BACTERIOPHAGE PRD1 AS A POTENTIAL SURROGATE FOR ADENOVIRUS IN DRINKING WATER DISINFECTION WITH FREE CHLORINE, LOW PRESSURE ULTRAVIOLET LIGHT, AND SUNLIGHT

BY

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THESIS

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ABSTRACT

Waterborne pathogens are increasingly a worldwide concern in drinking water because of their ability to cause high levels of morbidity and mortality. Especially in developing regions, a lack of access to safe drinking water, adequate sanitation, and resources to implement water treatment processes contributes to the spread of pathogens. Emerging pathogens are also of concern in water treatment for communities in developed regions as they can be highly resistant to specific treatment technologies. Viruses are of particular concern in water treatment not only because of their virulence and ability to have high resistance to inactivation, but also because of the limited knowledge available. Human pathogenic viruses are not easy to study in the laboratory or in the field because of strict biosafety regulations and the use of expensive cell culture methods that are time consuming. Often it is not practical to perform testing with human pathogens, and therefore surrogates can be used. Currently, there is a need to develop proper surrogates especially for adenovirus, a human enteric pathogen found globally in drinking water sources. Adenovirus is known to be highly resistant to disinfection technologies such as ultraviolet (UV) light, combined chlorine, and solar disinfection. A potential surrogate for adenovirus is the bacteriophage PRD1 because of its similar size, morphology, and genome replication mechanism. The objective of this research was to compare the inactivation kinetics of PRD1 with that of adenovirus when exposed to free chlorine, low pressure ultraviolet light, and solar disinfection to determine if PRD1 is an appropriate surrogate. Using PRD1 as a surrogate would enable field testing to determine the efficacy of current and emerging water treatment technologies, more rapid and non virulent laboratory experiments, and the use of a surrogate for determining the mechanisms of inactivation of adenovirus.

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CHAPTER 1 INTRODUCTION

Waterborne pathogens are increasingly a worldwide concern in drinking water because of their ability to cause high levels of morbidity and mortality. Especially in developing regions, a lack of access to safe drinking water, adequate sanitation, and resources to implement water treatment processes contributes to the spread of pathogens. Without adequate protection of drinking water sources, waters can become heavily contaminated by human and animal waste which contributes to the spread of a range of pathogens including viruses. The United Nations (UN) Millennium Development Goal (MDG) 7 seeks to "halve, by 2015, the proportion of people without sustainable access to safe drinking water and sanitation" and the indicator used is the "proportion of population using an improved drinking water source, urban, and rural" (UN, 2003). A recent update on MDG 7 by the World Health Organization (WHO) details that 884 million people still lack access to improved drinking water sources (WHO, 2010). The WHO report and a recent report by the UN's Independent Expert on the issue of human rights obligations related to access to safe drinking water and sanitation further emphasize that improved drinking water sources may not meet drinking water quality standards, and therefore the number of people worldwide who lack access to safe drinking water is indeed much greater (de Albuquerque, 2010). Because of the high prevalence of waterborne diseases, current and emerging technologies for water disinfection are important to study in these areas. Point-of-use disinfection technologies are a viable treatment method in developing regions and implementation has showed an improvement in health and the potential for a sustainable solution; however, many systems currently used are not always completely effective in these challenging surface waters common to developing regions. Small community scale systems are also common in developing regions, but sometimes do not have adequate disinfection steps to prevent the spread of disease.

Emerging pathogens are also of concern in water treatment for communities in developed regions as they can be highly resistant to specific treatment technologies. There have been recent changes in water disinfection regulations by the US Environmental Protection Agency

(USEPA) with the Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) and the Stage 2 Disinfectants and DBPs Rule (Stage 2 DBPR) (USEPA, 2006 a,b). An increasing number of utilities have since been switching from the common disinfection strategy of using free chlorine as a primary and residual disinfectant to the use of different disinfection strategies to comply with new regulations. The LT2ESWTR requires a more robust treatment for inactivating *Cryptosporidium parvum* oocysts, which are highly resistant to free chlorine. The use of ultraviolet (UV) light is an effective disinfectant for the oocysts; however, the UV dosages commonly used for oocysts would not be adequate to inactivate certain viruses. The Stage 2 DBPR has also influenced the movement away from free chlorine because the rule regulates several trihalomethanes and haloacetic acids that are mostly associated with free chlorine disinfection. The use of combined chlorine as a residual disinfectant for distribution systems has been implemented by utilities to lower their amount of regulated DBPs in the finished water. With the new regulations, viruses have become emerging pathogens because certain viruses can be highly resistant to both UV and combined chlorine treatment.

Viruses are of particular concern in water treatment not only because of their virulence and ability to have high resistance to inactivation, but also because of the limited knowledge available. Viruses pathogenic to humans are not easy to study in the laboratory or in the field because of strict biosafety regulations. Additionally, quantification of infectious human viruses typically require the use of cell cultures which are time consuming to propagate, expensive, easily contaminated, and require specific conditions for growth that can be nearly impossible to achieve in regions that have intermittent or no access to electricity. When studying viruses, pure cultures and proper biosafety standards can be effectively controlled in a laboratory setting; however, when working in the field, it is often not practical to perform testing with human pathogens so surrogates can be used. One of the most commonly used surrogates is the single-stranded RNA bacteriophage MS2, which does not show similar inactivation to many human viruses including adenovirus for a range of disinfectants. Because of these challenges, there is a need to identify appropriate viral pathogen surrogates for testing the robustness of treatment technologies in the field and laboratory.

A human pathogenic double-stranded DNA virus, adenovirus, is present globally in drinking water sources and can be spread through the fecal oral route or through aerosols (Jiang, 2006). There are 51 serotypes of human adenovirus that cause a variety of human health effects including gastroenteritis, respiratory disease, and conjunctivitis (Jiang, 2006). Adenovirus is known to be highly resistant to disinfection technologies such as ultraviolet (UV) light, combined chlorine, and solar disinfection (SODIS) (Sirikanchana et al., 2008). In contrast, a study characterizing the inactivation kinetics of adenovirus serotype 2 with free chlorine at a range of temperatures and pH (Page et al., 2009) has revealed that free chlorine is highly effective in controlling adenovirus. As the water industry moves away from free chlorine, further research is necessary on alternative disinfection schemes because the switch in technologies are making adenovirus, as well as several enteroviruses, emerging pathogens. Such research efforts should also focus on providing safe water in developing regions with prevalence of waterborne diseases. Currently, specific enteric viruses are not regulated; however, adenovirus is present on the EPA's Contaminant Candidate List 3 with a potential for future regulation (USEPA, 2009).

PRD1 was chosen as a potential surrogate for adenovirus because of their strikingly similar morphologies and genome replication mechanisms. Additionally, it has been hypothesized that the two viruses are evolutionarily related (Benson et al., 1999). PRD1 is a bacteriophage that infects gram-negative bacteria carrying P, N, or W incompatibility group plasmids. Accordingly, PRD1 has a range of hosts including *Escherichia coli*, *Salmonella typhimurium*, and *Pseudomonas aeruginosa*. The viruses have very similar icosahedral capsid structures and are the only virions to contain a pseudo T=25 lattice structure. PRD1 and adenovirus have comparable diameter sizes of 63 and 90nm, respectively. The coat proteins and vertex proteins have related structures, and each virus has fiber protrusions from each of their 12 vertices on the protein coat. Upon host infection, the DNA of both viruses is released through a vertex. Furthermore, both viruses have linear double-stranded DNA genomes that contain inverted terminal repeat sequences, and they both use a protein primed mechanism for genome

replication, and contain a 5' terminal protein. The main structural difference between the two viruses is that PRD1 contains an internal lipid membrane derived from the bacterial host membrane whereas adenovirus does not (Benson et al., 1999). Because of these similarities particularly in structure and genome, PRD1 could potentially exhibit similar inactivation kinetics as adenovirus when exposed to varying disinfectants for water treatment especially when compared to other surrogates currently used like the single-stranded RNA bacteriophage MS2.

The objective of this research was to investigate if PRD1 is a proper surrogate for adenovirus serotype 2 when exposed to chemical disinfectants, ultraviolet light, and sunlight through the comparison of inactivation kinetics. Since the two viruses have such similar capsid structures, using PRD1 as a surrogate for adenovirus may help to elucidate mechanisms of inactivation of adenovirus. Elucidating the mechanism of inactivation of the virus could then lead to the development of more robust drinking water disinfection technologies and the development of sensors to detect infective viruses in drinking water. If this surrogate-host combination is able to exhibit similar inactivation kinetics in the laboratory setting as the target viral pathogen, then PRD1 could be safely used in the field to determine the efficacy of existing and emerging disinfectant techniques against adenovirus. Identifying a surrogate would be exceptionally useful for furthering laboratory research and for improving drinking water disinfection systems globally.

CHAPTER 2 MATERIALS AND METHODS

2.1 PRD1 Propagation and Viability Assessment

Bacteriophage PRD1 (BAA-769-B1) and its bacterial hosts *Escherichia coli* K12 J53-1 (BAA-769) and *Salmonella typhimurium* LT2 (19585) were obtained from the American Type Culture Collection (Manassas, Virginia). The freeze dried *E. coli* was rehydrated with autoclaved Tryptic Soy Broth (TSB) and then inoculated into a 3 % TSB suspension and agar slants composed of 3 % TSB and 1.5 % Tryptic Soy Agar (TSA). The suspension and slants were incubated for 24 h at 37 °C. The slants were placed in a 4 °C cold room for long term storage. From the suspension, 2 mL of *E. coli* grown overnight was inoculated into two flasks containing 100 mL and 500 mL of TSB and incubated at 37 °C. The absorbance of the *E. coli* suspension was monitored over time, and upon reaching exponential growth, the 100 mL flask was placed into the 4 °C cold room for storage. At this time, the 500 mL flask was inoculated with PRD1 and incubated at 37 °C overnight to propagate the virus.

The *S. typhimurium* was split into three cryovials for long term storage and one streak plate which was incubated at 37 °C and then stored in a refrigerator. Multiple colonies of *S. typhimurium* were removed from the streak plate by a wire loop and inoculated into 500 mL of TSB. The suspension was grown overnight in a 37 °C shaker incubator. The suspension was inoculated with 0.5 mL PRD1 stock and was incubated at 37 °C overnight to allow for virus replication.

Stock solutions of PRD1 were purified and concentrated using centrifugation, microfiltration, and ultrafiltration to yield a high titer of PRD1. The virus-host suspensions were centrifuged at 5000 rpm for 10 minutes to separate the cellular debris into a pellet. The supernatant was passed through a Stericup sterile vacuum filter unit with a polyvinylidene fluoride membrane (PVDF) with a nominal pore size of 0.22 µm (Millipore, Billerica, MA) to remove debris that was not removed during centrifugation. To remove dissolved matter from the virus stock, a PVDF ultrafiltration (UF) membrane with nominal molecular weight cutoff of 30 kDa (HFM-100; Koch

Membrane Systems, Wilmington, MA) was mounted in a sterile Amicon stirring cell (Millipore, Billerica, MA) and pressurized using air or nitrogen gas. The membrane was preconditioned by passing 200 mL of nanopure water and 50 mL of 1 mM carbonate buffer solution (CBS) to flush the system. The filtrate from the microfilter was poured into the stirring cell and then three additions each of 200 mL of 1 mM CBS were filtered through the cell to continually remove the dissolved organics. When the volume in the cell chamber reached about 20 mL, the filtration was stopped, and the resulting virus solution was resuspended into 150 mL of 1 mM CBS and aliquoted into 50 mL glass jars. The resulting stock was stored at 4 °C until used for disinfection experiments. The final virus titer was about 1×10^9 plaque-forming units (PFU)/mL for PRD1 propagated and assayed with *E. coli* and 3.9×10^6 PFU/mL for PRD1 propagated with *S. typhimurium* and assayed with *E. coli*.

PRD1 viability assessment was measured using the double agar layer plaque technique (Adams, 1959). The *E. coli* was grown from a stock flask on the day of the experiment until it reached an exponential growth phase. Virus samples from experiments were serially diluted with TSB. In a 48 °C water bath, glass vials were filled with 3.5 mL of soft agar, 0.25 mL of freshly grown *E. coli*, and 0.8 mL of virus sample. The contents were gently swirled and quickly poured into a hard agar petri dish. The plates were placed into a 37 °C incubator, and plaques were enumerated between 18 and 48 h after plating. Virus titers were calculated by counting the number of plaques on plates containing 30-300 PFU/plate.

2.2 Adenovirus Propagation and Viability Assessment

Adenovirus serotype 2 (VR-846) was propagated using human lung A549 carcinoma cells (CCL-185) obtained from the American Type Culture Collection (Manassas, Virginia). Detailed methods for adenovirus propagation and viability assessment as well as cell culture growth were described previously (Sirikanchana et al., 2008). Monolayers of A549 cells were grown on flasks in a nutrient media containing a modified Ham's F12K growth media in a 5 % CO_2 incubator. The nutrient media also contained 10 % fetal bovine serum, a fungizone, and an antibiotic. Viruses were propagated by inoculating stock adenovirus solutions onto cell

monolayers, incubating the flasks, and were released using the freeze-thaw method. The solution was then purified using centrifugation, microfiltration, and ultrafiltration to obtain a high titer virus stock. Viability was measured using a plaque assay by plating adenovirus onto A549 cell monolayers with a nutrient mixture and a soft agar overlay technique. Plates were then incubated, and plaques were quantified seven to ten days after plating.

2.3 Chlorine Disinfection

Free chlorine disinfection experiments were performed in a batch reactor following with a similar procedure as that described by Page et al. (2009). Three batch reactors consisting of sterile glass amber jars were used in each experiment. The reactors contained a 100 mL volume of 1 mM CBS and were continuously stirred by magnetic mixing. The CBS was prepared with nanopure water and the addition of sodium bicarbonate. Experiments were performed at a pH ranging from 8.3 to 10.0 and temperatures of 1°C and 14°C. Experimental conditions can be seen in Table 1. Temperature was controlled by a recirculating water bath and was kept within ±0.5 °C of the desired temperature.

In the first batch reactor (R1), a solution of sodium hypochlorite was added to achieve the target dose of free chlorine. Throughout the time interval of the experiment, the chlorine concentration was analyzed to determine the free chlorine dose and to confirm that there was no chlorine demand in the reactor and therefore no decay over time. The pH in batch reactors R2 and R3 was adjusted by the addition of 0.1 M sodium hydroxide or by diffusing or stripping carbon dioxide. The pH was monitored before and after each experiment. The measured pH values are shown in Table 1 for each experiment. Reactors R2 and R3 were immersed in the water bath for about an hour to allow the experimental water to adjust to the target temperature. Virus stock was then added to reactors R2 and R3 to achieve the desired initial viral concentration, and the viruses were given additional time to acclimate to the pH and temperature conditions. The purpose of reactor R2 was to monitor the decay of free chlorine over the duration of the experiment. The same volume of sodium hypochlorite solution added to R1 was added to R2, and the amount of free chlorine present was analyzed throughout the

experiment beginning from about 10 s after addition. Reactor R3 was used to take viral samples throughout the experiment to determine the inactivation of the virus. A control sample (N_0) was taken approximately five minutes before the addition of sodium hypochlorite to quantify the initial virus concentration. The reactor was then dosed with the same volume of sodium hypochlorite solution as R1 and R2. Viral samples were taken from the reactor in 1 mL volumes, and the chlorine in the sample was quenched using 0.1 mL of a 0.1 % sodium thiosulfate solution. The time of quenching was recorded as the sample time. Samples were also taken from R3 to analyze the free chlorine concentration.

Free chlorine concentration was quantified using the DPD colorimetric method (APHA et al., 2005). Cuvettes with 1 cm path length were used to measure free chlorine concentrations in the range of 0.25-1.5 mg/L as Cl_2. Cuvettes with 5 cm path lengths were used to measure free chlorine concentrations in the range of 0.1-0.25 mg/L as Cl_2. It should be noted that in several experiments, free chlorine concentration was fully quantified by using the R3 reactor for viral samples and free chlorine concentration. For these experiments R2 was not used.

2.4 Low Pressure Ultraviolet Light Disinfection

Viruses were exposed to low pressure ultraviolet light using a collimated beam system (Calgon Carbon Corporation, Pittsburg, PA) containing a low-pressure Hg lamp. The incident light emits a narrow emission spectra centered at 254 nm. Light intensity was measured using a radiometer and the intensity was approximately I = 0.046 mW/cm². UV fluences were determined by radiometry (Bolton and Linden, 2003). A 6 cm diameter dish containing 15 mL of 1 mM CBS was placed under the collimated beam. The reactor was continuously mixed by magnetic stirring. Viruses were added to the reactor and samples were taken at various UV doses.

2.5 Solar Disinfection

Using a laboratory solar simulator, various filters were used to mimic conditions on a sunny day in equatorial latitudes. A 1000 W Xenon arc lamp (Newport, Stratford, CT) was used to produce a collimated light beam which was then passed through filters before reaching the reactor. The filters used included an Air Mass 0 + 1.5, a 25 % transmittance filter, and a >324 nm long pass filter. A UV-vis radiant power meter (Newport, Irvine, CA) measured intensity of the incident light. Experiments were performed in a 100 mL water-jacketed batch reactor kept at 25 °C by a recirculating water bath. The reactor contained 25 mL of 1 mM CBS and was continually mixed by magnetic stirring under the incident light. Viruses were added to the reactor and samples were taken over time.

CHAPTER 3 RESULTS AND DISCUSSION

3.1 Free Chlorine Disinfection

3.1.1 Free chlorine concentration and exposure

The dosage or exposure to free chlorine was measured in terms of the *CT* concept. For some reactors, the *CT* was quantified with the expression:

$$CT = \int_0^t C dt' = C_0 \int_0^t e^{-k_d t'} dt' = \frac{C_0}{k_d}(1 - e^{-k_d t})$$

where C_0 is the initial free chlorine concentration, k_d is the first order decay constant for free chlorine, t is the exposure time, and t' is an integration time variable (Page et al., 2009). This equation describes the free chlorine decay by first-order kinetics. However, it was observed that chlorine underwent two phases of decay. The first phase resulted in an initial rapid decay of chlorine. When the first chlorine sample was taken between 5-10 s after chlorine addition, the chlorine had already completed the initial rapid decay. Subsequent chlorine samples confirmed that the chlorine was undergoing a second phase of slower decomposition for the duration of the experiment. The *CT* values for these reactors were calculated using the equation above, and by using the value obtained by extrapolating to the intercept at t=0 to determine C_0. This predicted C_0 value was therefore lower than the initial dosage determined by R1, and is attributed to the free chlorine demand occurring in the first phase of decomposition. To support the validity of this approach, the r^2 value was determined for each experiment.

For some experiments, the concentration of free chlorine did not decay according to the above expression, and the chlorine remained constant throughout the duration of the experiment. In such cases an average *C* value was obtained. This occurred when a small volume of stock virus solution was added to the reactor resulting in negligible chlorine decay. For some other experiments, chlorine underwent two phases of decomposition, a first phase of initial rapid decay followed by a second phase with no decay (concentrations remained within ±0.027 mg/L

as Cl_2). When the first chlorine sample was taken between 5-10 s after chlorine addition, the initial phase of rapid chlorine decay was already completed. For these reactors, an initial C_0 value was taken as the average C value and was therefore lower than the applied dose determined in R1.

3.1.2 Effect of pH

The effect of pH on the inactivation kinetics of PRD1 at 1 °C and 14 °C is presented in Figures 1 and 2, respectively. For both temperatures, pH has a relatively strong effect on the inactivation of PRD1 with free chlorine where more rapid inactivation occurs at decreasing pH values. Experimental data at 1 °C was obtained for pH ranging from 8.3 to 10.0. Slower inactivation of PRD1 occurred at increasingly higher pH values. For pH values lower than 8.3, the inactivation kinetics were so rapid that more than 4 logs of inactivation occurred before an initial viral sample could be obtained. The data therefore could not be quantified. At high pH values, an initial lag phase occurs and this lag phase seems to extend further at increasingly high pH values. After the initial lag, at all pH values a more rapid inactivation phase occurs. The slope of this more rapid inactivation decreases with increasing pH. For some experimental data sets, a leveling off of inactivation is observed after 4-logs of inactivation. Current data suggests that this leveling off may be consistent at all pH's measured; however, it is currently unknown if the virus is undergoing a slower rate of inactivation or if there is no additional inactivation at this point. Variability was observed at different pH values, especially at pH 10. This is being attributed to a lack of sensitivity in the pH probe at low temperatures, and further experiments will be performed to verify data. Similar trends were observed at 14 °C over a narrower pH range of 9.2 to 10.

3.1.3 Effect of temperature

The effect of temperature on the inactivation of PRD1 was measured over a range of pH values at 1 °C and 14 °C. At the higher temperature, PRD1 underwent more rapid inactivation kinetics which was expected. Similar slopes of inactivation were observed when comparing the same pH values at the different temperatures.

3.1.4 Effect of initial virus and free chlorine concentrations

The effect of initial virus concentration (N_0) on the inactivation kinetics of PRD1 with free chlorine was determined by adding varying initial concentrations of viruses for reactors with the same pH and temperature conditions. The variability between experiments is similar to that obtained at constant N_0 and so it is not attributed to the concentration of viruses initially added in the reactors. The *CT* concept was verified by varying the initial chlorine concentration added to the reactors at the same pH and temperature conditions. A more comprehensive analysis will be done by performing additional experiments.

3.1.5 Comparison of PRD1 and adenovirus inactivation kinetics with free chlorine

The trends and overall behavior of the inactivation kinetics of PRD1 when exposed to free chlorine are similar to those of adenovirus as seen in Figure 3. For most conditions tested, PRD1 undergoes somewhat faster inactivation kinetics compared to adenovirus. Similar to adenovirus, at higher temperatures, PRD1 kinetics are quicker than at low temperatures. The *CT* value required for achieving 4 logs of inactivation for PRD1 and adenovirus is very low for the conditions tested and shows that they both have similarly rapid inactivation kinetics with free chlorine. Using PRD1 as a tool for elucidating the mechanisms of inactivation of adenovirus by free chlorine may be a viable option based on this data and especially considering their similar morphologies.

3.2 Low Pressure Ultraviolet Light Disinfection

PRD1 was inactivated using low pressure ultraviolet light. The resulting inactivation kinetics is shown in Figure 4 together with the kinetics for other viruses. The PRD1 viruses used were propagated using hosts *E. coli* K-12 and *S. typhimurium* LT2 to form different lipids within the virus. Viruses were only plated with host *E. coli* K-12 during experiments. For both virus types tested, a 4-log inactivation of PRD1 was achieved at about 40 mJ/cm². These data are consistent with data by Meng and Gerba (1996) which show a 4-log inactivation of PRD1 with a

dose of 31.6 mJ/cm². The host bacteria used by Meng and Gerba (1996) was *S. typhimurium* LT2. Data from a study by Shin et al. (2005) shows a 4-log inactivation of PRD1 with *S. typhimurium* LT2 as a host at an approximate dose of 105 mJ/cm². Shin et al. (2005) hypothesize that the differences between their study and the Meng and Gerba (1996) study in the sensitivity of PRD1 to low pressure ultraviolet light may depend on how the viruses were prepared and purified and if they were exposed to repeated freezing and thawing. Both studies used *S. typhimurium* LT2 as the host bacteria for propagation and enumeration, whereas in this study *E. coli* K-12 was used as the host bacteria for enumeration. The other main difference between this study and the previous studies is the PRD1 propagation method. Both previous studies inoculated PRD1 and host bacteria with molten agar onto pre-solidified TSA petri dishes, let incubate, and then harvested phages from the plates which required the addition of chloroform or Tris-buffered saline. Lastly, the test waters for the research by Shin et al. (2005) were different. PRD1 was suspended in coagulated and filtered surface water from the Greater Cincinnati Water Works utility in Cincinnati, Ohio. This may have also played a role in the different inactivation kinetic data as the water used in this study was nanopure. Further experiments will be performed to determine if the efficacy of PRD1 disinfection depends on the host bacteria during plating and the type of water used.

The inactivation kinetics of PRD1 with low pressure UV was compared to that of other DNA and RNA viruses. Figure 4 shows that PRD1 does not have similar inactivation kinetics to adenovirus serotype 2 (Sirikanchana et al., 2008). PRD1 reaches 4-log inactivation about four times faster than adenovirus. Interestingly, Figure 4 shows that the inactivation kinetics behaves very similarly to the single-stranded RNA Coxsackie virus B5 with about the same dosage required for 4-log inactivation (Vonder Haar, 2009). PRD1 would be an effective surrogate for this human enteric pathogen for low pressure UV. The typical bacteriophage surrogate used for low pressure UV is the single-stranded RNA genome bacteriophage MS2. As the figure shows, this phage does not exhibit similar inactivation kinetics as Coxsackie virus B5 or adenovirus serotype 2, and MS2 is more resistant to LPUV than PRD1 (Coronell, 2004).

3.3 Solar Disinfection

PRD1 shows a similar resistance to solar disinfection compared to adenovirus type 2 as shown in Figure 5. A preliminary adenovirus experiment revealed that after 2.6 h exposure to simulated sunlight, less than 0.5-log inactivation occurred. PRD1 was then exposed to 48 h of continuous simulated sunlight and only about 2.8-log inactivation was achieved. With 16 h of full sunlight exposure, PRD1 only reaches about 1-log inactivation. This further confirms that SODIS exposure for one to two days (8 to 16 h of sunlight) will not be effective for all viruses, and alternative disinfectants are necessary to achieve 4-log inactivation. PRD1 is potentially a good surrogate for adenovirus when exposed to sunlight.

Table 1: Experimental conditions for PRD1 with free chlorine

Experiment Number	Date	pH	pH range	Temperature (°C)	Virus dose N_0 (PFU/mL)	Chlorine conc. (mg/L as Cl_2)	
						Dose	Co
28	10/6/10	8.3	8.33-8.25	1	5.00E+05	0.166	0.167
27	10/6/10	8.5	8.54-8.39	1	7.25E+05	0.166	0.160
23	9/24/10	8.7	8.72-8.70	1	5.75E+05	0.307	0.305
32	10/15/10	8.85	8.84-8.88	1	1.69E+06	0.393	0.384
19	9/15/10	9.0	9.05-9.05	1	9.50E+05	0.610	0.595
21	9/20/10	9.0	9.01-9.10	1	1.45E+06	0.478	0.449
4	7/17/10	9.2	9.24-9.21	1	1.15E+06	0.461	0.311
22	9/22/10	9.2	9.24-9.20	1	1.06E+06	0.423	0.401
31	10/14/10	9.2	9.24-9.21	1	1.43E+06	0.415	0.373
12	8/13/10	9.6	9.62-9.65	1	4.28E+06	0.640	0.472
13	8/14/10	9.6	9.56-9.45	1	3.72E+06	0.640	0.446
24	9/27/10	9.6	9.60-9.55	1	7.00E+05	0.420	0.376
26	10/4/10	9.8	9.83-9.86	1	5.25E+05	0.377	0.342
29	10/7/10	9.8	9.83-9.89	1	1.13E+06	0.350	0.344
33	10/19/10	10.0	9.97-10.00	1	8.50E+05	0.390	0.391
37	11/1/10	9.2	9.20-9.17	14	1.70E+06	0.448	0.410
35	10/28/10	9.6	9.59-9.59	14	9.63E+05	0.404	0.398
36	11/1/10	9.6	9.59-9.54	14	1.51E+06	0.448	0.411
38	11/2/10	10.0	10.03-10.03	14	7.75E+05	0.508	0.501

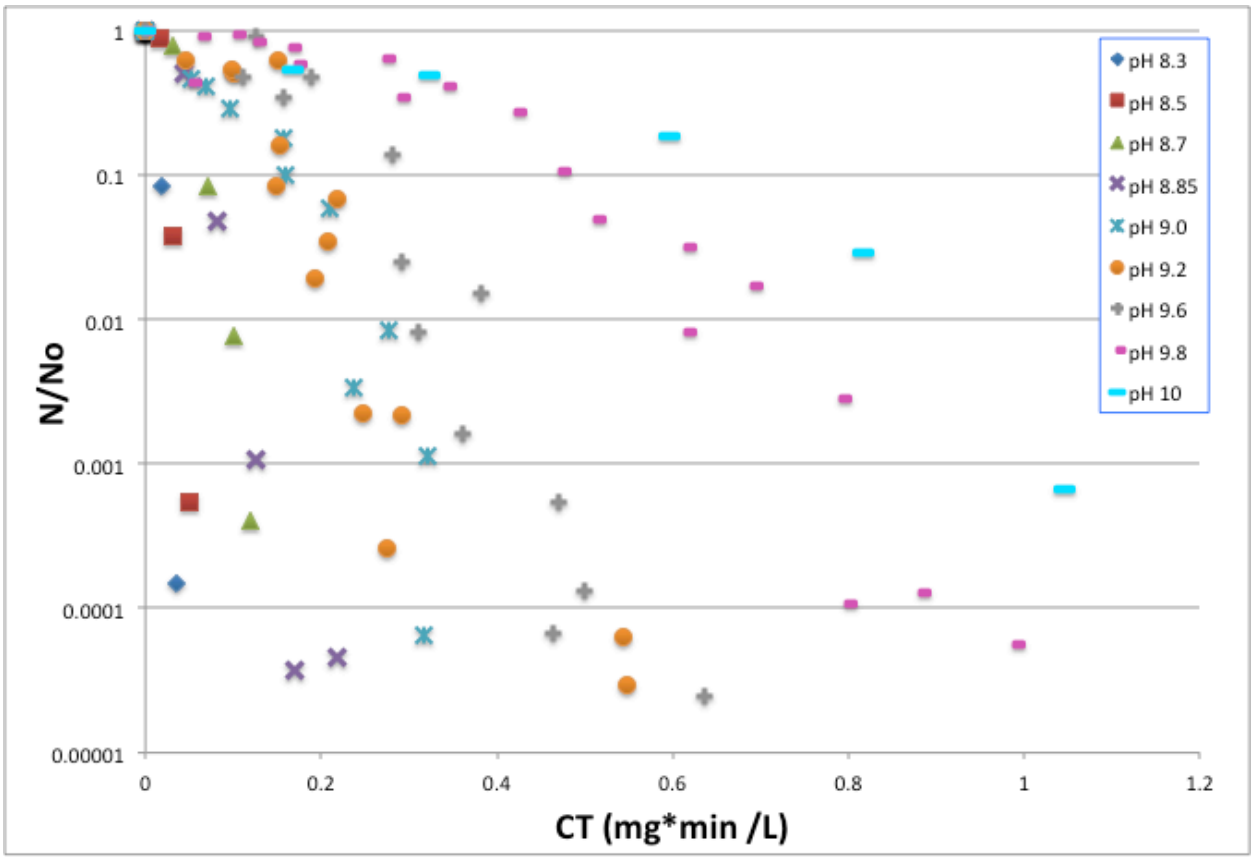

Figure 1: Inactivation kinetics of PRD1 with free chlorine at 1 °C

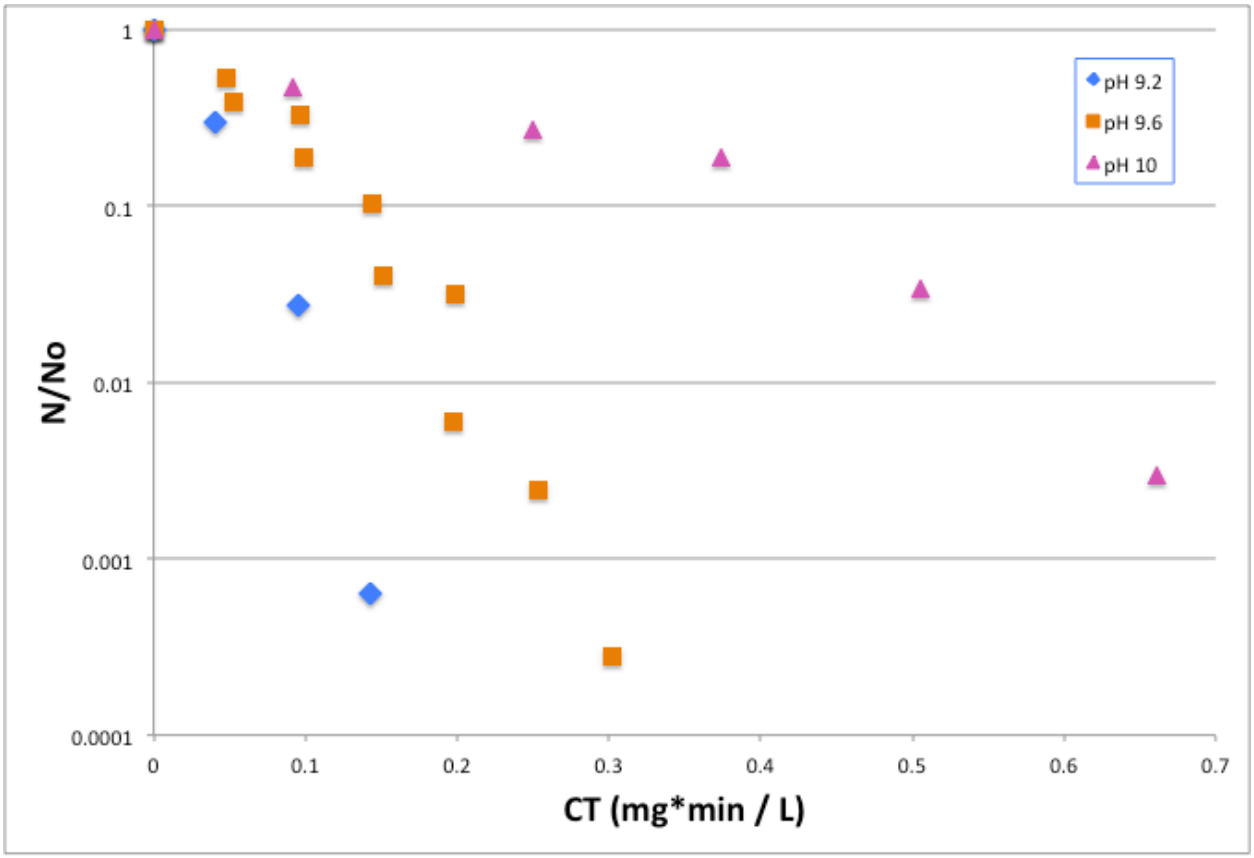

Figure 2: Inactivation kinetics of PRD1 with free chlorine at 14 °C

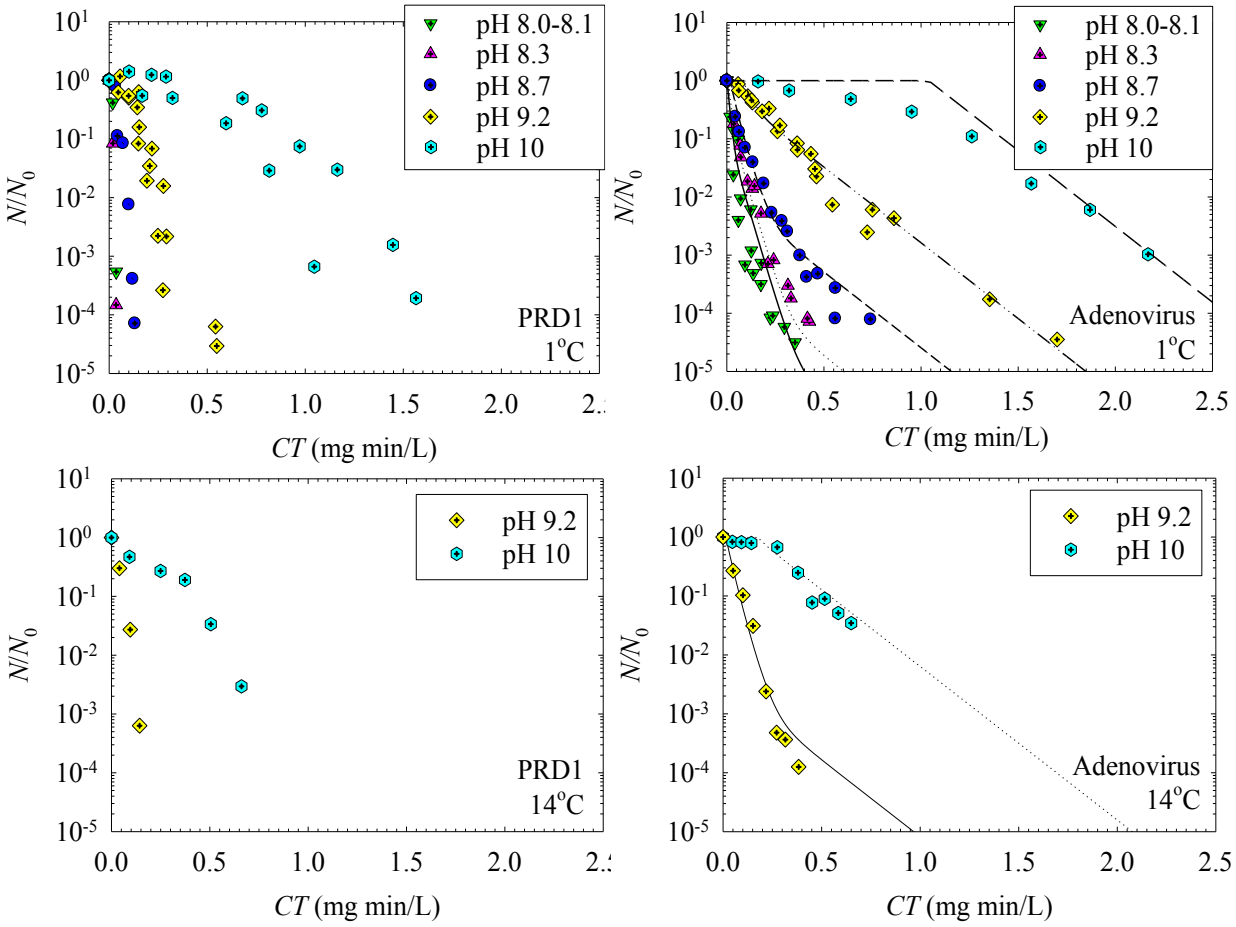

Figure 3: Comparison of inactivation kinetics of PRD1 and adenovirus 2 at 1 °C and pH 8.0-10, and at 14 °C and pH 9.2-10 (Adenovirus data from Page et al., 2009)

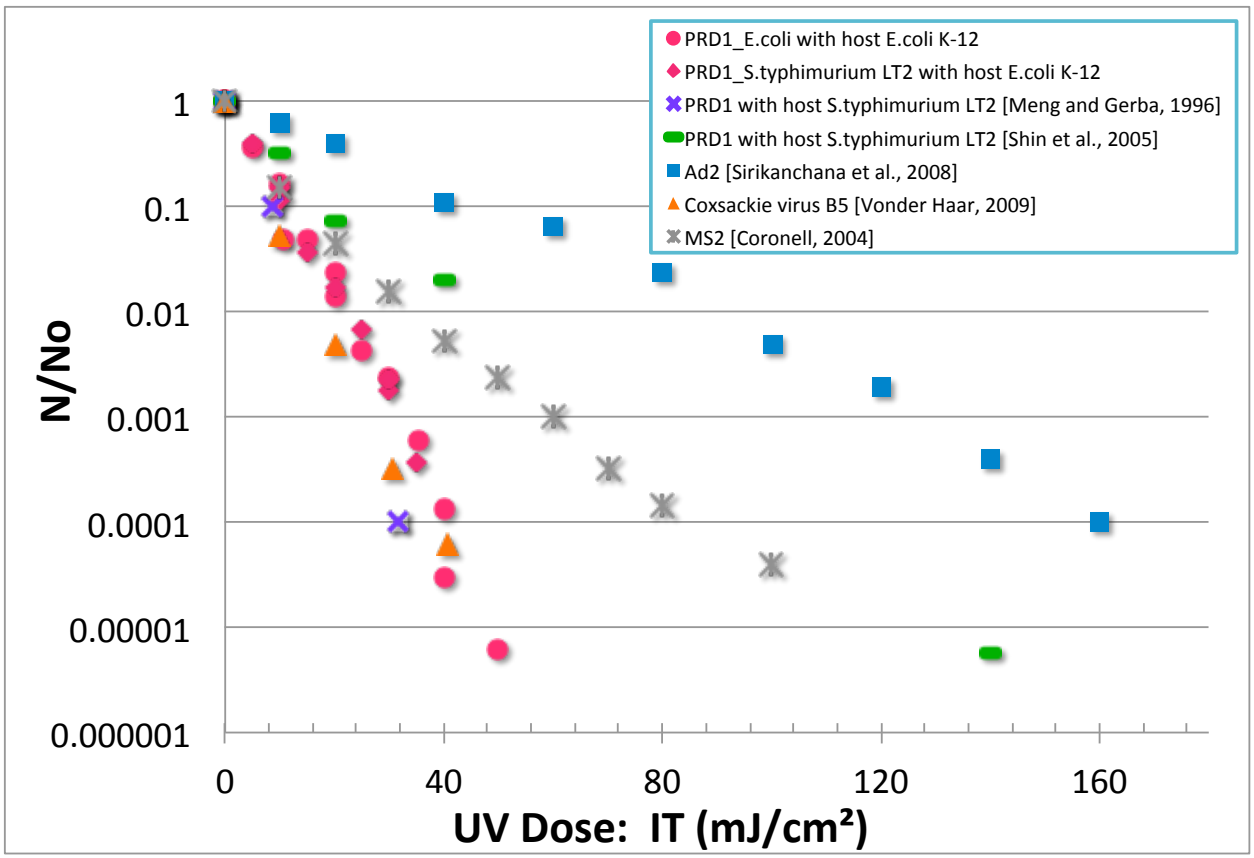

Figure 4: Comparison of inactivation kinetics of PRD1 to other viruses by low pressure ultraviolet light

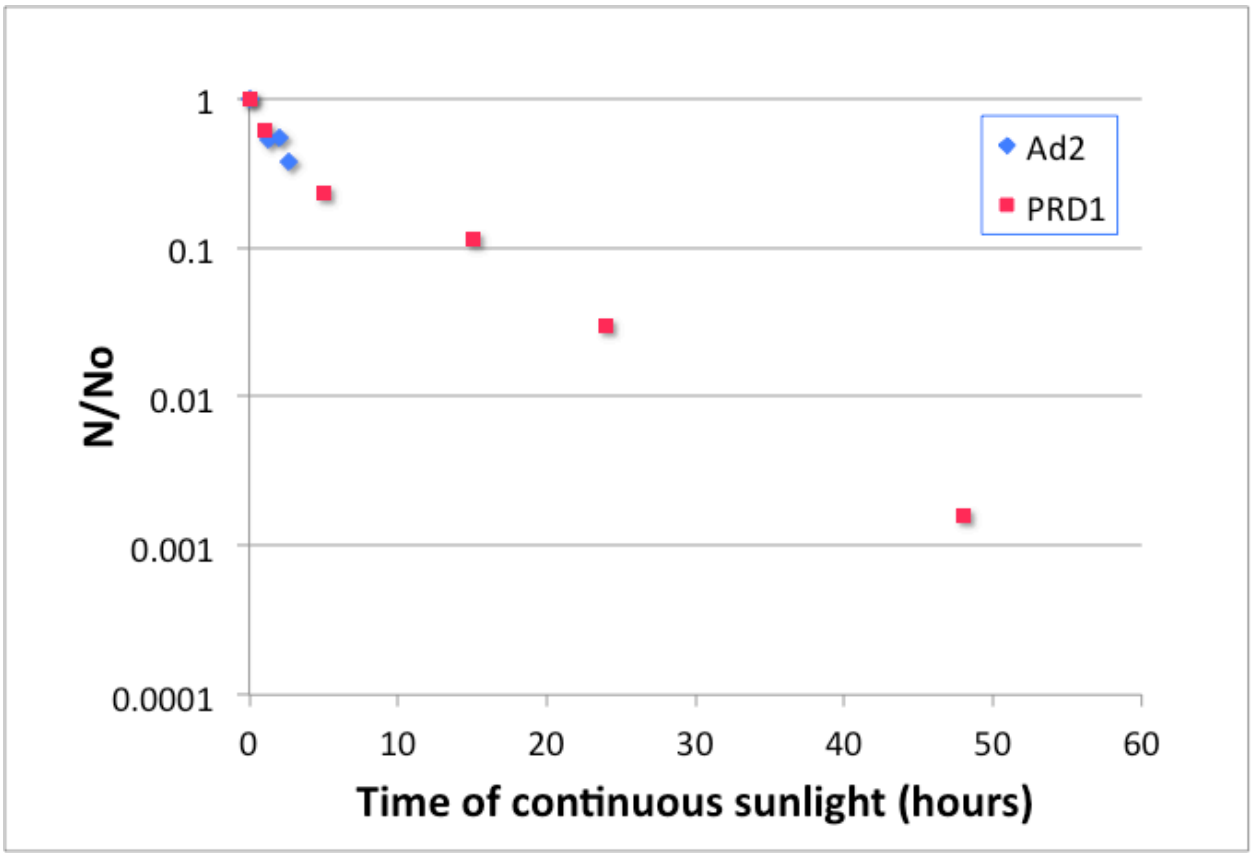

Figure 5: Comparison of inactivation of PRD1 and adenovirus with simulated solar disinfection

CHAPTER 4 CONCLUSIONS AND FUTURE WORK

This research has examined the inactivation kinetics of PRD1 in comparison to adenovirus for its use as a potential surrogate in laboratory and field studies. Inactivation kinetics data were presented for PRD1 when exposed to doses of free chlorine, low pressure ultraviolet light, and sunlight.

PRD1 appears to be a promising candidate surrogate for adenovirus for free chlorine disinfection even though their kinetics are somewhat different because the trends and overall behavior of the inactivation kinetics are very similar to those of adenovirus. Using PRD1 as a surrogate for adenovirus to elucidate the mechanism of inactivation by free chlorine may be a viable option especially considering their similar capsid structures. Furthermore, at higher temperatures such as those expected in developing regions, PRD1 may be a promising surrogate for fieldwork. Some variability in data at low temperatures is attributed to the pH probe lacking sensitivity at very low temperatures; therefore, more robust data sets will be produced in future research phases. A model to predict PRD1 inactivation when exposed to free chlorine will be developed and will be compared to that of adenovirus.

For low pressure ultraviolet light, PRD1 is a better surrogate for RNA viruses than for adenovirus. Adenovirus requires four times the low pressure UV dose that PRD1 requires to reach 4-logs of inactivation. Since low pressure UV has been suggested to affect the viral genome, the reason for PRD1 having similar inactivation kinetics as RNA viruses will need to be explored. It is also possible that different host cells may have the ability to repair DNA damage in the virus and restore its viability. Future experiments will be performed using *S. typhimurium* LT2 as the host to see if this host bacteria results in different inactivation kinetics. This could also be explored for the range of PRD1 host bacteria to see if there is an appropriate surrogate-host combination to exhibit similar inactivation kinetics as adenovirus.

Both adenovirus and PRD1 are highly resistant to solar disinfection. PRD1 can potentially be used as a surrogate in the field to assess disinfection technologies like SODIS in developing regions. Further research will be done to determine the inactivation kinetics of PRD1 when exposed to monochloramine in tandem with SODIS to determine if a synergistic relationship exists similarly to adenovirus (Page, 2009). If PRD1 is found to be a nonpathogenic surrogate for adenovirus disinfection with monochloramine and SODIS, such finding would be beneficial to laboratory and field testing.

An overall goal of this research is to obtain a better understanding of how disinfectants act on a molecular level. This will allow the development of simple, appropriate, and robust disinfection technologies to provide safe drinking water in developing regions. As more viruses are studied in the laboratory after exposure to various disinfectants, trends between virus inactivation kinetics are being observed which hopefully will aid in understanding the mechanism of inactivation of human viruses.

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APPENDIX A. RAW DATA: INACTIVATION OF PRD1 WITH FREE CHLORINE

Experiment Number 4

PRD1 vs. Free Chlorine

pH	9.2
pH range	9.24-9.21
Temp (°C)	1

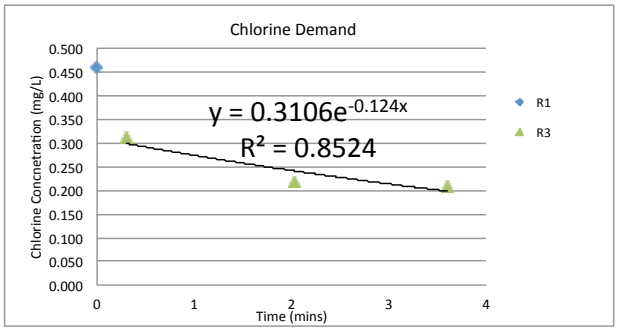
7/17/10
Cuvette size (cm) 1
Reactor: 100mL 1mM CBS
Virus added: 0.5mL
Substock: 100mL NP+0.9mL stock Cl. Added: 0.15mL

CHLORINE ANALYSIS

	Actual Time (mins)	DF	ABS	Chlorine Concentration (mg/L)
R1	0.250	1	0.098	0.466
	1.500	1	0.097	0.461
	4.000	1	0.096	0.456

Time (mins)	Dose (mg/L as Cl_2)
0.000	0.461

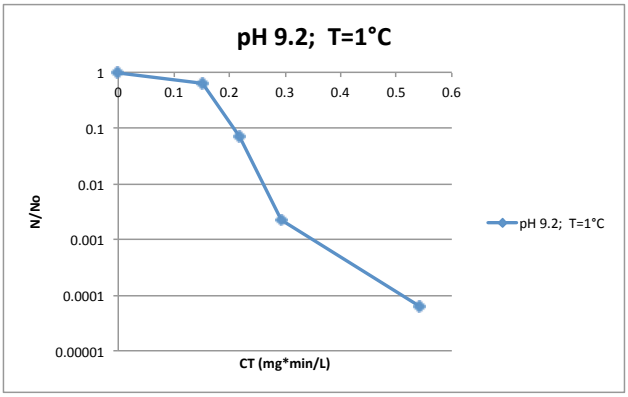
	Actual Time (mins)	DF	ABS	Chlorine Concentration (mg/L)
R3	0.300	1	0.066	0.314
	2.033	1	0.046	0.219
	3.600	1	0.044	0.209



Co (mg/L as Cl_2)	0.311
k_d (min^{-1})	0.124
R^2	0.852

VIABILITY

R3	Actual Time	0	-1	-2	-3	-4	-5	-6	N	N/No	CT (mg*min/L)
1	0.00					92	27	0	1150000	1	0
2	0.50	TNTC	TNTC	TNTC	TNTC	58	11	0	725000	0.63043478	0.150777598
3	0.73	TNTC	TNTC	TNTC	63	4	0		78750	0.06847826	0.218004527
4	1.00	TNTC	TNTC	20	1	0	0		2500	0.00217391	0.292490883
5	1.97	58	5	0	0				72.5	6.3043E-05	0.542764959
6	3.22	0	1	0	0						

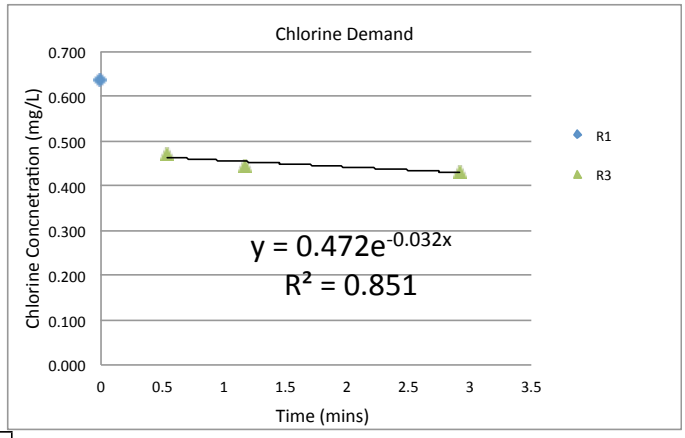


Experiment number 12

PRD1 vs. Free Chlorine

pH	9.6
pH range	(9.62-9.65)
Temp (°C)	1

8/13/10
Cuvette size (cm) 1
Reactor: 100mL 1mM CBS
Virus added: 0.5mL
Substock: 100mL NP+0.9mL stock Cl. Added: 0.18mL



CHLORINE ANALYSIS

R1			
Actual Time (mins)	DF	ABS	Chlorine Concentration (mg/L)
0.333	1	0.135	0.641
1.333	1	0.135	0.641
2.500	1	0.134	0.637

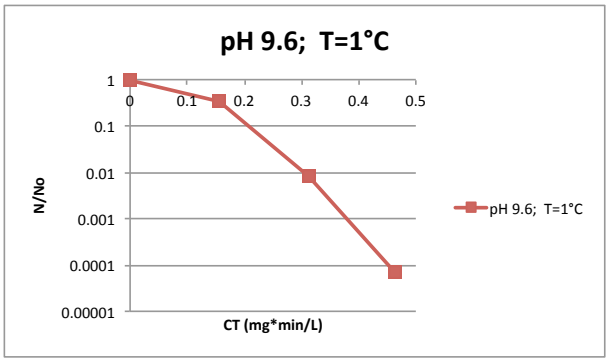
Time (mins)	Dose (mg/L as Cl_2)
0.000	0.640

R2				
Actual Time (mins)	DF	ABS	Chlorine Concentration (mg/L)	pH
0.333	1	0.096	0.456	9.59
1.000	1	0.083	0.394	
2.917	1	0.080	0.380	9.63

Co (mg/L as Cl_2)	0.472
k_d (min^{-1})	0.032
R^2	0.851

VIABILITY

R3	Actual Time	0	-1	-2	-3	-4	-5	N	N/No	CT (mg*min/L)
1	0.00			TNTC		304	38	4275000	1	0
2	0.33		TNTC	TNTC		118	6	1475000	0.34502924	0.156497198
3	0.67			355	21	0		35312.5	0.00826023	0.311333964
4	1.00		23	2	1	0		287.5	6.7251E-05	0.464527914
5	1.50	5	3	0	0	0				
6	2.00	4	0	0						
7	2.50	0	0	0						



Notes: For R3, the chlorine concentration at about 2:40 has an ABS of 0.100 which is much higher than the one R2 measured.
On the same day with the same water, a pH9.2 experiment was held (see 11). The data from that R2 is used in this experiment for C values.

Experiment number 13

PRD1 vs. Free Chlorine

pH	9.6
pH range	9.56-9.45
Temp (°C)	1

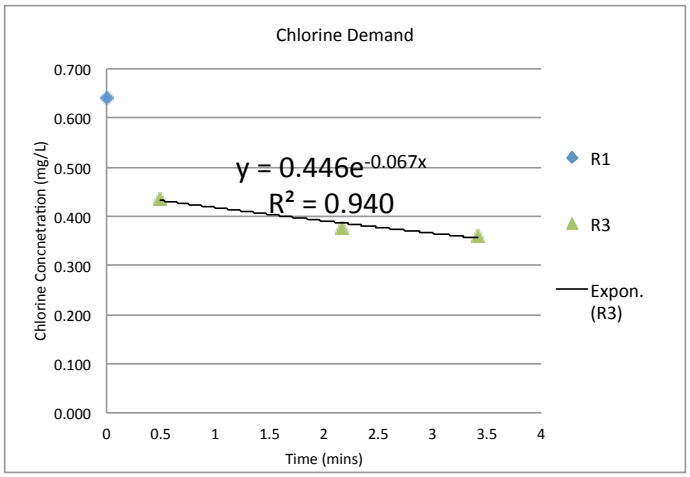
8/14/10
Cuvette size (cm) 1
Reactor: 100mL 1mM CBS
Virus added: 0.5mL
Substock: 100mL NP+0.9mL stock Cl. Added: 0.18mL

CHLORINE ANALYSIS

R1			
Actual Time (mins)	DF	ABS	Chlorine Concentration (mg/L)
0.333	1	0.136	0.646
1.500	1	0.134	0.637
2.500	1	0.134	0.637

Time (mins)	Dose (mg/L as Cl_2)
0.000	0.640

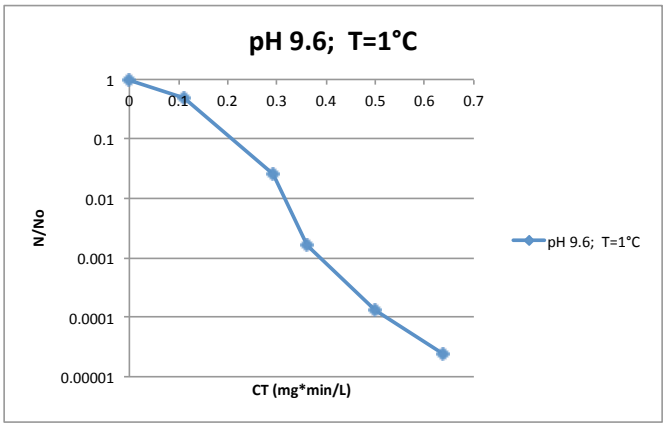
R3			
Actual Time (mins)	DF	ABS	Chlorine Concentration (mg/L)
0.500	1	0.092	0.437
2.167	1	0.079	0.375
3.417	1	0.076	0.361



Co (mg/L as Cl_2)	0.446
k_d (min^{-1})	0.067
R^2	0.94

VIABILITY

R3	Actual Time	0	-1	-2	-3	-4	-5	N	N/No	CT (mg*min/L)
1	0.00			TNTC		395	20	3718750	1	0
2	0.25		TNTC	TNTC		144	10	1800000	0.48403361	0.11057138
3	0.67		TNTC		74	3		92500	0.02487395	0.290790663
4	0.83	TNTC		48	0	1		6000	0.00161345	0.36148141
5	1.17		39	2	0	0		487.5	0.00013109	0.500516653
6	1.50	73	21	0	0	0		91.25	2.4538E-05	0.636481192
7	1.87	4	2	0	0	0				
8	2.47	0	1	0						



Experiment number 19

PRD1 vs. Free Chlorine

pH	9.05
Temp (°C)	1

9/15/10
Cuvette size (cm) 1
Reactor: 100mL 1mM CBS
Virus added: 0.1mL
Substock: 100mL NP+0.9mL stock Cl. Added: 0.17mL

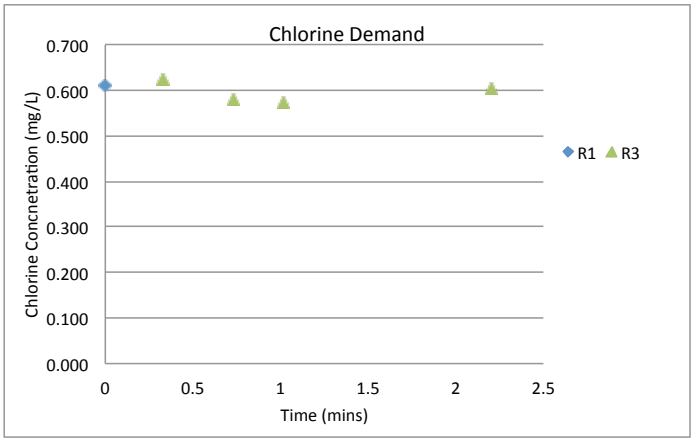
CHLORINE ANALYSIS

R1			
Actual Time (mins)	DF	ABS	Chlorine Concentration (mg/L)
0.333	1	0.130	0.618
1.250	1	0.128	0.608
2.583	1	0.127	0.603

Time (mins)	Dose (mg/L as Cl_2)
0.000	0.610

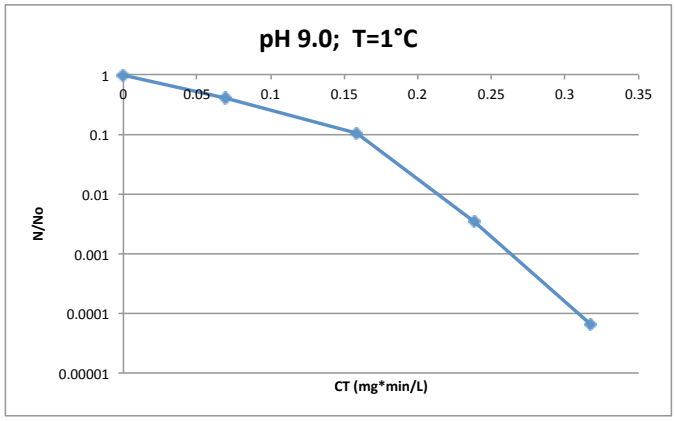
R3			
Actual Time (mins)	DF	ABS	Chlorine Concentration (mg/L)
0.333	1	0.131	0.622
0.733	1	0.122	0.580
1.017	1	0.121	0.575
2.20	1	0.127	0.603

Chlorine Concentration (mg/L)	0.595



VIABILITY

R3	Actual Time	0	-1	-2	-3	-4	-5	-6	N	N/No	CT (mg*min/L)
1	0.00			TNTC		76	1		950000	1	0
2	0.12	TNTC	TNTC	TNTC		31			387500	0.40789474	0.069418052
3	0.27	TNTC	TNTC	TNTC	77	9			96250	0.10131579	0.158669834
4	0.40	TNTC	255	23	5	0			3187.5	0.00335526	0.238004751
5	0.53	50	2	0	0				62.5	6.5789E-05	0.317339667
6	0.75	18	0	0	0						
7	0.93	0	0	0	0						
8	1.08	0	0	0	0						
9	1.33333333	2	1	0							
10	1.66666667	0	0								



Experiment number 21

PRD1 vs. Free Chlorine

pH	9
pH range	(9.01-9.10)
Temp (°C)	1

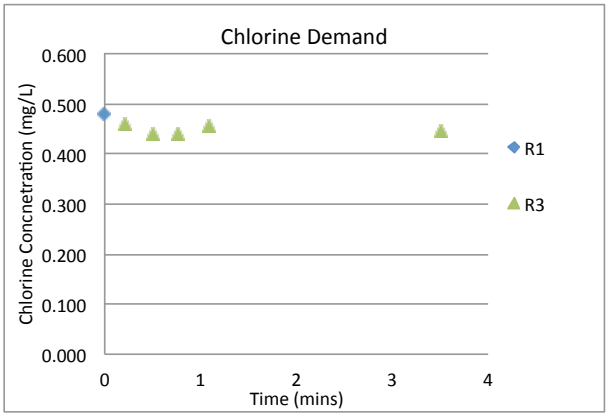
9/20/10

Cuvette size (cm) 1

Reactor: 100mL 1mM CBS

Virus added: 0.1mL

Substock: 100mL NP+0.9mL stock Cl. Added: 0.13mL



CHLORINE ANALYSIS

R1			
Actual Time (mins)	DF	ABS	Chlorine Concentration (mg/L)
0.333	1	0.101	0.480
1.383	1	0.100	0.475
2.550	1	0.101	0.480

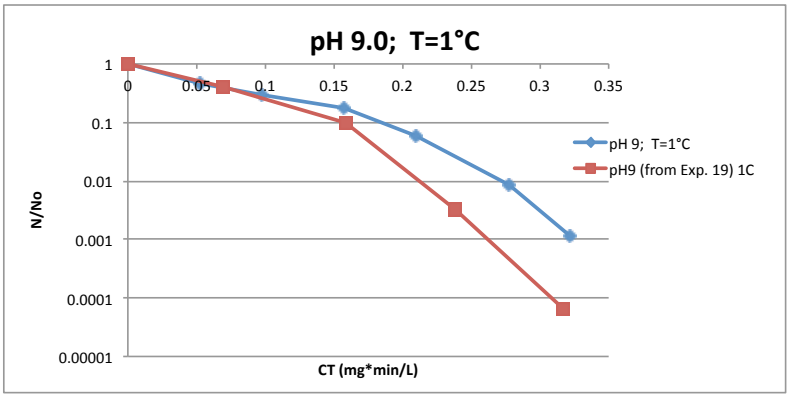
Time (mins)	Dose (mg/L as Cl_2)
0.000	0.478

	Actual Time (mins)	DF	ABS	Chlorine Concentration (mg/L)	pH
R2	0.217	1	0.097	0.461	8.99
	0.500	1	0.093	0.442	
	0.767	1	0.093	0.442	
	3.500	1	0.094	0.447	9.05
R3	1.083	1	0.096	0.456	

Chlorine Concentration (mg/L)	0.449

VIABILITY

R3	Actual Time	0	-1	-2	-3	-4	-5	N	N/No	CT (mg*min/L)
1	0.00			TNTC		116	18	1450000	1	0
2	0.12			TNTC		54	7	675000	0.46551724	0.052430721
3	0.22		TNTC		204	47		421250	0.29051724	0.097371338
4	0.35	TNTC	TNTC	TNTC	209	22		261250	0.18017241	0.157292162
5	0.47	TNTC	TNTC	TNTC	68			85000	0.05862069	0.209722882
6	0.62	TNTC	TNTC	97	2			12125	0.00836207	0.277133808
7	0.72	TNTC	131	4				1637.5	0.00112931	0.322074426

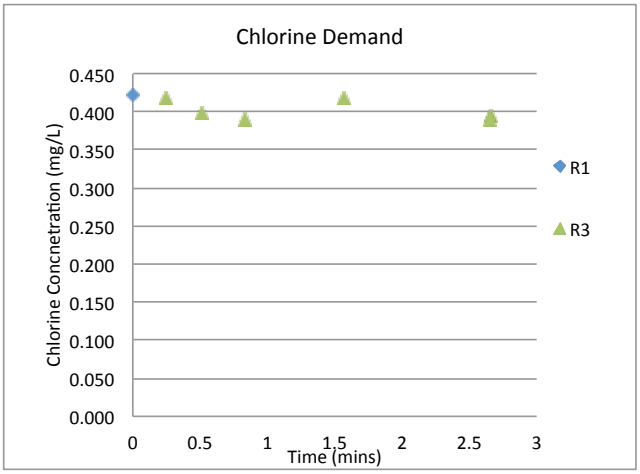


Experiment number 22

PRD1 vs. Free Chlorine

pH	9.2
pH range	9.24-9.20
Temp (°C)	1

9/22/10
Cuvette size (cm) 1
Reactor: 100mL 1mM CBS
Virus added: 0.1mL
Substock: 100mL NP+0.9mL stock Cl. Added: 0.11mL



CHLORINE ANALYSIS

R1			
Actual Time (mins)	DF	ABS	Chlorine Concentration (mg/L)
0.400	1	0.089	0.423
1.467	1	0.089	0.423
2.667	1	0.089	0.423

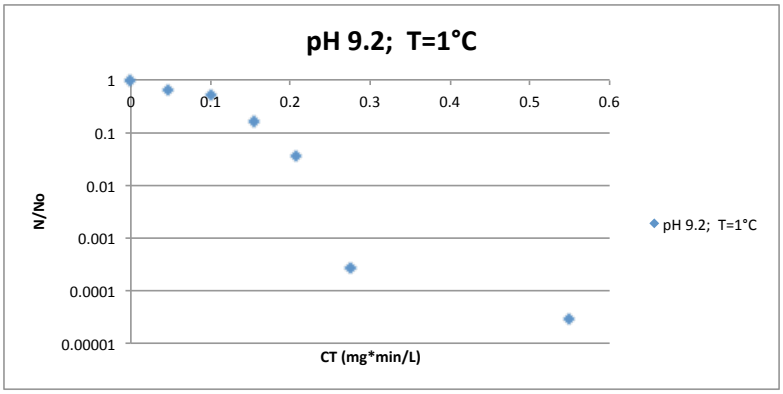
Time (mins)	Dose (mg/L as Cl_2)
0.000	0.423

	Actual Time (mins)	DF	ABS	Chlorine Concentration (mg/L)	pH
R2	0.250	1	0.088	0.418	9.16
	0.517	1	0.084	0.399	
	0.833	1	0.082	0.390	
	2.667	1	0.083	0.394	9.08
R3	1.567	1	0.088	0.418	
	2.650	1	0.082	0.390	

Chlorine Concentration (mg/L)	0.401

VIABILITY

R3	Actual Time	0	-1	-2	-3	-4	-5	N	N/No	CT (mg*min/L)
1	0.00			TNTC		85	6	1062500	1	0
2	0.12			TNTC		53	2	662500	0.62352941	0.046832937
3	0.25		TNTC	TNTC		43		537500	0.50588235	0.100356295
4	0.38		TNTC		135	10		168750	0.15882353	0.153879652
5	0.52	TNTC	TNTC	308	28	5		36750	0.03458824	0.207403009
6	0.68	318	13	12	1	1		280	0.00026353	0.274307205
7	1.02	15	1	0	0					
8	1.37	25	4	0	0			31.25	2.9412E-05	0.54861441
9	1.75	14	1	0	0					
10	2.25	1	0	0						



Note: Sample 6: The 13 plate had lots of surface bacteria growth which after wiping showed 20+ more very tiny plaques… difficult to read. Took average

Experiment number 23

PRD1 vs. Free Chlorine

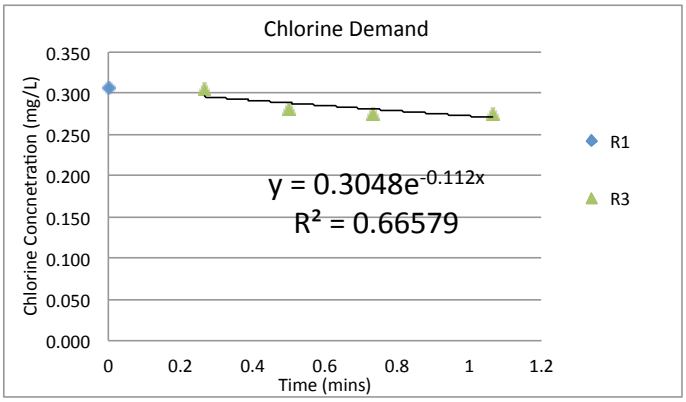
pH	8.7
pH range	8.72-8.70
Temp (°C)	1

9/24/10
Cuvette size (cm) 1
Reactor: 100mL 1mM CBS
Virus added: 0.1mL
Substock: 100mL NP+0.9mL stock Cl. Added: 0.09mL

CHLORINE ANALYSIS

R1			
Actual Time (mins)	DF	ABS	Chlorine Concentration (mg/L)
0.250	1	0.066	0.314
1.250	1	0.064	0.304
2.333	1	0.064	0.304

Time (mins)	Dose (mg/L as Cl_2)
0.000	0.307

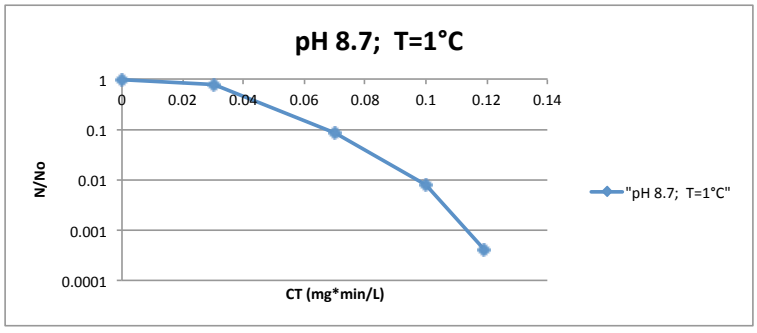


	Actual Time (mins)	DF	ABS	Chlorine Concentration (mg/L)	pH
R2	0.267	1	0.064	0.304	8.66
	0.500	1	0.059	0.280	
	0.733	1	0.058	0.276	8.66
R3	1.067	1	0.058	0.276	

Co (mg/L as Cl_2)	0.305
k_d (min^{-1})	0.112
R^2	0.666

VIABILITY

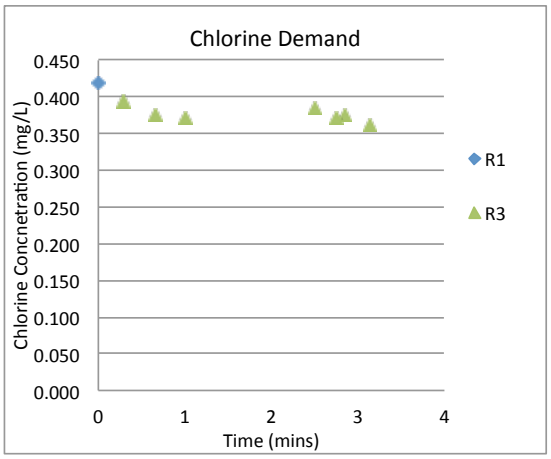
R3		Actual Time	0	-1	-2	-3	-4	-5	N	N/No	CT (mg*min/L)
	1	0.00			TNTC		46	1	575000	1	0
	2	0.10	TNTC	TNTC		460	27		456250	0.79347826	0.030329836
	3	0.23	TNTC	TNTC		39	5		48750	0.08478261	0.070244803
	4	0.33	TNTC	512	19	5			4387.5	0.00763043	0.099792287
	5	0.40	68	31	1	0			236.25	0.00041087	0.119307557
	6	0.52	12	4	0						
	7	0.68	0	0							



Experiment number 24

PRD1 vs. Free Chlorine
pH 9.6
pH range 9.60-9.55
Temp (°C) 1

9/27/10
Cuvette size (cn 1
Reactor: 100mL 1mM CBS
Virus added: 0.1mL
Substock: 100mL NP+0.9mL stock Cl. Added: 0.11mL



CHLORINE ANALYSIS

R1			
Actual Time (mins)	DF	ABS	Chlorine Concentration (mg/L)
0.250	1	0.089	0.423
1.250	1	0.088	0.418
2.367	1	0.088	0.418

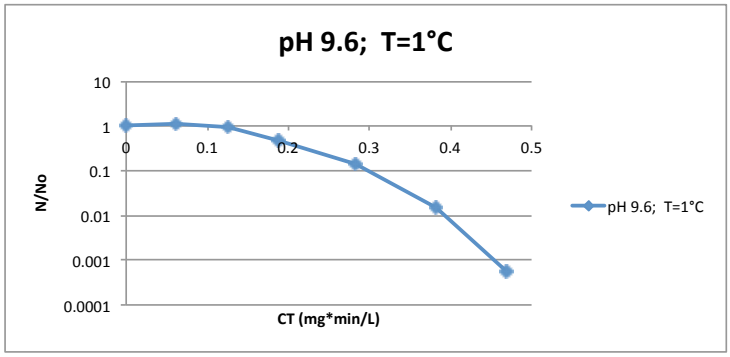
Time (mins)	Dose (mg/L as Cl_2)
0.000	0.420

	Actual Time (mins)	DF	ABS	Chlorine Concentration (mg/L)	pH	
R2	0.300	1	0.083	0.394	9.55	
	0.650	1	0.079	0.375		
	1.000	1	0.078	0.371		
	2.850	1	0.079	0.375		
	3.133	1	0.076	0.361	9.43? pH meter was responding very slowly	
R3	2.500	1	0.081	0.385		
	2.750	1	0.078	0.371		

Chlorine Concentration (mg/L)	0.376

VIABILITY

R3	Actual Time	0	-1	-2	-3	-4	-5	N	N/No	CT (mg*min/L
1	0.00			TNTC		56	1	700000	1	0
2	0.17		TNTC	TNTC		63		787500	1.125	0.06266259
3	0.33		TNTC	TNTC		52		650000	0.92857143	0.12532519
4	0.50		TNTC		268	20		335000	0.47857143	0.18798778
5	0.75	TNTC TNTC	TNTC		77	9		96250	0.1375	0.28198168
6	1.02	TNTC TNTC		86	11	1		10750	0.01535714	0.38224183
7	1.25	46	57	8	0			385	0.00055	0.46996946
8	1.50	22	0	0	0					
9	1.75	3	0	0						
10	2	0	0							



Experiment number 26

PRD1 vs. Free Chlorine

pH	9.8
pH range	9.83-9.86
Temp (°C)	1

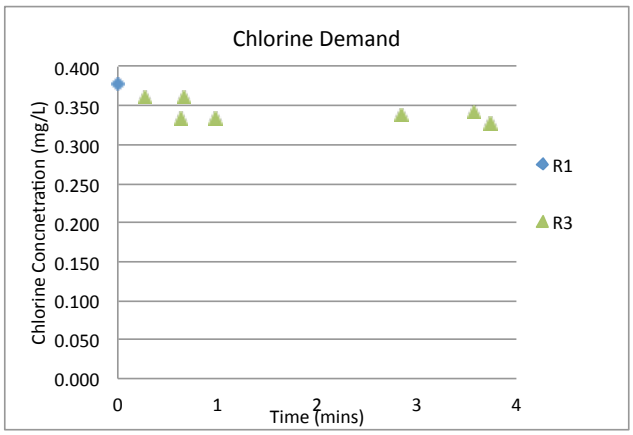
10/4/10

Cuvette size (cm) 1

Reactor: 100mL 1mM CBS

Virus added: 0.1mL

Substock: 100mL NP+0.9mL stock Cl. Added: 0.11mL



CHLORINE ANALYSIS

R1			
Actual Time (mins)	DF	ABS	Chlorine Concentration (mg/L)
0.367	1	0.080	0.380
1.333	1	0.079	0.375
2.383	1	0.079	0.375

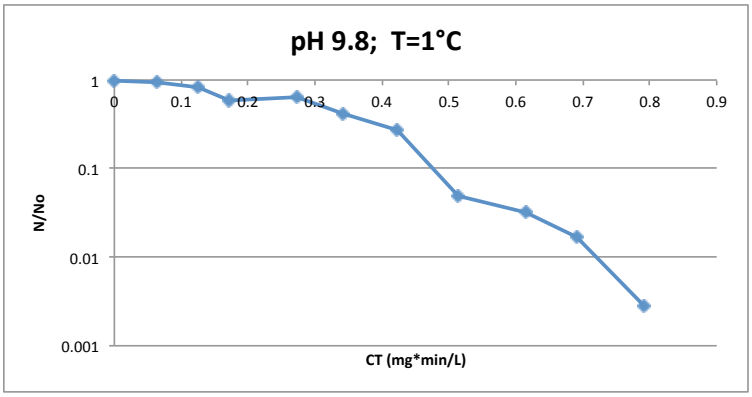
Time (mins)	Dose (mg/L as Cl_2)
0.000	0.377

	Actual Time (mins)	DF	ABS	Chlorine Concentration (mg/L)	pH
R2	0.267	1	0.076	0.361	9.84
	0.633	1	0.070	0.333	
	0.983	1	0.070	0.333	
	3.567	1	0.072	0.342	
	3.733	1	0.069	0.328	
R3	0.667	1	0.076	0.361	
	2.850	1	0.071	0.337	

Chlorine Concentration (mg/L)	0.342

VIABILITY

R3	Actual Time	0	-1	-2	-3	-4	-5	N	N/No	CT (mg*min/L)
1	0.00			TNTC		42	2	525000	1	0
2	0.18			TNTC		39	3	487500	0.92857143	0.062707838
3	0.37				490	35	3	437500	0.83333333	0.125415677
4	0.50		TNTC		249	26		311250	0.59285714	0.171021378
5	0.80	TNTC	TNTC		268	16		335000	0.63809524	0.273634204
6	1.00	TNTC	TNTC		174	16		217500	0.41428571	0.342042755
7	1.23	TNTC	TNTC		116			145000	0.27619048	0.421852732
8	1.50	TNTC	TNTC	41	37			25687.5	0.04892857	0.513064133
9	1.8	TNTC	TNTC	133				16625	0.03166667	0.61567696
10	2.01666667	TNTC	TNTC	71				8875	0.01690476	0.689786223
11	2.31666667	TNTC	118					1475	0.00280952	0.79239905

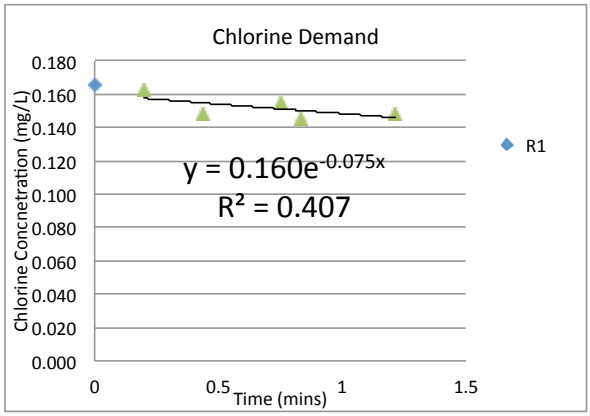


Experiment number 27

PRD1 vs. Free Chlorine

pH	8.5
pH range	8.54-8.39
Temp (°C)	1

10/6/10
Cuvette size (cm) 5
Reactor: 100mL 1mM CBS
Virus added: 0.1mL
Substock: 100mL NP+0.9mL stock Cl. Added: 0.07mL



CHLORINE ANALYSIS

R1			
Actual Time (mins)	DF	ABS	Chlorine Concentration (mg/L)
0.200	1	0.218	0.167
0.333	1	0.217	0.166
0.467	1	0.214	0.164

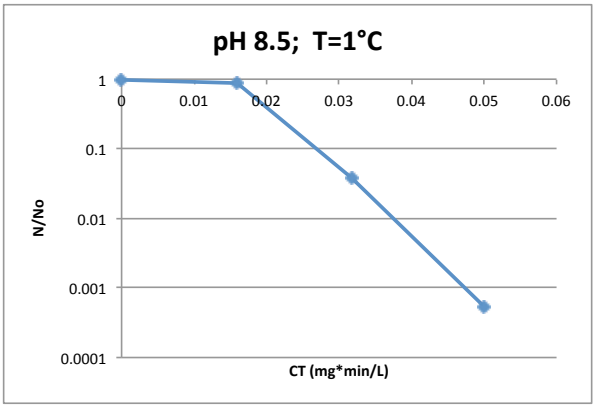
Time (mins)	Dose (mg/L as Cl_2)
0.000	0.166

	Actual Time (mins)	DF	ABS	Chlorine Concentration (mg/L)
R2	0.200	1	0.213	0.163
	0.433	1	0.194	0.148
	0.833	1	0.190	0.145
R3	0.750	1	0.203	0.155
	1.217	1	0.194	0.148

Co (mg/L as C	0.16
k_d (min^{-1})	0.075
R^2	0.407

VIABILITY

R3	Actual Time	0	-1	-2	-3	-4	-5	N	N/No	CT (mg*min/L)
1	0.00			TNTC		58	2	725000	1	0
2	0.10		TNTC	TNTC	TNTC	52		650000	0.89655172	0.01594015
3	0.20	TNTC	TNTC	222	18			27750	0.03827586	0.031761196
4	0.32	202	43	7				395	0.00054483	0.050069735
5	0.42	13	2							
6	0.52	4								



Experiment number 28

PRD1 vs. Free Chlorine

pH	8.3
pH range	8.33-8.25
Temp (°C)	1

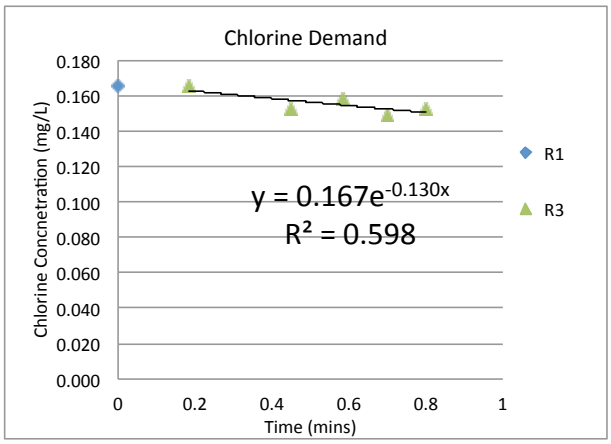
10/6/10

Cuvette size (cm) 5

Reactor: 100mL 1mM CBS

Virus added: 0.1mL

Substock: 100mL NP+0.9mL stock Cl. Added: 0.07mL



CHLORINE ANALYSIS

R1			
Actual Time (mins)	DF	ABS	Chlorine Concentration (mg/L)
0.200	1	0.218	0.167
0.333	1	0.217	0.166
0.467	1	0.214	0.164

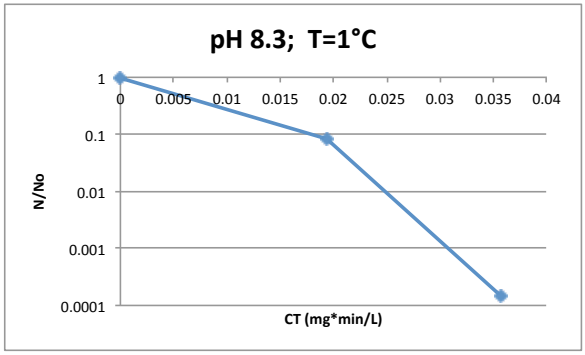
Time (mins)	Dose (mg/L as Cl_2)
0.000	0.166

	Actual Time (mins)	DF	ABS	Chlorine Concentration (mg/L)
R2	0.183	1	0.216	0.165
	0.450	1	0.199	0.152
	0.700	1	0.195	0.149
R3	0.583	1	0.207	0.158
	0.800	1	0.200	0.153

Co (mg/L as Cl_2)	0.167
k_d (min^{-1})	0.13
R^2	0.598

VIABILITY

R3	Actual Time	0	-1	-2	-3	-4	-5	N	N/No	CT (mg*min/L)
1	0.00			TNTC		40	1	500000	1	0
2	0.12	TNTC		337	30	5		42125	0.08425	0.019336329
3	0.22	59	24	1	1			73.75	0.0001475	0.035678502
4	0.32	15	3	0						
5	0.42	3	1							

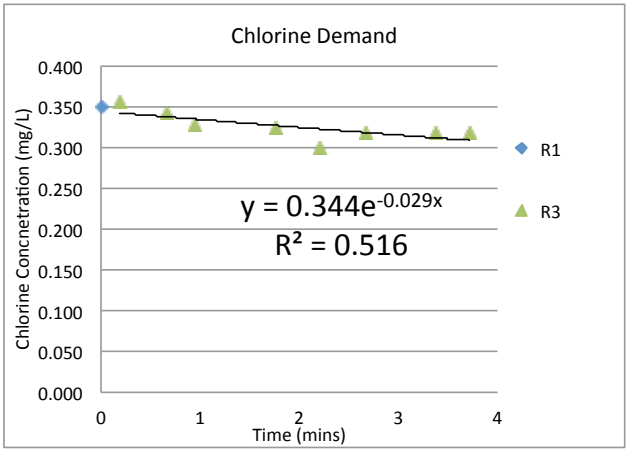


Experiment number 29

PRD1 vs. Free Chlorine

pH	9.8
pH range	9.83-9.89
Temp (°C)	1

10/7/10
Cuvette size (cm) 1
Reactor: 100mL 1mM CBS
Virus added: 0.11mL
Substock: 100mL NP+0.9mL stock Cl. Added: 0.11mL



CHLORINE ANALYSIS

R1			
Actual Time (mins)	DF	ABS	Chlorine Concentration (mg/L)
0.417	1	0.074	0.352
1.417	1	0.073	0.347
2.300	1	0.074	0.352

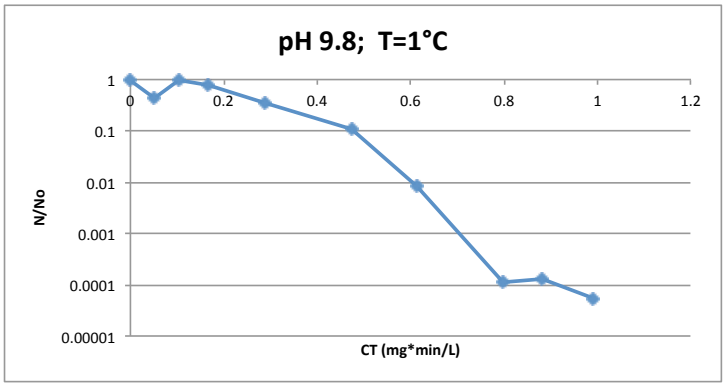
Time (mins)	Dose (mg/L as Cl_2)
0.000	0.350

	Actual Time (mins)	DF	ABS	Chlorine Concentration (mg/L)	pH
R2	0.183	1	0.075	0.356	9.77
	0.950	1	0.069	0.328	
	1.767	1	0.068	0.323	
	2.667	1	0.067	0.318	
	3.717	1	0.067	0.318	9.61
R3	0.667	1	0.072	0.342	
	2.217	1	0.063	0.299	
	3.38333333	1	0.067	0.318	

Co (mg/L as Cl_2)	0.344
k_d (min^{-1})	0.029
R^2	0.516

VIABILITY

R3	Actual Time	0	-1	-2	-3	-4	-5	N	N/No	CT (mg*min/L)
1	0.00			TNTC		90	5	1125000	1	0
2	0.15			TNTC		39	4	487500	0.43333333	0.051487933
3	0.30			TNTC		85	8	1062500	0.94444444	0.102752379
4	0.48		TNTC	TNTC		70		875000	0.77777778	0.16510684
5	0.85		TNTC		285	34		390625	0.34722222	0.2888256
6	1.40		TNTC		95	8		118750	0.10555556	0.471954496
7	1.83	TNTC		73	10			9125	0.00811111	0.614194654
8	2.40	98	4	1				122.5	0.00010889	0.797524238
9	2.66666667	114	20	0				142.5	0.00012667	0.882760047
10	3	50	5					62.5	5.5556E-05	0.988382038



Experiment number 31

PRD1 vs. Free Chlorine

pH	9.2
pH range	(9.24-9.21)
Temp (°C)	1

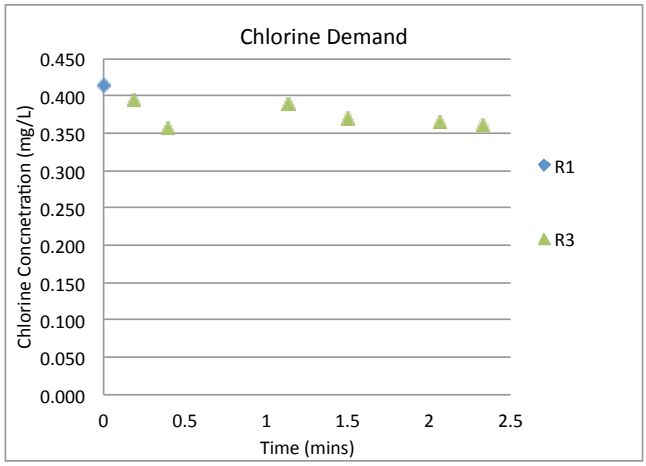
10/14/10

Cuvette size (cm) 1

Reactor: 100mL 1mM CBS

Virus added: 0.11mL

Substock: 100mL NP+0.9mL stock Cl. Added: 0.11mL



CHLORINE ANALYSIS

R1			
Actual Time (mins)	DF	ABS	Chlorine Concentration (mg/L)
0.267	1	0.088	0.418
1.217	1	0.087	0.413
4.017	1	0.087	0.413

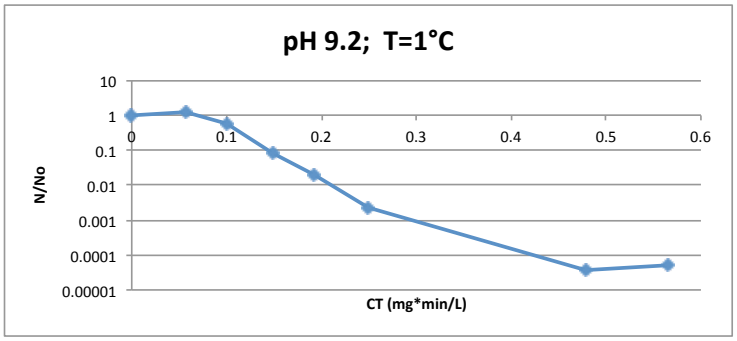
Time (mins)	Dose (mg/L as Cl_2)
0.000	0.415

	Actual Time (mins)	DF	ABS	Chlorine Concentration (mg/L)	pH
R2	0.183	1	0.083	0.394	9.20
	0.400	1	0.075	0.356	
	1.500	1	0.078	0.371	
	2.067	1	0.077	0.366	9.20
R3	1.133	1	0.082	0.390	
	2.333	1	0.076	0.361	

Chlorine Concentration (mg/L)	0.373

VIABILITY

R3	Actual Time	0	-1	-2	-3	-4	-5	N	N/No	CT (mg*min/L)
1	0.00			TNTC		114	12	1425000	1	0
2	0.15			TNTC		133	8	1662500	1.16666667	0.055938242
3	0.27		TNTC	TNTC		62		775000	0.54385965	0.099445764
4	0.40			135	177	18		119062.5	0.08355263	0.149168646
5	0.52		TNTC	221	10	0		27625	0.01938596	0.192676168
6	0.67	TNTC	254	12	0			3175	0.00222807	0.24861441
7	0.82	4	53	0						
8	1.00	16	1	0						
9	1.28333333	42	3					52.5	3.6842E-05	0.47858274
10	1.41666667	14	1							
11	1.51666667	55	0					68.75	4.8246E-05	0.565597783
12	1.73333333	14	5							

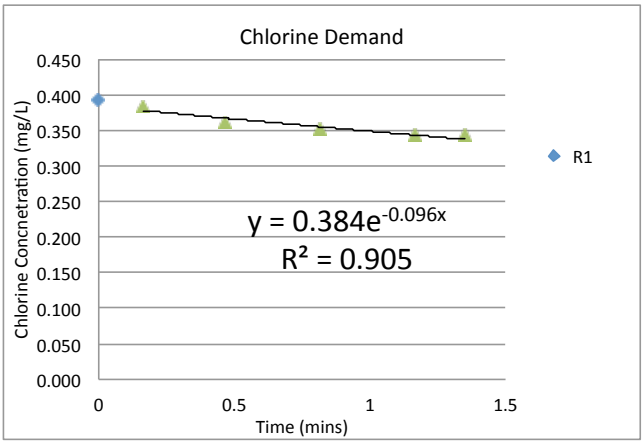


Experiment number 32

PRD1 vs. Free Chlorine

pH	8.85
pH range	8.84-8.88
Temp (°C)	1

10/15/10
Cuvette size (cm) 1
Reactor: 100mL 1mM CBS
Virus added: 0.11mL
Substock: 100mL NP+0.9mL stock Cl. Added: 0.11mL



CHLORINE ANALYSIS

R1			
Actual Time (mins)	DF	ABS	Chlorine Concentration (mg/L)
0.167	1	0.084	0.399
1.067	1	0.082	0.390
2.100	1	0.082	0.390

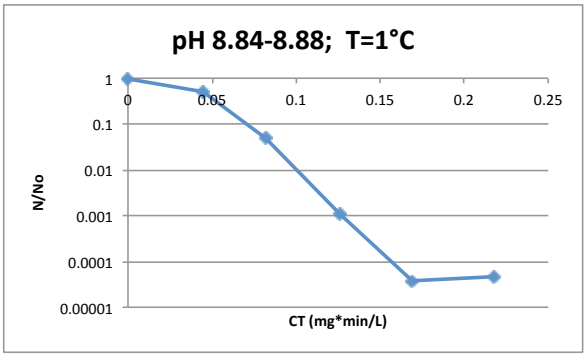
Time (mins)	Dose (mg/L as Cl_2)
0.000	0.393

	Actual Time (mins)	DF	ABS	Chlorine Concentration (mg/L)	pH
R2	0.167	1	0.081	0.385	8.88
	0.467	1	0.076	0.361	
	0.817	1	0.074	0.352	8.80
R3	1.167	1	0.072	0.342	
	1.350	1	0.072	0.342	

Co (mg/L as Cl_2)	0.384
k_d (min^{-1})	0.096
R^2	0.905

VIABILITY

R3	Actual Time	0	-1	-2	-3	-4	-5	N	N/No	CT (mg*min/L)
1	0.00			TNTC		135	5	1687500	1	0
2	0.12		TNTC	TNTC		68		850000	0.5037037	0.044550054
3	0.22	TNTC	TNTC		65	5		81250	0.04814815	0.082340688
4	0.33	TNTC	143	3	0	0		1787.5	0.00105926	0.125973672
5	0.45	51	6	0	0			63.75	3.7778E-05	0.169120692
6	0.58	61	2	1				76.25	4.5185E-05	0.217843456
7	0.72	9	1	0						
8	0.83	6	3							



Experiment number 33

PRD1 vs. Free Chlorine

pH	10
pH range	(9.97-10.00)
Temp (°C)	1

10/19/10

Cuvette size (cm) 1

Reactor: 100mL 1mM CBS

Virus added: 0.11mL

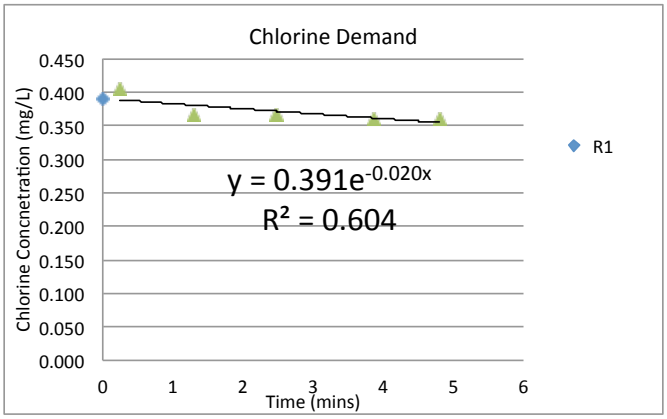
Substock: 100mL NP+0.9mL stock Cl. Added: 0.11mL

CHLORINE ANALYSIS

R1			
Actual Time (mins)	DF	ABS	Chlorine Concentration (mg/L)
0.333	1	0.083	0.394
1.333	1	0.082	0.390
2.750	1	0.081	0.385

Time (mins)	Dose (mg/L as Cl_2)
0.000	0.390

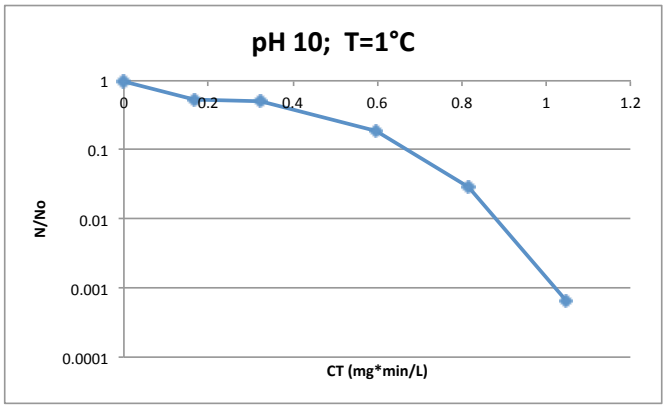
R3			
Actual Time (mins)	DF	ABS	Chlorine Concentration (mg/L)
0.250	1	0.085	0.404
1.300	1	0.077	0.366
2.483	1	0.077	0.366
3.867	1	0.076	0.361
4.800	1	0.076	0.361



Co (mg/L as C	0.391
k_d (min^{-1})	0.02
R^2	0.604

VIABILITY

R3		Actual Time	0	-1	-2	-3	-4	-5	N	N/No	CT (mg*min/L)
	1	0.00			TNTC		68	9	850000	1	0
	2	0.43				472	27		463750	0.54558824	0.168701239
	3	0.83				409	34		425000	0.5	0.323133078
	4	1.55		TNTC		126	4		157500	0.18529412	0.596752546
	5	2.13			196	8	0		24500	0.02882353	0.816588894
	6	2.75		45	8	2	0		562.5	0.00066176	1.046215358
	7	3.33		5	1	0					
	8	3.67	4	0	0						
	9	4.03333333	2	1	0						
	10	4.35	2	0							

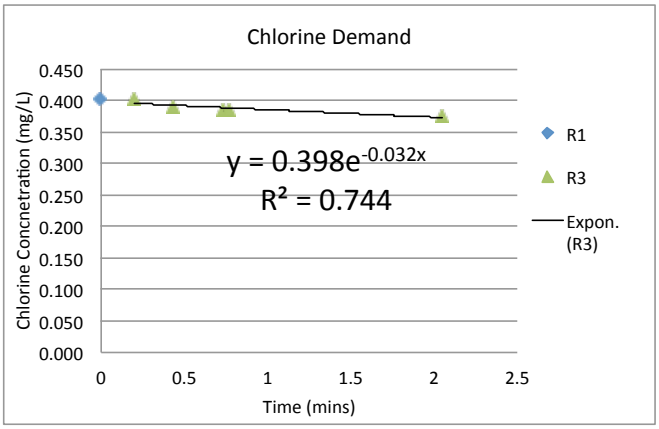


Experiment number 35

PRD1 vs. Free Chlorine

pH	9.6
pH range	9.59-9.59
Temp (°C)	14

10/28/10
Cuvette size (cm) 1
Reactor: 100mL 1mM CBS
Virus added: 0.11mL
Substock: 100mL NP+0.9mL stock Cl. Added: 0.13mL



CHLORINE ANALYSIS

R1			
Actual Time (mins)	DF	ABS	Chlorine Concentration (mg/L)
0.367	1	0.085	0.404
1.283	1	0.085	0.404
2.333	1	0.085	0.404

Time (mins)	Dose (mg/L as Cl_2)
0.000	0.404

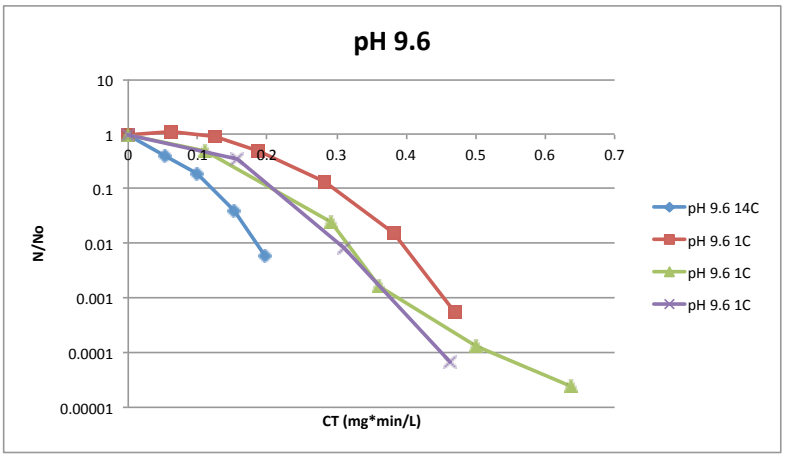
	Actual Time (mins)	DF	ABS	Chlorine Concentration (mg/L)	pH
R2	0.200	1	0.085	0.404	9.50
	0.433	1	0.082	0.390	
	0.733	1	0.081	0.385	
R3	0.767	1	0.081	0.385	
	2.050	1	0.079	0.375	

Co (mg/L as Cl_2)	0.398
k_d (min^{-1})	0.032
R^2	0.744

VIABILITY

R3

	Actual Time	0	-1	-2	-3	-4	-5	N	N/No	CT (mg*min/L)
1	0.00			TNTC		77	2	962500	1	0
2	0.13	TNTC	TNTC	TNTC	385	30		375000	0.38961039	0.052953619
3	0.25	TNTC	TNTC	TNTC	145	21		181250	0.18831169	0.099103059
4	0.38	TNTC	TNTC	228	39			38625	0.04012987	0.151634739
5	0.50	TNTC	319	46	3			5750	0.00597403	0.197416457
6	0.97	4	0	0						
7	1.18	0	0							
8	1.50	0	0							



Experiment number 36

PRD1 vs. Free Chlorine

pH	9.6
pH range	9.59-9.54
Temp (°C)	14

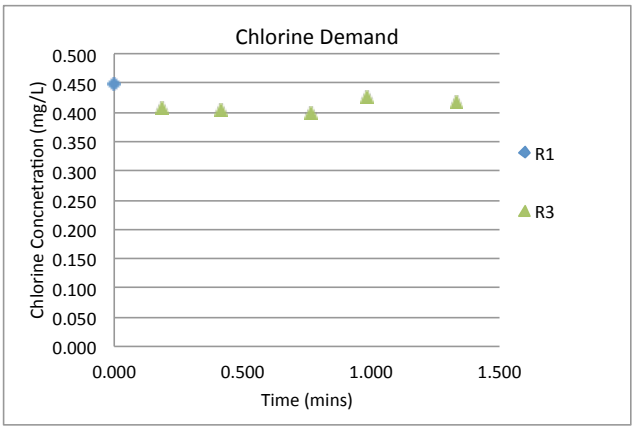
11/1/10

Cuvette size (cm) 1

Reactor: 100mL 1mM CBS

Virus added: 0.11mL

Substock: 100mL NP+0.9mL stock Cl. Added: 0.13mL



CHLORINE ANALYSIS

R1			
Actual Time (mins)	DF	ABS	Chlorine Concentration (mg/L)
0.433	1	0.094	0.447
1.400	1	0.094	0.447
2.417	1	0.095	0.451

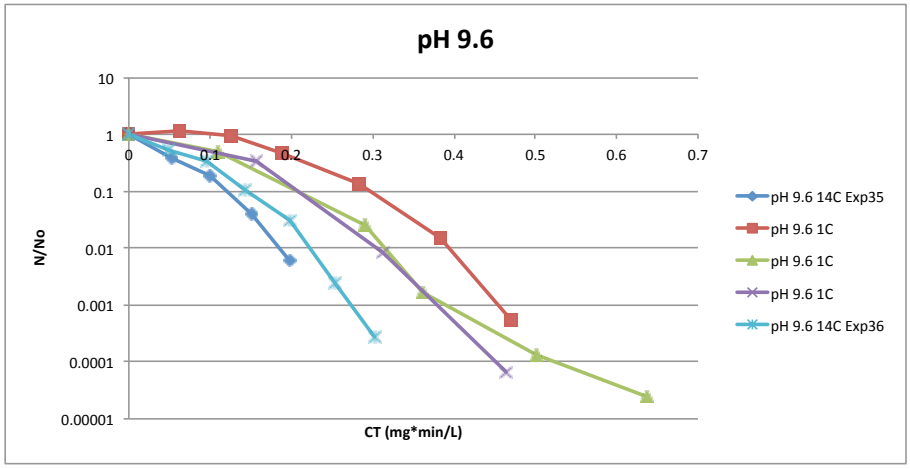
Time (mins)	Dose (mg/L as Cl_2)
0.000	0.448

	Actual Time (mins)	DF	ABS	Chlorine Concentration (mg/L)	pH
R2	0.183	1	0.086	0.409	9.50
	0.417	1	0.085	0.404	
	0.767	1	0.084	0.399	
R3	0.983	1	0.090	0.428	
	1.333	1	0.088	0.418	

Chlorine Concentration (mg/L)	0.411

VIABILITY

R3		Actual Time		0	-1	-2	-3	-4	-5	N	N/No	CT (mg*min/L)
	1	0.00				TNTC		121	11	1512500	1	0
	2	0.12			TNTC	TNTC		65		812500	0.53719008	0.04799683
	3	0.23			TNTC	TNTC		40		500000	0.33057851	0.09599367
	4	0.35		TNTC	TNTC		124			155000	0.10247934	0.1439905
	5	0.48	TNTC	TNTC	TNTC		38			47500	0.03140496	0.19884402
	6	0.62	TNTC		287	30				3668.75	0.00242562	0.25369755
	7	0.73		338	6					422.5	0.00027934	0.30169438
	8	0.85		9	0							



Experiment number 37

PRD1 vs. Free Chlorine

pH	9.2
pH range	(9.20-9.17)
Temp (°C)	14

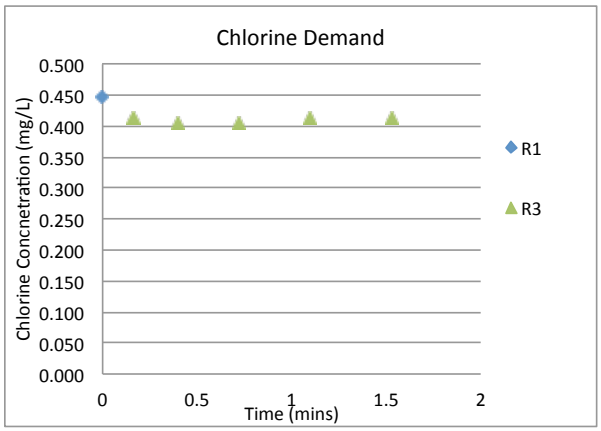
11/1/10

Cuvette size (cm) 1

Reactor: 100mL 1mM CBS

Virus added: 0.11mL

Substock: 100mL NP+0.9mL stock Cl. Added: 0.13mL



CHLORINE ANALYSIS

R1			
Actual Time (mins)	DF	ABS	Chlorine Concentration (mg/L)
0.433	1	0.094	0.447
1.400	1	0.094	0.447
2.417	1	0.095	0.451

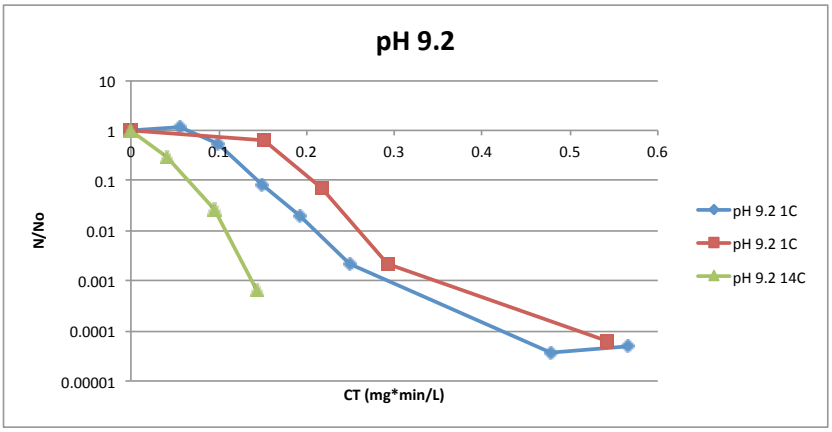
Time (mins)	Dose (mg/L as Cl_2)
0.000	0.448

	Actual Time (mins)	DF	ABS	Chlorine Concentration (mg/L)	pH
R2	0.167	1	0.087	0.413	9.20
	0.400	1	0.085	0.404	
	0.717	1	0.085	0.404	
R3	1.100	1	0.087	0.413	
	1.533	1	0.087	0.413	

Chlorine Concentration (mg/L)	0.410

VIABILITY

R3	Actual Time	0	-1	-2	-3	-4	-5	N	N/No	CT (mg*min/L)
1	0.00			TNTC		136	20	1700000	1	0
2	0.10	TNTC	TNTC	TNTC		41		512500	0.30147059	0.040950119
3	0.23	TNTC	TNTC	TNTC	37	6		46250	0.02720588	0.095550277
4	0.35	TNTC	86	11	2			1075	0.00063235	0.143325416
5	0.47	1	0	0	0					
6	0.60	0	0	0						
7	0.72	0	0							
8	0.83	0	0							
9	0.95	0								



Experiment number 38

PRD1 vs. Free Chlorine

pH	10.0
pH range	10.03-10.03
Temp (°C)	14

11/2/10

Cuvette size (cm) 1

Reactor: 100mL 1mM CBS

Virus added: 0.11mL

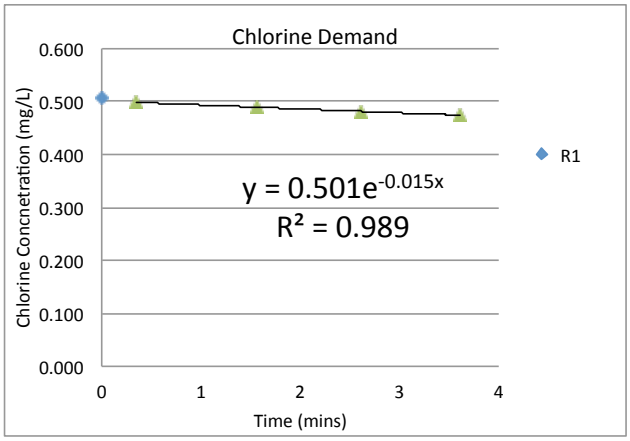
Substock: 100mL NP+0.9mL stock Cl. Added: 0.15mL

CHLORINE ANALYSIS

R1			
Actual Time (mins)	DF	ABS	Chlorine Concentration (mg/L)
0.450	1	0.108	0.513
1.317	1	0.106	0.504
2.433	1	0.107	0.508

Time (mins)	Dose (mg/L as Cl_2)
0.000	0.508

R3			
Actual Time (mins)	DF	ABS	Chlorine Concentration (mg/L)
0.350	1	0.105	0.499
1.567	1	0.103	0.489
2.617	1	0.101	0.480
3.617	1	0.100	0.475

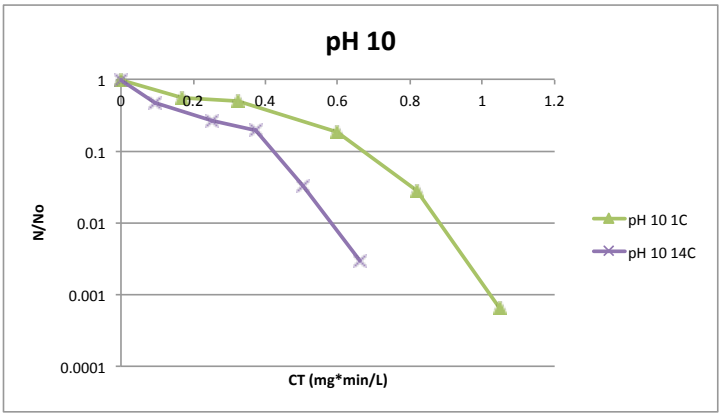


Co (mg/L as Cl_2)	0.501
k_d (min^{-1})	0.015
R^2	0.989

VIABILITY

R3

	Actual Time	0	-1	-2	-3	-4	-5	N	N/No	CT (mg*min/L)
1	0.00			TNTC		62	1	775000	1	0
2	0.18		TNTC		314	27		365000	0.47096774	0.091723822
3	0.50	TNTC	TNTC		168	19		210000	0.27096774	0.249562969
4	0.75	TNTC	TNTC	TNTC	118	14		147500	0.19032258	0.37364431
5	1.02	TNTC	TNTC	209	24	2		26125	0.03370968	0.505485874
6	1.33	TNTC	183	20	5			2287.5	0.00295161	0.661364312
7	1.80	11	1	1	0					
8	2.35	7	1	0						
9	2.78333333	1	0	0						
10	3.25	1	0							



APPENDIX B. RAW DATA: INACTIVATION OF PRD1 WITH LPUV

PRD1 propagated with *E.coli* plated on *E.coli*

PRD1 vs. LPUV
2/1/10

Reactor: 15mL 1mM CBS
Virus: 0.1mL of PRD1

Time (mins)	0	-1	-2	-3	-4	-5	-6	-7	Dose (mJ/cm^2)	PFU/mL	N/No
0					TNTC	137	22	0	0	2.23E+07	1
4:30	TNTC	TNTC	TNTC	TNTC	87	12	0	0	10.8	1.09E+06	0.048739496
9:01	TNTC	TNTC	TNTC	TNTC	25	2	0	1	20	3.13E+05	0.014005602
18:01	TNTC	53	6	2	0	0	2	0	40	6.63E+02	0.0000297

PRD1 vs. LPUV
10/25/10

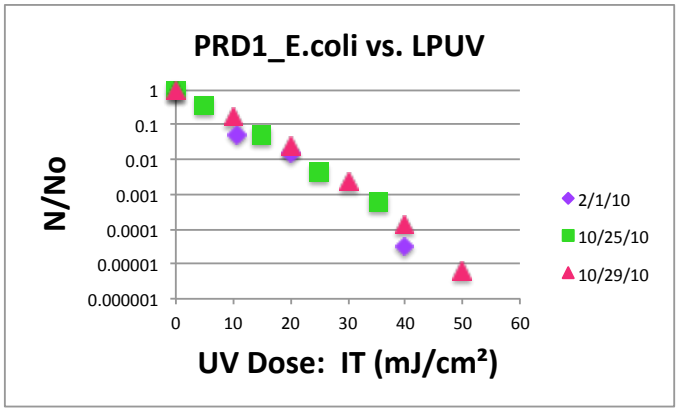
Reactor: 15mL 1mM CBS
Virus: 0.1mL of PRD1

Time (mins)	Dose (mJ/cm^2)	0	-1	-2	-3	-4	-5	-6	PFU/mL	N/No
0	0					TNTC	74	19	9250000	1
4.3166667	10				TNTC	121	17		1512500	0.163514
8.6333333	20			TNTC	169	16			211250	0.022838
12.95	30		TNTC	173	15				21625	0.002338
17.266667	40	TNTC	99	8					1237.5	0.000134
21.583333	50	45	2						56.25	6.08E-06

PRD1 vs. LPUV
10/29/10

Reactor: 15mL 1mM CBS
Virus: 0.01mL of PRD1

Time (mins)	Dose (mJ/cm^2)	0	-1	-2	-3	-4	-5	PFU/mL	N/No
0	0				TNTC	129	12	1612500	1
2.15	5			TNTC	TNTC	47		587500	0.364341
6.4666667	15		TNTC	TNTC	62	7		77500	0.048062
10.783333	25		TNTC	56	4			7000	0.004341
15.2	35.2	335	77	4				962.5	0.000597
19.416667	45	21	4						



PRD1 propagated with *S.typhimurium* plated on E.coli

PRD1_salmonella virus vs. LPUV
10/22/10

Reactor: 15mL 1mM CBS
Virus: 1mL of PRD1_salmonella

Time (mins)	Dose (mJ/cm^2)	0	-1	-2	-3	-4	-5	PFU/mL	N/No
0	0				142	22	1	177500	1
4.25	10	TNTC	TNTC	212	9	1		26500	0.149296
8.4833333	20	TNTC	241	26	0	0		3012.5	0.016972
12.733333	30	247	23	0	0	0		308.75	0.001739

PRD1_salmonella virus vs. LPUV
10/25/10

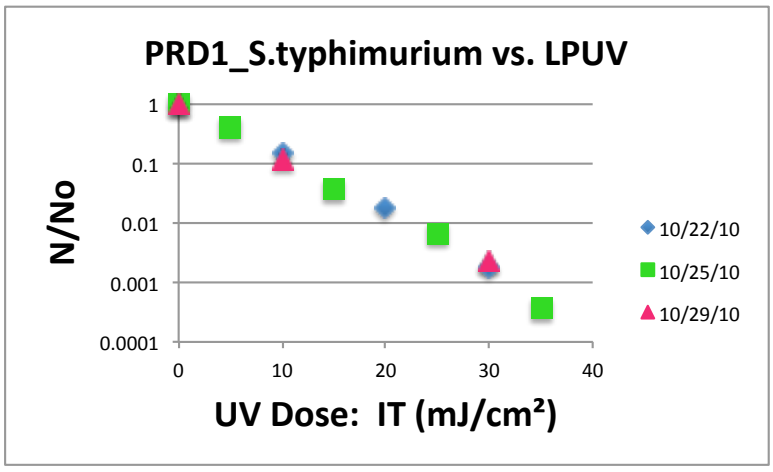
Reactor: 15mL 1mM CBS
Virus: 1mL of PRD1_salmonella

Time (mins)	Dose (mJ/cm^2)	0	-1	-2	-3	-4	-5	PFU/mL	N/No
0.00	0				193	17	2	241250	1
2.17	5			TNTC	77	9		96250	0.398964
6.48	15		TNTC	70	11			8750	0.036269
10.80	25	TNTC	129	6				1612.5	0.006684
15.12	35	69	10					86.25	0.000358
17.27	40	9	6						

PRD1_salmonella vs. LPUV
10/29/10

Reactor: 15mL 1mM CBS
Virus: 1.2mL of PRD1_salmonella

Time (mins)	Dose (mJ/cm^2)	0	-1	-2	-3	-4	-5	PFU/mL	N/No
0.00	0				207	23	3	258750	1
4.32	10			237	23	2		29625	0.114493
8.63	20		389	20	1				
12.95	30	334	46	2				575	0.002222
17.27	40	12	2						

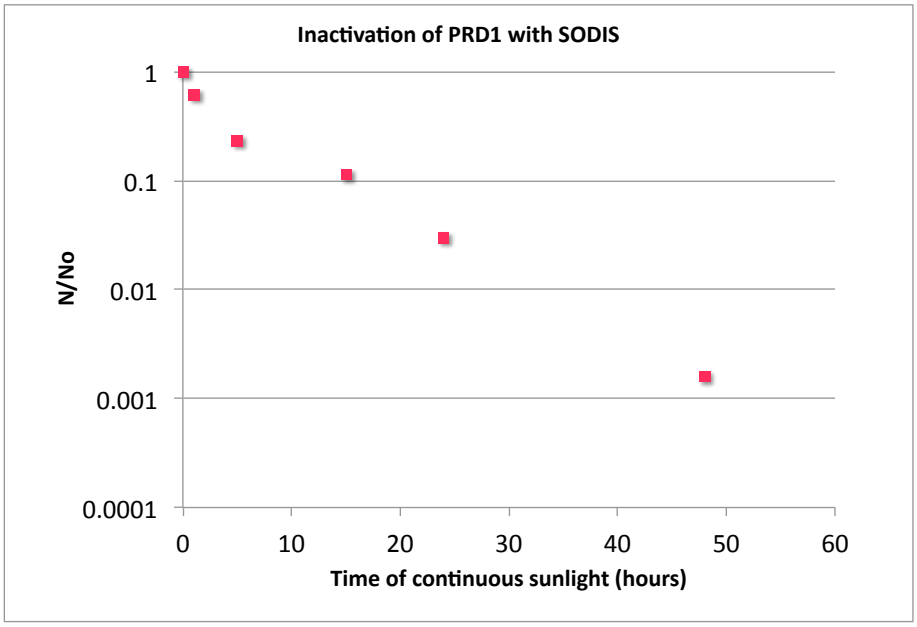


APPENDIX C. RAW DATA: INACTIVATION OF PRD1 WITH SODIS

PRD1 vs. SODIS

Reactor	25mL 1mM CBS
Filters:	AMO
	AM1.5
	25%T
	>324nm
PRD1 added:	0.10 mL
Temp	25°C
Intensity:	136.1mW

Sample No.	Time (hrs)	0	-1	-2	-3	-4	-5	-6	N	N/No
1	0					TNTC	63	8	7875000	1
2	1					TNTC	38	0	4750000	0.6031746
3	5				TNTC	147	16	1	1837500	0.23333333
4	15	TNTC	TNTC	TNTC	TNTC	71	9	2	887500	0.11269841
5	24	TNTC	TNTC	TNTC	189	15	4	0	236250	0.03
6	48	TNTC	TNTC	101	10	0	0	0	12625	0.00160317



APPENDIX D. RAW DATA: INACTIVATION OF ADENOVIRUS WITH SODIS

Ad2 vs. SODIS

pH	8.32
Reactor	25mL 1mM CBS
Filters:	AMO
	AM1.5
	25%T
	>324nm
Ad2 added:	0.10 mL
Temp	25°C
Intensity:	107 mW/cm²

	Time (hrs)	0	-1	-2	-3	-4	-5	-6	N	N/No
1	0					8	0	0	100000	1
2	0.66666667				83	0	0	0	103750	1.0375
3	1.33333333				42	0	0	0	52500	0.525
4	2			TNTC	43	0	0		53750	0.5375
5	2.66666667			TNTC	30	0	0		37500	0.375

