Photocatalytic Inactivation of Microbial Pathogens in Water

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Abstract

The inactivation of pathogens in drinking water has, for the last hundred years, been a major contributor to public health and well being. Chlorination of drinking water, introduced in the early nineteenth century, has contributed to the reduction of microbial pathogens in drinking water and numbers of those reporting illnesses associated with contaminated water.

Chlorination, however, has its own drawbacks, including the formation of trihalomethanes, and the fact that research suggests that it may be ineffective against protozoan, viral or biofilm forming organisms.

Over the last twenty years a new technology has been developed which has been shown to address the above concerns. Semiconductor photocatalysis is a process which requires no addition of chemicals to the water and therefore leaves no residual toxic by products.

This study involves the experimental assessment of the disinfection efficiency of a point of use photocatalytic system for the domestic market. Experiments were carried out using *E.coli* in deionised water and tap water, with a reactor coated with titanium dioxide, over a period of several weeks.

The results, while inconclusive, did indicate that the process has merits. On each occasion, the bacterial count was reduced substantially although the reduction was not as pronounced as other studies have demonstrated it to be.

Key words: photocatalysis; *E.coli*; titanium dioxide; water; disinfection.

Introduction

Water is fundamental to life and health. Everyone in the developed world uses large quantities of water every day washing, bathing, flushing toilets, and drinking. Business uses water to produce almost everything we use. Many people expect to have clean drinking water at will and take it for granted. Typical UK household water consumption was, in 1994, 475 litres per day, and automatic washing machines can use up to 110 litres every time they are used (Gray, 1994).

In order to safeguard public health the water supplied must be free from pathogenic organisms, high levels of harmful chemicals and other contaminants. Water sources are often contaminated with sewage runoff and nitrates from agricultural fertilisers. These can affect both surface and ground water sources and consequently water needs to be carefully screened and treated (Geldreich, 1990).

Several potentially fatal diseases have been and continue to be associated with contaminated drinking water. The incidence of typhoid fever has been almost eradicated in the developed world. However, the disease is still widespread in the developing world and results in the deaths of many thousands of people. This is a very common waterborne disease caused by the bacteria *Salmonella typhi* with a one in ten chance of mortality if untreated (BBC Health, 2006). Cholera is another example of water borne illness. This acute intestinal infection is caused by the toxin produced by the bacteria *Vibrio cholerae* and no longer occurs in the UK but *cholera gravis,* a severe form of the illness, is still claiming lives in South America, Africa and Asia (Health Protection Agency, 2008). Almost 20% of the world’s population still have no access to clean drinking water and “around 3.1m people died in 2002 as a result of diarrhoeal diseases and malaria, 90% of whom were children,” according to a report by the United Nations (U.N., 2006).

Since John Snow discovered that the source of a cholera outbreak in London in 1854 was a water pump, it has been clear that the provision of a supply of safe drinking water is fundamental to the safeguarding of public health. Indeed the lack of such supply contributes to many deaths throughout the world on a daily basis. As long ago as 1500BC the ancient Egyptians were using alum for the clarification of drinking water. In 1850, before Pasteur‘s theory of germs, slow sand filtration was becoming more common and in 1900 chlorination of drinking water was introduced (Royal Society of Chemistry, 2003).

The treatment of drinking water supplies in the developed world is carried out in a number of ways including coagulation and flocculation, clarification, ozonation, filtration and disinfection with chemicals or light (Fig.1).

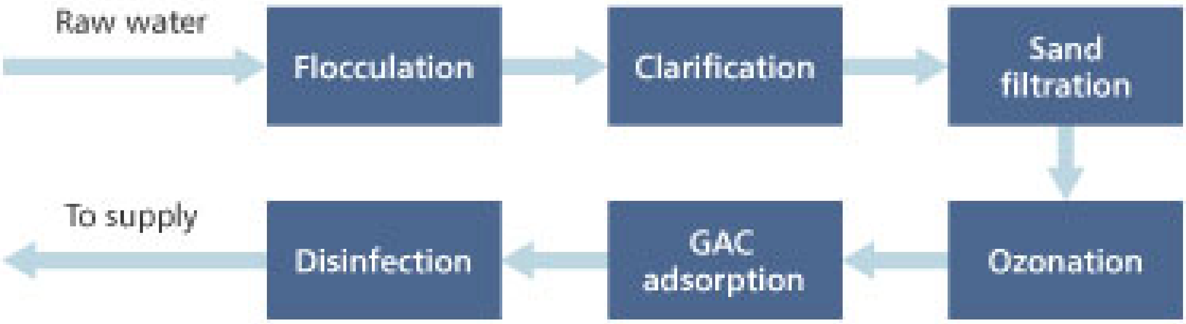


Fig.1

Granular activated carbon (GAC) has also been widely used as an effective method for the removal of organic precursors, thereby reducing the regrowth of bacteria after disinfection. It involves the removal of a compound from a solution by attachment to a surface adsorbent. This attraction may be physical, chemical or electrical (Black et al, 1992).

Disinfection is necessary to remove residual pathogens left behind after other treatments. The most common disinfection method is chlorination of the water with chlorine gas and hypochlorites (Rizzo et al, 2007). There is concern however that this treatment is ineffective against some pathogens, such as *Cryptosporidium* and *Giardia*, the oocysts and cysts of which have been found in fully treated drinking water. With an effective dose of between 10-100 cysts or oocysts, this is obviously an area of concern (Szewzyk et al, 2000). *Clostridium perfringens* spores are more resistant to chlorination than vegetative bacteria such as coliforms, (the indicator organism for faecal contamination) and bacterial susceptibility to inactivation by chlorination varies widely (Environment Agency, 2002).

European Union Council Directive 98/83/EC (1998) on the quality of water intended for human consumption states in Article 4 that the water shall be clean and wholesome if it is “free from any micro-organisms and parasites and from any substances which, in numbers or concentrations, constitute a potential danger to human health”, (EUR Lex, 2008). It appears from recent research that current methods of water treatment may not fully comply with this directive and that some by products of the chlorination disinfection process may indeed pose a risk to health.

Chlorination By-products

Chlorination results in the formation of several by-products such as trihalomethanes (THMs), which may be harmful to human health. THMs are the result of the reaction of chlorine and its derivatives with organic matter in the water.

Bofetta (2006), states that average measurements of THMs in the United States reached 10µg/L for chloroform, bromodichloromethane and chlorodibromomethane, and almost 5µg/L for bromoform. He cites a study which states that above 1µg/L may lead to an increased risk of bladder cancer while pointing out that there are several complicating factors including the level of organic matter in the water, the water source and contributory dietary factors. Other authors (Kim et al, 2002: Latifloglu, 2003) have found similar results in other areas of the world.

Treatment

The treatment of drinking water therefore has been shown to be highly effective and necessary but with some drawbacks as stated above. A new treatment was emerging in the 1960s with the discovery of the effect of oxide semiconductors which responded to light. This technique of water splitting into hydrogen and oxygen involved the application of titanium dioxide and led to heterogenous photocatalysis, using photoelectrochemistry without an external circuit. Subsequently, Frank and Bard (1977) examined the use of TiO_2 to decompose cyanide (Fujishima and Zhang, 2005).

Photocatalysis

In recent years a process has emerged which may address the concerns regarding the shortcomings of chemical disinfection. TiO_2 Photocatalysis is the treatment of water or air with light and a catalyst namely titanium dioxide. It is a novel technology which has been shown to be effective in inactivating pathogens in water (Blake et al, 1999). No chemicals are added to the water during the process and no toxic by-products are produced.

The process has received much attention since Matsunaga et al (1985) reported the concept as being successful in the destruction of *Lactobacillus acidophilus, Saccharomyces cerevisiae* and *Escherichia coli* in the mid 1980s. Ibanez et al (2003) demonstrated the efficacy of the catalyst when exposing the bacterium *enterobacter cloacae* to UVA light (to which it is known to be resistant). The experiment showed that sub lethal doses of UVA when combined with TiO_2 were highly effective in inactivating *E. cloacae*. Guimaraes and Baretta (2003) reported that the process is capable of inactivating spores of *Clostridium perfringens* by a factor of 98% after an exposure time of 152 seconds.

TiO_2

Although there are a number of successful catalysts used in the disinfection of drinking water including ZnO, ZrO_2, CeO_2, Fe_2O_3, and WO_3, TiO_2 is the most widely used because of its stability, photoactivity and lack of toxicity, (Benabbou et al, 2007). These authors report that "photocatalytic water disinfection seems to be a promising alternative for bacterial elimination"; with the provision that certain parameters are taken into account including the intensity of the UV light and the concentration of the TiO_2.

Photocatalysis, through the reduction of organic matter in drinking water, may assist in the reduction of trihalomethane production in treated water, as well as the obvious benefit of disinfection of the water itself. Murray and Parsons (2006) state that photocatalysis using TiO_2 can achieve a reduction of over 96% of ultra violet absorbing natural organic matter species and over 81% reduction of dissolved organic carbon.

Mode of action

TiO_2 in its crystalline form behaves as a semiconductor. The illumination of the TiO_2 with near UV light results in the generation of excess electrons and holes. At the TiO_2 surface the holes react with oxygen or hydroxyl groups to produce hydroxyl radicals. The excess electrons react with oxygen to form superoxide ions and consequently more hydroxyl radicals (Ireland et al, 1993). This group used titanium dioxide in its crystalline form in a flow through water reactor. Their experiments were carried out using dechlorinated tap water in an attempt to assess the efficacy of the reactor.

Killing mechanism

TiO_2 Photocatalysis relies on the production of hydroxyl radicals in its killing mechanism. Maness et al (1999) studied the killing mechanism of TiO_2 and discovered that the photocatalysis of *E. coli* and the consequent production of hydroxyl radicals resulted in the peroxidation of the lipid cell membrane initially and then in major cell membrane damage. This led to the loss of essential functions including respiration, and death of the cell. Similar results were reported by Nadtochenko et al (2005) using infra red spectroscopy and atomic force microscopy. Transmission electron microscopy shows the interaction of the TiO_2 with the bacterial cell surface.

Cho et al (2003) stated that an "excellent linear correlation" existed between the production of OH radicals and the inactivation of *E. coli* in a paper described as being the first quantitative study of this kind of correlation. The group go on to state that the OH radical is one thousand to ten thousand times as effective as chlorine and ozone.

Matsunaga et al (1985) also reported an oxidation of Coenzyme A resulting in respiratory activity inhibition. Sunada et al (2003) studied the destruction of *E. coli* endotoxin which is also a part of the cell membrane, suggesting that the "photokilling reaction is initiated by a partial decomposition of the outer membrane, followed by disordering of the cytoplasmic membrane, resulting in cell death."

Photo-electrochemistry

Several authors have reported the efficacy of an applied potential coupled with the photocatalytic system. Photo-electrochemistry has been proven to be effective in the disinfection of water contaminated with faecal indicators (Butterfield et al, 1997). This team used an electric field within the photochemical reactor investigating the destruction of *E. coli* and *Clostridium perfringens* reporting a 100% removal of *E. coli*. Dunlop et al (2002) compared different TiO_2 catalysts using anatase and rutile mixture and anatase alone, on thin films with and without an applied potential. Their findings showed that the photocatalysis was more effective with the anatase rutile mixture and the applied potential showed a significant disinfection rate increase on both films. This work reported a 99.996% reduction of colony forming units after 120 minutes of photocatalytic treatment.

Methods of application

There are two distinct methods for TiO_2 photocatalytic disinfection of bacteria in water. These are in suspension, i.e. slurries of TiO_2 suspended in the liquid which requires disinfection, and fixed TiO_2 where the TiO_2 is fixed on the surface of the reactor and the light fed from an external source. Both systems have limitations. The suspended TiO_2 reactor requires continuous stirring to ensure mixing and an end step where the TiO_2 must be removed from the water, while the fixed TiO_2 reactor suffers from lower reaction rates, possibly attributable to poor contact with the catalyst, especially in high bacterial concentrations. (Blake et al, 1999).

Point of use application

A point of use reactor such as the one used here is designed to treat the water at the end point, i.e. in the home. The reactor would be positioned under the sink, with the rising main flowing through it just before the water reaches the tap. The reactor would be treating the water for any residual chemicals such as trihalomethanes, hormones such as oestrogen, and any chlorine resistant bacteria before it reaches the consumer.

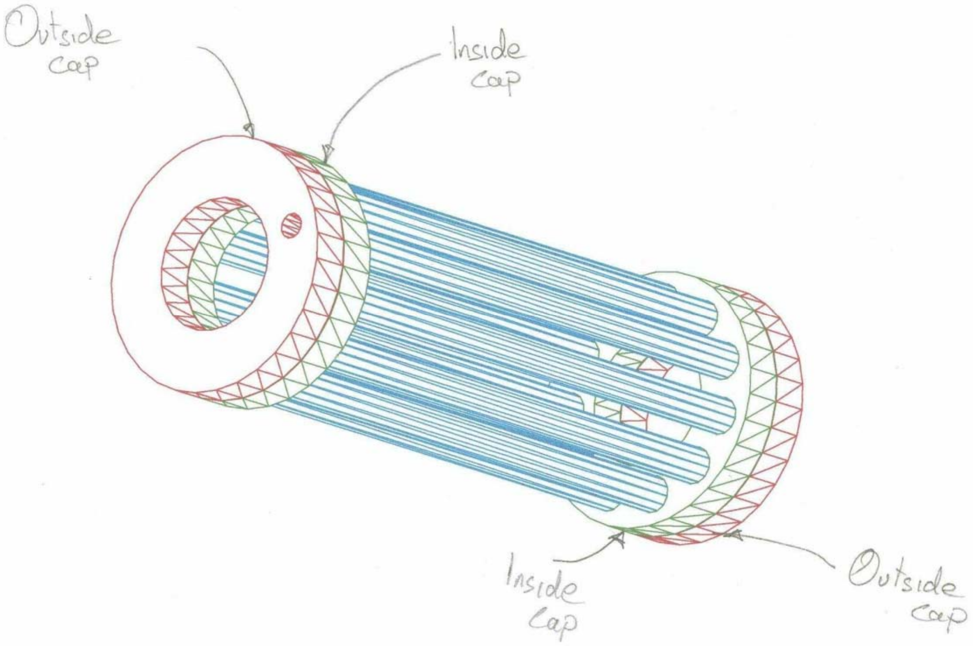
Although under the experimental conditions described below the initial bacterial load in the water sample each time was in excess of 10^4 CFU/ml these numbers are much higher than levels expected to be found in domestic potable drinking water and it should be stressed that this reactor is not designed or expected to inactivate bacteria at this concentration consistently.

Materials and methods

The photocatalytic reactor was designed by the Photocatalysis Research Group (PRG) attached to the Nanotechnology and Integrated Bioengineering Centre (NIBEC) for research at the University of Ulster at Jordanstown. The reactor prototype was constructed by the engineering department at the university. It consists of a cylindrical continuous glass tubing system (Figure 2) onto which TiO_2 powder Degussa P25 (75% anatase and 25% rutile) has been fixed. The reactor was attached to a peristaltic pump (Watson Marlow 101 U) and a UV light source consisting of a black light bulb (Sylvania 15W BLB UVA - peak emission ~365 nm) was inserted into the middle of the cylinder. The reactor was then enclosed in aluminium foil to ensure the maximum effect from the UV light source.

Experiments were carried out using a reactor with no catalyst i.e. UV light only control, (photolysis), using the TiO_2 reactor without UV light, dark control, (absorption only) and with both light and catalyst (photocatalysis). The test solution was pumped through the reactor at a pre- designated rate and collected in sterile glass jars which had been autoclaved at 121^0C for 15 minutes.

Fig. 2



E. coli K12 was cultured overnight from stock streak plates in Luria Bertani (LB) broth. In a typical experiment 50-60 non selective LB agar plates were prepared and incubated overnight to ensure a 'tacky' surface for the sample absorption.

The reactor was flushed with a 1% solution of H_2O_2 and then rinsed with sterile distilled water before each use. Optical density of the *E. coli* culture was determined by first 'blanking' the spectrophotometer and then placing the *E. coli* culture in the machine and reading the display at 600 nm. The culture was placed in a balance centrifuge (Centaur 2 MSE) at 4400 revolutions per minute for ten minutes. The broth was then aspirated from the culture and the pellet re-suspended in 10 ml Ringers solution.

This starting sample was diluted with further 9ml Ringers solutions to 10^7, 10^6, 10^5, 10^4, 10^3, 10^2 dilutions, assuming from the optical density readings a starting sample of approximately 10^8 Colony forming units (CFU). 100µl aliquots were withdrawn and plated onto non selective agar from the 10^4, 10^3, and 10^2 dilutions to establish numbers of CFU in the original sample.

Tap water was boiled to remove any microbial contaminants, collected in a sterile glass jar and allowed to cool. This water was inoculated with the diluted culture at various concentrations and thoroughly mixed. Samples of this solution were plated on non selective agar, and designated as 'stock'. To ensure optimum levels of oxygen in the stock sample for the reduction reaction to occur, the stock was then oxygenated with an aquarium pump for ten minutes before being passed through the reactor. Tubing from the aquarium pump entering the stock solution was disinfected prior to use.

The peristaltic pump speed was calculated using a measured volume of water and a stopwatch and set at 20ml per minute (pump speed 26). The stock solution was arranged at one end of the reactor, beside the pump, with silicone tubing used to feed the solution into the reactor. Tubing at the other end of the reactor was placed in a sterile glass jar to collect the treated solution. The pump was switched on and the stock pumped through the reactor.

Samples were taken for analysis following treatment. As the treated solution passed through the exit tube of the reactor, the first 15ml was collected and discarded (to remove the risk of residual contamination from the tubing). The next 10ml was collected in a sample jar and labelled ‘Pass 1’. The remainder of the stock was collected in the sterile glass jar. A 1ml sample from ‘Pass 1’ was again diluted with 9ml Ringers to suitable concentrations and three samples of each dilution plated onto LB agar.

The treatment process was continued, oxygenating between each pass through the reactor, to ensure optimum O_2 levels, up to a maximum of five passes. All plates were left for several hours then inverted, and incubated at 37.5 ^{0}C for 24 hours before colony forming units were visually identified and counting.

The disinfection experiment was carried out over a period of eight weeks, with one set of results obtained each week.

Results

In all but one of the experiments the initial stock concentration used was in the region of 10^4 CFU/ml and dilutions to 10^3 and 10^2 CFU/ml were prepared, to ensure that colonies could be easily counted – (approximately 20-200 CFU per plate).

The number of CFU/ml in the stock solution was significantly reduced by 1 Pass through the photocatalytic reactor (71.86%). Significant disinfection was not observed in the control experiments, no treatment (~3%) and UV light only (~3%), (Figure 3). To obtain complete disinfection the treatment was repeated for three passes (Figure 4). The percentage disinfection increased with successive treatments however complete disinfection (97.8% following 3 passes) was not obtained.

The results in this initial study were surprising given the research already carried out on the topic and the success of the various authors cited.

To determine if the initial results were an anomaly due to one or more parameters or variables, one experiment was carried out during which the water was subjected to five passes through the reactor, (Fig.5). The same aseptic technique was used and the water was aerated between each pass to ensure there was an adequate amount of oxygen present to enable hydroxyl radical production.

Inactivation of the bacteria from initial numbers has been successful; however, colonisation is evident after the second pass and is seen to increase after subsequent passes through the reactor, until the fifth pass which shows maximum inactivation at this dilution (98.77%). Although the re-appearance of the bacteria after this level of treatment is at very low levels, their presence in drinking water systems is unacceptable and therefore needs to be addressed.

In research of this nature it would be common practice to experiment until the bacteria have been inactivated and stop when no further growth is apparent. This study shows that while the *E.coli* numbers have been substantially reduced, there is a propensity for survival and re-growth.

There are several conditions which may explain why this re-growth is occurring including a possible fault with the reactor itself. Human error may be another factor, however the results, while unexpected, were consistent. The adsorption onto and subsequent release of the bacteria from the catalyst surface has also been considered but there is little evidence to support this.

Consideration was given to the possibility of bacterial re-contamination during the process, including contamination from the plastic tubing, glassware, pump, pipettes and the reactor. This was ruled out following subsequent experiments when the utmost caution was exercised to ensure aseptic techniques and similar results were achieved.

One possible explanation for the survival of bacteria following treatment could be the formation of persister cells. This type of cell was first described by Joseph Bigger in 1944 (Lewis, 2006). Persister cells are recalcitrant cells which appear to survive antibiotic and other disinfection methods. Lewis, (2007) describes them as “essentially invulnerable” and goes on to explain that they neither grow nor die in the presence of antimicrobials. Keren et al, 2003 described persister cells as “specialized survivor cells”. If indeed this type of persister cell was present within the water sample it could account for the remaining bacteria in the water after treatment. If however these bacteria were present in small enough numbers in treated water and there was a sufficient concentration of residual disinfectant the water may still be wholesome as the persister cells cannot grow in the presence of the antimicrobial but merely survive.

Without carrying out a number of further experiments, speculation would suggest that the parameters within which the research was carried out were not in fact optimal and that further work should be done. The investigation of the many parameters and variables associated with this study to the degree required, while initially an aim was in reality outside the scope of the study, considering the time constraints of a final year student project and the availability of the laboratory for further experimentation.

It should again be borne in mind that the initial bacterial loading investigated was ~ 10^4 CFU/ml. The reactor is designed to operate as a point of use device, i.e. used to ensure potable water entering is safe and therefore further experiments should be carried out at more realistic bacterial loadings.

Results for 17 October 2007

	Plate 1	Plate 2	Plate 3	Average	S Dev	S Error
No treatment T=0	237	219	248	234.6667	14.64013	8.452482
No treatment P1	230	219	241	230	11	6.350853
UV only T=0	204	197	210	203.6667	6.506407	3.756476
UV only P1	197	204	189	196.6667	7.505553	4.333333
Photocatalysis T=0	205	206	207	206	1	0.57735
Photocatalysis P1	56	43	40	46.33333	8.504901	4.910307

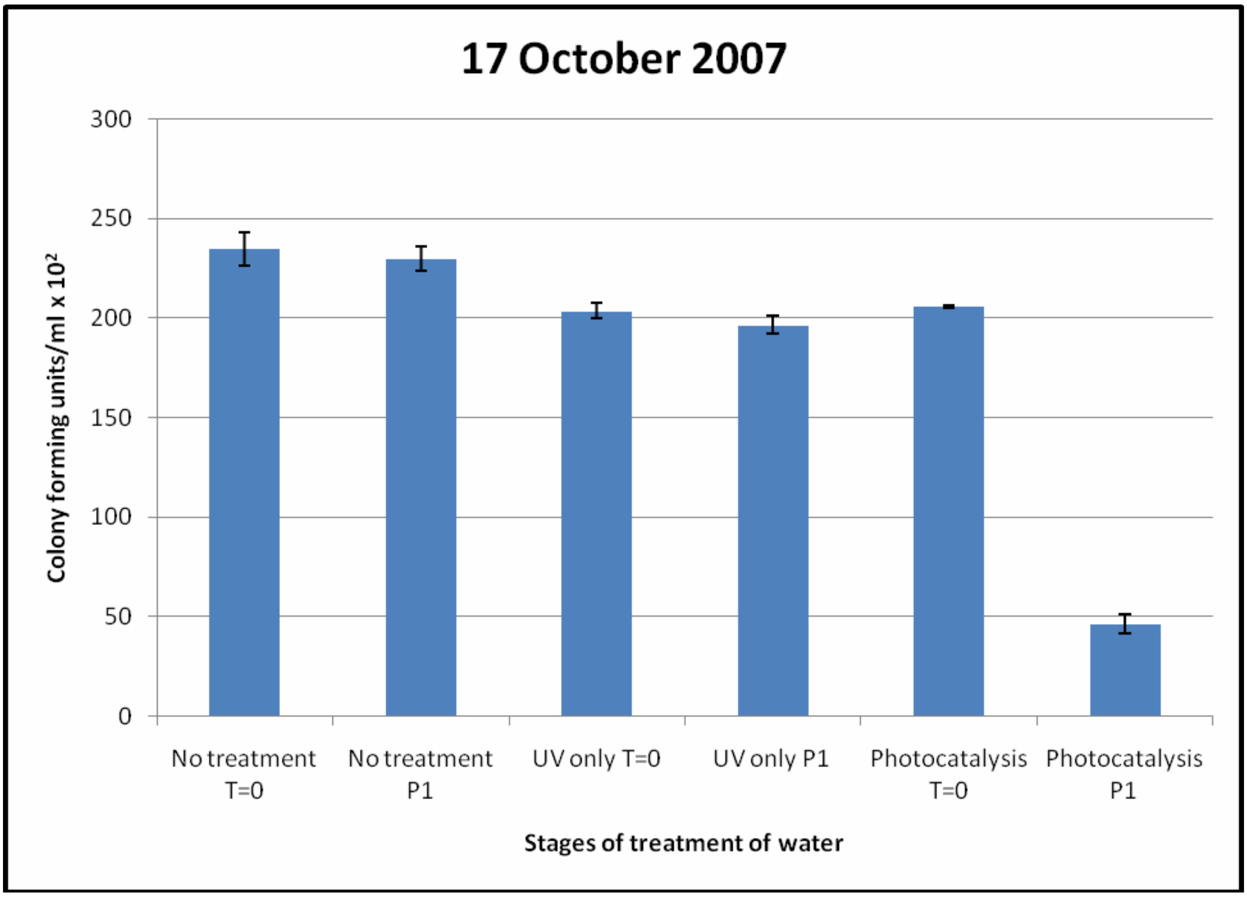


Fig. 3

Results for 31 October 2007

	Plate 1	Plate 2	Plate 3	Average	S Dev	S Error
Stock	118	140	147	135	15.13275	8.736895
T=0	130	159	158	149	16.46208	9.504385
P1	116	105	101	107.3333	7.767453	4.484541
P2 (T=0)	84	75	83	80.66667	4.932883	2.848001
P3	4	3	1	2.666667	1.527525	0.881917

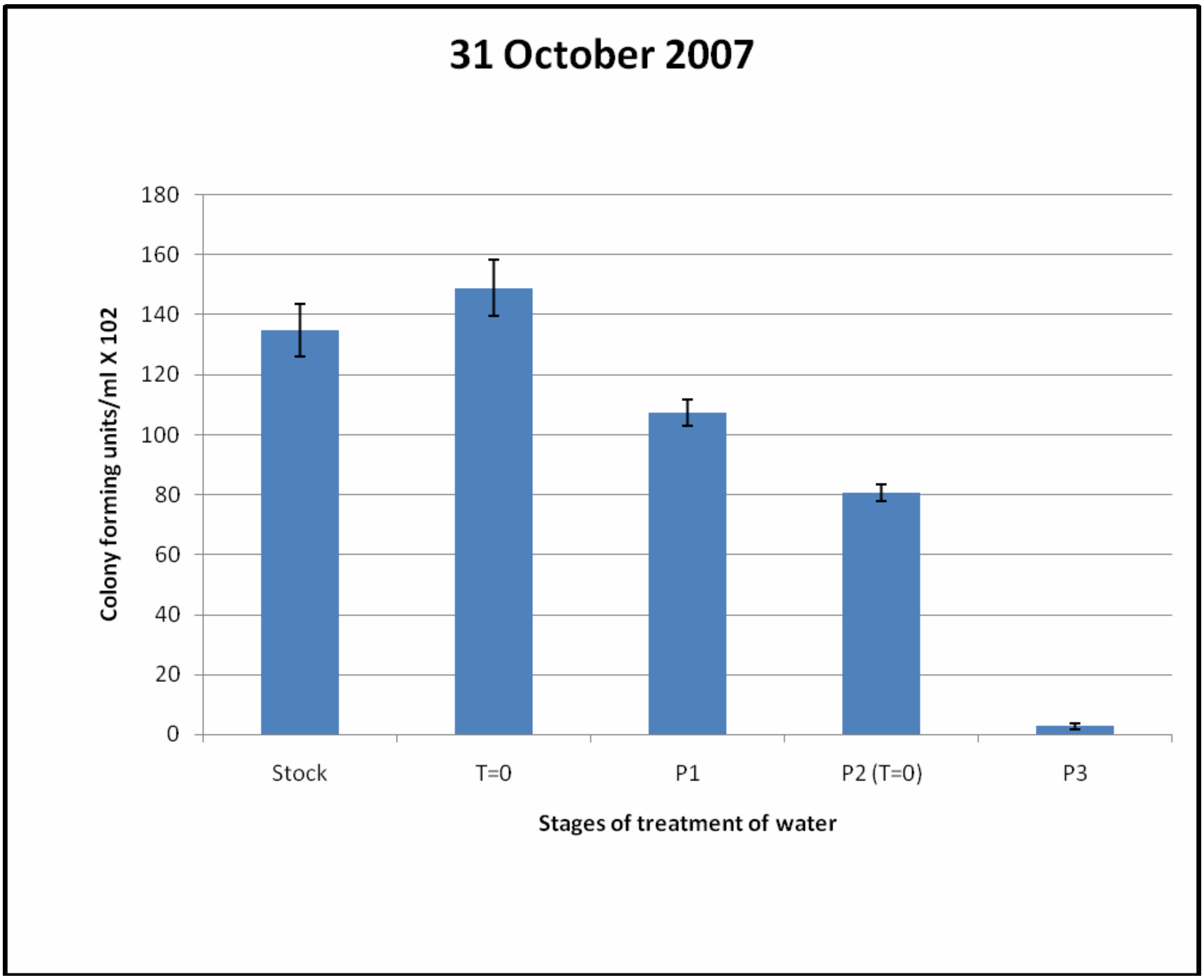


Fig. 4

Results for 14 November 2007

	Plate 1	Plate 2	Plate 3	Average	S Dev	S Error
Stock	152	174	162	162.6667	11.01514	6.359595
T=0	157	138	128	141	14.73092	8.504901
P1	138	133	151	140.6667	9.291573	5.364492
P2	3	1	1	1.666667	1.154701	0.666667
P3	5	5	3	4.333333	1.154701	0.666667
P4	5	5	4	4.666667	0.57735	0.333333
P5	2	0	0	0.666667	1.154701	0.666667

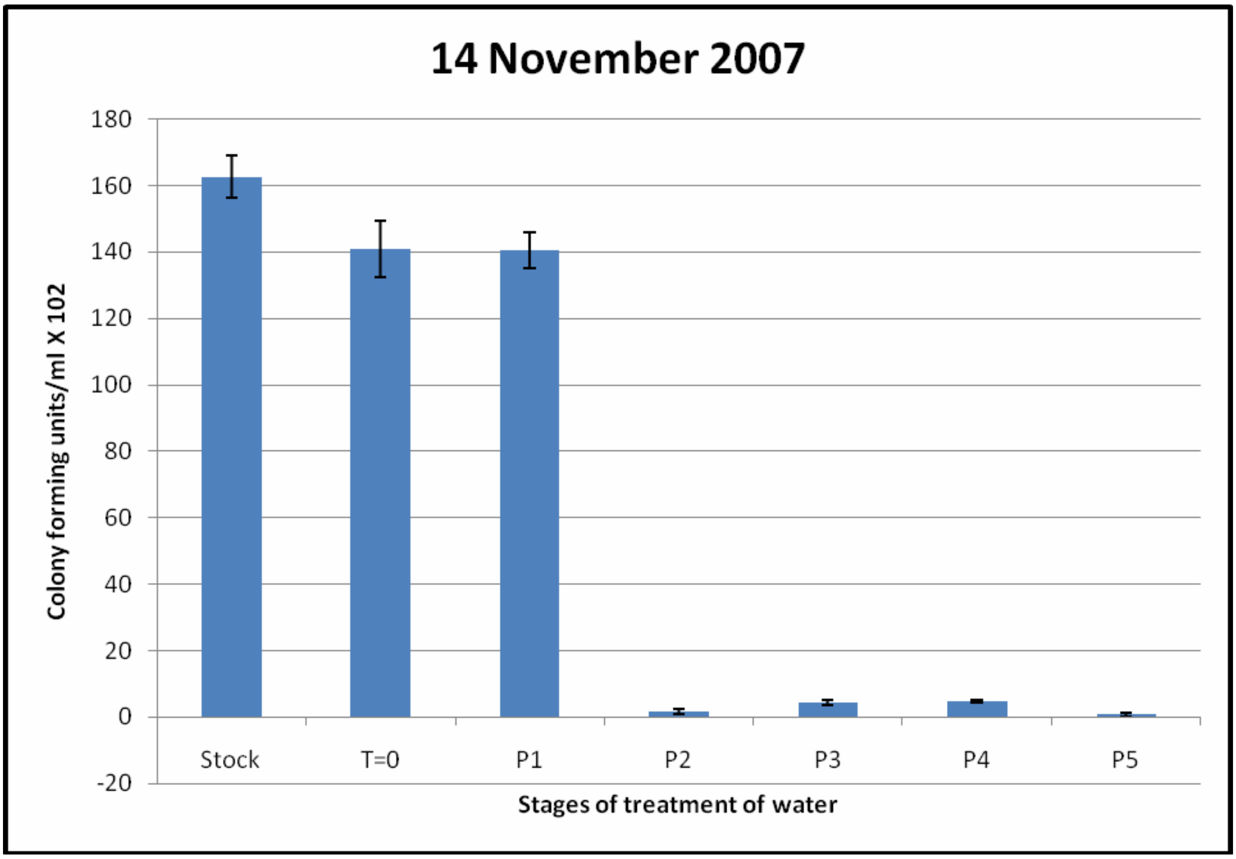


Fig. 5

Discussion and Conclusions

This study was designed to assess the efficiency of a point of use (POU) water treatment system using a novel technology to eradicate pathogenic bacteria from drinking water in a domestic setting.

The research has shown that the technology has a practical application and is effective in the inactivation of a wide range of bacterial micro-organisms. Benabbou et al (2007), show that photocatalysis can result in the total inactivation of E.coli at a certain concentration of TiO_2, but also say that at a concentration 10 times higher, the effect was markedly less efficient. Cho et al (2004) also showed a variety of results for different variables and demonstrated that the production of the hydroxyl radical showed a linear correlation with the inactivation of *E.coli*. These results show that the parameters and variables employed during the experimentation must be carefully monitored.

The previous statement is especially true where large numbers of people are using private water supplies. These supplies are particularly prone to contamination especially from agricultural run off and septic tank effluent and are cases where point of use systems could be particularly useful.

Even in public supplies, it has been shown that the water can become contaminated after treatment with chlorine at the disinfection stage, which is the last stage of treatment. According to Gray, (1994) contamination of the distribution system can occur through “air valves, hydrants, booster pumps, service reservoirs, cross connections” or through poor repairs to the plumbing. This author cites major outbreaks of giardiasis in Bristol (1985) because of a fractured main, and a typhoid outbreak in Switzerland in 1963 due to a sewage leak into the main. He also states that if there is a high organic or humic acid load it is difficult to maintain a sufficiently high level of residual chlorine in the water system.

These difficulties and those stated earlier regarding disinfection by products are testimony to the potential impact of this point of use water treatment device in the inactivation of pathogenic bacteria. Figures 3 and 4 clearly show the effect of photocatalysis on *E.coli* and if the correct parameters are applied this system could be an invaluable tool in providing clean safe water in the home.

The outcome of the experimental study carried out for this paper was a variety of results as seen above. This can be explained by the many variables to which the process is susceptible. The optimum parameters which must be achieved in order to render the process effective and efficient were not attained during this experimentation. TiO_2 has indeed been proven to demonstrate bactericidal activity when illuminated with UV light, however further work needs to be done.

Limitations and consideration for further research

This study was limited in that it was a final year project and time bound. The availability of the laboratory facilities was limited also and only eight sessions of experiments was possible over the course of the study.

These limitations have resulted in a variety of parameters as follows not being investigated as thoroughly as required;

1. Concentration of TiO_2; Benabbou et al, (2007) experimented with several concentrations of TiO_2 deciding that 0.25 g/L is the optimum at various concentrations of E.coli. This research could be verified with more experimentation in a slurry type reactor. In this fixed reactor however, the concentration of the TiO_2 cannot be so easily altered. The concentration in this reactor was $1mg/cm^{2,}$ determined as optimum for the removal of chemical pollutants (McMurray et al, 2004).

2. UV light intensity; the authors above discuss a doubling of time for inactivation following a decrease in UV light intensity at various concentrations of TiO_2. Again with more time this variable could have been explored in greater detail.

3. Pump speed and illumination time; the pump speed for the experiments was set at 20 ml/minute, based on previous experimentation and supervisor experience. Had this been reduced and therefore the time increased, the results may have been different. Huang et al (2000) reported that a four fold increase in illumination resulted in complete killing of all bacteria.

4. Concentration of bacteria; the initial concentration of the bacteria present in the sample will have a direct bearing on the outcome of the experiment. Very high concentrations of bacteria may reduce the effect of the catalyst on those in the middle of the water flow because of a shading effect and therefore a reduction in the production of hydroxyl radicals necessary for the inactivation of the bacteria.

5. The presence of persister cells as detailed above could account for bacteria remaining following treatment. This factor would require a great deal of time and further experimentation to detect.

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