# EFFECTS OF UV LIGHT DISINFECTION ON TETRACYCLINE RESISTANT BACTERIA IN WASTEWATER EFFLUENTS

A Thesis

by

HANNAH CHILDRESS

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2010

Major Subject: Biological and Agricultural Engineering

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Approved by:

| | |
|---|---|
| Chair of Committee, | Raghupathy Karthikeyan |
| Committee Members, | Cady Engler |
| | Terry Gentry |
| Head of Department, | Gerald Riskowski |

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# ABSTRACT

Effects of UV Light Disinfection on Tetracycline Resistant Bacteria in Wastewater Effluents. (August 2010)

Hannah Childress, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Raghupathy Karthikeyan

The ubiquitous use of antibiotics has led to an increasing number of antibiotic-resistant bacterial strains, including strains that are multidrug resistant, pathogenic, or both. Numerous studies have been conducted showing the presence of antibiotic-resistance genes (ARGs) and antibiotic-resistant and multidrug resistant bacteria in wastewater and drinking water treatment plants. There is also evidence to suggest that ARGs spread to the environment, and to humans and animals, through wastewater effluents. The overall objective of this study was to investigate the effect of UV light disinfection on ARGs and antibiotic resistant bacteria.

Wastewater effluent samples from a wastewater treatment plant (WWTP) in Texas were evaluated for differences in abundance and diversity of tetracycline resistant bacteria before and after UV treatment. The effects of photoreactivation or dark repair on the reactivation of bacteria present in WWTP effluent after UV disinfection were also examined. Culture based methods were used to characterize viable heterotrophic, tetracycline resistant heterotrophic, *E. coli*, and tetracycline resistant *E. coli* bacteria present before and after UV treatment. Molecular methods were used to characterize the

diversity of organisms present and to test for the presence of *tet*(Q), a tetracycline resistance gene associated with human origins.

UV disinfection was found to be as effective at reducing concentrations of resistant heterotrophs and *E. coli* as it was at reducing total bacterial concentrations. The lowest survival ratio following UV disinfection was observed in tetracycline-resistant *E. coli*, showing it to be particularly susceptible to UV treatment. Photoreactivation and dark repair rates were found to be comparable to each other for all bacterial populations. UV disinfection was found to significantly alter the community composition of tetracycline-resistant bacteria, though it did not have the same effect on the total bacterial community.

# ACKNOWLEDGEMENTS

I would like to thank my advisor and committee chair, Dr. Karthikeyan, and my committee members, Dr. Engler and Dr. Gentry, for their advice and support. I would also like to thank Bailey Sullivan for teaching lab procedures, and the operators at the wastewater treatment plant for their assistance.

I am grateful to the Department of Biological and Agricultural Engineering for providing a Graduate Research Assistantship and the Texas Water Resources Institute for providing the W.G. Mills Scholarship to conduct my research.

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# 1. INTRODUCTION

The ubiquitous use of antibiotics has led to an increasing number of antibiotic-resistant bacterial strains, including strains that are multidrug resistant, pathogenic, or both. Drug resistant strains were first identified in hospitals, with resistance for each antibiotic appearing not long after the antibiotic was introduced (Levy and Marshall, 2004). Antibiotic-resistant infections, and particularly those which are multi-drug resistant, are more difficult to treat and cost more lives than normal bacterial infections. Some strains of *E. coli* are resistant to six families of antibiotics; similarly, infections of *P. aeruginosa* and *A. baumanii* can be resistant to all or all but one of the antibiotic families (Levy and Marshall, 2004). Clearly, antibiotic resistance genes pose a major health threat. Additionally, it is estimated that the cost of treating antibiotic resistant infections in the United States ranges between $150 million and $30 billion per year (Levy and Marshall, 2004).

There is growing evidence that significant amounts of bacterial antibiotic resistance genes (ARGs) are present not only in hospital settings but in the environment as well. Human and agricultural activities have been found to contribute to the incidence of ARGs in the environment (Pei et al, 2006). One such human influence, wastewater treatment plants, is the focus of this study; in particular, the impact of ultraviolet light

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This thesis follows the style of *Water Research.*

disinfection on the concentration of antibiotic-resistant bacteria and antibiotic resistance genes released into the environment.

Resistance mechanisms have been identified for all of the major classes of antibiotics (Levy and Marshall, 2004). Mechanisms of antibiotic resistance include natural resistance in bacteria which lack the drug target or transport system and chromosomal- or plasmid-encoded antibiotic resistance genes. Plasmid-encoded resistance genes are thought to be the most common resistance mechanism to most currently used antibiotic drugs (Chee-Sanford et al., 2009). These resistance genes can be transferred through horizontal gene flow, which is thought to be a significant source for the spread of antibiotic resistance in environmental systems (Chee-Sanford et al., 2009). Horizontal gene transfer may occur by conjugation, transduction, or transformation (Chee-Sanford et al., 2009). Chromosomal or plasmid DNA, which are major sources of ARG, may be passed from one bacterial cell to another through conjugation. Transduction is a transfer of DNA by bacteriophages whereby foreign DNA may be incorporated into the host cell. Transformation is a process of transporting exogenous DNA into the cell if a cell is genetically competent, meaning that it is capable of uptaking and binding foreign DNA (Chee-Sanford et al., 2009).

This study focuses on tetracycline resistance because of the widespread use of tetracycline and high incidence of tetracycline resistance; it has been estimated that over 3 million kg/yr of tetracycline is used in the U.S. in agricultural applications alone (Chopra and Roberts, 2001). Tetracycline resistance is the most common kind of resistance found in bacteria isolated from the environment as well as from animals

(Billington et al, 2002); one study found 47-89% of bacteria in various soil and water environments were tetracycline resistant (Esiobu et al, 2002). The antibiotics in the tetracycline family are broad-spectrum agents that work against a variety of Gram-positive and Gram-negative bacteria by inhibiting protein synthesis. In addition, they are used for the prevention of malaria and at sub-therapeutic levels as livestock growth promoters (Chee-Sanford et al., 2009).

There are 29 known tetracycline resistance genes (Chopra and Roberts, 2001). The main mechanisms of tetracycline resistance are efflux genes and ribosomal protection proteins; there is also another mechanism which uses enzymes to inactivate tetracycline. Efflux genes reduce the concentration of tetracycline in the cell by producing membrane-associated proteins which export the drug. Ribosomal protection proteins protect the ribosomes from tetracycline; these confer a resistance to a wider spectrum of tetracyclines than do the efflux genes (Chopra and Roberts, 2001).

*Tet*(Q), a tetracycline resistance gene generally associated with human origin, is commonly found in wastewater treatment plants. It codes for a ribosomal protection protein and is found in both Gram-positive and Gram-negative species. Tet(Q) genes are frequently associated with conjugative transposons, which code for their own transfer (Chopra and Roberts, 2001). These transposons are capable of transporting plasmids between bacterial species.

Significant levels of ARGs have been detected in urban and agricultural environments, in wastewater and drinking water treatment plants, and even in treated wastewater and drinking water (Pruden et al., 2006). There is potential for antibiotic-

resistant bacteria to spread to humans because of their environmental presence. Hospital waste streams are a major source of antibiotics and resistance genes into the environment; however livestock production, particularly concentrated animal feeding operations (CAFOs), may also be a significant contributor. More than half of the antibiotics administered in the United States are given to livestock (Pruden et al., 2006), and approximately 75% of the antibiotics used in livestock production are excreted in waste (Chee-Sanford et al., 2009).

Selective environmental pressures lead to proliferation of antibiotic resistant bacterial strains. The extensive medical and agricultural use of antibiotics is an obvious selective pressure. During the development of antimicrobial agents, development of new drugs was nearly always followed by resistance to those drugs (Cohen, 1992). However, there are also organisms which naturally produce antibiotics and may contribute to the environmental presence of resistance genes (Singer et al., 2006). In addition, there is evidence of linkage between antibiotic resistance genes and other resistance genes, including those resistant to quaternary ammonium compounds and metals (Singer et al., 2006).

Numerous studies have been conducted showing the presence of ARGs and antibiotic-resistant and multidrug resistant bacteria in wastewater and drinking water treatment plants (Armstrong et al., 1981; Auerbach et al., 2007; Schwartz et al., 2002; Volkmann et al., 2004; Martins da Costa et al., 2006). There is also evidence to suggest that ARGs spread to the environment, and to humans and animals, through wastewater effluents. Wastewater treatment plants have been found to have higher concentrations

and more diversity of tetracycline resistance genes than natural waters (Auerbach et al, 2007). A study by Pruden et al (2006) showed the presence of *tet*(W) and *tet*(O) genes in treated wastewater, indicating that tetracycline resistance genes can be introduced into the environment through wastewater treatment plant effluents.

Ultraviolet light disinfection is being more and more commonly used as the final disinfection step during wastewater treatment. UV light reacts with bacterial DNA to cause the formation of pyrimidine dimers, thus inactivating the bacteria; this process is illustrated in Figure 1. However, many bacterial species can utilize the mechanisms of photoreactivation or dark repair to become reactivated. Photoreactivation occurs in UV-A or visible light; it is hypothesized that pyrimidine dimers form a complex with a photoreactivating enzyme which can then undergo photolysis that restores the original monomer (Masschelein, 2002). Photoreactivation is illustrated in Figure 2. Dark repair requires multiple enzymes to excise the dimers from the DNA, and considerably fewer bacteria can reactivate under dark conditions (Sanz et al, 2007); dark repair is shown in Figure 3.

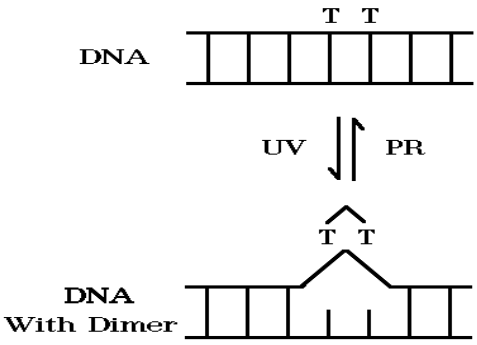


**Figure 1. UV inactivation of bacterial DNA (Montelone, 1998).**

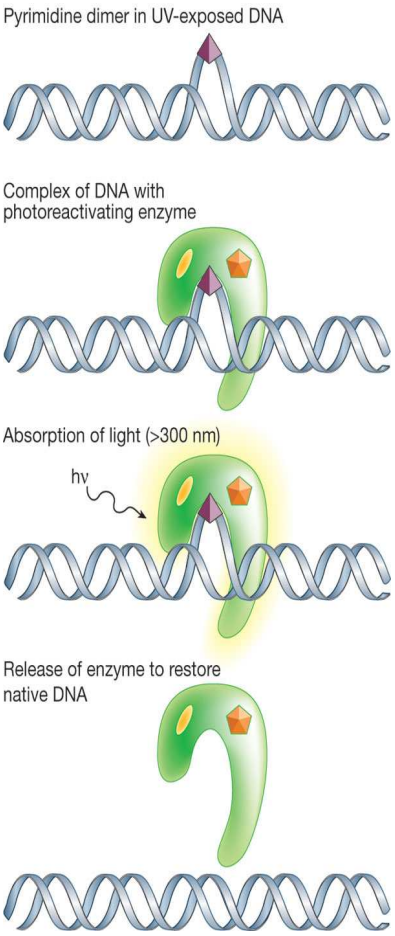


**Figure 2. Photoreactivation of inactivated bacterial DNA (Friedburg, 2003).**

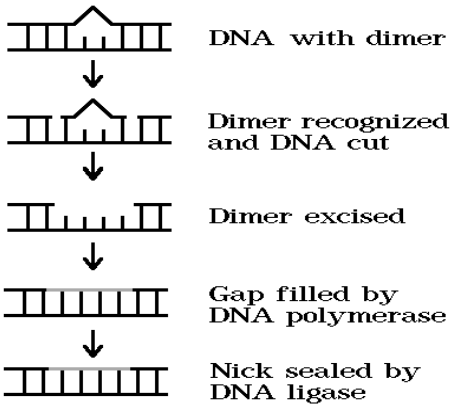


**Figure 3. Dark repair of inactivated bacterial DNA (Montelone, 1998).**

Little research has been done to date on the effect of UV light treatment on antibiotic resistance genes and antibiotic-resistant bacteria. One study indicates that UV disinfection does not decrease the number of tetracycline resistance genes present and suggested that treatment did not likely reduce the concentration of *tet*(Q) and *tet*(W) genes in effluent (Auerbach et al, 2007). However, this study did not take into account viable counts of resistant bacteria or the possible effects of photoreactivation.

The overall objective of this study was to investigate the effect of UV light disinfection on ARGs and antibiotic resistant bacteria. Wastewater effluent samples from a wastewater treatment plant (WWTP) in Texas were evaluated for differences in abundance and diversity of tetracycline resistant bacteria before and after UV treatment. Culture based methods were used to characterize viable heterotrophic, tetracycline resistant heterotrophic, *E. coli*, and tetracycline resistant *E. coli* bacteria present before and after UV treatment. Bacterial colonies from culture plates were isolated and sequenced in order to characterize the diversity of the organisms present before and after treatment. Polymerase chain reactions (PCR) were performed on the isolated bacterial DNA to test for the presence of a tetracycline resistance gene (*tet*(Q)) usually from human origin.

The effects of photoreactivation or dark repair on the reactivation of bacteria present in WWTP effluent after UV disinfection were also examined. The results of this study should suggest best management practices to minimize antibiotic resistance genes and resistant bacteria in effluent from WWTPs which use UV disinfection.

# 2. EFFECTS OF UV LIGHT DISINFECTION ON TETRACYCLINE RESISTANT BACTERIA IN WASTEWATER EFFLUENTS

## 2.1 Materials and Methods

### *2.1.1 Experimental Design*

Total heterotrophic bacteria, resistant heterotrophic bacteria, total *E. coli*, and resistant *E. coli* were enumerated using culture-based methods from wastewater samples taken before UV treatment and after UV treatment. One sample was taken from before UV treatment and two samples were taken from after UV treatment; one was kept under photoreactivation conditions and one was kept under dark repair conditions. At each plating time (detailed in section 2.1.3), three replicates were plated from each sample.

### *2.1.2 Wastewater Samples*

Samples were collected from a wastewater treatment plant in southeast Texas on four different dates, summarized in Table 1. Samples were collected in autoclaved 500 mL Pyrex© bottles, leaving approximately 100 mL headspace. On each collection date, one sample was collected from immediately before UV treatment and two samples were collected from immediately after UV treatment: one in a clear bottle and one in an aluminum-foil-covered bottle. All samples were stirred continuously on magnetic stir plates maintained at room temperature for 48 hours and kept near a window which allowed exposure to natural light.

*2.1.3 Bacterial Enumeration*

Heterotrophic bacteria, tetracycline-resistant heterotrophic bacteria, *E. coli*, and tetracycline-resistant *E. coli* were enumerated from each sample over a 48 hour period for heterotrophs and a 24 hour period for *E. coli.* Samples were plated at 0, 0.5, 1, 2, 4, 6, 12, and 24 hours for all samples and also at 48 hours for heterotrophic samples. Ten-fold serial dilutions were performed as required to obtain appropriate colony numbers, and samples were plated in triplicate by the spread plate method on either Difco© nutrient agar for heterotrophs or MacConkey agar for *E. coli.* For the enumeration of resistant bacteria, 14 mg/L tetracycline (bacterial $LD_{50}$ for tetracycline – Chopra and Roberts, 2001) was added to the agar after autoclaving. Plates were incubated for 24 hours and then counted.

*2.1.4 Photoreactivation and Dark Repair Kinetics*

Equation (1) was used to determine the specific growth rate for the exponential growth phase of each bacterial culture. The exponential growth phase was estimated graphically. These rates were used to model growth curves for the cultures.

$$\mu = \frac{\ln \frac{X}{X_0}}{t} \qquad (1)$$

where

$\mu$ = specific growth rate for exponential bacterial growth (1/min)

X = number of organisms at time t (CFU/mL)

$X_0$ = number of organisms at time 0 (CFU/mL)

t = time (min)

Reactivation can be represented as a function of survival ratio with respect to the initial bacterial concentration leaving UV disinfection treatment. The survival ratios were calculated in percentages using the following equation:

$$S = \frac{N_t}{N_0} \times 100 \quad (2)$$

where

S = survival ratio at time t (percent),

$N_t$ = concentration of microorganisms at time t after the beginning of reactivation (CFU/mL)

$N_0$ = concentration of microorganisms before disinfection (CFU/mL)

Equation (3) was used to quantify the photoreactivation or dark repair that occurred. This equation was proposed by Lindenauer and Darby (1994) and is used rather than a log increase formula because it explicitly accounts for the number of organisms before UV treatment. However, the modification of this formula proposed by Quek and Hu (2008), which uses log bacterial concentrations, was used in order to quantify the % repair.

$$\%Repair = \frac{\log N_t - \log N}{\log N_0 - \log N} * 100 \quad (3)$$

where

N = number of organisms surviving UV treatment (CFU/mL)

Rate of repair was calculated using equation (4), as presented by Quek and Hu (2008).

$$Rate = \frac{\log(N_t - N)}{t} \quad (4)$$

where

Rate = rate of repair (log/hr)

t = time interval between the two samples (hr)

*2.1.5 Statistical Analysis*

One-way ANOVA with α=0.05 was performed to test the statistical significance in treatment means within and among treatments. Rate of UV repair of various bacteria in municipal wastewater effluent was also analyzed using one-way ANOVA with α=0.05.

*2.1.6 Bacterial DNA Extraction and Sequencing*

One colony from each of 3 replicate plates from each sampling time between 0 and 4 hours was restreaked on the same type of agar and incubated for 48 hours. Samples from these colonies were then transferred to 2 mL test tubes containing LB broth and again incubated for 48 hours. DNA extraction was then performed on these samples using Qiagen© QIAamp DNA extraction kits, following protocol for cultured cells.

A 0.5 µL aliquot of the DNA, 22.5 µL Thermo Scientific 1.1X PCR Master Mix, 1 µL 100µM 16S rRNA forward primer (5'- AGA GTT TGA TCC TGG CTC AG -3'),

and 1 µL 100µM 16S rRNA reverse primer (5'- ACG GCT ACC TTG TTA CGA CTT -3'). PCR was performed on this mix in an Eppendorf Thermal Cycler using 40 cycles of 30 s at 95° C, 30 s at 54° C, and 1 min at 72° C. The resultant PCR product was sent to Agencourt Bioscience Corporation for sequencing and two reads per target were performed.

The 16S rRNA gene sequence data were filtered to remove sequences less than 500 bp in length, and the Ribosomal Database Project website (Cole et al, 2005) was used to assign putative identities to each sequence. The sequences were aligned using ClustalW with default settings and then trimmed to a common length. The DNAml program in PHYLIP 3.6 (Felsenstein, 2005) was used with default settings to create a phylogenetic tree using the maximum likelihood method. This tree was used in MOTHUR v.1.7.0 (Schloss et al, 2009) to perform a parsimony test with six groups; bacteria from before UV treatment, bacteria from after UV treatment kept in light, bacteria from after UV treatment kept in darkness, resistant bacteria from before UV treatment, resistant bacteria from after UV treatment kept in light, and resistant bacteria from after UV kept in darkness.

*2.1.7 Amplification of tet(Q) Gene*

A 5 µL aliquot of the DNA from each isolate from plates containing tetracycline was mixed with 12.5 µL Promega 2X GoTaq Green Master Mix, 0.25 µL 100µM *tet*(Q) forward primer (5'- AGA ATC TGC TGT TTG CCA GTG -3'), 0.25 µL 100µM *tet*(Q)

reverse primer (5'- CGG AGT GTC AAT GAT ATT GCA -3'), and 7 µL nuclease free water. PCR was performed on this mix in an Eppendorf Thermal Cycler using 40 cycles of 30 s at 95° C, 30 s at 52° C, and 1 min at 72° C. The resultant PCR product was used to perform gel electrophoresis.

Electrophoresis was performed using a Fisher Scientific model FB-SB-1316 horizontal electrophoresis system. Gels were cast using 1% w/v agarose and 1X TBE buffer. Ethidium bromide was used for staining gels. Each lane of the gel electrophoresis was run with a 25 bp ladder and a positive control for *tet*(Q). The electrophoresis was run at 100 V for approximately 30 minutes. Gels were photographed using Fotodyne FOTO/Analyst Investigator machine and software with an ethidium bromide filter.

## 2.2 Results and Discussion

### *2.2.1 Characteristics of Wastewater Treatment Plant Effluent*

Table 1 shows the flow, Total Suspended Solids (TSS), Total Dissolved Solids (TDS), and *E. coli* concentration for each date on which samples were taken, as well as the monthly averages for October and November. This data was obtained from WWTP operators. Total heterotrophs were enumerated from the sample taken on 10/28, resistant heterotrophs from the sample taken on 11/02, total *E. coli* from the sample taken on 11/11, and resistant *E. coli* from the sample taken on 11/18. While comparisons between total and resistant bacteria were not made from samples taken on the same date, flow conditions were similar and TSS were the same, therefore it was

assumed that bacterial concentrations were similar. Flow on all sampling dates was below the monthly averages, but the flows were relatively similar to each other (daily flows for October and November ranged from 4.84 to 12.6 MGD). TSS was the same for all sampling dates, while TDS varied. Most *E. coli* counts were below the monthly averages, however the count on the 11/11 sampling date was higher than the average and also much higher than the count on the 11/18 sampling date. Since these were the samples from which *E. coli* and resistant *E. coli* were enumerated, the comparison between total *E. coli* and resistant *E. coli* may have been affected.

**Table 1. WWTP Flow and Effluent Quality Data.**

| | Flow (MGD) | Total suspended solids TSS (mg/L) | Total dissolved solids TDS (mg/L) | *E. coli* (CFU/100 mL) |
|---|---|---|---|---|
| 10/28/09 | 6.28 | 2 | 520 | 17 |
| 11/02/09 | 5.27 | 2 | 620 | 26 |
| 11/11/09 | 5.96 | 2 | 640 | 42 |
| 11/18/09 | 5.75 | 2 | 580 | 17 |
| Oct. avg: | 6.75 | 3 | 550 | 28 |
| Nov. avg: | 6.08 | 3 | 597 | 28.23 |

UV disinfection efficiencies are shown in Figure 4. Close to or greater than 90% disinfection efficiency was observed for all samples except total *E. coli*. Similar efficiencies were reported in previous studies for total heterotrophic bacteria and E. coli (Lindenauer and Darby, 1994). UV treatment effectively disinfected tetracycline resistant heterotrophs and *E. coli*. However, the disinfection efficiency for tetracycline resistant heterotrophs was only 89%, lower than the 96% disinfection of total heterotrophs.

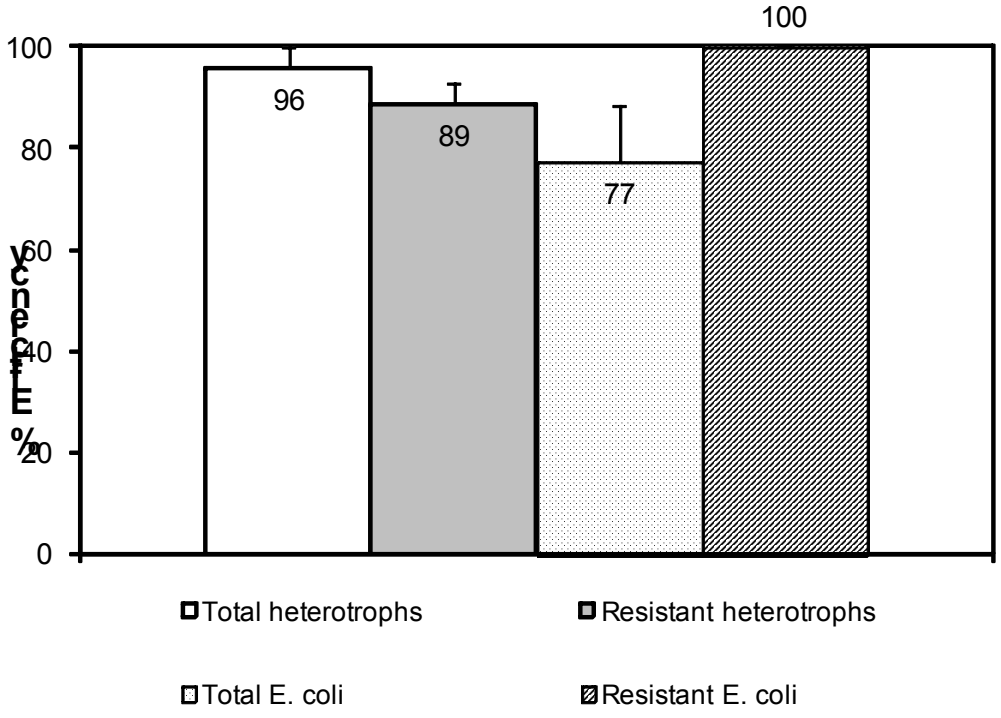


**Figure 4. UV disinfection efficiencies on wastewater bacteria.**

*2.2.2 Bacterial Growth Kinetics*

Figures 5 through 16 show the bacterial growth data for heterotrophs, resistant heterotrophs, *E. coli,* and resistant *E. coli.* Viable bacterial concentrations were shown only through 9 hr, approximately the end of the exponential phase for heterotrophs – the *E. coli* exponential growth phase ended sooner. For all the figures, n=3 at all data points, and the mean values from the replicate plates are plotted. Error bars were not included in the plots to avoid confusion.

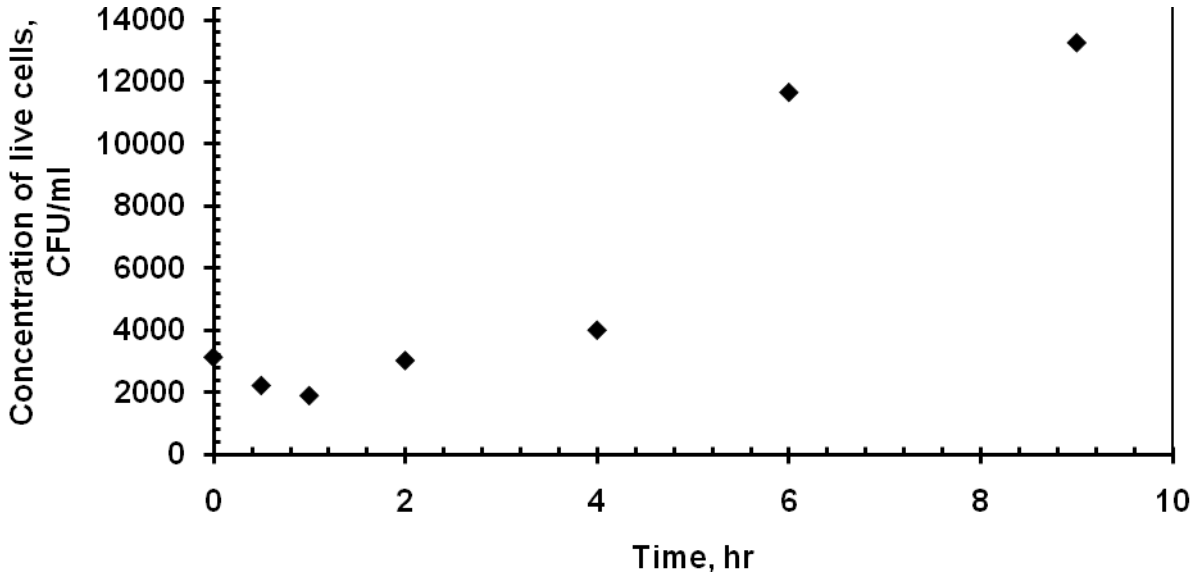


**Figure 5. Growth of heterotrophic bacteria in municipal wastewater before UV disinfection.**

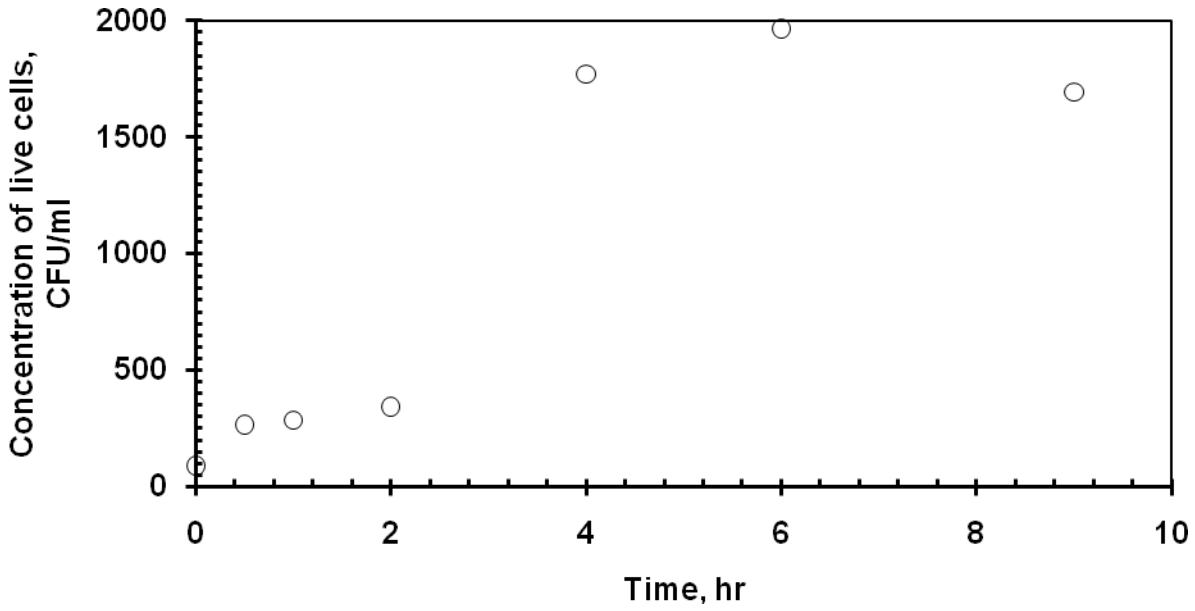


**Figure 6. Growth of heterotrophic bacteria in municipal wastewater effluent after UV disinfection and exposure to light.**

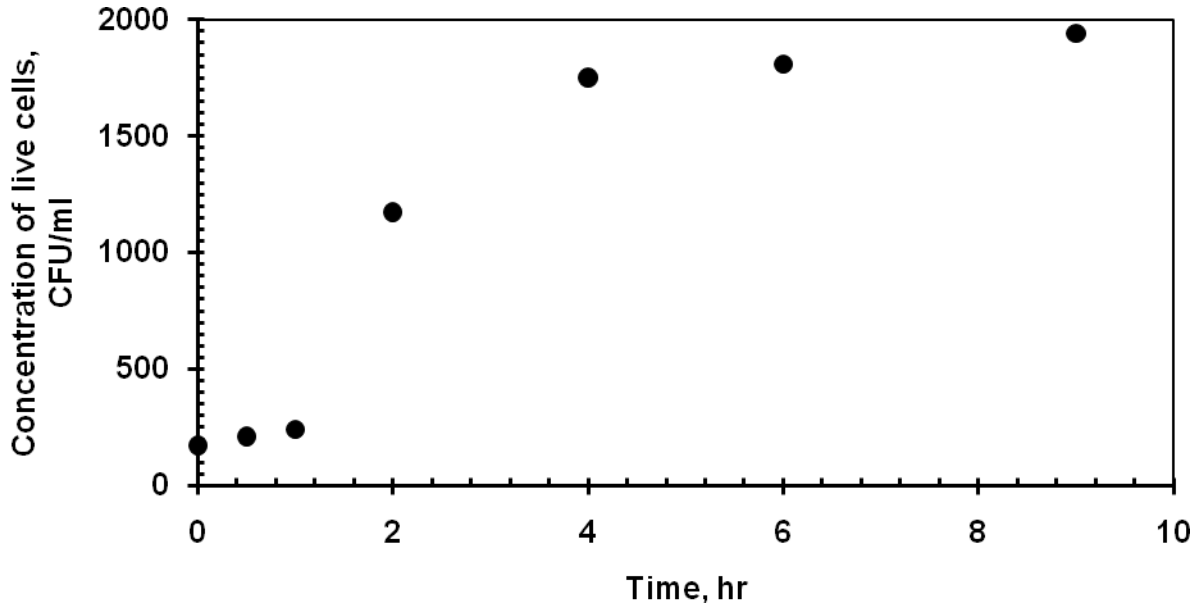


**Figure 7. Growth of heterotrophic bacteria in municipal wastewater effluent after UV disinfection and exposure to darkness.**

All wastewater samples cultured for heterotrophic bacteria showed similar growth trends, though concentration of viable bacteria before UV were much higher than after disinfection, as expected. Heterotrophic growth under all conditions appears to be in the lag phase until sometime between one and two hours. The maximum viable bacterial concentration in wastewater after UV kept in both light and dark was around 2000 CFU/mL. However the concentration of bacteria kept in darkness reached this maximum concentration at 9 hr while the concentration reached this maximum at 6 hr for the sample kept in light.

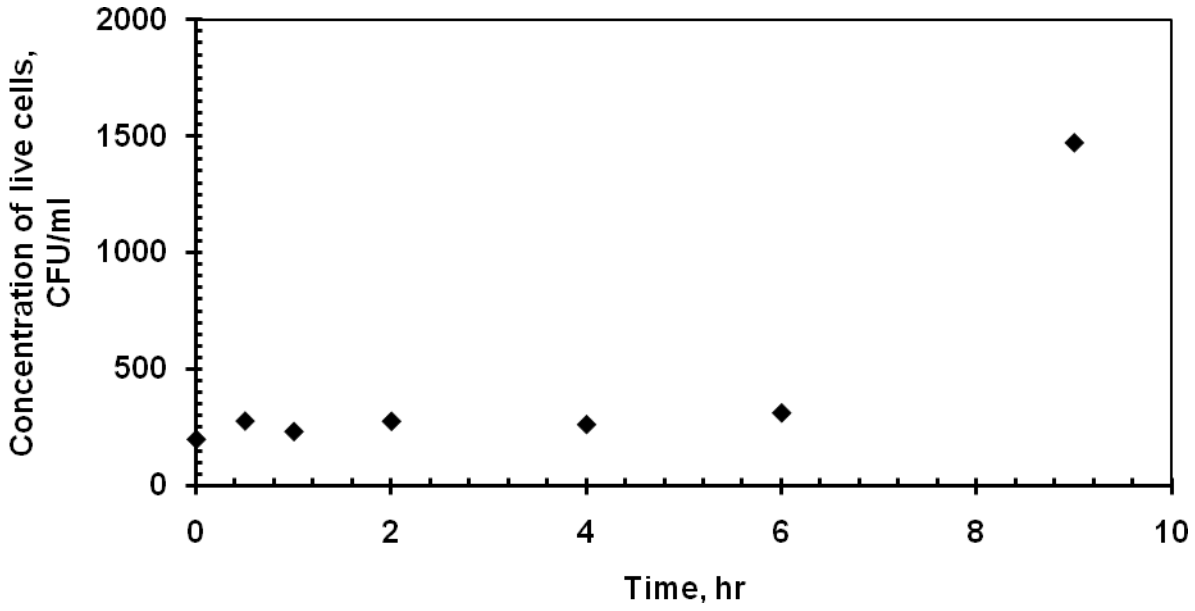


**Figure 8. Growth of tetracycline-resistant heterotrophic bacteria in municipal wastewater before UV disinfection.**

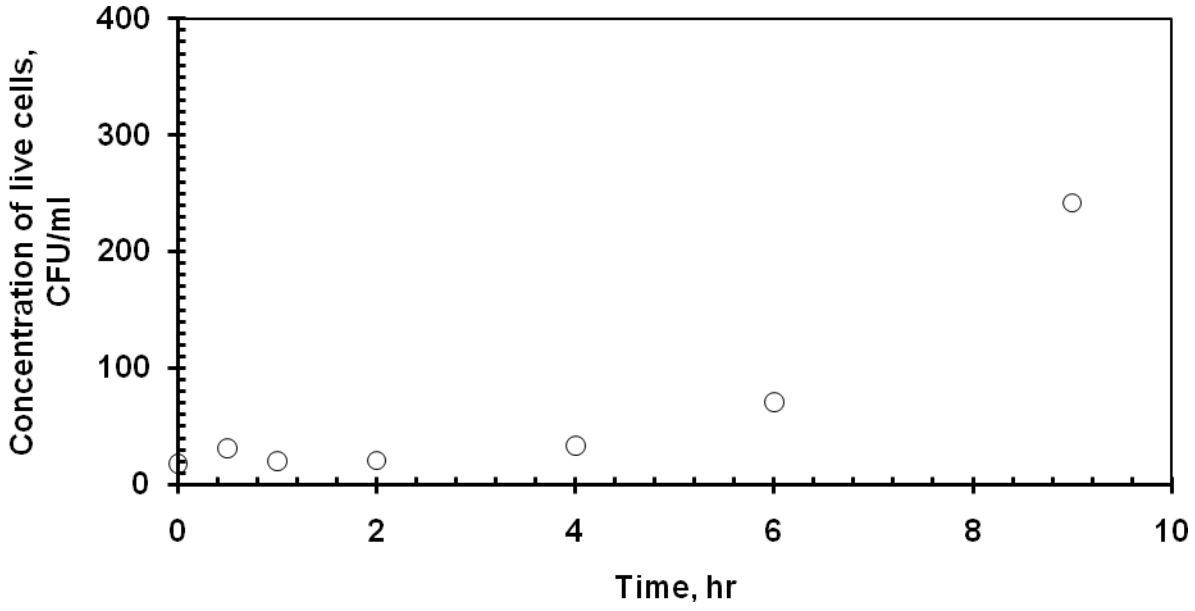


**Figure 9. Growth of tetracycline-resistant heterotrophic bacteria in municipal wastewater effluent after UV disinfection and exposure to light.**

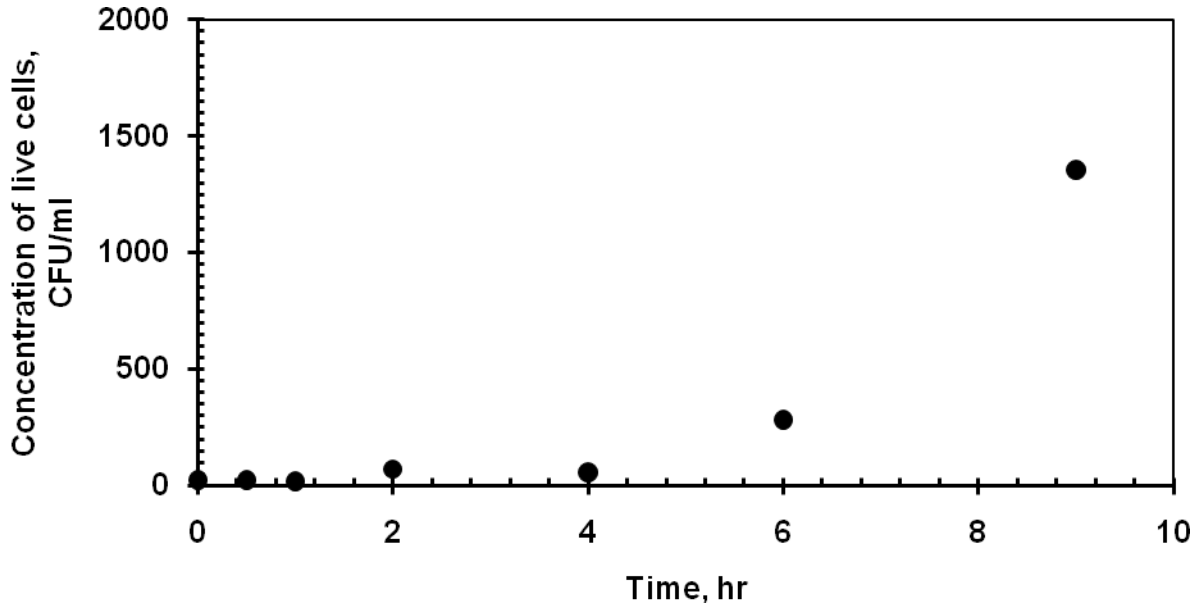


**Figure 10. Growth of tetracycline-resistant heterotrophic bacteria in municipal wastewater effluent after UV disinfection and exposure to darkness.**

Figures 8 through 10 show the growth curves for tetracycline-resistant heterotrophic bacteria. Lag phases in the growth for tetracycline-resistant heterotrophs (Figure 8-10) were longer than those for the total heterotrophic bacteria (Figures 5-7). The concentration of tetracycline-resistant heterotrophs in wastewater after UV kept in darkness reached approximately the same concentration by the end of 9 hrs as the sample from before UV. However, the sample from after UV disinfection kept in light had much lower numbers after the same time.

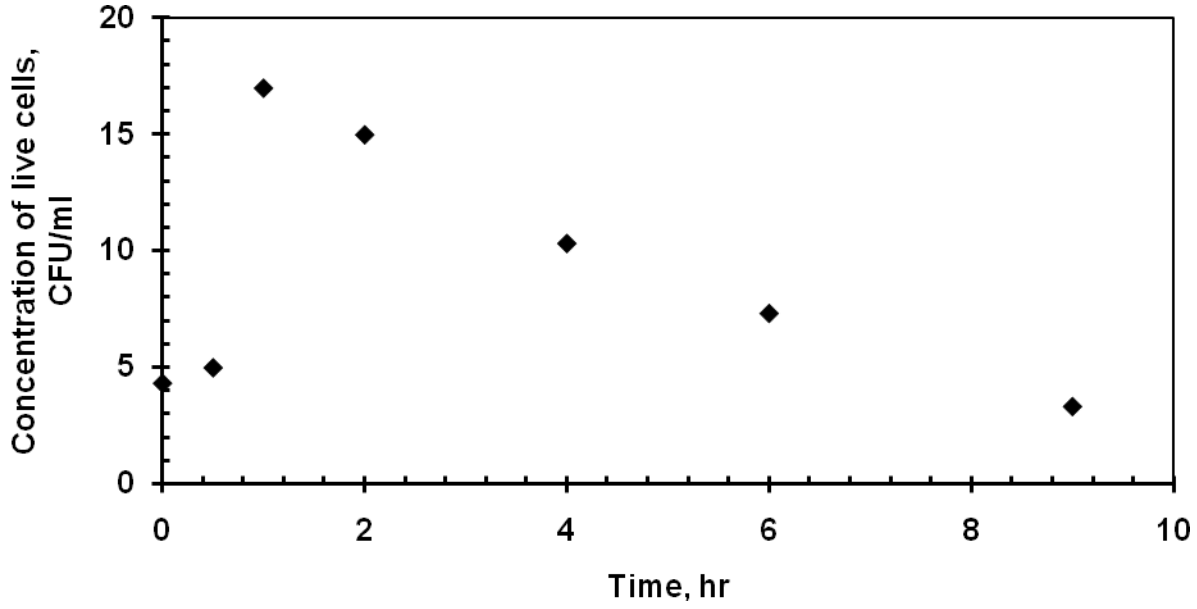


**Figure 11. Growth of *E. coli* in municipal wastewater before UV disinfection.**

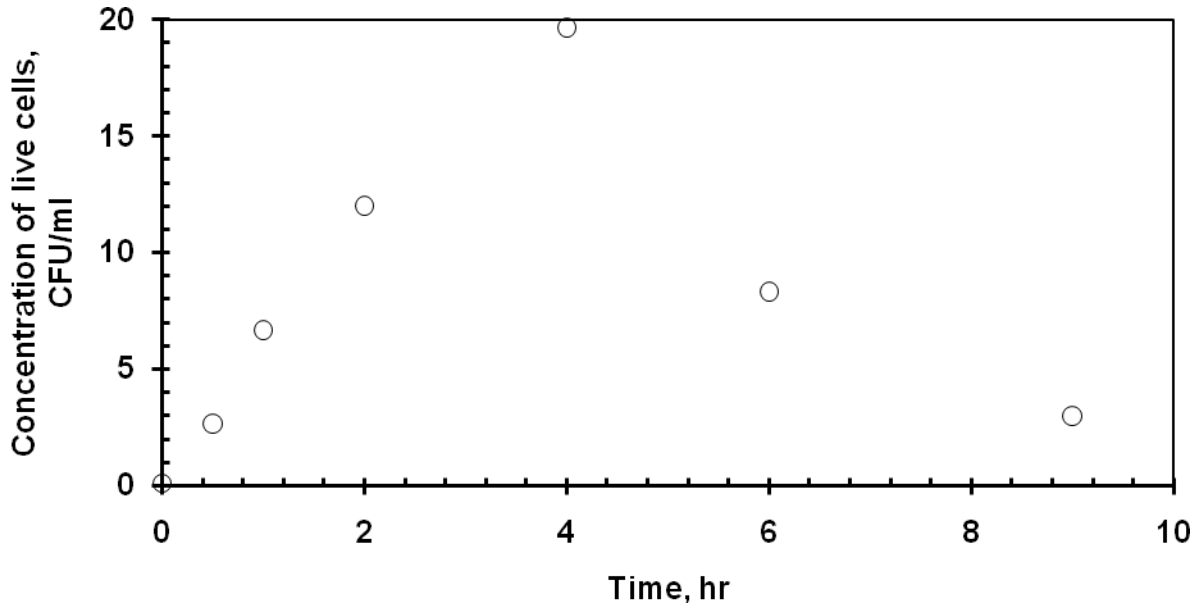


**Figure 12. Growth of *E. coli* in municipal wastewater effluent after UV disinfection and exposure to light.**

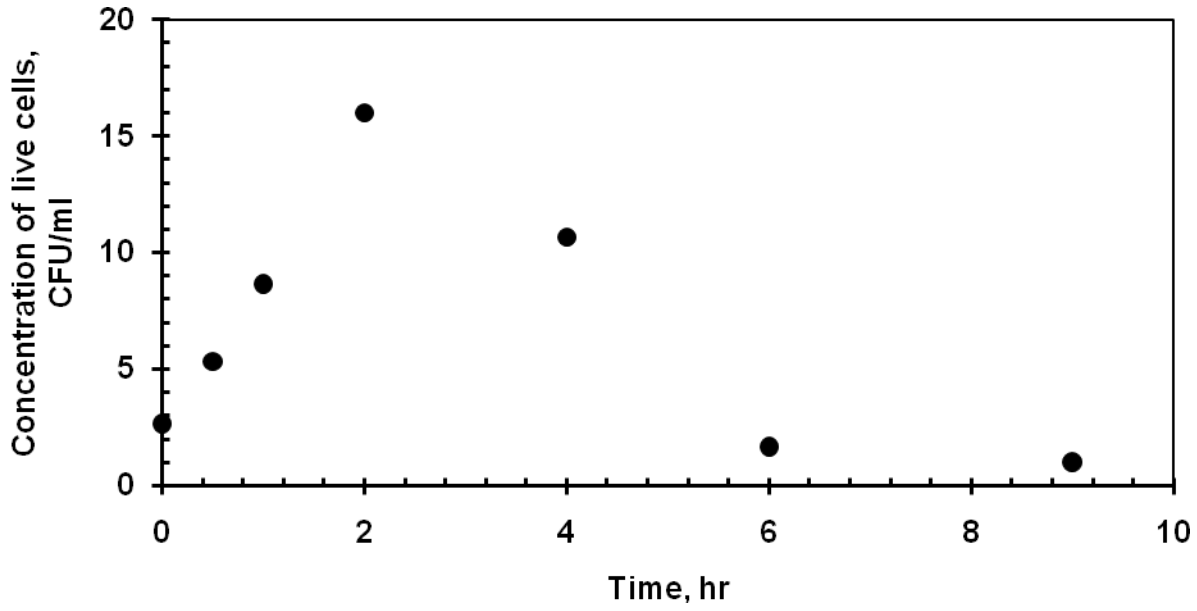


**Figure 13. Growth of *E. coli* in municipal wastewater effluent after UV disinfection and exposure to darkness.**

Figures 11 through 13 show the growth curves for total *E. coli.* The *E. coli* growth trend appears similar for all samples, with no lag phase and immediate exponential growth, followed by the decay phase well before the end of the 9 hr incubation period. The samples varied in the length of the exponential growth phase, with the sample from before UV having the shortest time of exponential growth and the sample after UV kept in darkness having the longest. Growth of *E. coli* from after UV kept in light had the highest cell concentration; however both samples from after UV treatment reached or exceeded the maximum concentration of the sample from before UV.

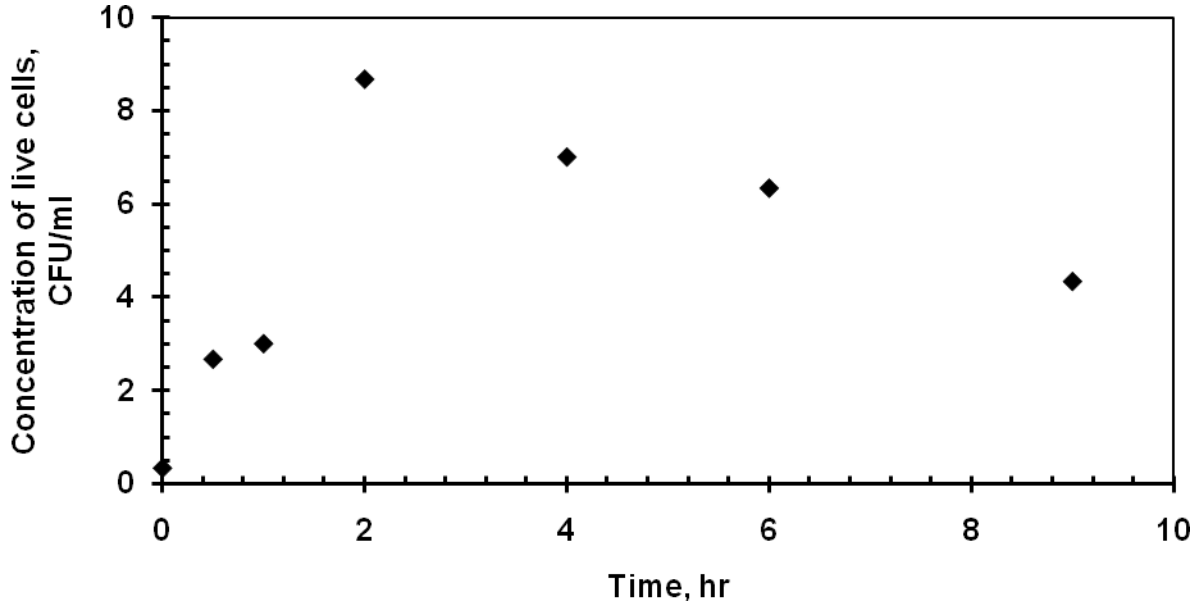


**Figure 14. Growth of tetracycline-resistant *E. coli* in municipal wastewater before UV disinfection.**

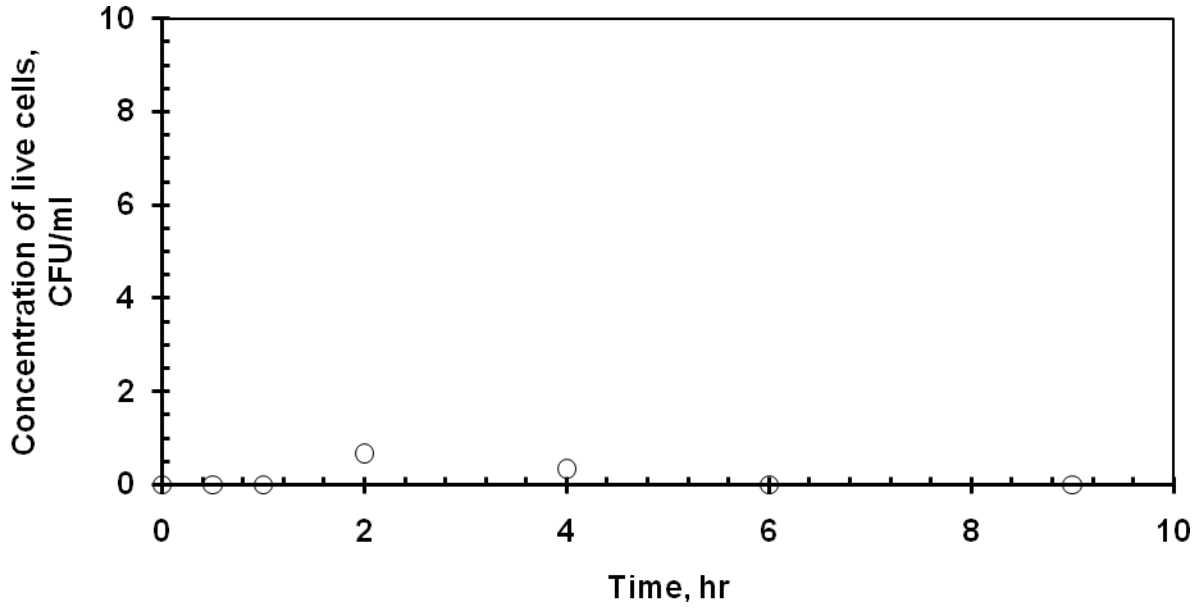


**Figure 15. Growth of tetracycline-resistant *E. coli* in municipal wastewater effluent after UV disinfection and exposure to light.**

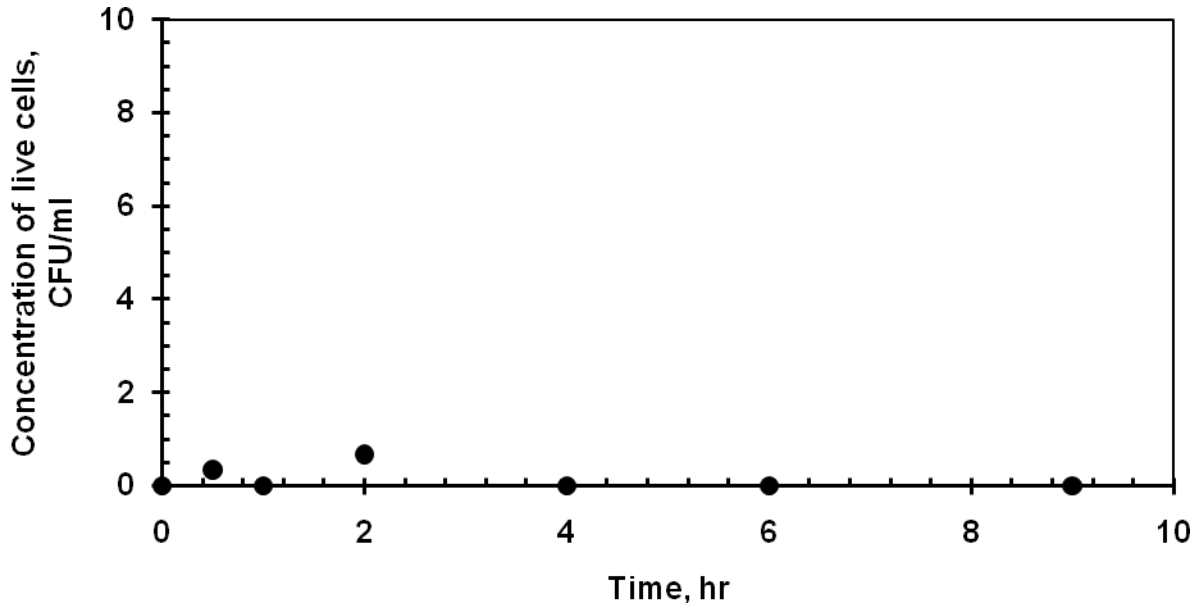


**Figure 16. Growth of tetracycline-resistant *E. coli* in municipal wastewater effluent after UV disinfection and exposure to darkness.**

Figures 14 through 16 show the growth curves for tetracycline-resistant *E. coli*. Growth of resistant *E. coli* in wastewater before UV treatment was similar to the growth observed for the total *E. coli* population, with immediate exponential growth followed by the decay phase after about 2 hrs. Growth of resistant *E. coli* in wastewater effluent after UV disinfection exposed to light or dark was negligible.

**Table 2. Specific Growth Rates (1/min)**

| | Heterotrophs | | *E. coli* | |
|---|---|---|---|---|
| | Total | Resistant | Total | Resistant |
| Before UV | 0.006 | 0.009 | 0.023 | 0.027 |
| After UV light | 0.007 | 0.007 | 0.022 | * |
| After UV dark | 0.011 | 0.011 | 0.015 | * |

*These cultures did not reach exponential phase growth.

Table 2 summarizes the specific growth rates for each of the 12 samples. Growth after UV treatment in dark was faster than either growth before UV treatment or growth after UV treatment in light for both heterotrophs and resistant heterotrophs. The resistant heterotrophic bacteria and total heterotrophic bacteria had the same growth rates after UV treatment both in light and in dark; however the resistant heterotrophs had faster growth before UV treatment than the total heterotrophs. Thus, it appears that the growth rates for heterotrophs and resistant heterotrophs are similar. For the total *E. coli* population, growth before UV treatment and after UV treatment in light was similar, while the growth after UV in the dark was slower. Growth rates for tetracycline-resistant *E. coli* could not be compared since these cultures did not achieve exponential growth.

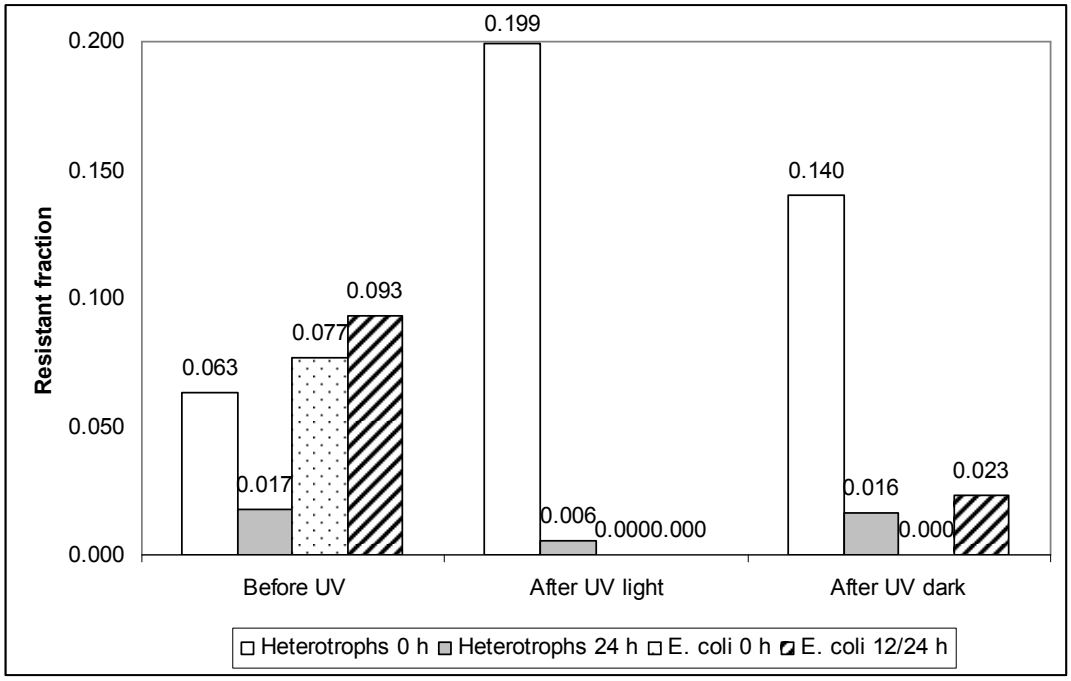


**Figure 17. Fractions of resistant bacteria.**

Figure 17 shows the fractions of the total bacteria that were tetracycline-resistant before UV disinfection and after UV disinfection and exposed to light or dark at 0 h and 12 or 24 h. For heterotrophs, all resistant fractions were lower at 24 h than at 0 h, which is likely due to the longer lag phase observed in the growth of the resistant heterotrophs. At 0 h, there was a higher fraction of resistant heterotrophic bacteria in the samples from after UV than before UV; at 24 h the fraction of resistant bacteria after UV was equal to or less than the fraction of resistant bacteria before UV, which is also consistent with the difference in kinetic constants between total heterotrophic bacteria and resistant heterotrophic bacteria. For *E. coli*, the resistant fraction was 0 or very small (2%) at both times after UV in light and dark. The fraction of resistant *E. coli* before UV treatment was slightly higher at 12 h than at 0 h.

*2.2.3 Comparison between and among Treatments*

Figures 18 through 21 compare the cell concentration in each of the three samples – before UV, after UV kept in light, and after UV kept in dark - at various times for heteretrophic bacteria, resistant heterotrophic bacteria, *E. coli,* and resistant *E. coli*. Bacterial cell concentrations are plotted on a log scale at five different sample times. For all the figures, n=3 at all data points, and the mean values from the replicate plates are plotted. Error bars are not presented to avoid confusion. Letter labels indicate significant differences detected using ANOVA.

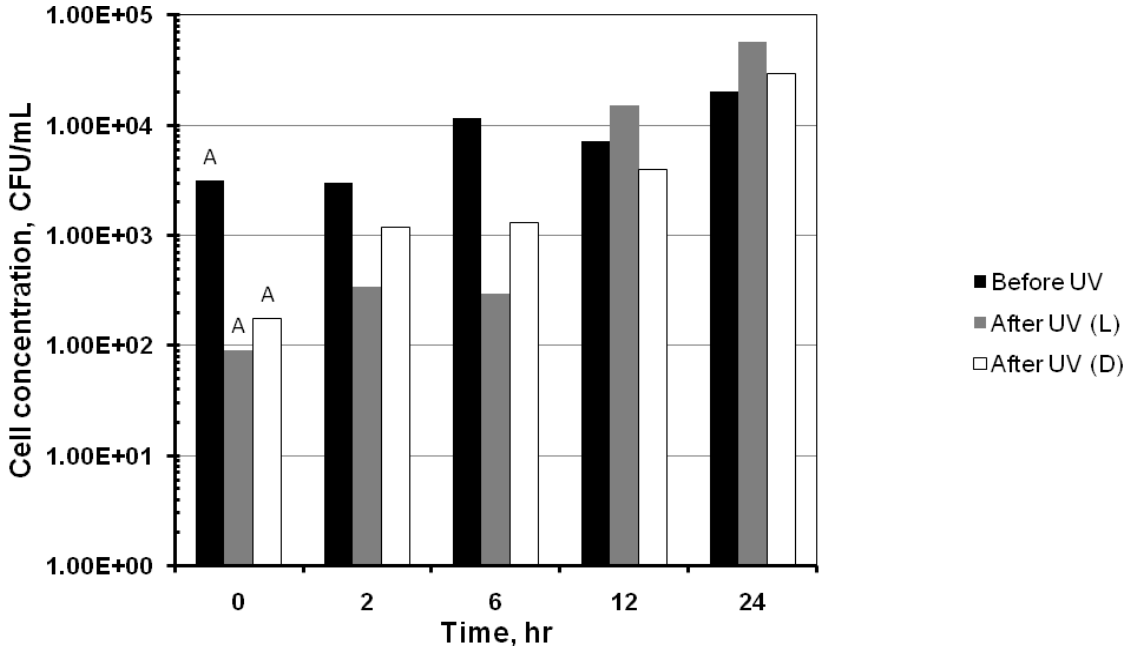


**Figure 18. Viable heterotrophic bacteria in wastewater before UV disinfection and after UV disinfection kept in light or dark over 24 hour period.**

One-way ANOVA performed on the data shown in Figure 18 with $\alpha$=0.05 did not show any significant difference among before UV, after UV kept in light, and after UV kept in dark for heterotrophic bacteria.

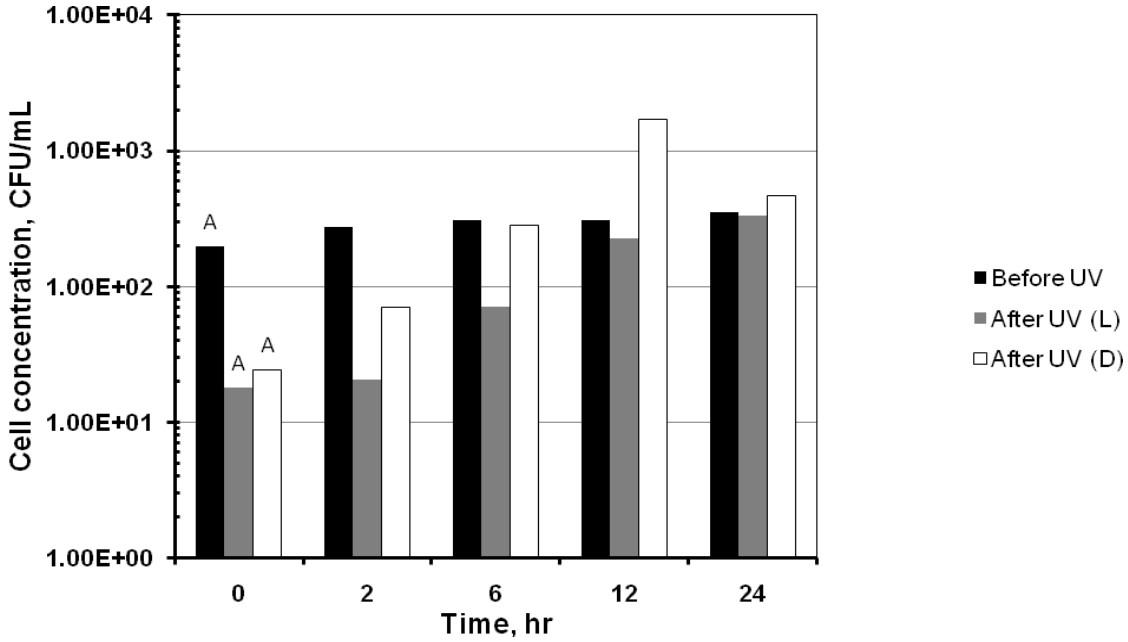


**Figure 19. Viable resistant heterotrophic bacteria in wastewater before UV disinfection and after UV disinfection kept in light or dark over 24 hour period.**

One-way ANOVA on the data shown in Figure 19 performed with $\alpha$=0.05 did not detect any significant difference among before UV, after UV kept in light, and after UV kept in dark for tetracycline-resistant heterotrophic bacteria.

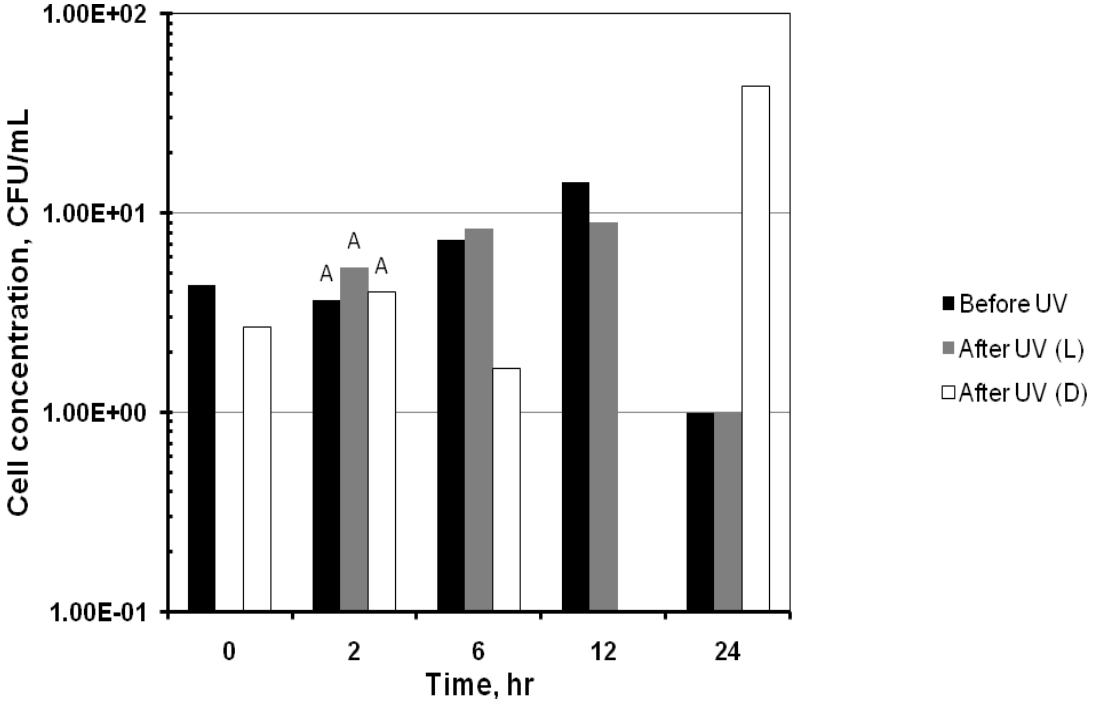


**Figure 20. Viable *E. coli* in wastewater before UV disinfection and after UV disinfection kept in light or dark over 24 hour period.**

One-way ANOVA performed on the data shown in Figure 20 with $\alpha$=0.05 did not detect any significant difference among before UV, after UV kept in light, and after UV kept in dark for *E. coli*.

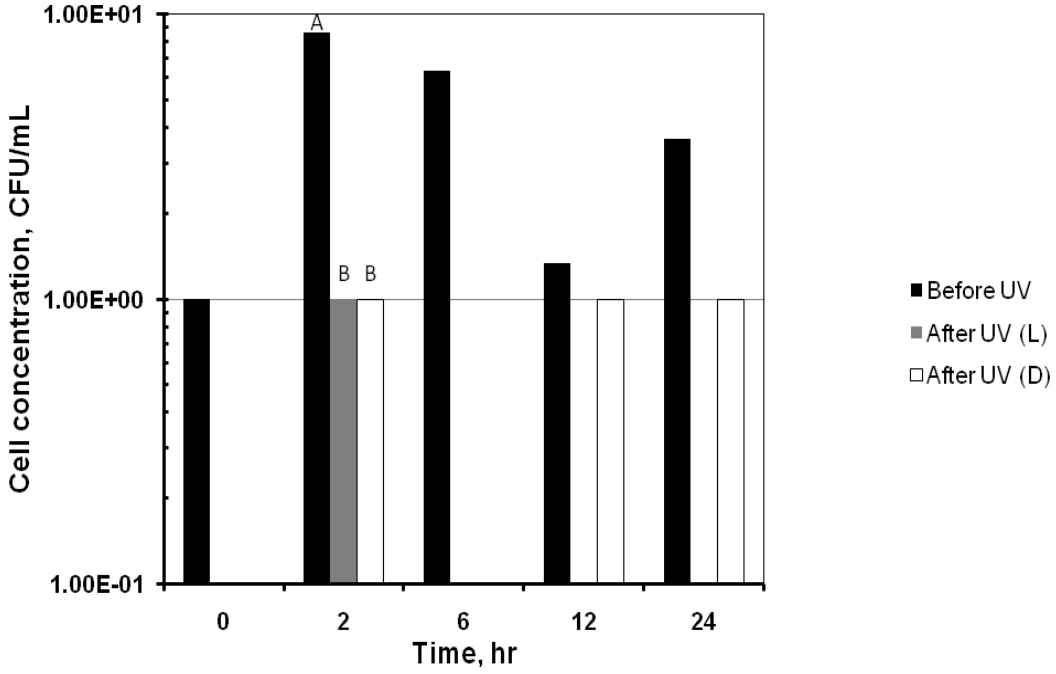


**Figure 21. Viable resistant *E. coli* in wastewater before UV disinfection and after UV disinfection kept in light or dark over 24 hour period.**

Figure 21 shows the concentrations for tetracycline-resistant *E. coli*. Resistant *E. coli* growth was observed for the sample from after UV treatment kept in light only at 2 hr. Little growth was observed after UV treatment for either sample at any sampling time. One-way ANOVA performed with $\alpha$=0.05 did detect significant difference among before UV, after UV kept in light, and after UV kept in dark for tetracycline-resistant *E. coli*. The concentration of resistant *E. coli* in the sample from before UV disinfection was found to be significantly higher than the concentration of resistant *E. coli* in both of the samples from after UV disinfection. This clearly shows that UV disinfection effectively reduced the concentration of tetracycline resistant *E. coli*.

Figures 22 through 24 compare the cell concentrations of the different sets of bacteria – heterotrophic, resistant heterotrophic, *E. coli,* and resistant *E. coli* – at various times between 0 and 24 hr for each treatment (Before UV, after UV and exposed to light, after UV and exposed to dark). Concentrations are plotted on a log scale at different sampling times. n=3 for all samples, however error bars are not presented to avoid confusion. Letter labels indicate significant differences detected using ANOVA.

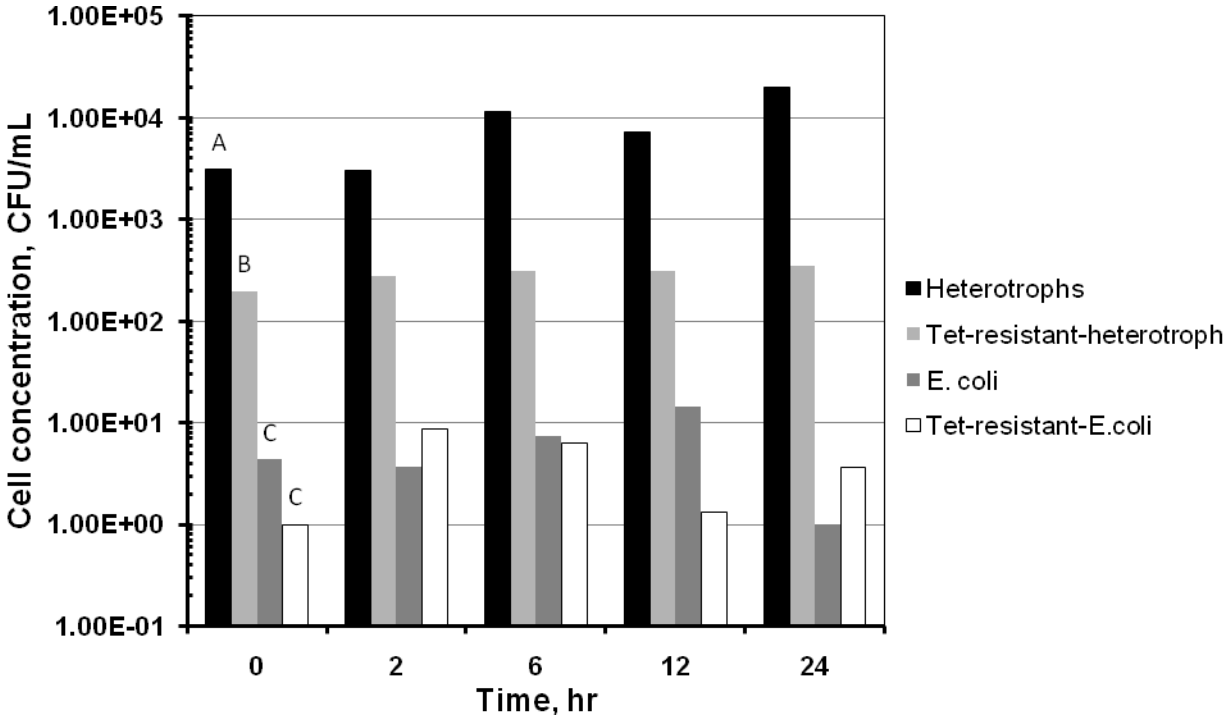


**Figure 22. Viable bacteria in wastewater before UV disinfection over 24 hour period.**

Figure 22 compares concentrations before UV disinfection. Concentrations of heterotrophs and resistant heterotrophs were consistently higher than concentrations of *E. coli* and resistant *E. coli.* Concentrations of total heterotrophs were also consistently

1 to 2 log higher than those for resistant heterotrophs; however, concentrations of *E. coli* and resistant *E. coli* were similar. One-way ANOVA performed at α=0.05 found total heterotrophs and resistant heterotrophs to be significantly different from all other treatments; however, total *E. coli* and resistant *E. coli* were not significantly different from one another.

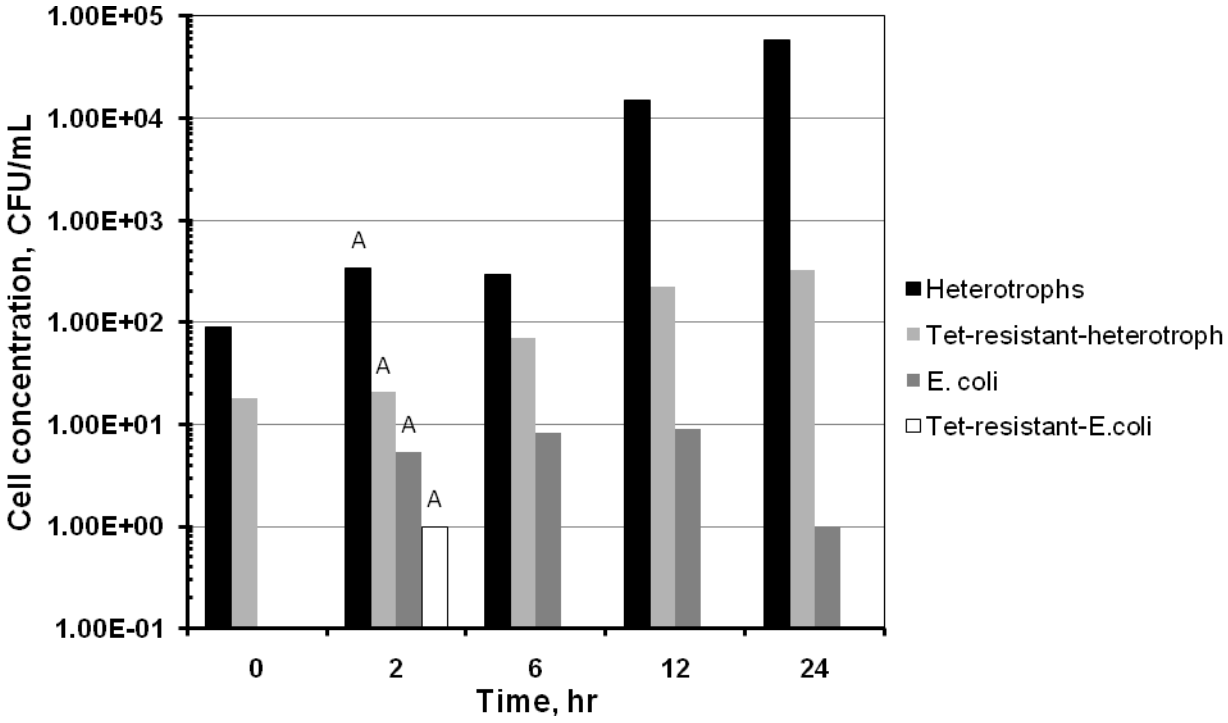


**Figure 23. Viable bacteria in wastewater after UV disinfection and exposed to light over 24 hour period.**

Figure 23 shows the concentrations after UV treatment with samples kept in light. No growth was observed for resistant *E.* coli except at 2 hr. One-way ANOVA performed at α=0.05 did not find any significant differences among different bacteria.

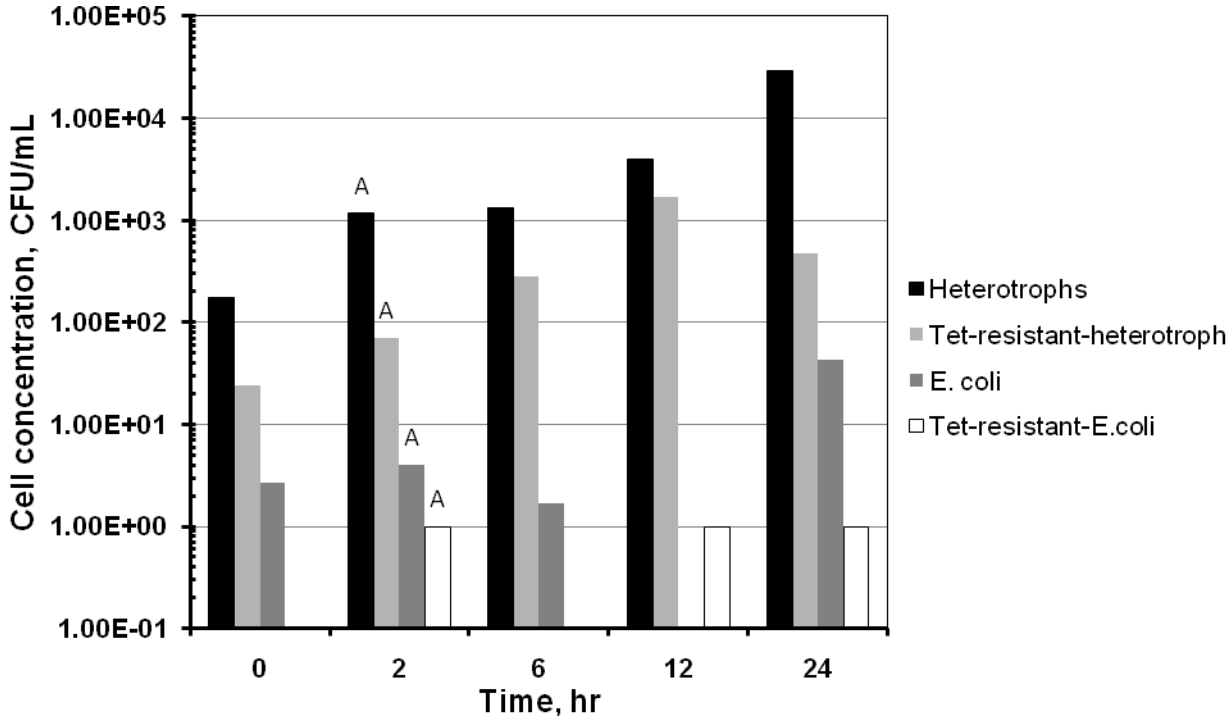


**Figure 24. Viable bacteria in wastewater after UV disinfection and exposed to darkness over 24 hour period.**

Figure 24 shows the concentrations after UV treatment kept in darkness; as for the after UV kept in light, there was little growth of tetracycline-resistant *E. coli*. One-way ANOVA performed at $\alpha$=0.05 did not find any significant differences among different bacteria.

*2.2.4 Survival and Repair*

Figures 25 through 28 show the survival ratio in percent for heterotrophic bacteria, resistant heterotrophic bacteria, *E. coli,* and resistant *E. coli.* For all data points, n=3, however error bars are not plotted to avoid confusion. Data points that were over 100% were not plotted, as a ratio over 100% clearly indicates that there was growth

occurring and not only survival.

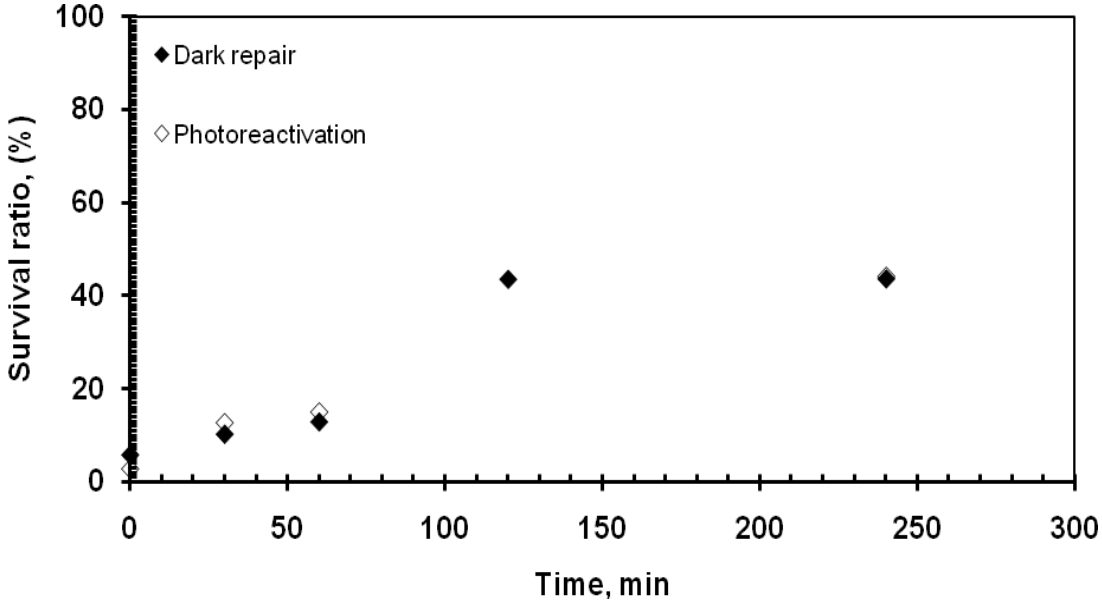


**Figure 25. Survival ratio versus time of exposure to light or darkness for heterotrophic bacteria in municipal wastewater effluent.**

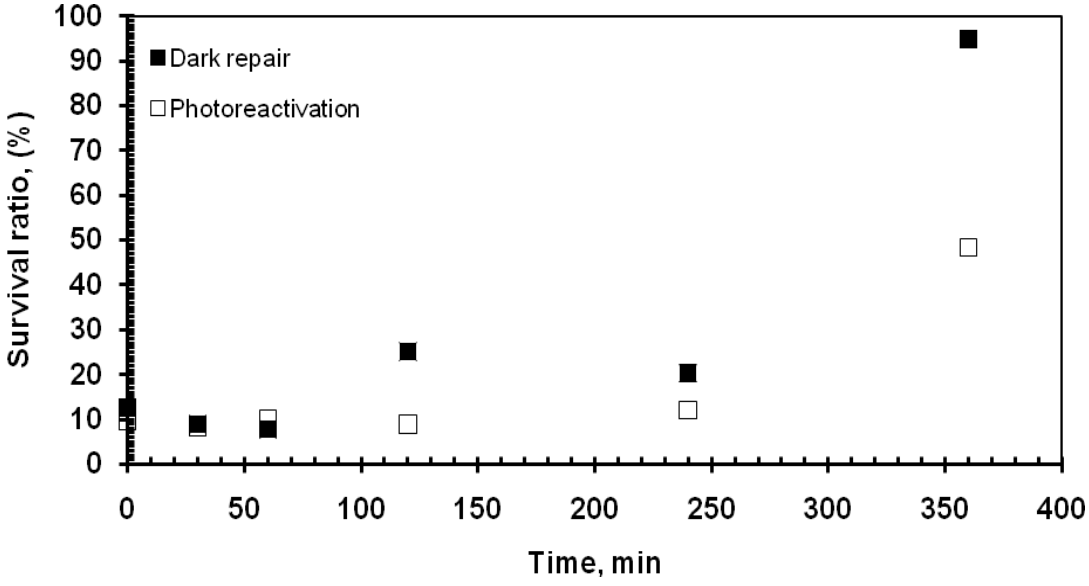


**Figure 26. Survival ratio versus time of exposure to light or darkness for tetracycline-resistant heterotrophic bacteria in municipal wastewater effluent.**

Figures 25 and 26 show the survival ratios under photoreactivation or dark repair conditions from 0 to 360 minutes for heterotrophs and tetracycline-resistant heterotrophs. For total heterotrophs, the photoreactivation and dark repair survival ratios were the same or very similar at all sampling times except 120 min, where the data for photoreactivation was over 100% and thus was omitted. The survival photoreactivation and dark repair survival ratios were identical at 240 minutes, therefore the photoreactivation data point cannot be seen on the plot. For resistant heterotrophs, photoreactivation and dark repair survival ratios are similar up to 240 minutes, but the dark repair survival ratio is higher at 360 min. The maximum survival ratio achieved for total heterotrophic bacteria was 44%, while for resistant heterotrophic bacteria the maximum survival ratio for photoreactivation was 48% and the maximum survival ratio for dark repair was 94%.

Survival ratios under dark repair conditions for both heterotrophic resistant and non-resistant bacteria were considerably higher than those reported in previous studies, including those by Lindenauer and Darby (1994) and Sanz et al (2007). A possible explanation is that these studies enumerated either pure cultures or indicator organisms, while these data were acquired from the entire culturable heterotrophic community in the wastewater effluent, and thus could potentially include organisms with higher rates of dark repair than those used in the previous studies.

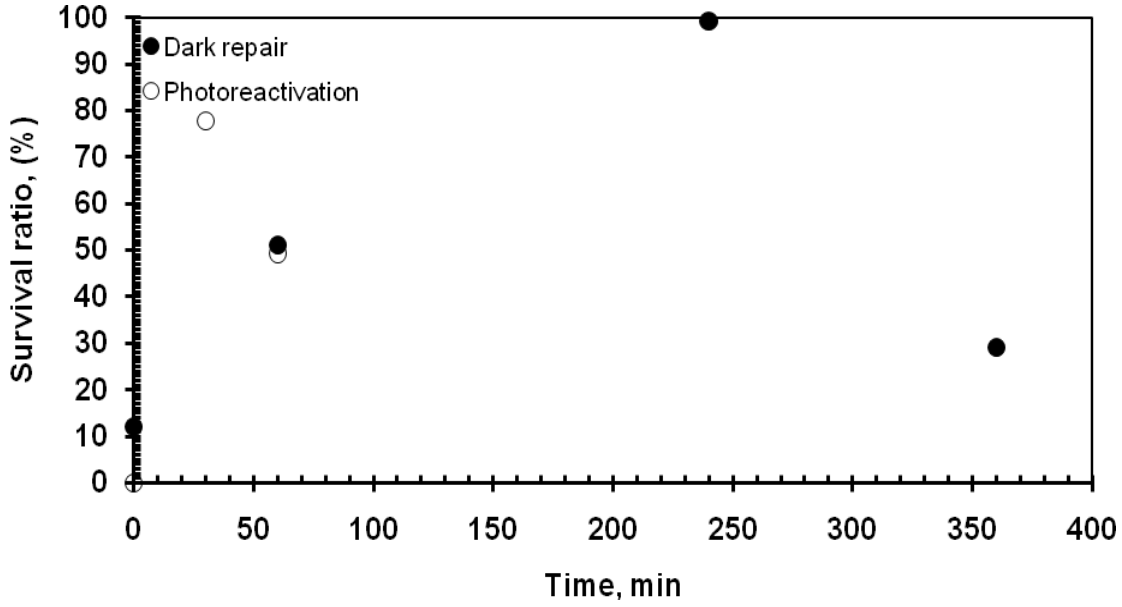


**Figure 27. Survival ratio versus time of exposure to light or darkness for *E.coli* in municipal wastewater effluent.**

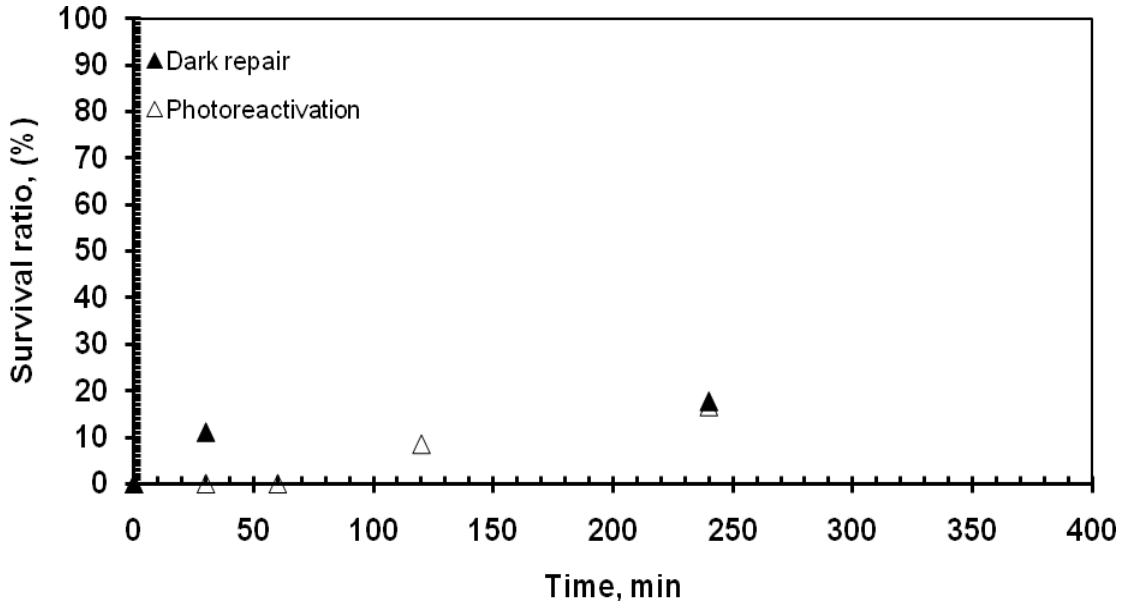


**Figure 28. Survival ratio versus time of exposure to light or darkness for tetracycline-resistant *E.coli* in municipal wastewater effluent.**

Figures 27 and 28 show the survival ratios under photoreactivation and dark repair conditions for *E. coli* and resistant *E. coli.* The photoreactivation survival ratio of *E. coli* was 80% after 30 min and had risen beyond 100% by 120 min; thus photoreactivation was only plotted through 60 min. The maximum dark repair survival ratio for total *E. coli* was 100%. Tetracycline resistant *E. coli* survival ratios reached the maximum at around 17% for both photoreactivation and dark repair; as previously mentioned, resistant *E. coli* showed little regrowth after UV disinfection. Survival ratios for total *E. coli* were much higher than survival ratios for resistant *E. coli* under both photoreactivation and dark repair conditions.

A study by Quek and Hu (2008) found the photoreactivation after exposure to Low Pressure (LP) UV lamps of various pure strains of *E. coli* to range from 40 – 80% after four hours; the dark repair after the same time period was found to range from about 12 – 25%. The maximum photoreactivation survival ratios shown in Figure 24 are similar to those found by Quek and Hu (2008); however, the dark repair survival ratios were much higher. Survival ratios for resistant *E. coli* under dark repair conditions were similar to those reported by Quek and Hu (2008), while photoreactivation survival ratios were lower. The Quek and Hu (2008) study used pure *E. coli* cultures at a concentration of about 1 x $10^8$ CFU/mL, much higher than the concentrations in the WWTP effluent.

Figures 29 through 32 show the % log repair under photoreactivation and dark repair conditions for heterotrophs, resistant heterotrophs, *E. coli*, and resistant *E. coli*. Data were plotted from 0 hr to the time at which % log repair increased to over 100%, as this is the point at which there is clearly growth occurring and not only reactivation or

repair. The plot for total *E. coli* is an exception, as this % log repair was over 100% by 1 hr; these data were therefore plotted up to 200% for comparison purposes. n=3 for all data points, however error bars were not included to avoid confusion. Data points more than three standard deviations from the mean were omitted from the plots.

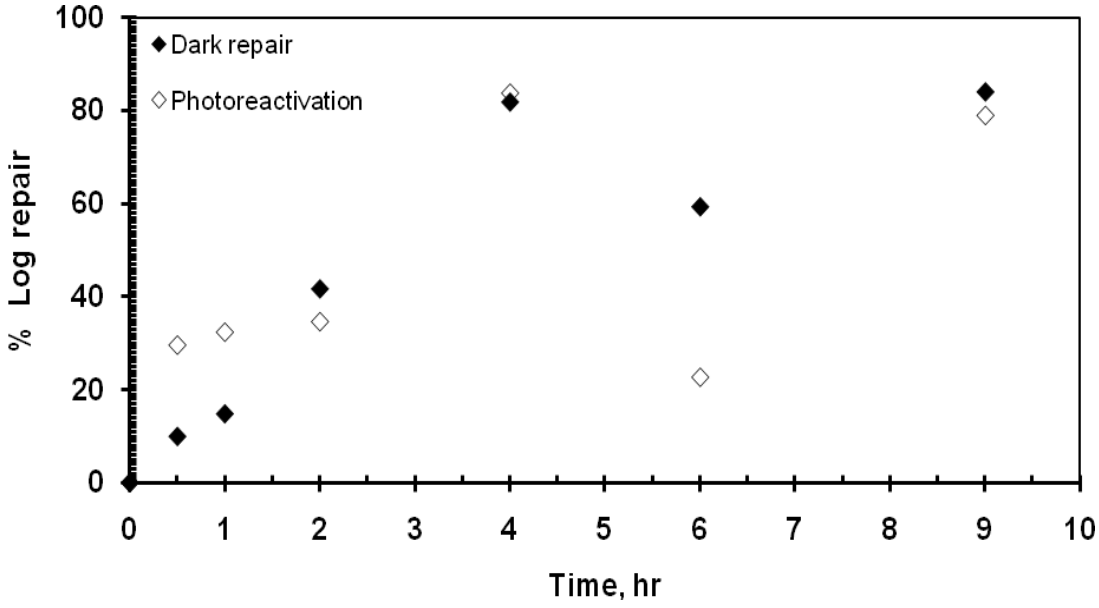


**Figure 29. Percentage log repair of heterotrophic bacteria in wastewater effluent after UV disinfection and exposed to light or darkness.**

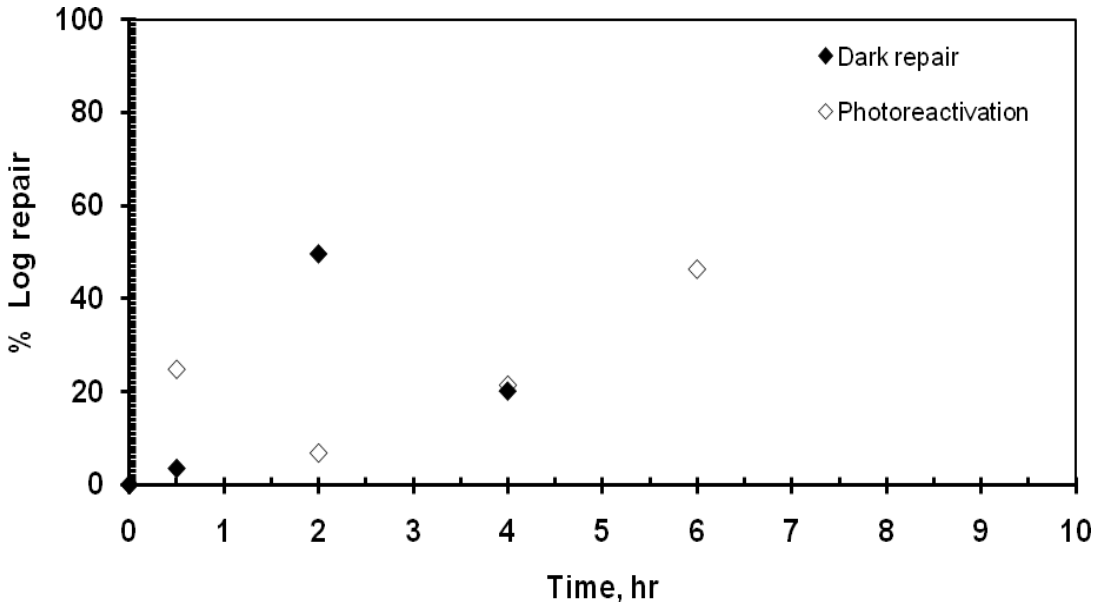


**Figure 30. Percentage log repair of tetracycline-resistant heterotrophic bacteria in wastewater effluent after UV disinfection and exposed to light or darkness.**

Figures 29 and 30 show the % log repair of heterotrophic and resistant heterotrophic bacteria under photoreactivation and dark repair conditions. Heterotrophic bacteria reached about 80% log repair after 9 hrs under both photoreactivation and dark repair conditions. For resistant heterotrophic bacteria, log repair was over 100% by 9 hrs. The maximum % log repair achieved for resistant bacteria before exceeding 100% was 46% for photoreactivation and 50% for dark repair.

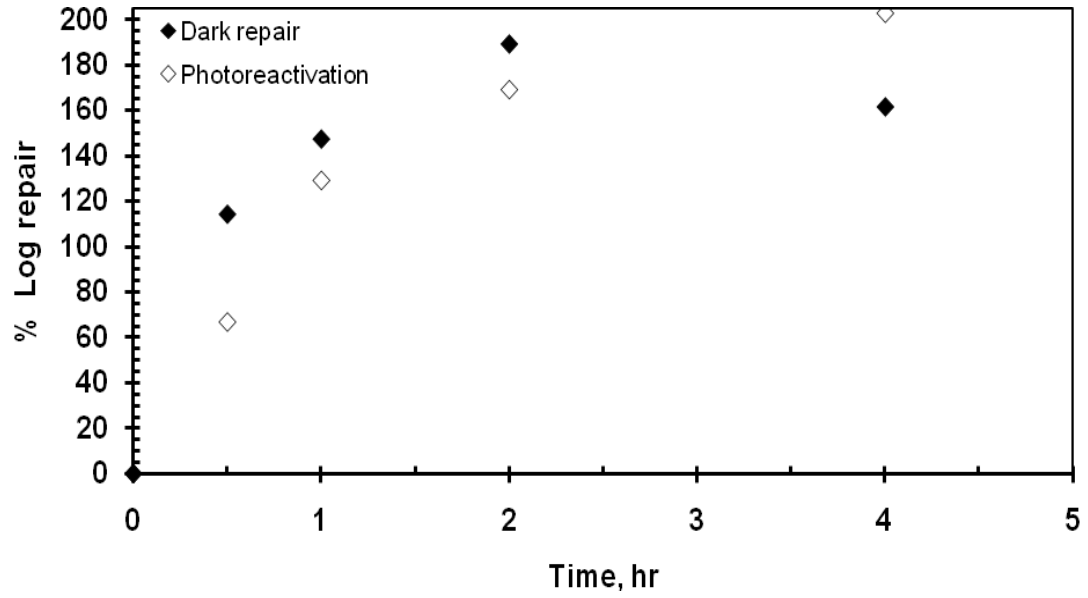


**Figure 31. Percentage log repair of *E. coli* in wastewater effluent after UV disinfection and exposed to light or darkness.**

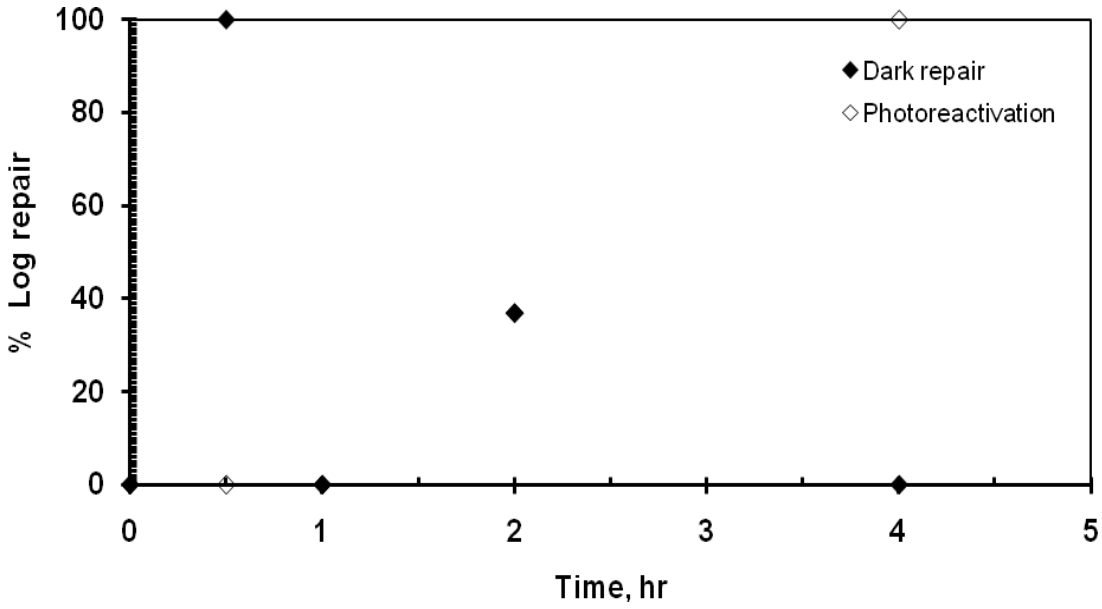


**Figure 32. Percentage log repair of tetracycline-resistant *E. coli* in wastewater effluent after UV disinfection and exposed to light or darkness.**

Figures 31 and 32 show the % log repair for *E. coli* and tetracycline-resistant *E. coli* under photoreactivation and dark repair conditions. Log repair for total *E. coli* had reached 67% for photoreactivation and 114% for dark repair by the 0.5 hr sampling time, and photoreactivation was above 100% log repair by the 1 hr sampling time; thus, % log repair for total *E. coli* was plotted through 4 hr for comparison to resistant *E. coli*. Log repair reached 100% at 0.5 hr for dark repair and at 4 hr for photoreactivation in resistant *E. coli*; however, dark repair decreased to 0% log repair by 4 hr.

Log repair rates for various strains of *E. coli* reported in a study by Quek and Hu (2008) reached a maximum of 80% for photoreactivation and 25% for dark repair after 4 hrs. *E. coli* in this study showed a much faster increase in % log repair under photoreactivation conditions, and a higher maximum % log repair under both photoreactivation and dark repair conditions. The study by Quek and Hu (2008) used lab cultivated *E. coli* strains, while the *E. coli* in the present study were those found in a WWTP, which may account for the difference in % log repair.

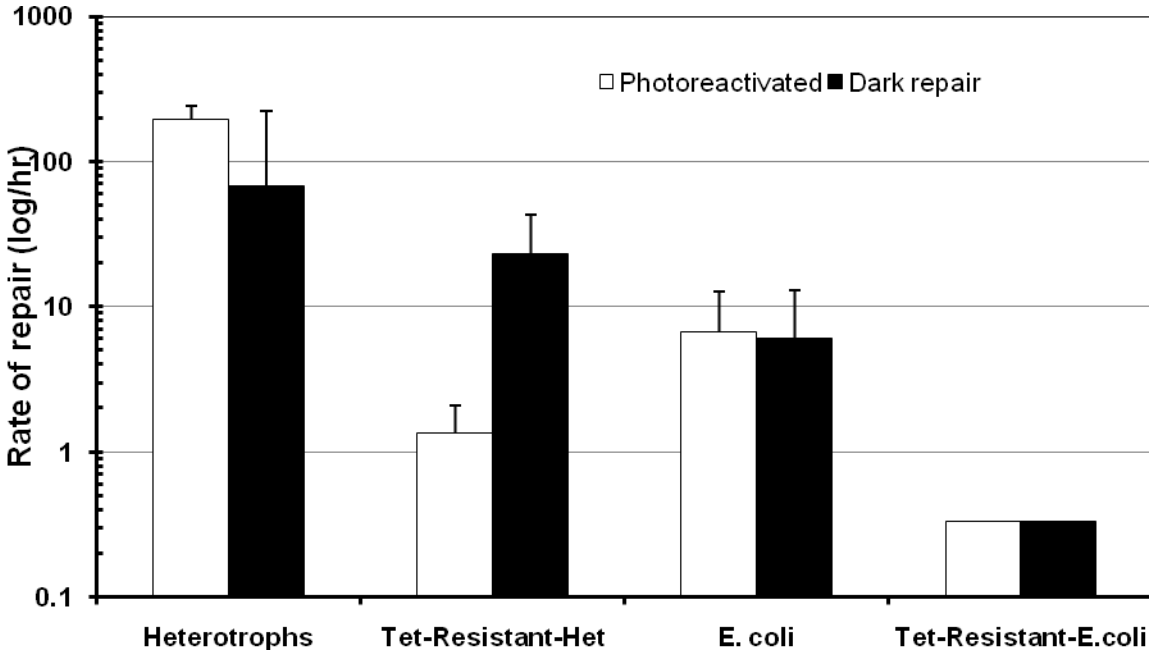


**Figure 33. Repair rates of various bacteria in municipal wastewater effluent after UV disinfection and exposed to light or darkness.**

Figure 33 shows the rate of repair (log/hr) under photoreactivation and dark repair conditions for heterotrophs, resistant heterotrophs, *E. coli*, and resistant *E. coli*; error bars represent one standard deviation. Rates of photoreactivation and dark repair were similar for *E. coli* and were the same for tetracycline resistant *E. coli.* One-way ANOVA with $\alpha$=0.05 found a significant difference between the rate of photoreactivation for total heterotrophs and the rate of photoreactivation for all other bacteria, and no significant difference in rates of dark repair for the different types of bacteria. No significant difference was found between the photoreactivation and dark repair rates for any category of bacteria.

The study by Quek and Hu (2008) found photoreactivation rates of 1 to 3 log/hr for various lab strains of *E. coli*; the study did not present dark repair rates. The rate of photoreactivation presented in Figure 30 is at least twice that of the rate found in the Quek and Hu study (2008). As for the % log repair, it is possible that the difference may be accounted for by differences between the lab strains of *E. coli* and the WWTP strains.

*2.2.5 Cultured Population Composition*

Table 3 shows the relative abundance of the six cultured populations by class (sequences obtained from isolates from nutrient and MacConkey agars were combined for community comparisons). All cultured populations with the exception of resistant bacteria after UV treatment kept in light or kept in dark were composed primarily of gammaproteobacteria. Small numbers of bacilli and betaproteobacteria were also identified. Cultured populations of resistant bacteria from after UV treatment, both kept in light and kept in dark, had mostly unclassified bacteria and unclassified root sequences.

**Table 3. Classification of Bacterial Isolates.**

| | Total | | | Resistant | | |
|---|---|---|---|---|---|---|
| **Class** | **Before** | **After Light** | **After Dark** | **Before** | **After Light** | **After Dark** |
| Bacilli | 2 | 2 | 5 | 0 | 1 | 0 |
| Betaproteobacteria | 0 | 1 | 1 | 0 | 2 | 2 |
| Gammaproteobacteria | 20 | 24 | 24 | 23 | 4 | 2 |
| Unclassified bacteria | 3 | 0 | 0 | 3 | 4 | 3 |
| Unclassified root | 4 | 0 | 0 | 0 | 6 | 10 |
| **Total** | 29 | 27 | 30 | 26 | 17 | 17 |

The parsimony test performed using MOTHUR showed a significant difference in cultured population composition between resistant bacteria before UV treatment and resistant bacteria after UV treatment, with a p-value of $<0.001$ for both after UV kept in

light and after UV kept in dark compared with before UV. Additionally, cultured populations of resistant bacteria at each sampling location were significantly different from the total cultured populations, with p-values of 0.005 for before UV, 0.002 for after UV kept in light, and <0.001 for after UV kept in dark. However, no significant difference was detected between the total cultured population before UV treatment and the total cultured population after UV treatment for either photoreactivation or dark repair conditions.

Table 4 shows the closest putative identities assigned by Seqmatch in RDP to isolates from plates containing tetracycline. *Citrobacter freundii* was the only species identified in isolates both before and after UV treatment. Isolates from before UV treatment plated on nutrient agar were predominantly *Aeromonas* and *Providencia* species. The majority of isolates from after UV treatment kept in dark were unidentifiable.

**Table 4. Putative Identities of Resistant Bacterial Isolates.**

| | Nutrient agar | | | MacConkey agar | | |
|---|---|---|---|---|---|---|
| **Species** | **Before** | **After light** | **After dark** | **Before** | **After light** | **After dark** |
| Acetobacter pasteurianus | | 1 | 2 | | | |
| Acidovorax sp. | | | 2 | | | |
| Acinetobacter sp. | 1 | | | | | |
| Aeromonas punctata | 1 | | | 1 | | |
| Aeromonas sp. | 4 | | | | | |
| Bacillus anthracis | | 1 | | | | |
| Buttiauxella agrestis | 1 | | | | | |
| Citrobacter freundii | 1 | | | 2 | 2 | |
| Citrobacter sp. | | | | 2 | | |
| diazotroph str. | | 1 | | | | |
| Enterobacter sp. | | 1 | | | | 1 |
| Escherichia coli | | | | 1 | | |
| Halomonas sp. | | 1 | | | | |
| Klebsiella pneumoniae | | | | | | 1 |
| Pectobacterium atrosepticum | | | 1 | | | |
| Pectobacterium carotovorum | | 1 | | | | |
| Providencia alcalifaciens | 6 | | | | | |
| Providencia rettgeri | 2 | | | | | |
| Pseudomonas sp. | | 2 | | | | |
| Raoultella ornithinolytica | | | | 1 | | |
| Shewanella frigidimarina | | 1 | | | | |
| Shigella flexneri | | | | 3 | | |
| soybean epiphytic bacterium | | | | 1 | | |
| Vogesella sp. | | 1 | | | | |
| unidentified | | 4 | 9 | | | |

Thus, it appears that the cultured population of tetracycline-resistant bacteria both before and after UV treatment differs in composition from the total bacterial cultured population. It also appears that UV treatment significantly altered the composition of the tetracycline-resistant bacterial cultured population, although it did not have the same effect on the total bacterial cultured population.

*2.2.6 Qualitative PCR to Detect tet(Q) Gene*

Qualitative PCR and gel electrophoresis analysis showed that 100% of tetracycline-resistant heterotrophic and *E. coli* bacterial isolates contained the *tet*(Q) gene. Since the WWTP where samples were collected is a municipal plant serving a small city, it was expected that a high number of resistant bacteria would contain a human origin resistance gene.

# 3. CONCLUSIONS

The overall objective of this study was to investigate the effect of UV light disinfection on ARGs and antibiotic resistant bacteria. Wastewater effluent samples from a wastewater treatment plant (WWTP) in Texas were evaluated for differences in abundance and diversity of tetracycline resistant bacteria before and after UV treatment. Culture based methods were used to characterize viable heterotrophic, tetracycline resistant heterotrophic, *E. coli*, and tetracycline resistant *E. coli* bacteria present before and after UV treatment. The effects of photoreactivation or dark repair on the reactivation of bacteria present in WWTP effluent after UV disinfection were also examined. Bacterial colonies from culture plates were isolated and sequenced in order to characterize the diversity of the organisms present before and after treatment. Polymerase chain reactions (PCR) were performed on the isolated bacterial DNA to test for the presence of a tetracycline resistance gene (*tet*(Q)) usually from human origin.

Overall, UV disinfection was found to be at least as effective for reducing concentrations of tetracycline-resistant heterotrophs and *E. coli* as it was for reducing concentrations of total heterotrophs and *E. coli*. UV disinfection was found to be most effective at eliminating resistant *E. coli*, as resistant *E. coli* achieved a maximum survival ratio after photoreactivation or dark repair of only 17%, much lower than the survival ratios for the other types of bacteria.

Survival ratios of heterotrophic bacteria and % log repair of *E. coli* under dark repair conditions were found to be much higher than those reported in previous studies

(Lindenauer and Darby, 1994; Sanz et al, 2007; Quek and Hu, 2008). Survival ratios and % log repair under dark repair conditions in the present study were found to be comparable to survival ratios and % log repair under photoreactivation conditions. This finding suggests that the bacterial strains found in WWTPs may have higher rates of dark repair than the lab strains used in the previous studies.

UV light disinfection was found to significantly alter the cultured population composition of tetracycline-resistant bacteria under both photoreactivation and dark repair conditions; this was in contrast with the total bacterial cultured population, which was not significantly altered by UV disinfection. Furthermore, tetracycline-resistant bacterial cultured population was found to differ significantly from total bacterial cultured population at the same location. All bacterial isolates from culture plates containing tetracycline were found to possess the human origin *tet*(Q) gene, which is consistent with the influent sources for the WWTP studied.

# 4. RECOMMENDATIONS

The high rates of dark repair found in this study indicate that further research is needed to investigate dark repair rates of bacteria found in wastewater treatment plants to determine whether they are commonly much higher than dark repair rates found in laboratory strains. UV disinfection was found to be effective at reducing resistant bacteria; however, further research would be required in order to determine whether other wastewater treatment technologies or best management practices might more effectively reduce resistant bacteria and thus minimize the amount of resistance genes introduced into the environment.

# REFERENCES

Armstrong, J.L., Shigeno, D.S., Calomiris, J.J., and Seidler, R.J. (1981) Antibiotic-resistant bacteria in drinking water. Appl. Environ. Microbiol. 42, 277-283.

Auerbach, E.A., Seyfried, E.E., and McMahon, K.D. (2007) Tetracycline resistance genes in activated sludge wastewater treatment plants. Wat. Res. 41, 1143-1153.

Billington, S.J., Songer, J.G., and Jost, B.H. (2002) Widespread distribution of a tet W determinant among tetracycline-resistant isolates of the animal pathogen *Arcanobacterium pyogenes.* Antimicrobial Agents and Chemotherapy 46(5), 1281-1287.

Chee-Sandford, J.C., Mackie, R.I., Koike, S., Krapac, I.G., Lin, Y., Yannarell, A.C., Maxwell, S., and Aminov, R.I. (2009) Fate and transport of antibiotic residues and antibiotic resistance genes following land application of manure waste. J. Environ. Qual. 38, 1086-1108.

Chopra, I. and Roberts, M. (2001) Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiology and Molecular Biology Reviews 65(2), 232-260.

Cohen, M.L. (1992) Epidemiology of drug resistance: implications for a post-antimicrobial era. Science 257, 1050-1055.

Cole, J.R., Chai, B., Farris, R.J., Wang, Q., Kulam, S.A., McGarrell, D.M., Garrity, G.M., and Tiedje, J.M. (2005) The ribosomal database project (RDP-II): sequences and tools for high-throughput rRNA analysis. Nucl. Acids Res. 33, D294–D296.

Esiobu, N., Armenta, L., and Ike, J. (2002) Antibiotic resistance in soil and water environments. International Journal of Environmental Health Research 12, 133-144.

Felsenstein, J. (2005) PHYLIP (*Phylogeny Inference Package*) Version 3.6. Distributed by the author. Department of Genome Sciences, University of Washington: Seattle.

Friedburg, E.C. (2003) DNA damage and repair. Nature 421, 436-440.

Levy, S.B. and Marshall, B. (2004) Antibacterial resistance worldwide: causes, challenges, and responses. Nature 10(12), S122-S129.

Martins da Costa, P., Vaz-Pires, P., and Bernardo, F. (2006) Antimicrobial resistance in *Enterococcus* spp. isolated in inflow, effluent and sludge from municipal sewage water treatment plants. Wat. Res. 40(8), 1735-1740.

Masschelein, W.J. (2002) Ultraviolet Light in Water and Wastewater Sanitation. Boca Raton, FL: Lewis Publishers.

Montelone, B.A. (1998) Mutation, mutagens, and DNA repair. http://www-personal.ksu.edu/~bethmont/mutdes.html#repair Accessed May 6, 2010.

Pei, R., Cha, J., Carlson, K.H., and Pruden, A. (2007) Response of antibiotic resistance genes (ARG) to biological treatment in dairy lagoon water. Environ. Sci. Technol. 41, 5108-5113.

Pei, R., Kim, S.C., Carlson, K.H., Pruden, A. (2006) Effect of river landscape on the sediment concentrations of antibiotics and corresponding antibiotic resistance genes (ARG). Wat. Res. 40, 2427-2435.

Pruden, A., Pei, R., Storteboom, H., and Carlson, K.H.. (2006) Antibiotic resistance genes as emerging contaminants: studies in northern Colorado. Environ. Sci. Technol. 40, 7445-7450.

Sanz, E.N., Dávila, I.S., Balao, J.A.A., and Alonso, J.M.Q. (2007) Modeling of reactivation after UV disinfection: effect of UV-C dose on subsequent photoreactivation and dark repair. Wat. Res. 41, 3141-3151.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., et al. (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75(23), 7537-7541.

Schwartz, T., Kohen, W., Jansen, B., and Obst, U. (2002) Detection of antibiotic-resistant bacteria and their resistance genes in wastewater, surface water, and drinking water biofilms. FEMS Microbiology Ecology 43, 325-335.

Singer, R.S., Ward, M.P., and Maldonado, G. (2006) Can landscape ecology untangle the complexity of antibiotic resistance? Nature 4, 943-942.

Volkmann, H., Schwartz, T., Bischoff, P., Kirchen, S., and Obst, U. (2004) Detection of clinically relevant antibiotic-resistance genes in municipal wastewater using real-time PCR (TaqMan). J. Microbiol. Methods 56(2), 277–286.

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