *Escherichia coli* reduction rate variation to acid conditions. From the data obtained significant disinfection was not observed for the dark control (photocatalyst only with no UV), UVA irradiation alone (in the absence of photocatalyst) or with the sample stirred in the presence of photocatalyst. Process taking place during dark control was explained as adsorption-desorption of the *Escherichia coli* from the surface of the photocatalyst.

Photocatalysis is a promising technology in water purification with possible and practical opportunities existing for small-scale point-of-use water purification units where potable water could be treated for disinfection of pathogens or trace priority pollutants remaining in (or entering) the water distribution network following conventional treatment; for example, chlorine-resistant pathogens, endocrine-disrupting chemicals (EDCs) or pharmaceuticals and personal care products (PPCPs). Our earlier work (unpublished) showed that our photocatalyst composite was effective in removal of heavy metals and organochlorine pesticides which are some of the pollutants that are not removed by chlorination. The green reactions

of photocatalysis are ideal in energy saving and emission reductions.

Research Highlights

This work focused on exploring the potential of nanoparticle photocatalysis in use for water purification. *Escherichia coli* was used as model bacteria to explore disinfection kinetics of the nanocomposites used and the optimum working conditions of pH, initial catalyst amount and time factors. The findings can be applied to point-of-use water purification especially for poor communities living in the tropics where solar energy can be harvested and harnessed to effect pathogen removal.

Limitations

The major limitation in this work was the subsequent requirement to filter the nanoparticles after each test. This was because the tests were performed with dispersed and stirred nanoparticles in water. Anchoring procedure for the nanoparticles on immobilized support will be undertaken later.

Recommendations

The author recommends more work to be carried out to establish the efficacy of the photo catalyst in treatment of industrial effluents from other types of bacteria, fungi, algae, viruses and toxic inorganics like phthalates; work which was not carried out in this project. Also, a step by step determination of degradation products for the bacteria was not monitored in this work and it is recommended that more work should be carried out to monitor their photocatalytic degradation with time.

Anchoring of the nanoparticles on immobilized supports is another area which needs to be explored. This will eliminate the process of filtration required after each test using expensive filters. Another area which requires to be explored is on the energy storage capacities of the nano particles. This will determine whether the nano particles can be used in solar cells.

The nanoparticles should also be tested against air purification and on self-cleaning surfaces and their performance be compared to that of Titanium (IV) Oxides which has already been tested. This will help in determining the optimum applications. Results on X-Ray fluorescence indicated that the type of doping achieved was primarily Wolframyl or surface doping and that no solid solution was achieved under the conditions of this work. This means that the composites had the WO_3 nanoparticles adsorbed on the surface of TiO_2 . It is recommended that more work be done that will

effect lattice doping or the incorporation of WO_3 into TiO_2 matrix.

This will give a comparative study between the composited and lattice-doped products which will help in deciding on the better performing products for similar applications in water purification. It is recommended that the composites be anchored on different supports and their efficacy in pollutant removal be tested and compared to the free dispersions of the nanoparticles in water. This will help in eliminating the problem of filtration which has to be done at the end of each experiment.

The author also recommends that studies should be carried out using the filtered composites to establish whether they can subsequently be re-used after initial application, and the performance efficiency at each re-use step. This will inform on whether they can be incorporated into water filters and also help in establishing the lifespan of those filters.

Funding and Policy Aspects

From the findings of this research work, it is recommended that policy makers take into consideration the potential contributions of scientific research into policy implementation in water purification processes. In Kenya, the right to clean drinking water is embedded in the constitution as a right to every citizen. This can only be realized if clean and energy efficient technologies are adopted and incorporated into policies that guide water purification and distribution. The relevant Kenya Government research funding bodies should work with research institutions through funding water purification research, and coordinate the implementation of research findings to target communities.

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References

Feng P., Weagant S., Grant M., 2002. Enumeration of *Escherichia coli* and the Coliform Bacteria: Bacteriological Bacteriological Analytical Manual, 8th Edition, Revision A: Chapter 4.

Grieken R., Marugan J., Sordo C., Martinez P. and Pablos C., 2009. Photo catalytic Inactivation of Bacteria in Water Using Suspended and Immobilized Silver-TiO₂. *Applied Catalysis B* 93, 112-118.

Gumy D., Rincon A.G., Hajdu, R., Pulgarin C., 2006. Solar photocatalysis for detoxification and disinfection of water: different types of suspended and fixed TiO₂ catalysts study. *Solar Energy*, 80(10), 1376–1381.

Habibi M.H., Mojtaba Nasr-Esfahani, Giti E., and Baharak H., 2010. Nanostructure Thin Films of Titanium Dioxide Coated on Glass and Its Anti UV Effect for Living Organisms. *Current Nanoscience*, 2010, 6, 324-329.

Heyde M. and Portalier R., 1990. Acid Shock Proteins of *Escherichia coli*. Federation of European Microbiological Societies. *Microbiology Letters* 69, 19.

Ke D.N., Liu H.J., Peng T.Y., Liu X., and Dai K., 2008. Preparation and Photo catalytic Activity of WO₃/TiO₂ Nanocomposite Particles. *Materials Letters* 62(3), 447-450.

Kwon Y.T., Song K.Y., Lee W.I., Choi G.J. and Do Y.R., 2000. Photo catalytic Behavior of WO₃-Loaded TiO₂ in an Oxidation Reaction. *Journal of Catalysis* 191, 192-199.

Masakazu A. and Masato T., 2001. Design and development of second-generation titanium oxide photo catalysts to better our environment: Approaches in Realizing the Use of Visible Light. *International Journal of Photoenergy* 3, 92-94.

Pin-Ching M., Sharon S., Daniel M. B., Zheng H., Wolfrum E. J. and William A. J., 1999. Bactericidal Activity of Photo catalytic TiO₂ Reaction: Toward an Understanding of its Killing Mechanism. *Applied and Environmental Microbiology* volume 65(9), 4094-4098.

Sobana, N., Selvam K., and Swaminathan, M. 2008. Optimization of photocatalytic degradation conditions of direct red 23 using nano-Ag doped TiO₂. *Separation and Purification Technology*, 62, (3), 648–653,

United States Environmental Protection Agency, 2010. State of the Science Literature Review: Nano-Titanium (IV) Oxide Dioxide Environmental Matters. *STREAMS Final Report* pp 44.

Watterworth, L.A. and Schraft, H. 2005. Enumeration of heterotrophs, fecal coliforms, and *Escherichia coli* in water: comparison of 3M Petrifilm plates with standard plating procedures. *Journal of Microbiological Methods*, 60, 335-342.