EPA/600/R-10/003

Impact of Wet-Weather Peak Flow Blending on Disinfection and Treatment: A Case Study at Three Wastewater Treatment Plants

By

R. Boris Rukovets and Brian J. Mitchell
Interstate Environmental Commission
311 West 43rd St., Suite 201
New York, NY 10036

Contract Number: EP06C000010

Project Officers
Richard Field and Mary K. Stinson
National Risk Management Research Laboratory
Water Supply and Water Resources Division
U.S. Environmental Protection Agency
Edison, New Jersey 08837

National Risk Management Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, Ohio 45268

Notice

The U.S. Environmental Protection Agency (EPA) through its Office of Water, Office of Policy, Economics and Innovation, and Office of Research and Development has financially supported and collaborated in the research described herein. It has been subjected to the Agency's peer and administrative review and has been approved for publication as an EPA document. Any mention of a trade name or commercial product does not constitute endorsement or recommendation by the EPA for use. The views expressed in this report are those of the individual authors and do not necessarily reflect the views and policies of the EPA.

Foreword

The U.S. Environmental Protection Agency (EPA) is charged by Congress with protecting the Nation's land, air, and water resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, EPA's research program is providing data and technical support for solving environmental problems today and building a science knowledge base necessary to manage our ecological resources wisely, understand how pollutants affect our health, and prevent or reduce environmental risks in the future.

The National Risk Management Research Laboratory (NRMRL) is the Agency's center for investigation of technological and management approaches for preventing and reducing risks from pollution that threaten human health and the environment. The focus of the laboratory's research program is on methods and their cost-effectiveness for prevention and control of pollution to air, land water and subsurface resources; protection of water quality in public water systems; remediation of contaminated sites, sediments and ground water; prevention and control of indoor air pollution; and restoration of ecosystems. NRMRL collaborates with both public and private sector partners to foster technologies that reduce the cost of compliance and to anticipate emerging problems. NRMRL's research solutions to environmental problems by: developing and promoting technologies that protect and improve the environment; advancing scientific and engineering information to support regulatory and policy decisions; and providing the technological support and information transfer to insure implementation of environmental regulations and strategies at the national, state, and community levels.

This publication has been produced as part of the Laboratory's strategic long-term research plan. It is published and made available by EPA's Office of Research and Development to assist the user community and to link researchers with their clients.

Sally Gutierrez, Director
National Risk Management Research Laboratory

Abstract

This research project was administered by the EPA Office of Research and Development and funded by Office of Water; Office of Policy, Economics and Innovation; and Office of Research and Development.

Blending is the practice of diverting a part of peak wet-weather flows at wastewater treatment plants (WWTPs), after primary treatment, around biological treatment units and combining effluent from all processes prior to disinfection and subsequent discharge from a permitted outfall. For combined sewer systems, EPA's 1994 Combined Sewer Overflow (CSO) Policy encourages delivery of maximum flows to WWTPs, while ensuring that bypasses do not result in National Pollution Discharge Elimination System (NPDES) permit exceedences. Consistent with that principle, blending of flows at WWTPs serving combined sewer systems presents one of the more technically practicable and economically feasible alternatives. In addition, in December 2005, the EPA proposed, for public comment, a new policy for addressing peak flow events at municipal WWTPs served by separate sewer systems, also through flow maximization.

This project's intent was to determine the microbiological impact of blending primary effluent flows that are in excess of secondary treatment capacity with the secondary effluent prior to disinfection at large municipal WWTPs. This approach is typically used by a number of municipal WWTPs within the Interstate Environmental Commission's (IEC) jurisdiction during wet weather to maximize the flow to the WWTP and reduce CSO events. The primary objective of the study was to evaluate the effect of wet-weather blending on the concentration of fecal coliform and *Enterococcus* indicator bacteria, total residual chlorine, protozoa and viruses in the WWTP final effluent. Three New York City WWTPs were monitored for this project. The project was important for better predicting and understanding the impact of blending on CSO pollution control and receiving water quality.

The results showed that during blending, the sampled WWTPs remove, on average, between 97% and 99% of coliphage and enteric viruses; approximately 71% of *Cryptosporidium;* and between 40% and 88% of *Giardia*. The geometric mean for fecal coliform effluent concentrations during blending at the three WWTPs ranged from 520 to 19,000 MPN/100 ml and the corresponding geometric mean for *Enterococcus* effluent concentrations ranged from 870 to 17,000 MPN/100 ml. During blending, effluent BOD and TSS concentrations remained below 30 mg/l (a monthly average permit limit for both parameters) at two out of three WWTPs; the third WWTP, that had results above 30 mg/l for both parameters, was undergoing a partial construction at the time of sampling.

A sample maceration procedure was conducted to determine if bacteria occluded by particulate matter could be enumerated. Maceration was accomplished using a commercial Waring™ blender, which breaks apart particulate matter exposing bacteria within the particle interstices. After a statistical evaluation, it was shown that the maceration of effluent samples resulted in an increase in both fecal coliform and *Enterococcus* concentrations when compared to unmacerated samples.

The measurement of flow rates was made for WWTP influent flows at all three WWTPs during both dry-weather, non-blending events and wet-weather blending events. During wet-weather blending events, the exact measurement of the flow through secondary treatment systems was made only at one WWTP (i.e., WWTP 1). Based on knowledge of the blending process and associated flow rates of primary flow treated versus secondary flow treated, flows through the secondary system at the two other WWTPs (WWTP 2 and WWTP 3) were estimated.

The strength of this study is that it gathered information at three full-scale WWTPs functioning as usual during actual dry-weather non-blending and wet-weather blending operation. Also, this study represents a first detailed effort to analyze the impact of blending during wet weather.

The limitation of the study is that it represents only one geographical location for the three plants studied and the wet-weather blending ratios or flow rates were measured in only one of the three plants. Thus, the geographical closeness and the limited number of facilities evaluated during the study suggest that these results should be viewed as plant – specific.

Additional studies are recommended at a variety of WWTPs to provide reinforcement of the data obtained in this study.

Contents

Figures

Tables

Glossary of Terms and Acronyms

AdV	Adenovirus
BGM	Buffalo Green Monkey cell line
BCS	Biological Consulting Services
BOD_5	Five-Day Biochemical Oxygen Demand
CaCo-2	Human intestinal cell line
Cell line	Cells of a human or animal that are grown in a laboratory and used for detection of the presence of a particular organism. In this study, four cell lines, i.e., BGM, MA104, PLC/PRF/5 and CaCo-2, were used for detection of the enteric viruses.
$CBOD_5$	Five-Day Carbonaceous Biochemical Oxygen Demand
DESA	Division of Environmental Science and Assessment
EPA	Environmental Protection Agency
EV	Enterovirus
h	Hours
HAV	Hepatitis A Virus
IEC	Interstate Environmental Commission
MA104	Cell line derived from Rhesus monkey kidney
mg/l	Milligram per Liter
MGD	Million Gallon per Day
min	Minutes
MPN	Most Probable Number
ND	Non-detect
NPDES	National Pollutant Discharge Elimination System
NYC	New York City
NYC DEP	New York City Department of Environmental Protection
NYS DEC	New York State Department of Environmental Conservation
ORD	Office of Research and Development
PLC/PRF/5	Human hepatoma cell line
REO	Reovirus
rpm	Revolutions Per Minute
RV	Rotavirus
s	Seconds
SP	Sampling point
TRC	Total Residual Chlorine
TSS	Total Suspended Solids
WWTP	Wastewater Treatment Plant

Acknowledgments

This is to acknowledge the significant contributions to this document made by the following fellow professionals**:**

Mohammed Billah, Donald Brady and Kevin Weiss, U.S. EPA-Office of Wastewater Management

Jennifer Cashdollar, Christopher Impellitteri, Daniel Murray, PE, Mark Rodgers, Mano Sivaganesan (Statistician), U.S EPA-Office of Research and Development

Stephen Schaub, Ph.D., U.S. EPA-Office of Science and Technology

Deborah Szaro, Ruth Sykes and Irwin Katz, U.S. EPA- Region 2 Laboratory (Edison, NJ)

Sarah Garman, U.S. EPA-Office of Policy, Economics and Innovation

James Olander, U.S. EPA- Region 2

Troy Scott, Ph.D. and George Lukasik, Ph.D., Biological Consulting Services

Claudio Ternieden, Water Environment Research Foundation

Special thanks to the staff of the New York City Department of Environmental Protection for valuable assistance with the study design and during the on-site sampling.

Last but not least, we thank Richard Field, PE, Project Officer, and Mary Stinson, Associate Project Officer, both from the U.S. EPA Office of Research and Development, for providing the invaluable contributions to this report and important guidance throughout the entire project.

Chapter 1. Introduction

1.1 Project Background

Blending is the practice of diverting a part of peak wet-weather flows at wastewater treatment plants (WWTPs) after primary treatment around biological treatment units and combining effluent from all processes prior to disinfection and subsequent discharge from a permitted outfall. The U.S. Environmental Protection Agency's (EPA) 1994 CSO Policy provides guidance for anticipated bypasses for WWTPs served by combined sewers. The aforementioned CSO Policy encouraged delivery of maximum flows to the WWTPs, while ensuring that bypasses do not result in effluent water quality violations. Consistent with that principle, blending of flows at WWTPs serving combined sewer systems, in many instances, presents one of the more technically practicable and economically reasonable approaches. To demonstrate the viability of this approach, the impact of blending during peak wet weather on the microbiological quality of the effluent was the subject of this study.

The project was conducted by the Interstate Environmental Commission (IEC) under the direction of EPA. IEC is a tri-state (NY, NJ, CT) quasi-governmental regulatory pollution control agency.

The major purpose of the project was to analyze the efficacy of microbiological treatment and disinfection of blending primary effluent flows that are in excess of secondary treatment capacity, with the secondary effluent prior to disinfection at large municipal WWTPs. This approach is typically used by the New York City Department of Environmental Protection (NYC DEP), and a number of other municipal WWTP operators, during wet-weather events to maximize the flow to the treatment WWTP and reduce the number and flow volume of CSOs. Analyses of the effluent concentrations of Total Residual Chlorine (TRC), fecal coliform, *Enterococcus*, protozoa and viruses during wet-weather blending are methods used to determine the impact of blended flows on the disinfection process and final effluent quality. The results of the project are important for predicting and understanding the impact of blending on CSO water pollution control and on water quality improvement.

While the latest Peak Wet-Weather Policy[1] proposed by EPA in December 2005 addresses flow maximization only for the separate sewer system, flow maximization for the combined sewer system is no less challenging. While both system types may require flow maximization to decrease the amount of flow that is discharged untreated into waterways, the need to maximize the flow to the WWTPs for separate sewer systems during wet-weather events is primarily driven by infiltration and inflow to sanitary lines, which can frequently be reduced through operation and maintenance, rehabilitation and other capital investments. Given the differences in design of the existing infrastructure and resulting peak volumes that occur during wet weather in combined sewer system, the need for the flow maximization and, consequently, bypass of the secondary treatment units during wet-weather events at WWTPs generally require greater capital

[1] National Pollutant Discharge Elimination System (NPDES) Permit Requirement for Peak Wet-Weather Discharges from Publicly Owned Treatment Works Treatment WWTPs Serving Separate Sanitary Sewer Collection Systems. Federal Register, Vol. 70, No. 245, December 22, 2005.

investments to reduce blending. Research on blending in combined sewer systems can potentially lead to results that would apply to both combined sewer systems and separate sewer systems as flow rates and sanitary wastewater loadings will tend to have similarities during wet weather events.

In the project area, 12 out of 14 New York City (NYC) WWTPs receive influent predominantly from a combined sewer system; most of these WWTPs use a flow maximization approach similar to the one described in this report. The two remaining WWTPs receive influent primarily from a separate sewer system and have sufficient hydraulic capacity to handle wet-weather events. Since blending is typically not used at these two WWTPs, they were not chosen for this study. Hence, the three WWTPs selected for this project were WWTPs with combined sewer systems.

Since this study and its results represent only one geographic location, they cannot be used for drawing definitive conclusions of overall impact of blending on a national scale. Also, conclusions drawn from the results are dependent on the analytical protocol used. Classical microbiological methods for enumerating viable bacteria rely on collection of a representative sample. For consistent enumeration, microbes must be homogeneously distributed within such samples (APHA, 2005). However, work conducted by several researchers has shown that microbes can adsorb and/or adhere to particles within water samples (Wellings, et. al., 1976; Hoff, 1978; Hoff and Akin, 1986). Interestingly, some recent work conducted by Perdek and Borst (2000) suggests that for combined sewer overflow samples, recovery of indicator organisms can be improved by macerating for 2 minutes in a blender at 22,000 rpm with a mixture of additives described by Camper, et al. 1985. This mixture included a buffer, a chelating agent, and a surfactant which was found to be effective for maximizing the recovery of culturable heterotrophic bacteria from granular activated carbon. Work conducted on blended effluents by Camper, et al. suggested that macerating samples may enhance recovery of culturable bacteria consistent with the findings of Perdek and Borst. However, the effectiveness of maceration, and/or surfactants for enhanced recovery of viruses, bacteriophage and protozoans needs to be evaluated to determine if such sample handling techniques improves the recovery, and therefore the accuracy and precision, of enumeration techniques for pathogens.

1.2 Treatment Processes at New York City WWTPs

As stated above, the three WWTPs evaluated during this project, receive most of their flow from combined sewer systems. Figure 1 depicts the process schematic at WWTP 3. The process is typical of NYC DEP WWTPs and includes coarse screening and degritting, primary treatment, secondary treatment, disinfection, and sludge treatment.

Preliminary treatment begins by the wastewater flowing through bar screens located 1 to 3 inches apart, which remove large pieces of trash including sticks, rags, bottles, plastic cups and other items. This part of the treatment process protects the main pumps that deliver the wastewater to the WWTP.

The wastewater then flows through a primary settling tanks, where the flow of wastewater is slowed, allowing heavier solids to settle to the bottom and the lighter material to float. The

lighter material that floats is skimmed from the top of the tank water surface. The heavier solids (primary sludge) are pumped through cyclone degritters that use centrifugal force to separate out sand, grit, and gravel; which are removed and disposed of by landfilling. The degritted sludge is then sent to the sludge treatment facility. The primary setting tank effluent flows to the secondary treatment system.

Secondary treatment at all three WWTPs consists of two sections: aeration tanks and final settling tanks. Biological treatment by the activated sludge takes place in aeration tanks, where air and settled activated sludge from a second set of settling tanks (final settling tanks) is mixed. The air mixes the wastewater and activated sludge, which in turn stimulates the growth of oxygen-using (aerobic) bacteria in the wastewater. These microorganisms consume most of the remaining organic pollutants, leaving a residual of heavier particles that settle later in the final settling tanks.

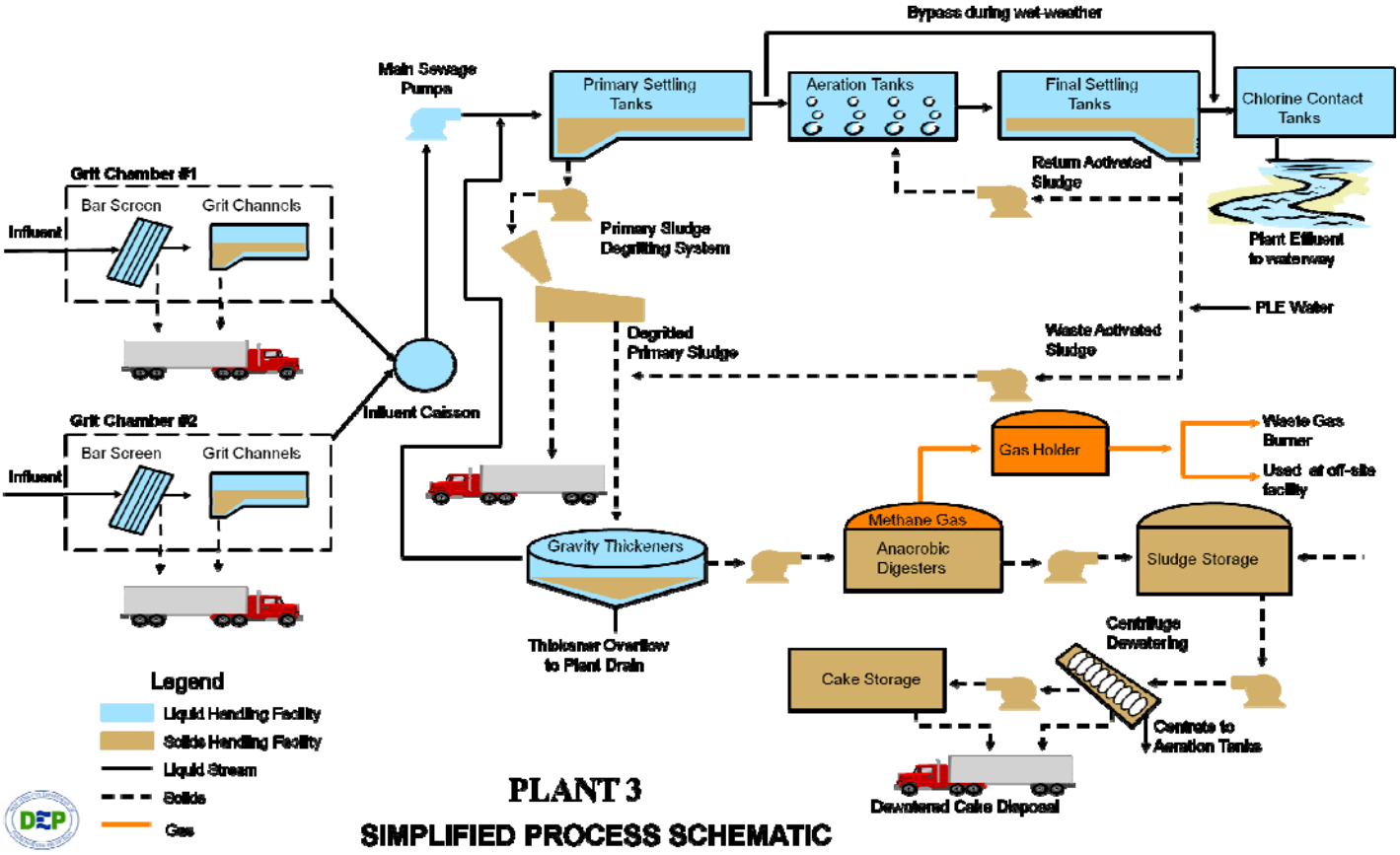


Figure 1. Schematic of the Treatment Process at WWTP 3
Source: NYC DEP

The flow from the aeration tanks then goes into the final settling tanks. In these tanks, similar to the process in primary settling tanks, the heavier particles settle to the bottom and are then

removed as secondary sludge. The majority of the secondary settled sludge is returned to the aeration tanks where it is used as the previously described seed sludge; a smaller portion of the secondary settled sludge is sent to the sludge treatment facility. The treated water then proceeds to the chlorine contact tanks for disinfection.

While primary and secondary treatment removes most of the solids and reduces the microorganism content of wastewater, microorganisms that could cause disease remain in the secondary effluent. Concentrations of these microorganisms must be reduced before the wastewater is released to the waterways and has the potential to come into contact with humans. The disinfection process used at all NYC WWTPs is chlorination by sodium hypochlorite. Hypochlorite, dissolved in the treated water, is held for 15-30 minutes within contact tanks to inactivate pathogens. The treated effluent is then released to local waterways.

A separate phase of treatment is sludge treatment. Sludge is removed from primary and secondary treatment systems. This sludge is 99% water and must be concentrated before further treatment. First, the sludge is sent to the sludge thickening tanks where it settles. The supernatant is sent back to the head of the WWTP, while the thickened sludge is sent to the digesters. The digesters are oxygen free tanks that are heated to 95º F and hold the sludge for two to three weeks. Methane, which is used as an energy source at some WWTPs, is one of the byproducts of the digestion process. The digested sludge is then pumped to a dewatering facility that dries the liquid sludge to a total solids concentration of about 26%.

During dry-weather flow conditions, the above-mentioned procedure is the wastewater flow process through the WWTP. During wet-weather flow, the process will remain the same as that for dry-weather flow, up to the point when the high flow through the WWTP reaches the threshold of 1.5 times the design flow. As wet-weather flowrates increase (typically, up to two times design flow), all flows entering the WWTP will still continue through preliminary and primary treatment, but only the portion of the flow equal to 1.5 times design flow (according to NYC DEP's Wet Weather Operation Manual) will be allowed to reach the secondary treatment. All other primary effluent flow is diverted around secondary treatment and recombines or "blends" with the secondary effluent for disinfection. This process is called "blending." Additional wet-weather flows that are beyond the capacity of the WWTP are discharged from overflow points in the collection system with no or partial treatment.

1.3 Key Research Questions

This study's objective was to answer to following questions:

Question #1: During wet-weather blending events at the three WWTPs studied what were BOD_5 and TSS levels in the blended effluent?

Question #2: During wet-weather blending events at the three WWTPs studied, what were the fecal coliform and *Enterococcus* levels in the blended effluent?

Question #3: For the three WWTPs studied, was there evidence for the removal of protozoa (*Cryptosporidium*, infectious *Cryptosporidium* and *Giardia*) during wet-weather blending?

Question #4: For the three WWTPs studied, was there evidence for the removal of viruses (Adenovirus, Astrovirus, Enterovirus, Rotavirus, Reovirus, Norovirus, Hepatitis A and male-specific and somatic coliphages as an indicator for viruses) during wet-weather blending?

Question #5: For the three WWTPs studied, to what extent did maceration of disinfected effluent samples change the levels of fecal coliform and *Enterococcus*?

Question #6: For the three WWTPs studied, what were pollutant levels in dry-weather effluent?

In conjunction with Question #1, the study also evaluated the effect of wet-weather blending on percent removal at the three WWTPs studied. However, it is important to point out that the discharge permits for NYC DEP WWTPs require compliance with the 85% removal requirement for both $CBOD_5$ and TSS WWTPs based on a 30-day arithmetic mean, which does not include the wet-weather data points. The NPDES permits for NYC WWTPs specifically state that *"During periods of wet- weather which causes the plant to exceed plant flows over the permitted flow for a calendar day, the $CBOD_5$ and TSS influent and effluent results for that day shall not be used to calculate 30-day arithmetic mean percent removal limitations. However, all concentrations shall be used in the calculation of the arithmetic mean value concentration limitations."*

The exclusion of wet-weather results applies solely to the calculation of 30-day percent removal and not to any of the calculations of the 30-day arithmetic mean for $CBOD_5$, BOD_5, TSS or fecal coliform effluent concentrations. Hence, all historical NYC DEP data presented in this report, except for the average monthly percent removal portions of Table 6 and Table 7, include data from periods of wet-weather flow. Additionally, all IEC results presented in this report include data from periods of wet-weather flow, without any exceptions. The summary of effluent limitations for NYC DEP WWTPs is given in Table 1.

There was high variation in many of the operational parameters among the three WWTPs in this study. Therefore, the key research questions were addressed separately for each WWTP. Due to the limited number of samples collected, especially for dry weather periods, the results should be interpreted and applied to other operations with caution. It should also be noted that while influent flow data were recorded for all three WWTPs, it was only at one plant (WWTP 1) that wet-weather secondary flow was recorded during the study. Because of the project team's knowledge of the blending process, they were able to estimate an approximate flow throughout the secondary system at the remaining two plants.

Table 1 - $CBOD_5$, BOD_5, and TSS Effluent Limitations for NYC DEP WWTPs

Parameter	Type	Limitation (mg/l)	Required by
$CBOD_5$	Monthly Average	25	NYS DEC
$CBOD_5$	7-day arithmetic average	40	NYS DEC
BOD_5	Monthly/30-day Average	30	IEC*
BOD_5	7-day arithmetic average	45	IEC*
BOD_5	6 consecutive hour average	50	IEC
TSS	Monthly/30-day Average	30	NYS DEC/IEC
TSS	7-day arithmetic average	45	NYS DEC and IEC
TSS	Daily Maximum**	50	NYS DEC
TSS	6 consecutive hour average	50	IEC

* Not directly listed in the permit, but is incorporated by reference in the permit.

** According to the permit, during periods of wet weather, which results in an instantaneous WWTP influent flow that exceeds twice the permitted flow, the TSS Daily Maximum limit of 50 mg/l shall neither apply for the day of measured flow nor for the succeeding day.

Chapter 2. Project Summary

The summary of all completed sampling events is presented in Table 2.

Table 2 - Sampling Events: Completed / Planned

WWTP	Permit Flow [2]	Dry-Weather Events	Wet-Weather Blending Events
1	120 MGD	1/1	4/4
2	60 MGD	1/1	4/4
3	275 MGD	2/1	4/4
Total:		**4/3**	**12/12**

The breakdown of the number of collected effluent samples as compared to the planned number of samples is shown in Table 3 and Table 4.

Table 3 – Dry-Weather Effluent Samples: Collected / Planned

WWTP	Bacteria	Macerated Effluent Samples	*Giardia*	*Crypto*	Infectious *Crypto*	Virus
1	3/3	3/2	3/3	3/3	3/3	3/3
2	3/3	2/2	3/3	3/3	3/3	3/3
3	6/3	3/2	---	---	---	---
Total:	**12/9**	**8/6**	**6/6**	**6/6**	**6/6**	**6/6**

Table 4 - Wet-Weather Effluent Samples: Collected / Planned

WWTP	Bacteria	Macerated Effluent Samples	*Giardia*	*Crypto*	Infectious *Crypto*	Virus
1	15/12	9/8	12/9	12/9	12/9	9/9
2	10/12	8/8	7/9	7/9	7/9	7/9
3	12/12	8/8	---	---	---	---
Total:	**37/36**	**25/24**	**19/18**	**19/18**	**19/18**	**16/18**

[2] Permit Flow is a 12-month rolling average (average of the current month with the eleven previous months) flow rate limit for the WWTP. The 12-month rolling average is calculated using total influent flow. During wet-weather, the NYC DEP WWTPs can typically handle 2 x Permit Flow. For NYC DEP WWTPs, permit flow typically equals the design flow rate.

The difference between the collected and planned number of samples presented in Tables 2 and 3 is due to the fact that there were two additional WWTP visits (an extra dry-weather run at WWTP 3 and an extra wet-weather blending run at WWTP 1) to make up for the portion of the samples lost during the preceding sampling events. There were also two occasions when the IEC's field staff had to interrupt sampling and, therefore, missed the last of the three wet-weather time-variable sample collections at WWTP 2 because blending stopped (first occasion) and due to a hazardous flooding condition (second occasion).

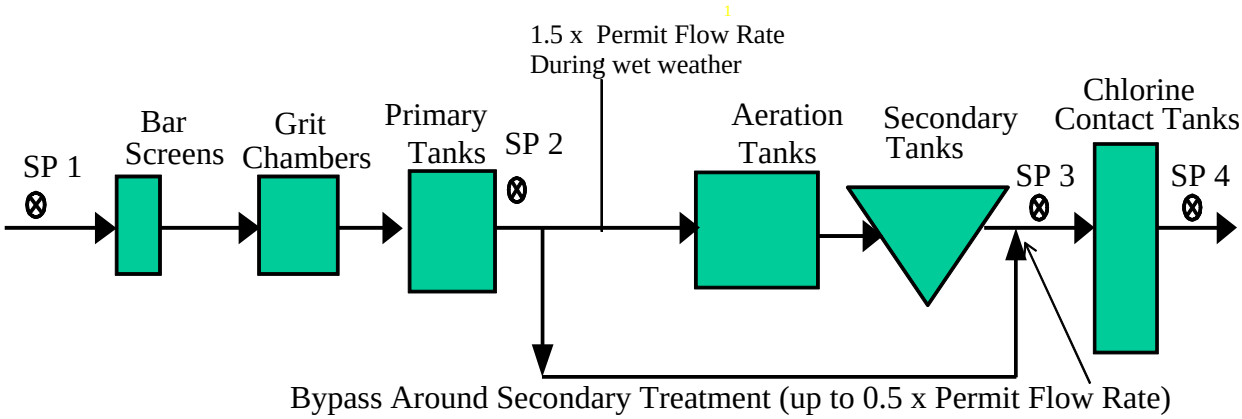
More detailed description of the number and type of samples that were targeted for collection throughout the entire WWTP, not just the effluent portion of it, is presented in Chapter 3.

Chapter 3. Project Design

3.1 Bacteria Sampling

During each sampling event, IEC field staff collected three grab influent (SP1, prior to any treatment), three grab primary effluent (SP2), three grab pre-chlorinated effluent (SP3, prior to chlorine contact tank) and three grab effluent (SP4, post chlorination) samples at 45-minute intervals. (See Figure 2 for sampling locations). The samples were analyzed at the IEC laboratory for fecal coliform (SM, 20th Edition: Method 9221 A, B, C & D) and *Enterococcus* (SM, 20th Edition: 9230 A & B) using the most probable number (MPN) method. There were a total of five sampling events per WWTP with at least one dry-weather (non-blending) event and four wet-weather (blending) events. The dry-weather event was used for comparison purposes. The dry-weather event, by definition, took place on a day when there was no precipitation and no precipitation during the preceding 48 hours. During wet-weather events, all samples were collected at peak flows, after WWTPs started bypassing secondary treatment.

IEC had also considered sampling a non-blending wet-weather event, for comparison purposes. This option was not added to the scope of work because of financial constraints. However, IEC analyzed available historical effluent data to derive additional comparative information about the impact of non-blending wet-weather events on the fecal coliform effluent concentration (Section 4.6 of the report).



Notes:

1) *Permit FlowRate = 12 Month Rolling Average Flow*
2) *SP1, SP2, SP3, SP4 – IEC Sampling Location*

Figure 2. WWTP Wet-Weather Blended and Dry-Weather Non-blended Sampling Locations

At each of the WWTPs, sampling for the wet-weather events occurred during precipitation of at least 0.25 inches of rain or higher that was heavy enough to cause a portion of the flow to bypass the secondary treatment (for New York City WWTPs, this typically happens after the influent flow exceeds one and a half times the permit flow limit for that WWTP). The sampling summary is in Table 5 below.

Table 5 – Sampling Summary

Event	WWTP 1			WWTP 2			WWTP 3		
	Bacteria	WWTP Macer-d Effluent Samples	Protozoa/ Virus	Bacteria	WWTP Macer-d Effluent Samples	Protozoa/ Virus	Bacteria	Macer-d Effluent Samples	Protozoa/ Virus
Wet 1	X	X + O	X						--
Wet 2	X	X		X	X	X			--
Wet 3	X	X	X	X	X		X	X	--
Wet 4				X	X	X	X	X	--
Wet 5	X	X	X				X	X	--
Wet 6				X	X	X	X	X	--
Dry 1	X	X + O	X						--
Dry 2				X	X	X	X	X	--

Notes: X – Sampling event
O - Maceration Optimization Procedure (see Section 3.3)

3.2 Protozoa and Virus Sampling

In addition to the bacteria sampling at the WWTPs, IEC performed four sampling events at two WWTPs (three during wet-weather blending and one during dry weather) for protozoa (*Giardia* and *Cryptosporidium*), virus (adenovirus, astrovirus, enterovirus, reovirus, rotavirus, norovirus and Hepatitis A) and male-specific coliphage. Due to budget constraints, only two of the three New York City WWTPs – WWTP 1 and WWTP 2 – that were used for the bacteria portion of the study were selected for the protozoa, virus and male-specific coliphage sampling.

Each sampling event at one WWTP was comprised of ten grab samples for protozoa analyses and ten grab samples for viral analyses. The protozoan sampling consisted of three grab influent (SP1, prior to any treatment), one primary effluent (SP2), three grab pre-chlorinated effluent (SP3, prior to chlorine contact tank) and three grab effluent (SP4, post chlorination) samples collected at 45-minute intervals.[3] Viral sampling during the same event included a total of ten samples - three grab influent (SP1, prior to any treatment), one primary effluent (SP2), three grab pre-chlorinated effluent (SP3, prior to chlorine contact tank) and three grab effluent (SP4, post chlorination) samples collected at 45-minute intervals.

After the completion of each sampling run, IEC delivered the protozoa samples to the EPA Region 2 Division of Environmental Science and Assessment (DESA) Laboratory located in Edison, NJ, where analyses for *Giardia* and *Cryptosporidium* were performed. On one occasion when the EPA DESA Laboratory had other work commitments, a contractual laboratory, Biological Consulting Services (BCS), was used for protozoan analyses.

[3] One additional influent sample was also collected to be used as a matrix spike for QA/QC purposes.

For the protozoan portion of the study, the EPA DESA Laboratory detected and enumerated *Giardia* and *Cryptosporidium* using EPA Method 1623. Since this method provides for quantification of *Giardia* and *Cryptosporidium*, but does not allow for viability or infectivity determination, the laboratory split the sample after processing.

Ten split samples per WWTP were sent out to BCS Laboratories to perform infectivity analyses for *Cryptosporidium* using a published method with some modification (Slifko et al., 1999; Rochelle et al., 2002). Following infectivity determination, molecular confirmation of the genotype was ascertained by PCR analysis for all samples, except for the positive control (Quintero-Betancourt et al., 2003).

For the virus portion of the study, ten virus samples per WWTP were also shipped to BCS for further analyses. BCS performed quantitative viral analyses on all of the aforementioned samples using EPA ICR Methodology (EPA/600/R-95/178).

In addition, BCS performed analyses for the detection and quantification of male-specific and somatic coliphage on all samples using a soft agar overlay procedure (Snustad and Dean, 1971).

3.3 Maceration

Maceration breaks apart particles, thereby exposing the particle-associated, occluded, and aggregated bacteria to the water column. The study consisted of two parts: 1) Maceration optimization, described below, and 2) Maceration of final effluent samples (SP4) to determine if maceration had any impact on the results described in Section 4.10.

Maceration Optimization

The optimization procedure was used for the disinfected final effluent samples (SP4) prior to analyses for fecal coliform and *Enterococcus*.

The maceration optimization procedure was conducted during one wet-weather blending event and one dry-weather event at WWTP 1 to determine the optimum speed (revolutions per minute [rpm]) and time (seconds [s]) of the multi-speed (3,500-22,000 rpm) laboratory blender[4] that was used for maceration of effluent samples during the subsequent wet- and dry-weather runs. Based on the assumptions and results of the previous studies conducted by the EPA, the optimum speed and time corresponds to the largest increase in the concentration of bacteria (*Enterococcus* and fecal coliform) of the macerated sample compared to the corresponding unmacerated sample.

Procedure Description: During the dry-period and wet-weather blending event, one final effluent sample collected from the SP4 location and the eleven replicates of this sample – a total of 12 100 ml samples – were macerated at four different speeds (3500 rpm, 7000 rpm, 14,500 rpm, and 22,000 rpm) and at three different time intervals (30s, 60s, and 90s) in an autoclaved Waring blender (see Table 6, below).

[4] TBD Model No. 7012S/7012G, Waring Products, New Hartford, Connecticut

Since none of the three WWTPs involved in the study dechlorinate their effluent, SP4 samples were dechlorinated by IEC field staff (using sodium thiosulfate) immediately upon collection. The maceration was conducted in the IEC laboratory before inoculation and within a 6-hour holding time after a sample was collected.

This optimization procedure was subsequently repeated for two additional final effluent samples taken at 45-minute intervals, to ensure replicability.

Table 6 - Number of Analyses for Maceration Optimization

Time/Speed	3,500 rpm	7,000 rpm	14,500 rpm	22,000 rpm
30 s	3	3	3	3
60 s	3	3	3	3
90 s	3	3	3	3
Total Macerated Analyses:				36

In summary, the project team selected 36 dry-weather and 36 wet-weather macerated analyses to be performed for fecal coliform and the same number of analyses (36 dry- and 36 wet-weather analyses) to be performed for *Enterococcus.* Each of the three final effluent samples involved in the experiment was analyzed as a regular unmacerated sample for comparison purposes.

Following the completion of the analyses, the optimum speed and time for wet-weather blended and dry-weather samples was selected based on the largest increase in the bacterial concentration of the macerated sample compared to the corresponding unmacerated sample. This selection was done based on analysis of optimization results for each of these three individual effluent samples and their geometric means. Selected optimization parameters—speed of 22,000 rpm and time of 60 s—were then used for fecal coliform and *Enterococcus* analyses of macerated samples during subsequent dry- and wet-weather blended events.

Chapter 4. Discussion of Project Results

4.1 Analyses of Historical WWTP Performance

NYC DEP's monitoring reports were examined for all three WWTPs for the 12-month period from May 2006 to April 2007 (Tables 7, 8 and 9). Over that period of time, all three WWTPs showed very good operational performance with monthly average effluent values consistently below 20 mg/l and frequently in single digits for both $CBOD_5$ and TSS parameters. Except for one instance at WWTP 3, percent removal[5] was consistently above 85% for both $CBOD_5$ and TSS parameters at all three WWTPs. Monthly geometric mean values for fecal coliform were in double digits for WWTP 2 and WWTP 3 and ranged from double digits to low triple digits for WWTP 1, consistent with dry-weather results obtained in this study.

The project team also looked at the results of IEC's compliance monitoring inspections conducted at all three WWTPs over the last two years. During that period of time, IEC carried out four unannounced inspections at each of the three WWTPs, i.e., a total of 12 inspections. Most of these inspections took place during dry weather, at which time both WWTP 1 and WWTP 2 were in compliance with their respective permit; only one out of six hourly samples[6] for fecal coliform at WWTP 3 (also collected during dry weather) was in violation during one of the inspections. However, when IEC inspected WWTP 1 on August 18, 2006, the WWTP began blending and five out of six hourly samples collected by IEC on the same day were above the permit limit,[7] (i.e., >16,000; 3,000; 5,000; >16,000; 5,000) with geometric average of >5,300 MPN/100 ml, consistent with wet-weather results of this study.

[5] As mentioned in Section 1.3, 30-day percent removal for $CBOD_5$ and TSS are the only permit parameters that are calculated without including wet-weather data.
[6] All six hourly samples were collected on the same day.
[7] The permit contains, among other parameters, IEC's fecal coliform limits of instantaneous maximum of 2,400 No/100 ml and 6-hour geometric mean of 800 No/100 ml.

Table 7. Average Monthly Effluent TSS and % Removal

Month/ Year	WWTP 1		WWTP 2		WWTP 3	
	TSS (mg/l)	Removal (%)	TSS (mg/l)	Removal (%)	TSS (mg/l)	Removal (%)
May 2006	14	94	6	96	8	94
Jun 2006	16	95	5	97	8	95
Jul 2006	14	94	4	97	7	94
Aug 2006	11	96	4	97	6	94
Sep 2006	18	93	4	98	9	94
Oct 2006	16	93	4	97	7	94
Nov 2006	17	92	6	96	10	93
Dec 2006	13	94	4	97	11	90
Jan 2007	10	94	4	97	13	88
Feb 2007	9	95	5	97	18	83
Mar 2007	9	95	7	96	14	86
Apr 2007	15	92	6	97	16	89
AVERAGE	**14**	**94**	**5**	**97**	**11**	**91**

Table 8. Average Monthly Effluent $CBOD_5$ and % Removal

Month/ Year	WWTP 1		WWTP 2		WWTP 3	
	$CBOD_5$ (mg/l)	Removal (%)	$CBOD_5$ (mg/l)	Removal (%)	$CBOD_5$ (mg/l)	Removal (%)
May 2006	9	95	5	96	5	96
Jun 2006	8	96	5	96	6	95
Jul 2006	12	93	5	96	5	95
Aug 2006	9	96	6	96	5	95
Sep 2006	14	93	5	96	5	96
Oct 2006	13	94	15	89	5	96
Nov 2006	15	92	5	95	6	96
Dec 2006	12	94	5	95	7	93
Jan 2007	8	96	5	95	8	92
Feb 2007	7	96	5	95	11	89
Mar 2007	8	95	6	<95	7	92
Apr 2007	12	<93	5	<96	9	92
AVERAGE	**11**	**94**	**6**	**95**	**7**	**94**

Table 9. 30-Day Geometric Mean Monthly Effluent Fecal Coliform Values [8] (MPN/per 100 ml)

Month/Year	WWTP 1		WWTP 2		WWTP 3	
	Geom. Mean	Highest Daily Value (during monthly period)	Geom. Mean	Highest Daily Value (30-Day Period)	Geom. Mean	Highest Daily Value (30-Day Period)
May 2006	25	246	4	540	27	640
Jun 2006	52	224	6	800	21	440
Jul 2006	89	480	4	4000	27	4000
Aug 2006	52	544	4	1000	6	600
Sep 2006	133	1200	4	400	9	300
Oct 2006	112	4000	6	400	19	260
Nov 2006	42	800	63	4000	27	278
Dec 2006	30	240	11	4000	85	680
Jan 2007	27	355	20	4000	85	4000
Feb 2007	53	324	14	52	45	600
Mar 2007	83	415	13	4000	67	4000
Apr 2007	80	4000	23	4000	74	2640

4.2 BOD_5 and TSS Results

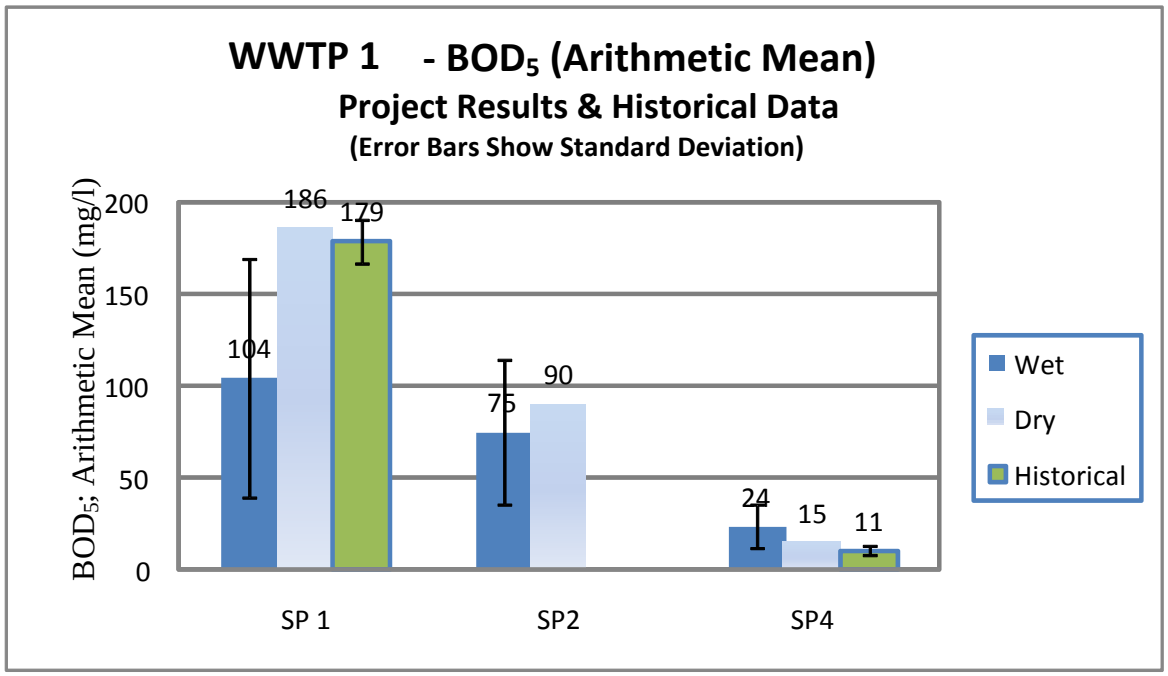
Analyses of BOD_5 and TSS were performed in the IEC's Laboratory using the Standard Methods SM 5210 B and SM 2540 D, respectively. The study results indicate that during blending, effluent BOD_5 [9] and TSS concentrations at WWTP 1 and WWTP 2 were, on average, below 30 mg/l, and effluent BOD_5 and TSS concentrations at WWTP 3 were, on average, above 30 mg/l; the arithmetic averages for both parameters (See Figure 3 and Table 10) were calculated based on five composite samples (one per sampling event) at WWTP 1, and four composite samples at both WWTP 2 and WWTP 3. The comparison of standard deviations from historical data vs. project wet-weather results (Table 7 & 8) shown in Table 10 indicates that these standard deviations are comparable, with the standard deviation being higher for the project wet-weather results vs. the historical ones (historical results included a combination of both dry- and wet-weather data).

Table 10. Comparison of Standard Deviations for Effluent BOD_5 and TSS

WWTP	Standard Deviation BOD_5 (mg/l)		Standard Deviation TSS (mg/l)	
	Historical	Project Wet-Weather	Historical	Project Wet-Weather
1	2.7	12.0	3.1	4.7
2	2.9	9.6	1.1	5.9
3	1.9	7.1	3.9	8.6

[8] Permits for NYC DEP WWTPs include effluent limit for fecal coliform of 30-day geometric mean of 200 No/100ml; 7-day geometric mean of 400 No/100 ml. They also include IEC limitations of 6-hour geometric mean of 800 No/100 ml; and instantaneous maximum of 2,400 No/100 ml.

[9] BOD_5 is an effluent criteria used by IEC, as opposed to $CBOD_5$, a criteria by the New York State Department of Environmental Conservation, a state regulatory agency that issues the permits for NYS WWTPs – See Table 1.



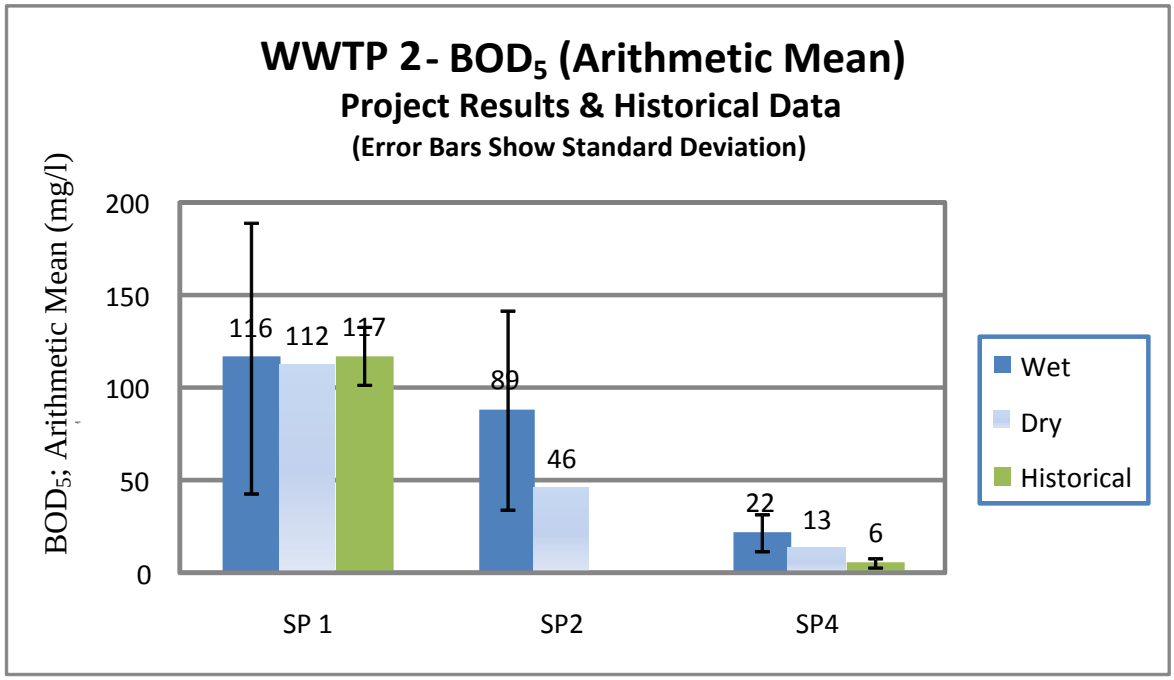
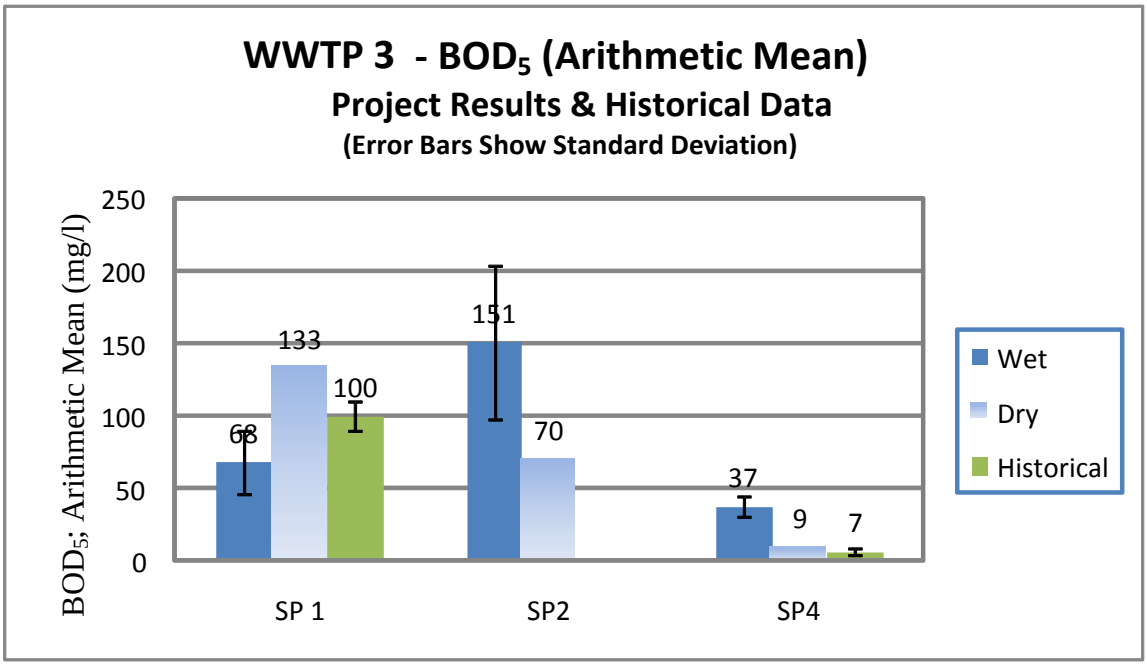


Figure 3. BOD_5 and TSS Results with Error Bars[10]

[10]Error bars were calculated using standard deviation. No error bars shown for dry-weather results, since there were only one or two dry-weather runs consisting of composite samples during the study.



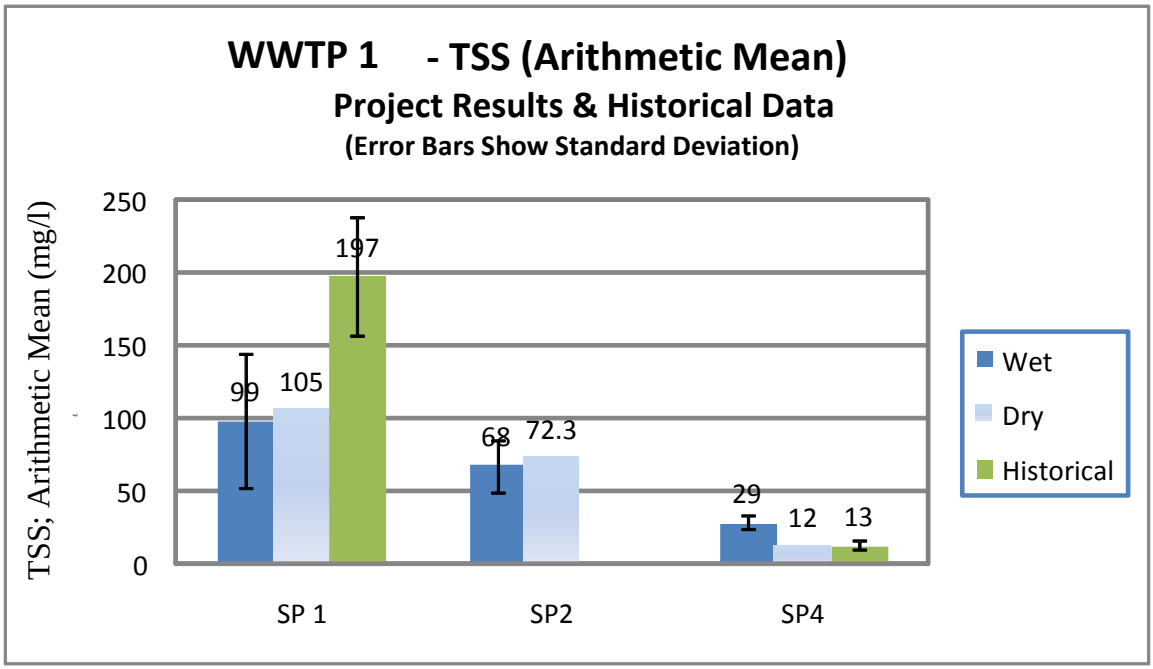
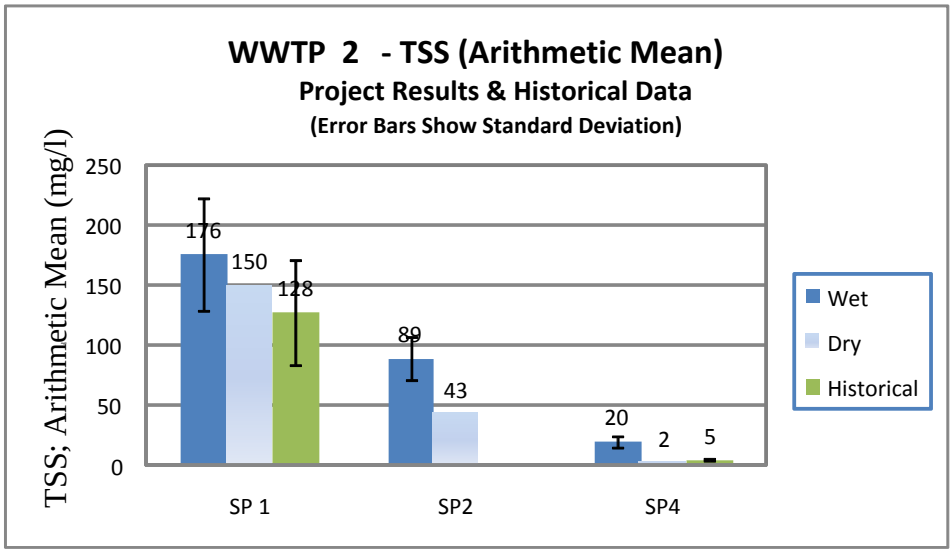


Figure 3. BOD_5 and TSS Results (cont.)



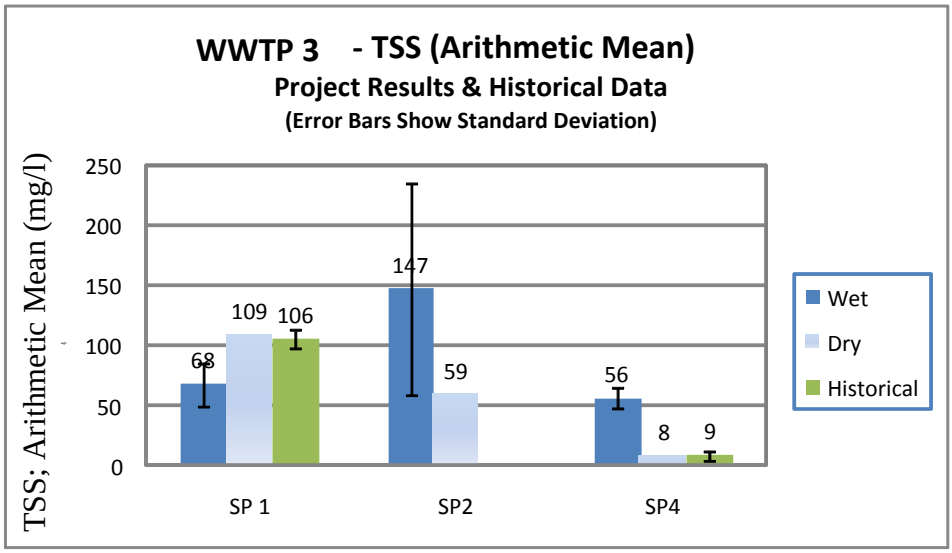


Figure 3. BOD_5 and TSS Results (cont.)

BOD_5 and TSS removal during blending remained, on average, above 80% at WWTP 2, above 70% at WWTP 1 and showed a greater decline at WWTP 3 where it was 45% for BOD_5 and 17% for TSS (Table 11).

Both BOD_5 and TSS effluent concentrations, and percent removal for WWTP 3, were of lower quality than those of WWTP 1 and WWTP 2 during the wet-weather events. IEC staff looked into the potential reasons for this, and discovered that during the time of the sampling, a major upgrade was being performed at WWTP 3.

Specifically at WWTP 3, at various times during the project, as much as 3 out of 13 aeration tanks and 8 out of 39 final tanks were out of service for the upgrade. This reduction of capacity at the WWTP could have had an adverse impact on the treatment quality, especially during

blending at peak wet-weather flows. Therefore, the results of sampling at WWTP 3 should be interpreted in this context.

Additional breakdown of percent removal for primary and secondary treatment for WWTP 1 and WWTP 2 during both dry-weather and wet-weather sampling is shown in Table 12. Table 12 demonstrates why during wet-weather blending total percent removal values for both WWTPs were slightly below the expected average of 85% (except for TSS at WWTP 2). The possible limiting factor was the percent removal in the primary treatment portion of the WWTPs. For primary treatment during wet-weather conditions, the percent removal values for BOD_5 at WWTP 2 and TSS at WWTP 1 were slightly below the typical range of removals of 25-40% for BOD_5 and of 50-60% for TSS during standard primary treatment operations. [11]

Table 11. BOD_5 and TSS Total Percent Removal [12]

WWTP	BOD_5 - Dry Weather (%)	BOD_5 - Wet Blending (%)	TSS - Dry Weather (%)	TSS - Wet Blending (%)
1	92	77	89	71
2	88	81	99	89
3	93	45	93	17

Table 12. BOD_5 and TSS Percent Removal for Primary and Secondary Treatment

WWTP	Parameter	Operating Mode	Primary Treatment (%)	Secondary Treatment (%)	Total WWTP (%)
1	BOD_5	Dry Weather	52	83	92
		Wet Weather	28	68	77
	TSS	Dry Weather	31	83	89
		Wet Weather	31	57	71
2	BOD_5	Dry Weather	59	72	88
		Wet Weather	23	75	81
	TSS	Dry Weather	71	95	99
		Wet Weather	49	78	89

The percent removals for Table 12 are calculated by the equations below for both BOD_5 and TSS. For the wet-weather events, SP4 concentrations include the secondary influent that also already received the blended primary effluent.

[11] Tchobanoglous et al, 1991 and Peavy et al., 1985

[12] Table 11 shows percent removal for the samples collected by IEC for this project, as opposed to Table 7 and Table 8 that include the data collected by NYC DEP as part of its permit monitoring requirements.

% Removal for Primary Treatment	=	(SP1-SP2) (100) / SP1
% Removal for Secondary Treatment	=	(SP2-SP4) (100)/ SP2
% Removal for Total WWTP	=	(SP1-SP4) (100)/ SP1

4.3 Bacteria Sampling Results

For both fecal coliform and *Enterococcus* at WWTP 3 and for fecal coliform at WWTP 1, the difference between wet-weather blending and dry-weather effluent concentrations was between a half and one order of magnitude. Effluent fecal coliform and *Enterococcus* levels were three orders of magnitude higher during wet-weather blending vs. dry-weather for both parameters at WWTP 2 and for *Enterococcus* at WWTP 1.

It is worth noting that wet-weather blending effluent concentrations were higher than the corresponding dry-weather effluent concentrations for both fecal coliform and *Enterococcus* at all three WWTPs. In addition, the order of magnitude reduction between influent and effluent (the "kill") was at least two orders of magnitude stronger in most (five out of six) cases at all three WWTPs during dry weather as compared to wet-weather blending (Table 13). Both phenomena can be explained, in part by an increase in hydraulic load, partial treatment, and reduction in chlorine contact time at subject WWTPs during wet-weather blending.

All results of quality control samples analyzed for both fecal coliform and *Enterococcus*, during the timeframe of the blending project, fell within the manufacturer-determined acceptance ranges. The average percent recoveries of the quality control samples run during the timeframe of the blending project were calculated; the fecal coliform quality control samples averaged 83% and the *Enterococcus* quality control samples averaged 139%. [13]

[13] These acceptance ranges are determined from interlaboratory studies. Though 139% is greater than 100%, but in the world of bacteriology (especially for MPN results) it is still considered within the acceptance limits.

Table 13. Fecal Coliform and *Enterococcus* Concentrations

WWTP	Average Flow (MGD)	Operating Mode	Fecal Coliform –Geometric Mean (MPN/100ml)			*Enterococcus* –Geometric Mean (MPN/100ml)		
			Influent	Effluent	Order of Magnitude Reduction	Influent	Effluent	Order of Magnitude Reduction
1	239	Wet Blending	4,200,000	4,900	10^3	890,000	17,000	10^2
	122	Dry	19,000,000	890	10^4	470,000	20	10^4
2	125	Wet Blending	1,100,000	19,000	10^2	260,000	14,000	10
	31	Dry	5,000,000	16	10^5	220,000	3	10^5
3	469	Wet Blending	1,600,000	520	10^3	280,000	870	10^3
	238.5	Dry	5,600,000	31	10^5	1,700,000	120	10^4

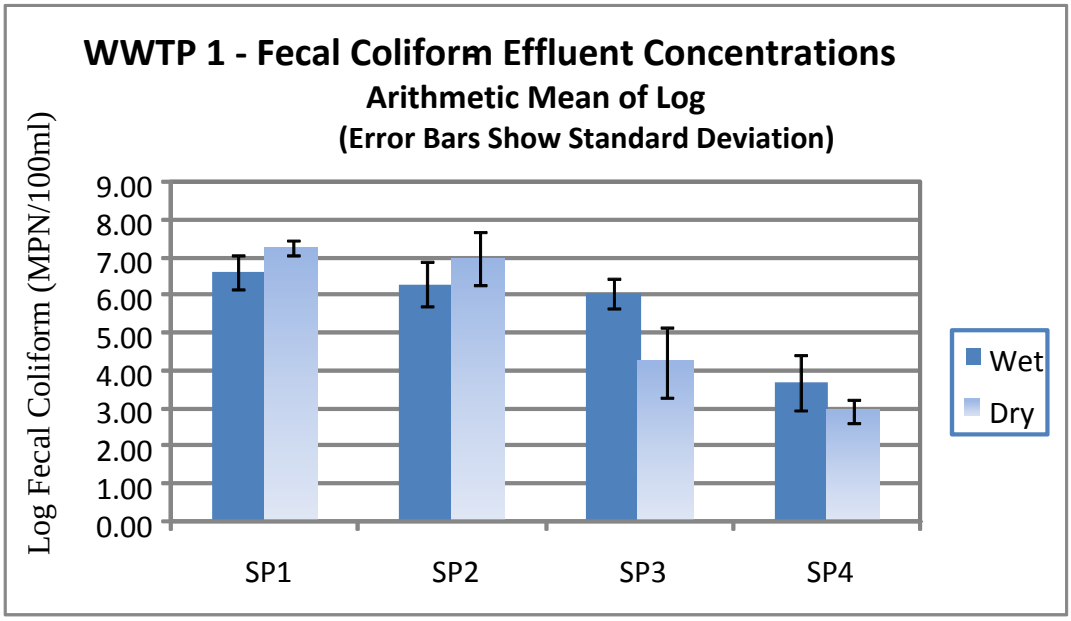
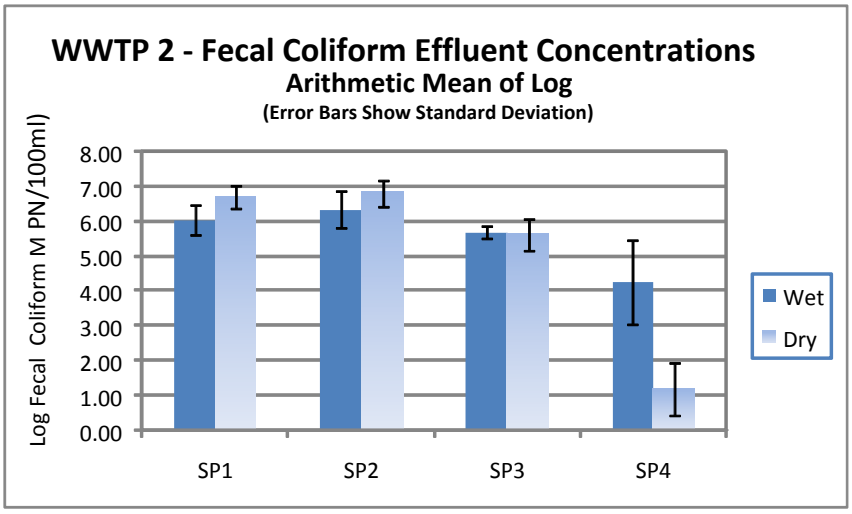
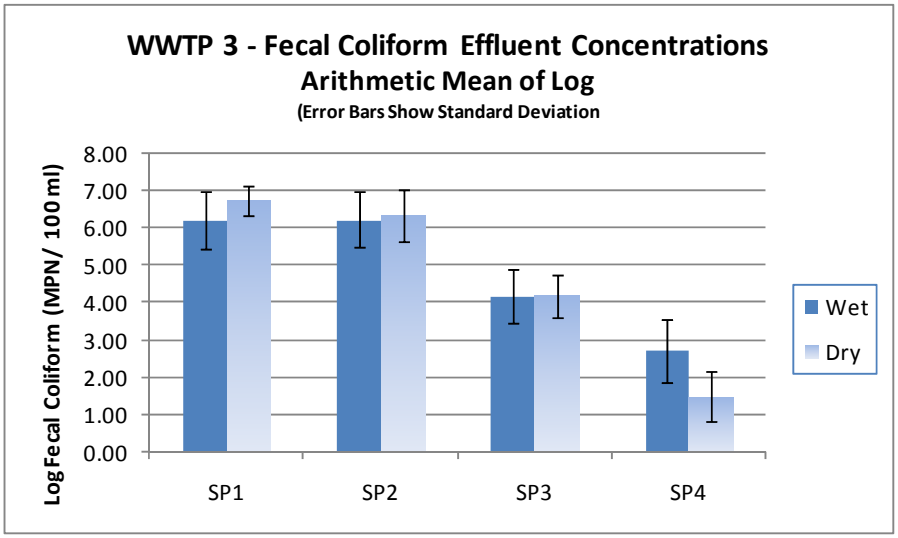


Figure 4. Fecal Coliform and *Enterococcus* Effluent Concentrations





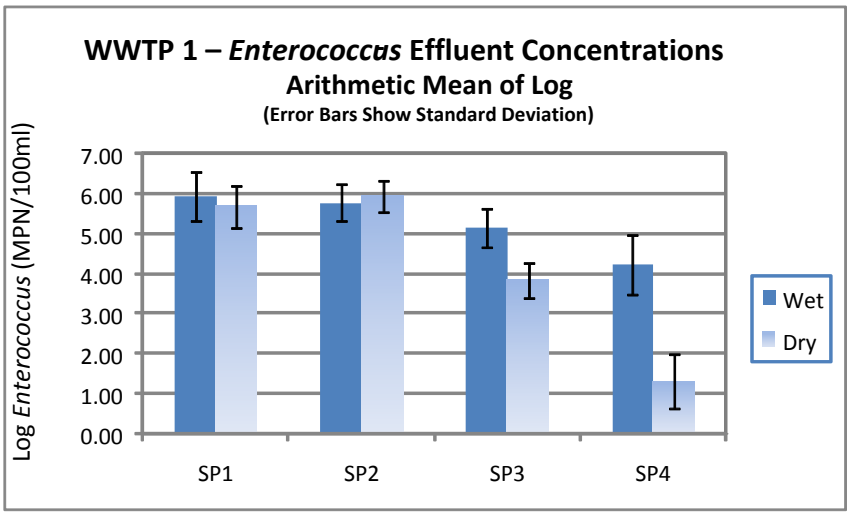
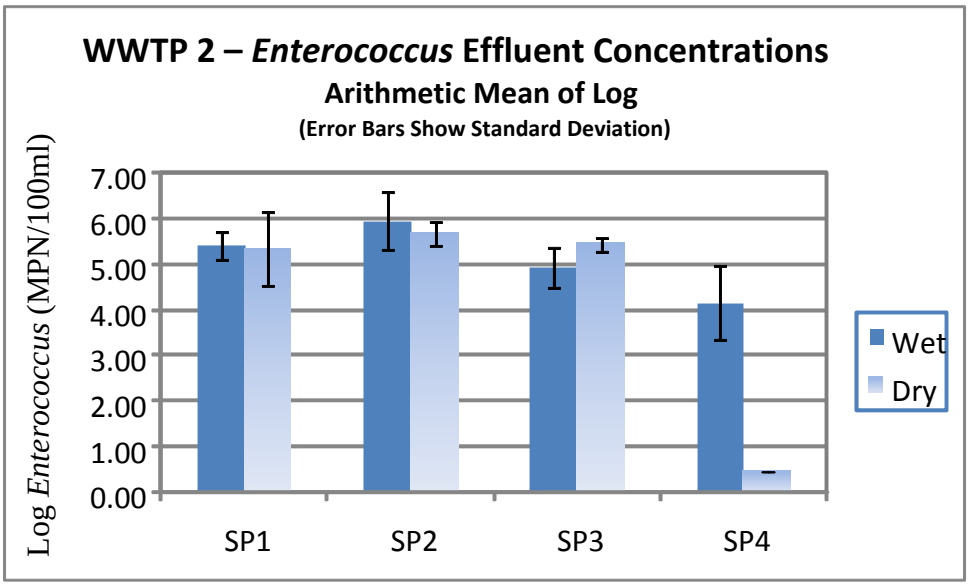


Figure 4. Fecal Coliform and *Enterococcus* Effluent Concentrations (cont.)



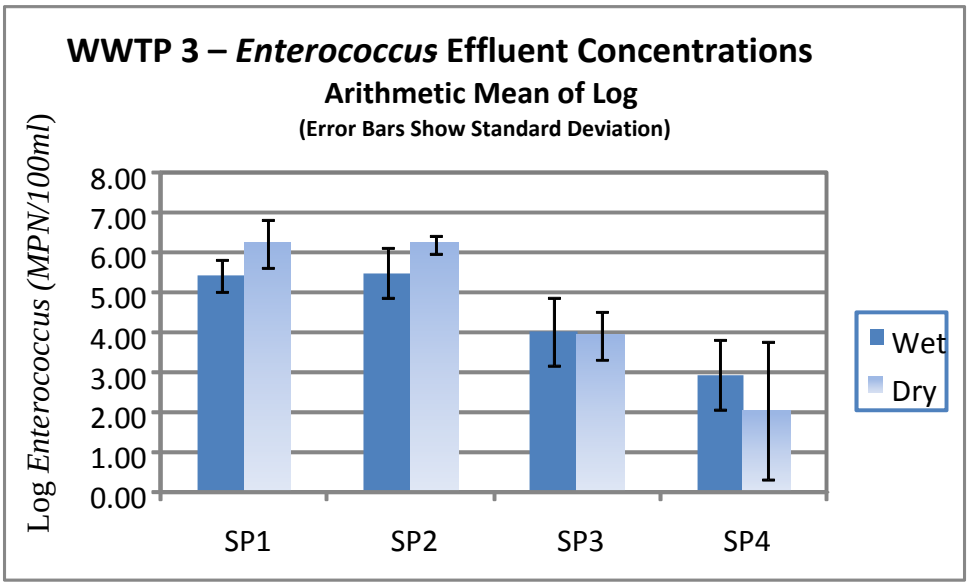


Figure 4. Fecal Coliform and *Enterococcus* Effluent Concentrations (cont.)

4.4 Blending Flow Ratio

The project team collected data for the WWTP influent flow rates at all three WWTPs during both dry- and wet-weather blending sampling events. At WWTP 1, during wet-weather, the flow rate through the secondary treatment system was recorded. While the exact flow rate through the secondary treatment system was not recorded for WWTP 2 or WWTP 3 because they did not have the necessary flowmeters, both of these WWTPs started blending at approximately 1.5 Permit Flow, i.e. at 90 MGD and 412.5 MGD, respectively.

A summary of the flow rate data is provided in Appendix C.

This information allowed the project team to estimate the blending ratio, i.e., the ratio of a flow bypassing secondary treatment to influent flow for all three WWTPs. At WWTP 1, this ratio ranged from 9% to 29%, with an average value of 22%. This was in line with the usual 25% ratio for blending at the NYC DEP WWTPs, i.e.:

Blending Ratio= Flow Bypassing Secondary Treatment / Target WWTP Wet-Weather Influent Flow

For a NYC DEP WWTP accepting a maximum of 2 x Permit Flow, the ratio is:

Blending Ratio = 0.5 **x** Permit Flow / 2 **x** Permit Flow = 25% (see Figure 2, page 9)

Using the estimated 1.5 x Permit Flow values as the threshold for the beginning of the bypass of secondary treatment for both WWTP 2 and WWTP 3, the project team approximated 29% as the average blending ratio for WWTP 2 and 11% as the average blending ratio for WWTP 3. This is important, since the higher blending ratio results in higher flow bypassing secondary treatment, and could potentially lead to lower effluent quality. These results could also partially explain why fecal coliform and *Enterococcus* results for WWTP 3, which had the lowest average blending ratio (11%), were less affected during wet-weather blending (see Section 4.3).

4.5 Total Residual Chlorine (TRC) Results

There was no correlation between TRC vs. fecal coliform and/or plant flow (using both multiple and two-variable regressions).

Chlorine residual concentrations were, on average, lower during wet-weather blending vs. dry weather at WWTP 2 and higher at WWTP 1 and WWTP 3 (Figure 5). While not measured by the project team, the contact times were shorter during blending events due to higher flow rates.

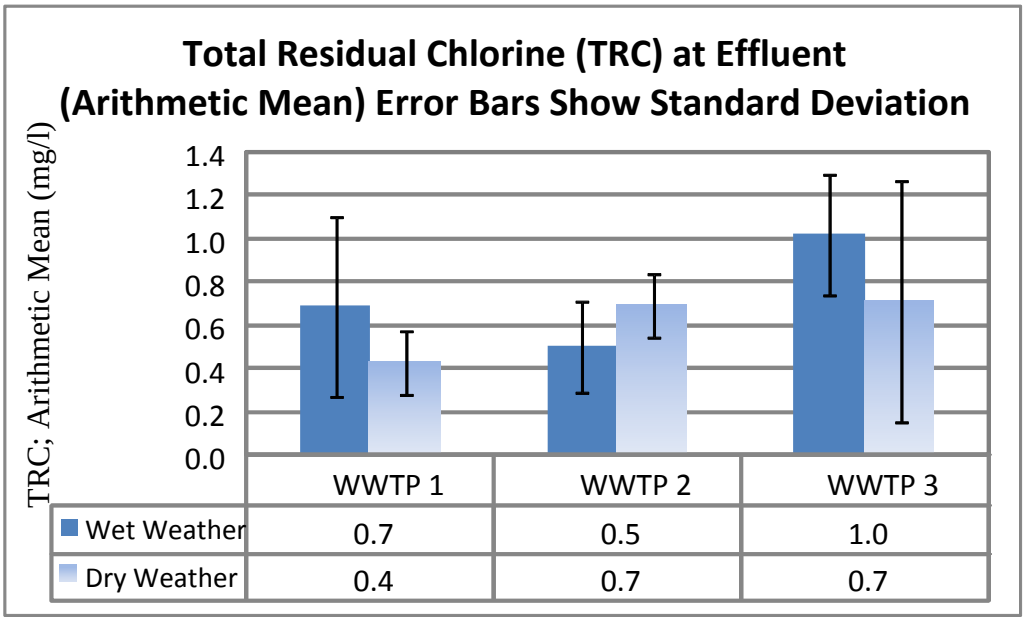


Figure 5. Total Residual Chlorine at Effluent

As background information, all three WWTPs have chlorine contact tanks and do not utilize a dechlorination process following chlorination.

For TRC measurement, the project field staff used IEC's standard operating procedure based on Hach Method 8167 and SM 18-20 4500-Cl G, which is a colorimetric version of the N, N-diethyl-p-phenylenediamine (DPD) method. The method is based on the reaction of DPD with chlorine to produce red color. The intensity of the color produced is proportional to the concentration of total chlorine in the sample. The Hach Pocket Colorimeter instrument was calibrated according to manufacturer's instructions to measure the total chlorine content in aqueous samples from 0.1 mg/l to 2.00 mg/l; samples in excess of the higher detection limit are diluted as appropriate.

The chlorine contact time was not directly measured during sampling. However, for WWTP 1, the chlorine contact time was later estimated using the WWTP flow rate values collected during sampling, as follows:

Contact Time (min) = Total Volume of Chlorine Contact Tanks / Flow Rate

The average contact time values are summarized in Table 14; individual contact time values are shown in Appendix C.

Table 14. Chlorine Contact Time, WWTP 1

Operating Mode	Estimated Average Chlorine Contact Time (min)	Range (min)
Dry weather	29.4	25.8 – 32.1
Wet weather	15.1	14.3 – 20.8

4.6 Analysis of Existing Monitoring Data – Wet-Weather Non-Blending Events

Though contracted to this research project, IEC's principal function was to conduct regular 6-hour inspections at NYC DEP treatment WWTPs to check compliance with both the NPDES permit requirements and IEC's Water Quality Regulations.

Examination of IEC monitoring data for the period 2001-2007 for the three WWTPs revealed two mixed wet-weather events (each including both blending and non-blending samples) and a partial wet-weather non-blending event with very low ("trace", i.e., less than 0.01 in.) precipitation at WWTP 1. Linear regression analysis of the fecal coliform vs. flow rate data for these three events (a total of 18 data points; 6 data points per event), showed a trend of moderate magnitude with R^2= 0.46 (Figure 6 and Table 15). This demonstrates that with increase in flow rate, the values of effluent results for fecal coliform also show an upward trend.

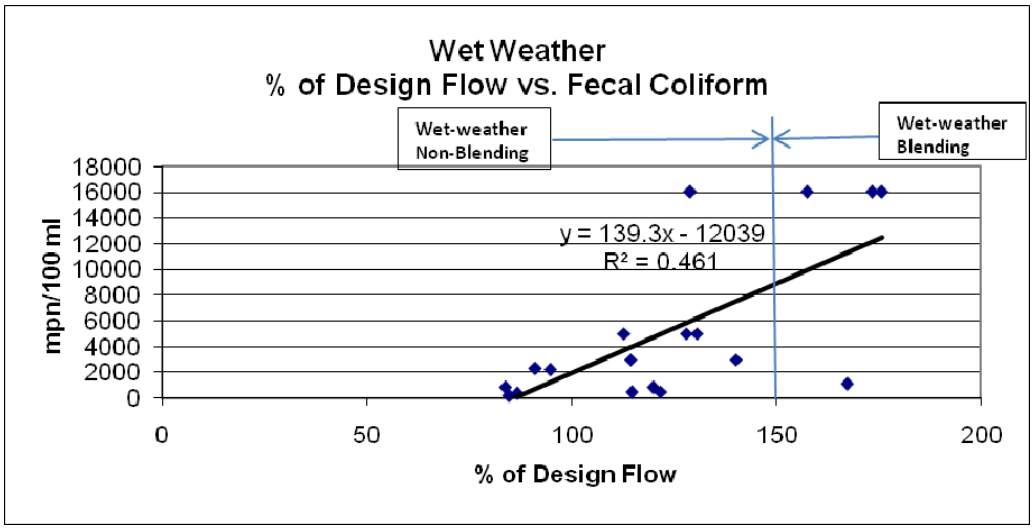


Figure 6. Historical IEC Wet-Weather Results – Percent of Design Flow vs. Fecal Coliform Count

Table 15. Historical IEC Wet-Weather Results (Non-Blending and Blending) (fecal coliform, MPN/ per 100 ml)

WWTP	Date	Flow [14]	% of Design Flow	Fecal Coliform	TRC (mg/l)	Daily Precipitation (in.) [15]
1	2/5/2001	102	85	170	0.9	0.5
1	2/5/2001	104	87	340	0.7	
1	2/5/2001	144	120	800	0.5	
1	2/5/2001	146	122	500	0.7	
1	**2/5/2001**	**211**	**176**	**16,000**	**0.7**	
1	**2/5/2001**	**208**	**173**	**16,000**	**0.7**	
1	5/18/2004	135	113	5,000	0.8	Trace
1	5/18/2004	137	115	3,000	0.8	
1	5/18/2004	138	115	450	0.9	
1	5/18/2004	114	95	2,200	0.8	
1	5/18/2004	109	91	2,300	0.8	
1	5/18/2004	101	84	800	0.7	
1	**8/28/2006**	**201**	**167**	**1,100**	**0.7**	0.35
1	**8/28/2006**	**189**	**158**	**16,000**	**0.7**	
1	8/28/2006	168	140	3,000	0.8	
1	8/28/2006	154	128	5,000	0.8	
1	8/28/2006	155	129	16,000	0.8	
1	8/28/2006	157	131	5,000	0.7	

4.7 Protozoa Results

Effluent concentrations of *Cryptosporidium* were higher during wet-weather blending at one WWTP (WWTP 1) when compared to dry weather. The *Cryptosporidium* effluent results during wet weather were mostly in single or low double digits, with an average percent removal of 71% at WWTP 1. Average percent removal for WWTP 2 could not be estimated, since less than three detectable results were reported (Figure 7 and Table 16).

Infectious *Cryptosporidium* values at both WWTPs were mostly low or non-detectable. Only two out of nineteen infectious *Cryptosporidium* effluent samples showed a detectable value; only one of these two samples showed the presence of *C. Parvum* Genotype II.

Effluent values of *Giardia spp.* were one order of magnitude higher during wet-weather blending vs. dry weather at both WWTP 1 and WWTP 2. The geometric mean of *Giardia* effluent results during wet weather were in the low triple digits, with 88% removal, at WWTP 1 and with 40% removal, at WWTP 2. While no estimation of infectivity of *Giardia* was performed for this study, it is logical to assume that, similar to *Cryptosporidium*, a portion of remaining *Giardia* should be non-infectious. [16]

[14] Blending samples with WWTP flow exceeding 150% of the design flow are shown in bold (blending typically occurs when WWTP flow exceeds 150%)
[15] Central Park, NY
[16] Studies showed that *Giardia* infectivity is generally more sensitive to hypochlorite than *Cryptosporidium*, which already demonstrated low infectivity in effluent concentrations obtained in this study.

Table 16. Range of Final Effluent Concentrations for Protozoa

WWTP	Operating Mode	*Giardia* cysts/ l (enumerated values)		*Cryptosporidium* oocysts/ l (enumerated values)		Infectious *Cryptosporidium* MPN/l	
		Range	Geometric Mean	Range	Geometric Mean	Range	Geometric Mean
1	Dry	6 – 21	12	1 – 8	2	<0.2	NA
	Wet Blending	40 – 720	148	<0.2 – 52	8	<0.3 - <9.2	NA
2	Dry	2 – 4	3	2 – 4	2	<0.2	NA
	Wet Blending	7 – 720	105	<0.2 – 2	NA	<0.5 – <2.4	NA

Note: NA =Less than three detectable results were reported, hence geometric mean could not be calculated

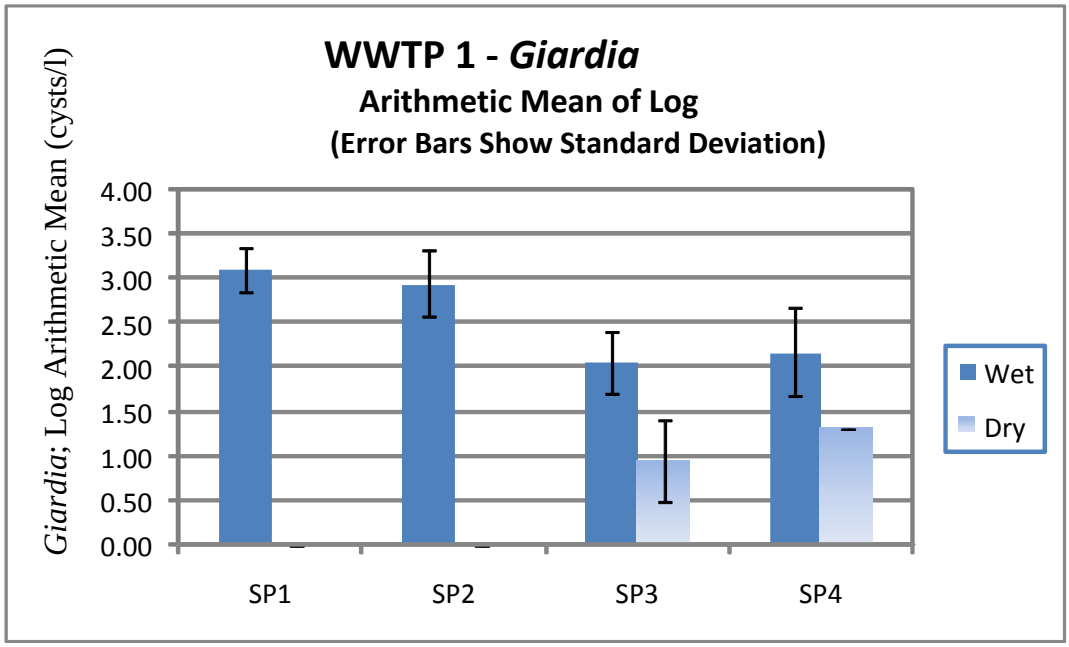
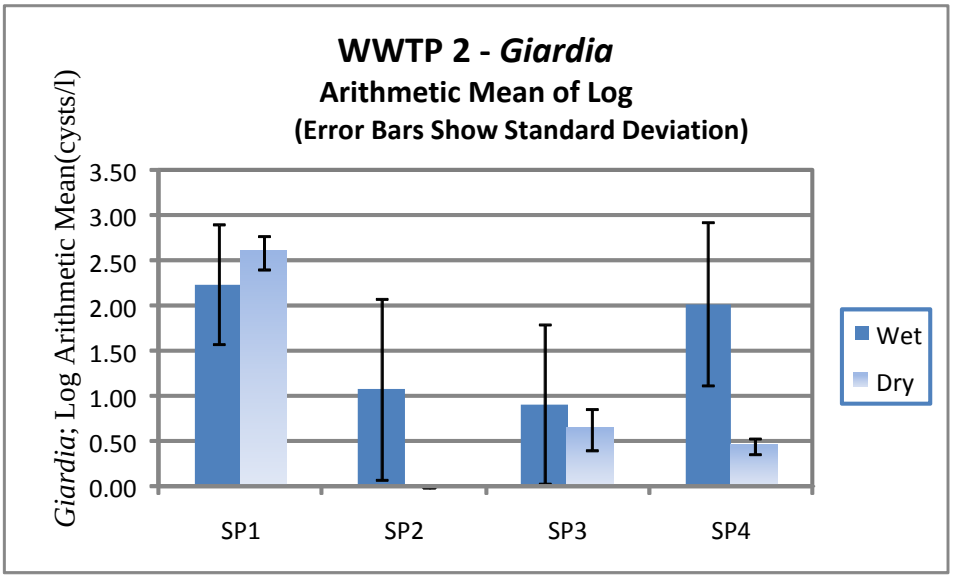


Figure 7. WWTPs 1 & 2 *Giardia*[17] & *Cryptosporidium* Effluent Concentrations[18] with Error Bars[19]

[17] Less than three detectable *Giardia* results were reported for both SP1 and SP2 locations at WWTP 1 and SP2 location at WWTP 2 during dry weather, hence the geometric mean could not be calculated.

[18] Dry-weather results for SP1 and SP2 locations at WWTP 1 were affected by clogged filters and, therefore prevented the full detection of *Giardia* and *Cryptosporidium*, therefore, dry-weather SP1 and SP2 results for WWTP 1 should not be used for deriving conclusions for this study.

[19] For these charts, the Arithmetic mean of the log of results were calculated. The error bars were then calculated by taking the standard deviation.



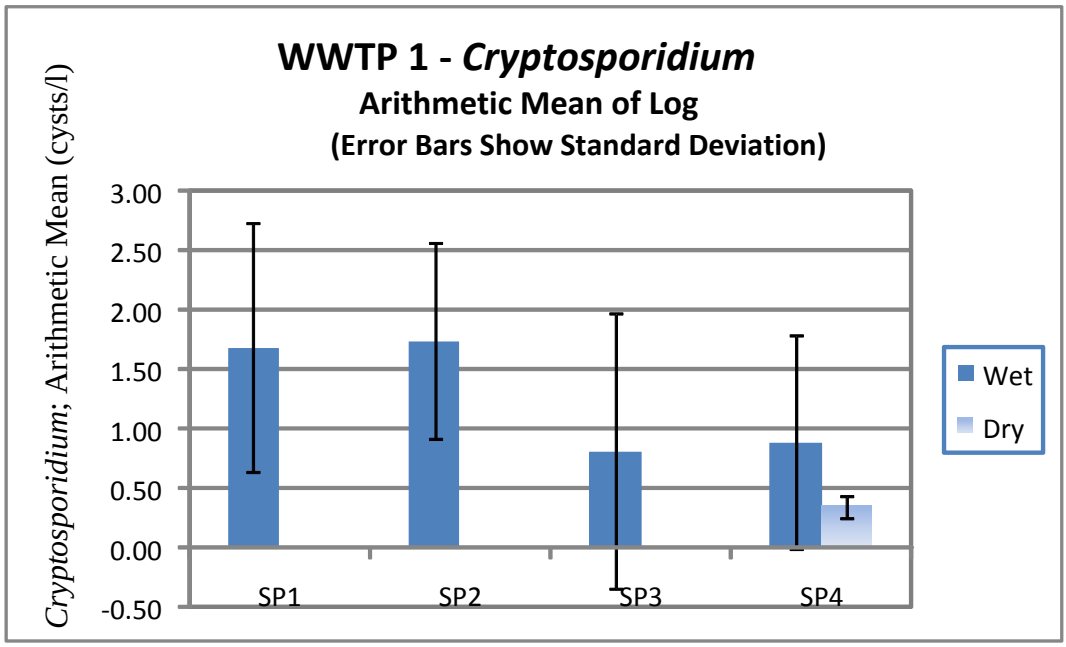


Figure 7. WWTPs 1 & 2 *Giardia* and *Cryptosporidium*[20] Effluent Concentrations (cont.)

[20] Less than three detectable *Cryptosporidium* results were reported for the SP1and SP2 locations at WWTP 1 during dry weather, hence geometric mean could not be calculated. At WWTP 2, all of the sampling points, except SP3 and SP4 for dry-weather had less than three detectable *Cryptosporidium* results hence geometric mean for these results could not be calculated.

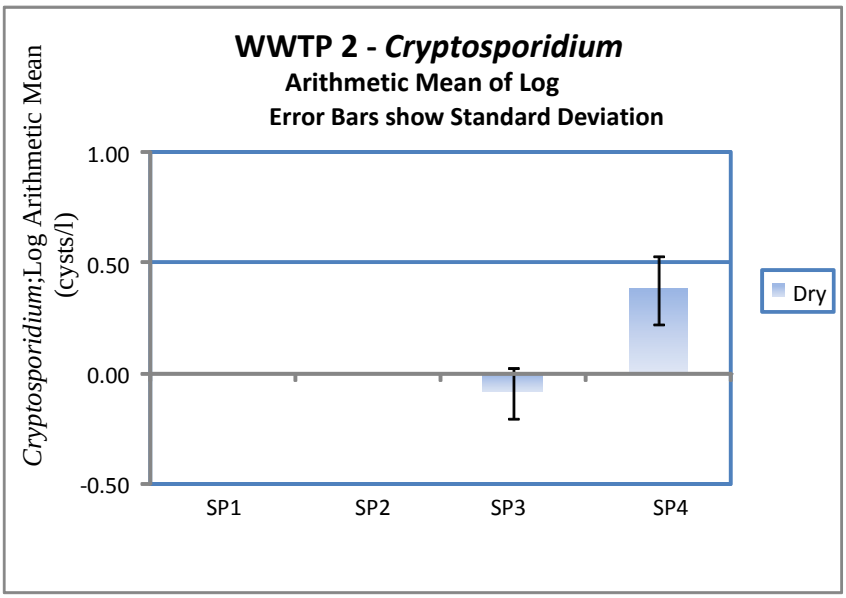


Figure 7. WWTPs 1 & 2 *Giardia* and *Cryptosporidium*[21] Effluent Concentrations with Error Bars (cont.)

Table 17. *Giardia* and *Cryptosporidium* Average Percent Removal

WWTP	Weather Condition	Removal of *Giardia* (%)	Removal of *Cryptosporidium* (%)
1	Wet	88	71
2	Wet	40	NA
1	Dry	NA	NA
2	Dry	99	NA

Note: NA =Less than three detectable values were reported at the influent and/or effluent of the WWTP, hence average percent removal could not be calculated.

4.8 Virus Results

With relatively high WWTP influent results during wet-weather blending, the effluent results for enteric viruses were, on average, in single digit or low double-digit infectious units/l in final effluents, with average removal between 98% and 99% for WWTP 1 and 99% for WWTP 2

[21] Less than three detectable *Cryptosporidium* results were reported for the SP1and SP2 locations at WWTP 1 during dry weather, hence geometric mean could not be calculated. At WWTP 2, all of the sampling points, except SP3 and SP4 for dry-weather had less than three detectable *Cryptosporidium* results hence geometric mean for these results could not be calculated.

(Table 21). The results were generally consistent, with some variability, across all four cell lines[22] (BGM, MA104, PLC/PRF/5 and CaCo-2), as demonstrated in Table 18 and Figure 8.

Table 18. Range of Final Effluent Results for Viruses

WWTP	Operating Mode	BGM Infectious Units/l		MA-104 Infectious Units/l		PLC/PRF/5 Infectious Units/l		CaCo-2 Infectious Units/l	
		Range	Geom. Mean	Range	Geometric Mean	Range	Geom. Mean	Range	Geom. Mean
1	Dry	1 – 3	2	1 – 5	2	2 – 5	3	<1 – 1	NA
	Wet	3 – 143	14	<2 – 393	21	9 – 32	18	1 – 34	4
2	Dry	3 – 6	5	5 – 14	8	4 - 5	5	<3 – 8	NA
	Wet	<1– 25	6	<1 – 12	4	<1- 104	11	<1 – 21	5

Note: NA =Less than three detectable results were reported, hence geometric mean could not be calculated

Enterovirus, Reovirus and Adenovirus were the "Top Three" viruses detected in WWTP samples; on a few occasions, Rotavirus was also detected (Table 20). Only Enterovirus, Reovirus, and Adenovirus were detected in final effluent samples. The cell lines that were used for detection of the enteric viruses using ICC-PCR and/or PCR shown in Table 19.

Table 19. Detection by Cell Lines

Cell Line	Viruses Targeted for PCR Detection
BGM:	Enterovirus, Reovirus
MA104:	Enterovirus, Rotavirus, Reovirus
PLC/PRF/5	Adenovirus
CaCo-2	Enterovirus, Astrovirus

Norovirus and Hepatitis A were analyzed by the direct RT-PCR method and neither was detected in samples.

In the cases when several viruses were detected in one sample, individual MPN concentrations for each virus group could not be generated due to the limitations of the EPA-ICR method.

Overall, the relative recoveries for virus samples were approximately 50%. The majority of recoveries were consistently 50%. The matrix spikes were performed with poliovirus as per the method described in EPA 600/R-95/178.

[22] Cell line – Human or animal cells that are grown in a laboratory and used for detection of the presence of a particular organism. In this study, four cell lines, i.e., BGM (Buffalo Green Monkey cell line), MA104 (cell line derived from Rhesus monkey kidney), PLC/PRF/5 (human hepatoma cell line) and CaCo-2 (human intestinal cell line), were used for detection of the enteric viruses.

Table 20. Detected Viruses

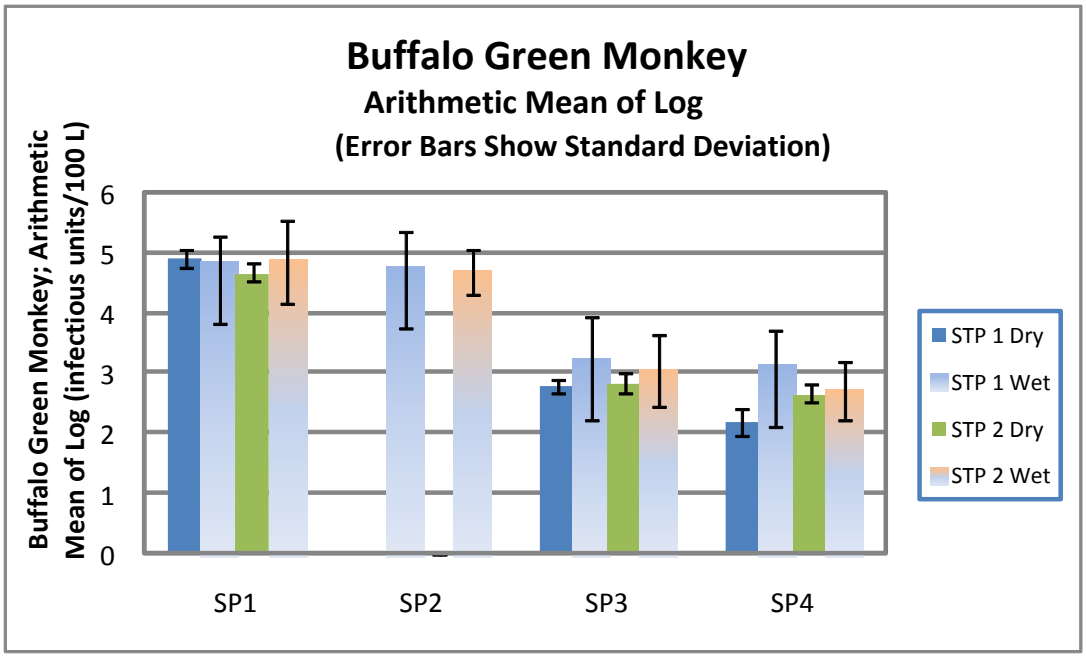
a) Dry weather

WWTP	Sampling Location	BGM	MA-104	PLC/PRF/5	CaCo-2
1	SP1	EV	EV,REO,RV	EV, AdV	EV
	SP2	EV	EV,REO,RV	EV	EV
	SP3	EV	EV,REO	---	---
	SP4	EV	REO	---	---
2	SP1	REO, EV	EV, REO	EV, AdV	EV
	SP2	EV	EV,REO	EV, AdV	EV
	SP3	EV	REO	EV, AdV	---
	SP4	---	REO	---	---

b) Wet weather

WWTP	Sampling Location	Event #	BGM	MA-104	PLC/PRF/5	CaCo-2
1	SP1	1	EV	EV,RV	EV, AdV	EV
		2	EV, REO	EV,RV	EV, AdV	EV
		3	EV, REO	EV, REO, RV	EV, AdV	EV
	SP2	1	EV, REO	EV,RV	EV, AdV	EV
		2	EV	EV, REO	EV, AdV	EV
		3	EV, REO	EV,REO	AdV	EV
	SP3	1	EV	REO	AdV	EV
		2	EV	EV, REO	AdV	---
		3	EV	REO	AdV	EV
	SP4	1	EV, REO	REO	AdV	---
		2	EV	REO	AdV	---
		3	---	---	AdV	---
2	SP1	1	EV, REO	EV, RV, REO	EV, AdV	EV
		2	EV, REO	EV, RV, REO	EV, AdV	EV
		3	EV, REO	EV, RV, REO	EV, AdV	EV
	SP2	1	EV	EV, RV	EV, AdV	EV
		2	EV, REO	EV, RV	EV, AdV	EV
		3	EV, REO	EV, RV, REO	EV, AdV	EV
	SP3	1	EV	RV, REO	AdV	EV
		2	EV, REO	RV	AdV	EV
		3	EV	REO	EV, AdV	---
	SP4	1	EV	RV, REO	---	---
		2	---	---	---	---
		3	EV	REO	AdV	---

Note: AdV = Adenovirus EV = Enterovirus RV = Rotavirus REO = Reovirus



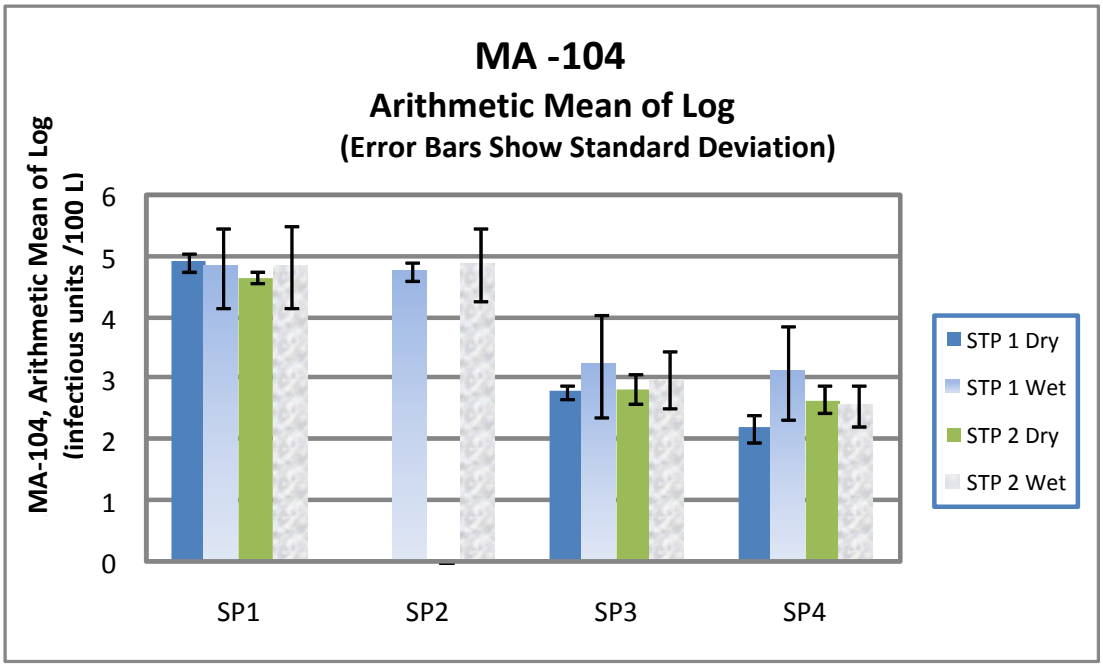
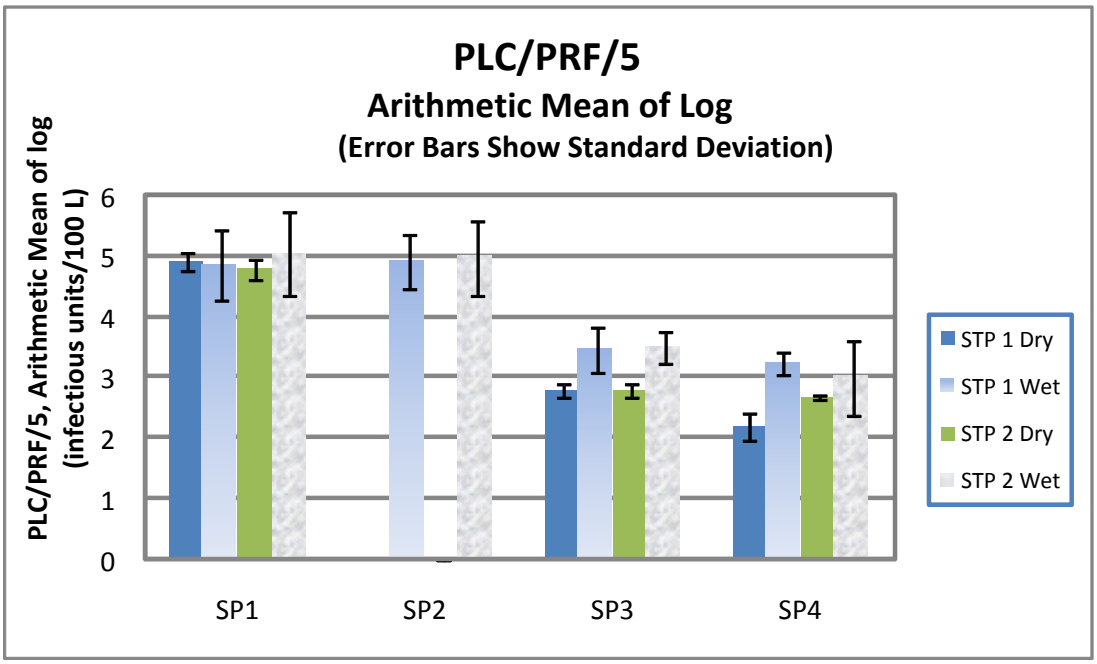


Figure 8. Virus Concentrations[24] by Cell Lines (cont.)

[23] Less than three detectable virus concentrations were reported for the SP2 location for both WWTP 1 and WWTP 2 for all of the four cell lines during dry weather, hence geometric mean values could not be calculated

[24] Less than three detectable virus concentrations were reported for the SP2 location for both WWTP 1 and WWTP 2 for all of the four cell lines during dry weather and for SP4 location for the CaCo-2 cell line during dry weather, hence geometric mean values could not be calculated



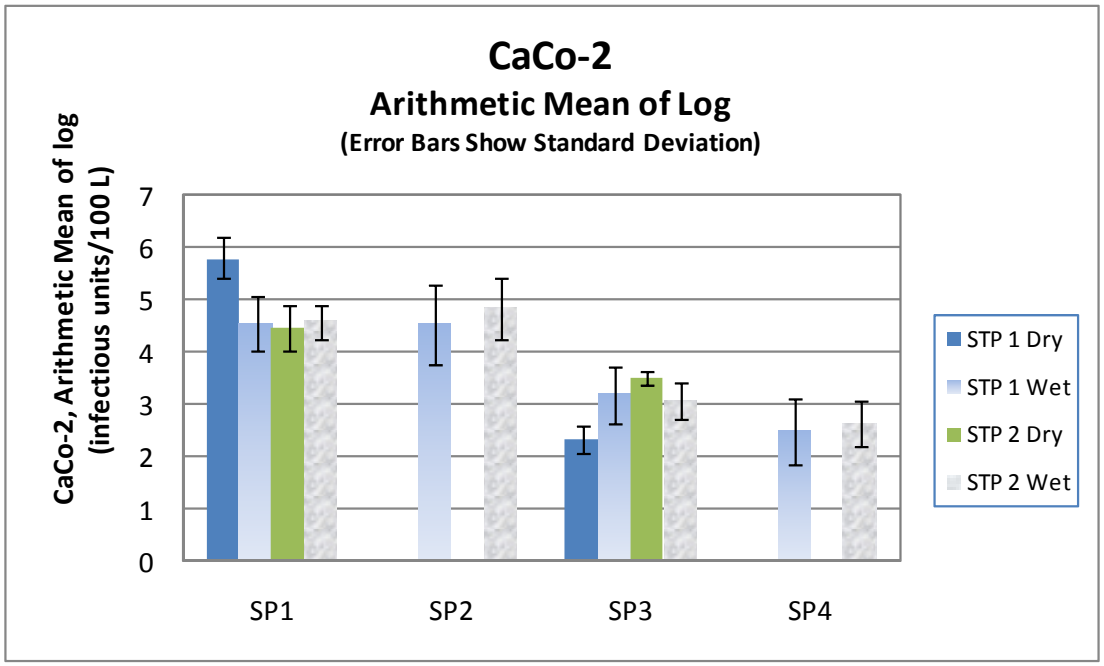


Figure 8. Virus Concentrations[25] by Cell Lines (cont.)

[25] Less than three detectable virus concentrations were reported for the SP2 location for both WWTP 1 and WWTP 2 for all of the four cell lines during dry weather and for SP4 location for the CaCo-2 cell line during dry weather, hence geometric mean values could not be calculated

Table 21. Average Percent Removal of Viruses during Wet-Weather

WWTP	BGM Removal (%)	MA-104 Removal (%)	PLC/PRF/5 Removal (%)	CaCo-2 cells Removal (%)
1	98	98	98	99
2	99	99	99	99

4.9 Coliphage Results

Analysis of the concentrations for the two WWTPs, at which the coliphage[26] samples were collected, shows that male-specific coliphage was generally lower during wet-weather blending vs. dry-weather conditions throughout WWTP 1. At both SP3 and SP4 for WWTP 2, there were no major differences in the geometric means between wet-weather blending and dry-weather conditions.

Effluent concentrations for both coliphage parameters - Famp and C3000 (a plaque assay on *E. coli*) - during wet weather were mostly in single digits, with average percent removal of 99% for both C3000 and Famp at WWTP 1, and 97% removal for Famp at WWTP 2. (Table 23). The average percent removal of C3000 for WWTP 2 could not be calculated, since less than three detectable results were reported for that parameter.

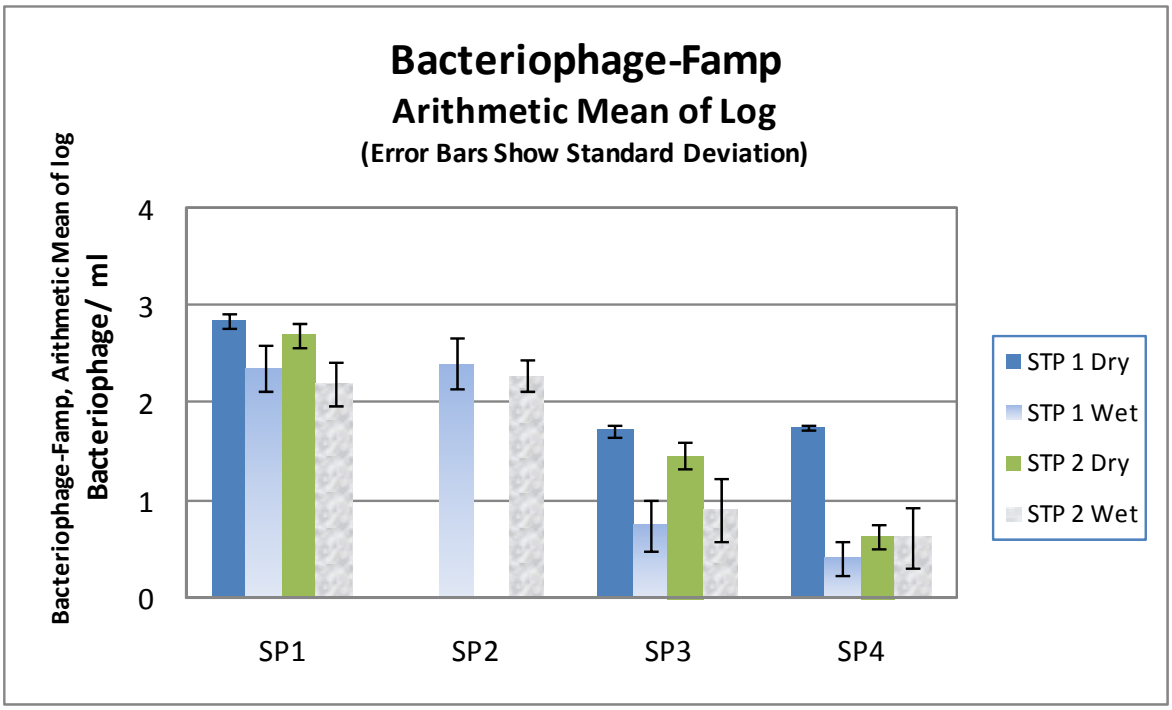
Table 22. Range of Final Effluent Concentrations for Coliphage

WWTP	Operating Mode	Famp bacteriophage / ml		C3000 bacteriophage / ml	
		Range	Geometric Mean	Range	Geometric Mean
1	Dry	53 – 62	57	32 – 56	43
	Wet	1 – 5	2	2 – 7	3
2	Dry	3 – 6	4	3 – 8	5
	Wet	2 – 12	4	1 – 3	NA

Note: NA =Less than three detectable results were reported, hence geometric mean could not be calculated

Here and thereafter, Famp represents F+ (male-specific) phage and C3000 represents both male-specific and somatic phage.

[26] Coliphages are bacteriophages that infect *Escherichia coli* (*E. coli*). They are frequently viewed as alternate bacterial indicators, representing enteric virus contamination.



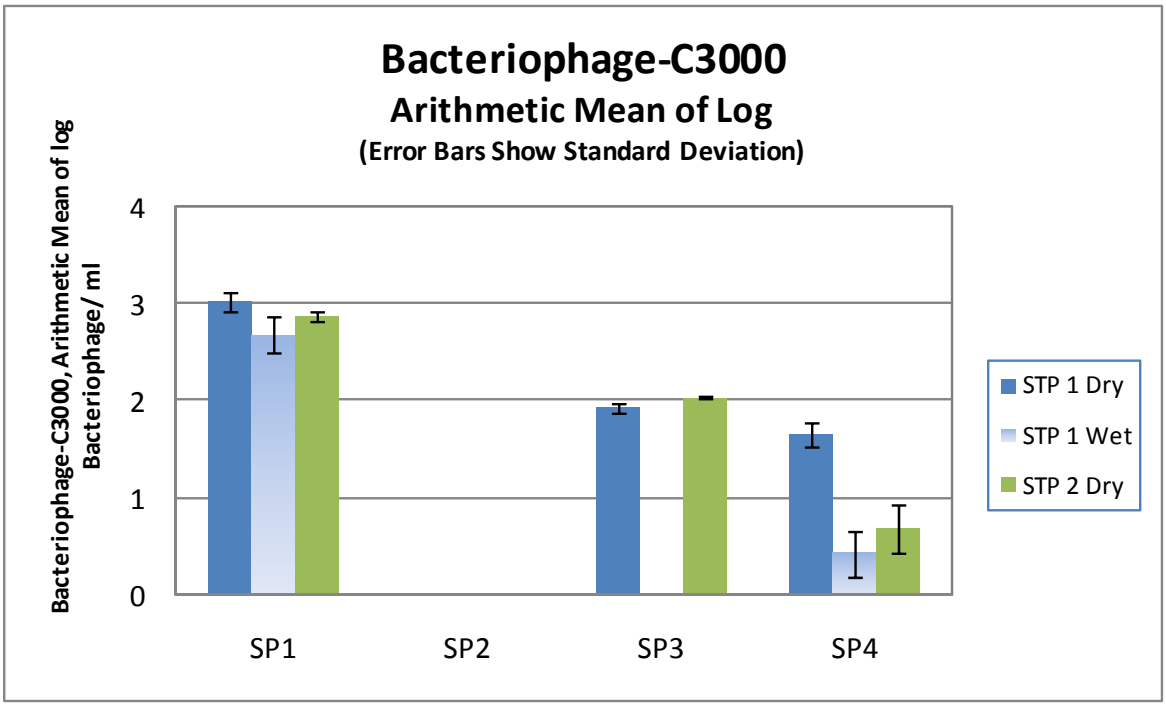


Figure 9. Coliphage Concentrations - Famp and C3000[27]

[27] Less than 3 detectable C3000 results were reported for the SP2 location at WWTP 1 during wet-weather and for all of the four locations at WWTP 2 during wet-weather, hence geometric mean values could not be calculated.

Table 23. Coliphage Average Percent Removal during Wet Weather

WWTP	Famp Removal (%)	C3000 Removal (%)
1	99	99
2	97	NA

Note: NA =Less than 3 detectable values for C3000 were reported at the each of the four locations at WWTP 2 during wet weather throughout the WWTP, hence percent removal values could not be calculated.

4.10 Maceration Results

The project team investigated the impact of maceration on the detection and enumeration of fecal indicator levels in chlorinated effluents. Previous research (Perdek and Borst, 2000) suggests that conventional MPN/MF methods fail to adequately measure bacteria within clusters of bacteria or particles. In an effort to account for such cluster and particle occlusion/association, two effluent samples per WWTP were collected from the post-chlorinated final effluent (SP4) during the first three wet-weather events and one dry-weather event. These samples were macerated at a pre-determined proper contact time and speed to investigate the effect of penetration of the disinfectant into the mix of blended primary and secondary effluent followed by disinfection. After the maceration, these samples were further analyzed for *Enterococcus* and fecal coliform and the results were compared to regular (unmacerated) effluent samples.

Since none of the three WWTPs involved in the study dechlorinate their effluent, SP4 samples were dechlorinated by IEC field staff using 0.025 N sodium thiosulfate immediately upon collection. The maceration was conducted in the IEC laboratory within a 6-hour holding time after sample was collected.

During the dry-weather run on July 17, 2006, at the WWTP 1, IEC collected 34 additional samples and 6 duplicates at SP4 to perform maceration optimization for fecal coliform and *Enterococcus*. The maceration optimization analyses indicated that for this round of sampling the optimum combination of blending speed and time was 22,000 rpm and 60 s, respectively, in order to obtain the highest fecal coliform and *Enterococcus* count.

The first wet-weather event took place on September 14, 2006, at the WWTP 1. Similar to the dry-weather run, 36 additional samples and 6 duplicates were collected from the SP4 location to perform maceration optimization for bacteria. The maceration optimization analyses indicated that for this round of sampling, there were two combinations that were potentially the optimum. These combinations were 3,500 rpm at 90 s and 22,000 rpm at 60 s. On October 20, 2006, during a wet-weather run at the WWTP 1, a second mini-optimization was performed with these combinations of speed and time repeated. This second run showed that 22,000 rpm at 60 s is the optimum setting for maceration for wet-weather events.

Since this portion of the study is purely a comparison of macerated and unmacerated results, which is independent of location and weather conditions, and to give it a greater data pool, all of the results comprising of 64 data points were combined. To get a more statistically precise

number all results with greater than (< 3) or less than (> 24,000) value and one outlier were removed. This still left 21 data points for fecal coliform and 24 for *Enterococcus.*

It was expected that maceration would result in higher fecal coliform and *Enterococcus* concentrations, because maceration exposes bacteria occluded in larger particles. A statistical evaluation of the data points performed for the macerated and unmacerated dry and blended disinfected effluent laboratory analyses revealed that the increase in macerated concentrations for fecal coliform and for *Enterococcus* was statistically significant. The statistical method used was analysis of variance (ANOVA). A three-way ANOVA was used to compare two weather types (Wet, Dry), three treatment plants (WWTP 1,WWTP 2, WWTP 3) and two treatments (macerated and unmacerated).

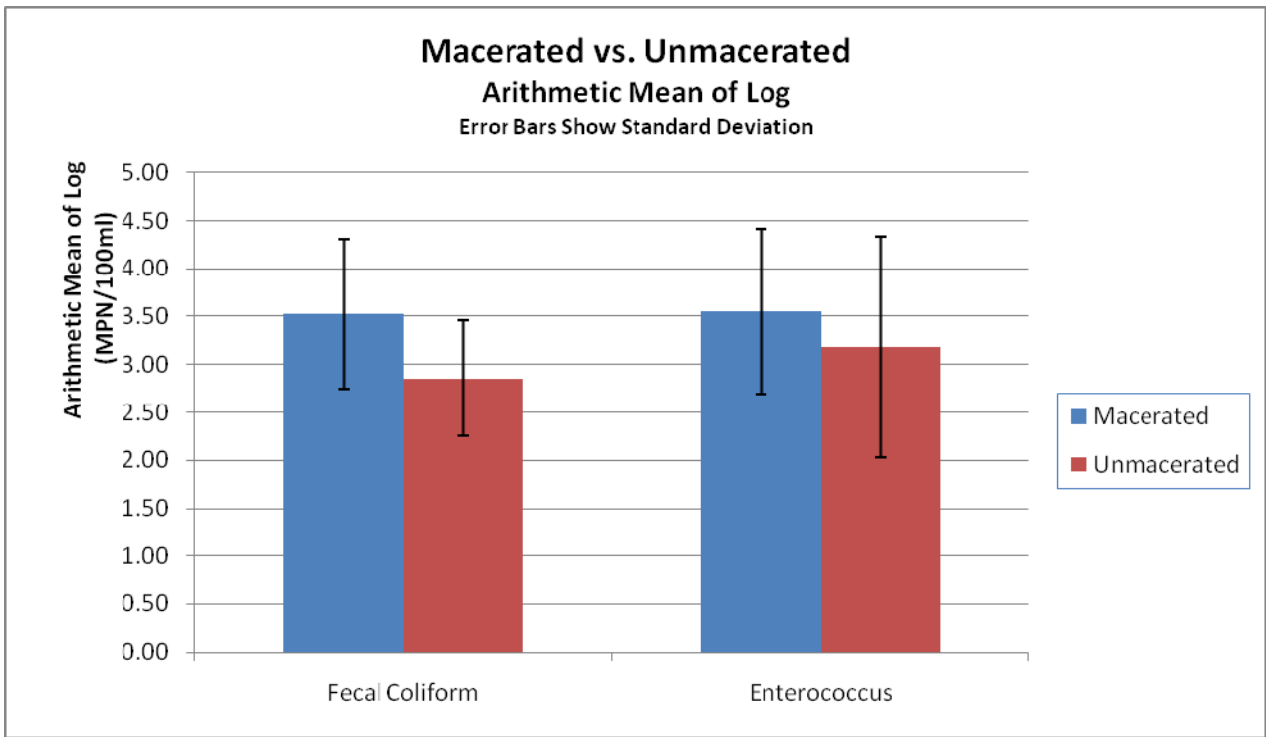


Figure 10. Macerated vs. Unmacerated Concentrations

Detailed maceration optimization charts are included in Appendix E.

Chapter 5. Conclusions and Recommendations

5.1 Conclusions and Observations

Samples of effluent from the three WWTPs that blended wet-weather flows were analyzed for key pathogens, pathogen indicators, TSS and BOD. During the sampling period, a major upgrade was being performed at WWTP 3, with several aeration and final tanks being out of service. These temporary modifications to the treatment facility likely had an adverse impact on the treatment quality, especially during blending at peak wet-weather flows. Based on this, the results of sampling for WWTP 3, should be interpreted in this context.

Effluent from the three WWTPs operating under dry-weather conditions with all flows receiving secondary treatment and disinfection was sampled for pathogens, pathogen indicators,TSS and BOD.

In combined sewer systems, microorganism removal during wet-weather blending events may vary from WWTP to WWTP depending on a number of different factors including design, operation, maintenance, and rainfall, resulting in a variable flow into the plant.

The limitation of the study is that it represents only one geographical location for the three plants studied and the wet-weather blending ratios or flow rates were measured in only one of the three plants. Thus, the geographical closeness and the limited number of facilities evaluated during the study suggest that these results should be viewed as plant–specific. Additional studies are recommended at a variety of WWTPs to provide reinforcement of the data obtained in this study.

1) **Question #1:** During wet-weather blending events at the WWTPs studied what were BOD and TSS levels in the blended effluent?

The average of BOD_5 wet-weather blending effluent concentrations were 24 mg/l at WWTP 1 (values ranged from 9.5 to 37 mg/l), and 22 mg/l at WWTP 2 (values ranged from 9 to 30 mg/l). The average of TSS wet-weather blending effluent concentrations were 29 mg/l at WWTP 1 (values ranged from 22 to 33 mg/l) and 20 mg/l at WWTP 2 (values ranged from 13 to 26 mg/l).

All of these average effluent values were below the IEC effluent value of 30 mg/l for a 30 day average. [28]

The average removal values for wet-weather blending samples collected during the study at WWTP 1 were 77% for BOD_5 and 71% for TSS; and at WWTP 2 were 81% for BOD_5 and 89% for TSS.

Since the total percent removal during blending at both WWTPs was slightly below the expected average of 85%, further analyses of percent removal data during blending showed that the possible limiting factor was the percent removal in the primary treatment portion of the WWTPs. The average percent removal values for primary treatment during wet-weather blending events at

[28] Since this study is a research project, the comparison with effluent limitations is used here only as a convenient benchmarking tool

WWTP 1 were 28% for BOD5 and 31% for TSS and at WWTP 2 were 23% for BOD5 and 49% for TSS. The aforementioned results for BOD5 at WWTP 2 and for TSS at WWTP 1 were slightly below the preferred range of removals of 25 to 40% for BOD5 and of 50 to 60% for TSS during standard primary treatment operations. Another reason for the lower removals is that a portion of the flow did not receive secondary treatment.

During the time of the sampling, a major upgrade was being performed at WWTP 3, with several aeration and final tanks being out of service, this likely had an adverse impact on the treatment quality, especially during blending at peak wet-weather flows. Based on this, the results of sampling for WWTP 3, should be used with caution. At WWTP 3, average effluent concentrations for both BOD_5 and TSS (average of 37 mg/l and 56 mg/l, respectively) were higher than the other two WWTPs, and the percent removal was notably lower than the other two WWTPs.

2) **Question #2**: During wet-weather blending events at the WWTPs studied, what were the fecal coliform and *Enterococcus* levels in the blended effluent?

The fecal colifom effluent concentrations had a geometric mean of 4,900 MPN/100 ml at WWTP 1 during wet weather, 19,000 MPN/100 ml at WWTP 2 during wet weather and 520 MPN/100 ml at WWTP 3 during wet weather.

The *Enterococcus* effluent concentrations had a geometric mean of 17,000 MPN/100 ml at WWTP 1 during wet weather, 14,000 MPN/100 ml at WWTP 2 during wet weather and 870 MPN/100 ml at WWTP 3 during wet weather.

3) **Question #3:** For the WWTPs studied, was there evidence for removal of protozoa (*Cryptosporidium*, infectious *Cryptosporidium* and *Giardia*) during wet-weather blending?

The total *Cryptosporidium* enumerated (non-infectious) effluent results during wet weather were mostly in single or low double digits, with removal of 71% for WWTP 1. Average percent removal for WWTP 2 could not be estimated, since less than three detectable results were reported.

During wet-weather blending, infectious C*ryptosporidium* showed a detectable value in only two of nineteen effluent samples (both results were in single digit and at WWTP 1). Only one of these nineteen effluent samples showed the presence of *C. parvum* Genotype II. [29]

The geometric mean of *Giardia* effluent results during wet weather were in the low triple digits, with 88% removal, at WWTP 1 and with 40% removal, at WWTP 2. No estimation of infectivity of *Giardia* was performed for this study.

4) **Question #4:** For the WWTPs studied, was there evidence for removal of viruses (Adenovirus, Astrovirus, Enterovirus, Rotavirus, Reovirus, Norovirus, Hepatitis A and male-specific and somatic coliphages as an indicator for viruses) during wet-weather blending?

[29] *C. Parvum* Genotype II can infect both human and non-human hosts.

Effluent results for enteric viruses during wet weather were mostly in single digits, with average removal between 98% and 99% for WWTP 1 and 99% for WWTP 2. The presence of Reovirus, Norovirus and Hepatitis A in the effluent was not detected at all.

During wet-weather blending, effluent results for both coliphage parameters—as measured by *E. coli* plaque analysis using Famp (male-specific) and C3000 (represents both male-specific and somatic)—were mostly in single digits, with average percent removal of 99% for both C3000 and Famp at WWTP 1 and 97% removal for Famp at WWTP 2. The average percent removal of C3000 for WWTP 2 could not be calculated, since less than three detectable results were reported for that parameter.

5) **Question #5:** For the WWTPs studied, to what extent did maceration of disinfected effluent samples change the enumerated levels of fecal coliform and *Enterococcus*?

After a statistical evaluation, the results showed that the maceration of effluent samples resulted in an increase in both fecal coliform and *Enterococcus* concentrations.

6) **Question #6:** For the WWTPs studied, what were the pollutant levels in dry-weather effluent?

The dry-weather effluent concentrations for BOD_5 were 15 mg/l at WWTP 1, 13 mg/l at WWTP 2 and 9 mg/l at WWTP 3. The dry-weather effluent concentrations for TSS were 12 mg/l at WWTP 1, 2 mg/l at WWTP 2 and 8 mg/l at WWTP 3.

The average percent removal for dry-weather samples collected during the study was 92% for BOD_5 and 89% for TSS at WWTP 1, 88% for BOD_5 and 99% for TSS at WWTP 2 and 93% for BOD_5 and 93% for TSS at WWTP 3. All of these dry-weather results exceeded the expected 85% removal.

Additional Observations

There was no correlation between total residual chlorine (TRC) vs. fecal coliform and/or WWTP flow (using multiple and two-variable regressions).

Enteric virus results were generally consistent, with some variability, across all four cell lines (BGM, MA104, PLC/PRF/5 and Caco-2).

Based on these findings, one of the additional implications of the study is that a pathogen removal for wet-weather blending is WWTP specific. Although not analyzed in this study, it is also clear that the design and operational characteristics of the individual WWTPs are factors affecting the ability of individual WWTPs to adequately handle peak flow.

It is important to emphasize that the findings from this research study, conducted in a single geographic area with a limited number of data points, are not meant to draw conclusions on a national scale to directly support any future nor existing EPA policy guidelines or regulations. Additional data collection is recommended at the WWTPs studied in this project, WWTPs with

separate sewer systems and WWTPs located in other locations to improve our understanding on impacts of wet weather flows on combined sewer and sanitary sewer system WWTP operations.

5.2 Future Research

A. Increase the understanding of the fate and transport of pathogens and related indicators being discharged from WWTPs during blending by:
    a. evaluating sampling protocols and test methods (including maceration, sonication, and tissue homogenization) for determining more accurate concentrations of specific microorganisms in WWTP effluents during normal dry weather conditions and blending,
    b. determining the major factors impacting fate and transport of pathogens in blended effluents, including die-off and after-growth potential of specific microorganisms in waters receiving effluents from WWTPs during blending, and
    c. assessing the effects of the discharge of effluents from WWTPs during blending, on risks to human health, especially in receiving waters used for recreation.

B. Characterize the effectiveness of treatment plant optimization and the application of additional treatment technologies for managing increased wet-weather flows by:
    a. obtaining information on municipal wastewater treatment plants regarding:
        i. WWTPs that experience increased wet-weather flows and the frequency of blending;
        ii. WWTPs that have conducted “stress tests” to determine the peak, wet-weather flow treatment capacity of their plant;
        iii. WWTPs that have tested or acquired commercial treatment technology, retrofitted existing technology, or otherwise treat side stream flows; and
        iv. WWTPs that have formal institutionalized monitoring protocols and decision-making processes for peak, wet-weather flow situations
    b. developing and validating treatment plant stress-testing protocols for determining peak, wet-weather flow capacities of WWTPs;
    c. characterizing the ability of retrofit, side-stream and other technology and process modifications to meet secondary treatment regulatory standards and, when coupled with appropriate disinfection, to remove key pathogens, including;
        i. physical-chemical processes, such as, chemical addition and ballasted flocculation, tube and plate settlers, fine-mesh screening and filtration, and dissolved-air floatation;
        ii. enhanced biological treatment with high-rate parallel facilities, such as, deep-bed, honey-comb plastic media trickling filtration; and series treatment by switching from conventional activated sludge to contact stabilization;
        iii. high-rate disinfection and related process modifications, such as, increased mixing intensity, increased disinfectant concentrations, more rapid and effective disinfectants (oxidants and ultraviolet light), and multi-stage dosing; and
        iv. innovative and advanced technologies, such as, activated carbon, high-gradient magnetic separation, and fluidized-bed biological treatment.

C. Provide technical guidance on the use of "stress tests," commercially available parallel-treatment units and other monitoring and treatment strategies by WWTPs to determine and augment the peak, wet-weather flow capacity of current treatment plants.

References

American Public Health Association. 2005. Standard Methods for the Examination of Water and Wastewater. 21st Edition, Washington, D.C.

Camper, A.K., LeChevallier, M.W., Broadway, S.C. and McFeters, G.A. 1985. Evaluation of procedures to desorb bacteria from granular activated carbon. Journal of Microbiological Methods. 3:187-198.

Chauret, C., S. Springthorpe, and S. Sattar. (1999). Fate of *Cryptosporidium* oocysts, G*iardia* cysts, and microbial indicators during wastewater treatment and anaerobic sludge digestion. *Canadian Journal of Microbiology*. 45:257-262.

Escalante, A.A., R. J. Montali, R. Fayer, and A.A. Lal (1999). Phylogenetic Analysis of *Cryptosporidium* Parasites Based on the Small-Subunit rRNA Gene Locus. *Applied and Environmental Microbiology* 65(4):1578–1583.

Grimm, A.C., J.L. Cashdollar, F.P. Williams, and G.S. Fout. (2004). Development of an astrovirus RT-PCR detection assay for use with conventional, real-time, and integrated cell culture/RT-PCR. *Canadian Journal of Microbiology*. 50: 269-278.

Hoff, J. C. The Relationship of Turbidity to Disinfection of Potable Water. In: Evaluation of the Microbiology Standards for Drinking Water (C. H. Hendricks, Ed.). EPA - 570/9-78-00C, U.S. Environmental Protection Agency, Washington, DC, 1978, pp 103-117.

Hoff, J. C. and Akin, E.W. 1986. Microbial resistance to disinfectants: mechanisms and significance. Environmental Health Perspectives. 69**:**7-13.

Oberste, M.S., K. Maher, A.J. Williams, N. Dybdahl-Sissoko, B.A. Brown, M.S. Gookin, S. Penaranda, N. Mishrik, M. Uddin, and M.A. Pallansch. (2006). Species-specific RT-PCR amplification of human enteroviruses: a tool for rapid species identification of uncharacterized enteroviruses. *Journal of General Virology* 87:119-128.

Quintero-Betancourt, W., A. L. Gennaccaro, T. M. Scott, and J. B. Rose. (2003). Assessment of Methods for Detection of Infectious *Cryptosporidium* Oocysts and *Giardia* Cysts in Reclaimed Effluents. *Applied and Environmental Microbiology* 69(9): 5380–5388.

Peavy, H.S., D. R. Rowe and G. Tchobanoglous (1985), Environmental Engineering, Mc Graw-Hill, Inc., New York, NY, 1985.

Perdek, J.M. and M. Borst (2000). Particle Association Effects on Microbial Indicator Concentrations for CSO Disinfection. *ASCE's Joint Conference on Water Resources Engineering and Water Resources Planning & Management,* July – August 2000, Minneapolis, MN.

Reynolds, K.A. (2004). Integrated cell culture/PCR for detection of enteric viruses in environmental samples. *Methods in Molecular Biology*. 268: 69-78.

Rochelle, P.A., M.M. Marshall, J.R. Mead, A.M. Johnson, D.G. Korich, J.S. Rosen, and R.D. Leom. (2002). Comparison of In Vitro Cell Culture and a Mouse Assay for Measuring Infectivity of *Cryptosporidium parvum. Applied and Environmental Microbiology* 68 (8):3809-3817

Sedmak, G., D. Bina, J. MacDonald, and L. Couillard. (2004). Nine-Year Study of the Occurrence of Culturable Viruses in Source Water for Two Drinking Water Treatment WWTPs and Influent and Effluent of a Wastewater Treatment WWTP in Milwaukee, Wisconsin (August 1994 through July 2003). *Applied and Environmental Microbiology*. 71:1042-1050

Slifko, T.R., D.E. Friedman, and J.B. Rose. (1999). A most-probable-number assay for the enumeration of infectious *Cryptosporidium parvum* oocysts. *Applied and Environmental Microbiology* 65(9):3936-3941.

Slifko T.R., D.E. Friedman D, J.B. Rose JB, and W. Jakubowski. (1997). An in vitro method for detecting infectious *Cryptosporidium* oocysts with cell culture. *Applied and Environmental Microbiology* 63 (9):3669-75.

Snustad, S.A., and D.S. Dean. (1971). *Genetic Experiments with Bacterial Viruses*, W.H. Freeman & Co. San Francisco; 1971.

Spinner, M.L. and G.D. DiGiovanni. (2001). Detection and identification of mammalian reoviruses in surface water by combined cell culture and reverse transcription-PCR. *Applied and Environmental Microbiology* 67: 3016-3020.

Tchobanoglous, G. and F.L. Burton (1991), Wastewater Engineering: Treatment, Disposal, and Reuse, Metcalf & Eddy, Inc., McGraw-Hill, New York, 1991.

van Heerden J., M.M. Ehlers, A. Heim, and W.O. Grabow. (2005). Prevalence, quantification and typing of adenoviruses detected in river and treated drinking water in South Africa. *Applied and Environmental Microbiology* 99: 234-242

Wellings, F.M., Lewis, A.L. and Mountain, C.W. 1976. Demonstration of solids-associated viruses in wastewater and sludge. Applied and Environmental Microbiology, 31**:**354-358.

Xiao, L., I. Sulaiman, R. Fayer, and A.A. Lal (1998). Species and Strain-specific Typing of *Cryptosporidium* Parasites in Clinical and Environmental Samples. *Memórias do Instituto Oswaldo Cruz* 93(5): 687-692.

Bibliography

1. U.S. Environmental Protection Agency, 2001. EPA Requirements for Quality Assurance Project Plans (QA/R-5), U.S. EPA Office of Environmental Information.

2. U.S. Environmental Protection Agency, 2001. Guidance on Environmental Data Verification and Data Validation (QA/G-8), U.S. EPA Office of Environmental Information.

3. U.S. Environmental Protection Agency, 1994. Guidance for the Data Quality Objectives Process (QA/G-4), U.S. EPA Office of Environmental Information.

4. AMERICAN PUBLIC HEALTH ASSOCIATION. 1992, Standard Methods for the Examination of Dairy Products, 18th ed., American Public Health Association, Washington. D.C.

5. AMERICAN PUBLIC HEALTH ASSOCIATION. 1970. Recommended procedures for the examination of Sea Water and Shellfish, 4th ed. American Public Health Association, New York.

6. AMERICAN PUBLIC HEALTH ASSOCIATION. American Water Works Association, and Water Environmental Federation, 1992. Standard Methods for the Examination of Water and Wastewater, 20th ed., Washington, D.C.

7. Interstate Environmental Commission, January 2004. Standard Operating Procedures Manual of the Interstate Environmental Commission for Sampling, Sample Preservation, Analyses, and Quality Control, New York, N.Y.

8. Interstate Environmental Commission, January 2002. Quality Control Manual of the Interstate Environmental Commission, New York, N.Y.

9. Interstate Environmental Commission, January 1983. Quality Assurance/Control Procedures for Microbiological Testing and Analysis, New York, N.Y.

Appendix A

Detailed Sampling Results

Plant Operating Mode	Plant	Location	Date	Fecal Coliform mpn/100 ml	*Entero* mpn/100 ml	*Giardia* cysts/ L	*Crypto* oocysts/ L	MPN/ L Infectious *Crypto*	BGM		MA-104		PLC/PRF/5		CaCo-2 cells		Famp bacteriophage / ml	C3000 bacteriophage / ml	BOD (composite) mg/l	TSS (composite) mg/l	Residual Chlorine mg/l
									Infectious Units/L	Virus Detected	Infectious Units/L	Virus Detected	Infectious Units/L	Virus Detected	Infectious Units/L	Virus Detected					
Dry	Plant 1	Influent	7/17/06	>24,000,000	130,000	<0.4	<0.4	<1.0	----	----	----	----	----	----	----	----	----	----	185.9	105.4	----
				>24,000,000	540,000	27.2	1.4	<0.9	----	----	----	----	----	----	----	----	----	----			----
				11,000,000	1,500,000	1.6	<0.4	<1.0	----	----	----	----	----	----	----	----	----	----			----
			8/14/06	----	----	----	----	----	1,156		713		788		7,880		832	936	----	----	----
				----	----	----	----	----	786	EV	233	EV,REO,RV	154	EV, AdV	2,412	EV	600	1344	----	----	----
				----	----	----	----	----	582		970		484		13,920		712	888	----	----	----
		PE	7/17/06	1,500,000	2,400,000	<0.33	<0.33	<0.9	----	----	----		----		----		----	----	90	72.3	----
				>24,000,000	600,000	----	----	----	----	----	----		----		----		----	----			----
				>24,000,000	430,000	----	----	----	----	----	----		----		----		----	----			----
			8/14/06	----	----	----	----	----	356	EV	356	EV,REO,RV	418	EV	2,200	EV	425	800	----	----	----
		SE (pre-chlor)	7/17/06	2,300	2,300	24.9	5.4	<0.2	----	----	----	----	----	----	----	----	----	----	----	----	----
				15,000	9,300	3	<0.09	<0.2	----	----	----	----	----	----	----	----	----	----	----	----	----
				150,000	15,000	9.1	3.8	<0.2	----	----	----	----	----	----	----	----	----	----	----	----	----
			8/14/06	----	----	----	----	----	7		11		13		3		52	95.2	----	----	----
				----	----	----	----	----	7	EV	6	EV,REO	7	---	3	---	61	72.8	----	----	----
				----	----	----	----	----	4		6		4		1		47.2	89.6	----	----	----
		Final Effluent	7/17/06	390	4	20.7	7.5	<0.2	----	----	----		----		----		----	----	15.1	11.85	0.55
				1,200	93	12	1.4	<0.2	----	----	----		----		----		----	----			0.45
				1,500	23	6.4	1.1	<0.2	----	----	----		----		----		----	----			0.26
			8/14/06	----	----	----	----	----	1		5		5		<1		52.8	56	----	----	0.32
				----	----	----	----	----	1	EV	1	REO	3	---	1	---	62.4	32.4	----	----	0.46
				----	----	----	----	----	3		1		2		1		56.8	52.8	----	----	0.46
	Plant 2	Influent	8/14/06	2,400,000	430,000	356	<1.0	<2.4	477		478		412		197		400	680			----
				4,600,000	27,000	618	4	<2.4	334	REO, EV	771	EV, REO	671	EV, AdV	145	EV	480	720	111.8	150	----
				11,000,000	930,000	276	<1.0	<2.4	667		578		823		912		680	840			----
		PE	8/14/06	11,000,000	280,000	536	4	<2.4	----		----		----		----		----	----			----
				11,000,000	430,000	446	<1.0	<2.4	211	EV	564	EV,REO	679	EV, AdV	1,970	EV	360	560	45.9	42.5	----
				2,400,000	930,000	----	----	----	----		----		----		----		----	----			----
		SE (pre-chlor)	8/14/06	460,000	230,000	5.7	0.7	<0.2	11		14		7		39		42	108.8	----	----	----
				1,100,000	230,000	6.2	1.1	<0.2	6	EV	8	REO	4	EV, AdV	24	---	26.4	105.6	----	----	----
				150,000	430,000	2.3	0.7	<0.2	6		5		7		39		22.8	113.6	----	----	----
		Final Effluent	8/14/06	<3	<3	2.8	2.0	<0.2	3		8		4		<3		3.2	2.8			0.74
				93	<3	2.3	1.9	<0.2	6	---	14	REO	4	---	<3	---	4.8	8	13.2	1.9	0.8
				15	<3	3.5	3.6	<0.2	6		5		5		8		5.6	6.4			0.52
	Plant 3	Influent	8/23/06	2,400,000	750,000	No Protozoa, Virus and Coliphage samples were collected at Plant 3 as per scope of work requirements													123.6	110.5	----
				2,400,000	750,000																----
				4,600,000	930,000																----
			4/24/07	>24,000,000	>24,000,000														141.4	108	----
				11,000,000	930,000																----
				4,600,000	2,400,000																----
		PE	4/24/07	2,400,000	930,000														70.4	58.7	----
				11,000,000	2,100,000																----
				430,000	2,400,000																----
		SE (pre-chlor)	8/23/06	93,000	15,000														----	----	----
				23,000	4,300														----	----	----
				23,000	2,300														----	----	----
			4/24/07	<3000	<3000														----	----	----
				4,000	9,000														----	----	----
				23,000	93,000														----	----	----
		Final Effluent	8/23/06	4	<3														2	1.02	1.81
				15	<3																0.38
				15	<3																0.28
			4/24/07	230	4,300														15.6	14.4	0.59
				150	2,100																0.64
				30	9,300																0.53

PE - Primary effluent
SE (pre-chlor) - Secondary effluent prior to chlorination
EV = Enterovirus
AdV = Adenovirus
RV = Rotovirus
Av = Astrovirus
REO = Reovirus

Plant Operating Mode	Plant	Location	Date	Fecal Coliform mpn/100 ml	*Entero* mpn/100 ml	*Giardia* cysts/ L	*Crypto* oocysts/ L	MPN/ L Infectious *Crypto*	BGM		MA-104		PLC/PRF/5		CaCo-2 cells		Famp bacteriophage / ml	C3000 bacteriophage / ml	BOD (composite) mg/l	TSS (composite) mg/l	Residual Chlorine mg/l
									Infectious Units/L	Virus Detected	Infectious Units/L	Virus Detected	Infectious Units/L	Virus Detected	Infectious Units/L	Virus Detected					
Wet	Plant 1	Influent	9/14/06	4,600,000	390,000	----	----	----	2,111	EV	1,741	EV, RV	1,213	EV, AdV	603	EV	500	620	159.4	159.6	----
				4,600,000	11,000,000	----	----	----	1,279		1,279		435		985		340	420			----
				4,600,000	4,600,000	----	----	----	2,111		792		870		1,741		180	220			----
			10/20/06	4,600,000	430,000	820	280	<9.2	572	EV, REO	914	EV, RV	1,677	EV, AdV	254	EV	320	600	186	137	----
				2,400,000	430,000	1,200	20	<9.2	914		5,425		6,606		254		500	760			----
				11,000,000	4,600,000	880	24	9.2 (1.3-68)	914		1,520		1,677		108		220	500			----
			11/8/06	>24,000,000	2,100,000	790	1,000	18.4 (2.5-136)	----	----	----	----	----	----	----	----	----	----	68	65	----
				>24,000,000	640,000	----	----	----	----	----	----	----	----	----	----	----	----	----			----
				11,000,000	2,400,000	----	----	----	----	----	----	----	----	----	----	----	----	----			----
			3/2/07	4,600,000	93,000	----	----	----	----	----	----	----	----	----	----	----	----	----	35.4	57.2	----
				1,200,000	150,000	----	----	----	----	----	----	----	----	----	----	----	----	----			----
				930,000	2,400,000	----	----	----	----	----	----	----	----	----	----	----	----	----			----
			4/27/07	1,500,000	230,000	1,100	100	<2.4	92	EV, REO	143	EV, REO,RV	110	EV, AdV	297	EV	160	ND*	72.4	74	----
				930,000	430,000	4,300	<1.0	<2.4	738		54		114		1,424		110	ND*			----
				4,600,000	930,000	1600	<1.0	<2.4	222		4,069		686		54		130	ND*			----
		PE	9/14/06	11,000,000	2,400,000	----	----	----	----	EV, REO	----	EV, RV	----	EV, AdV	----	EV	----	----	138.2	89.6	----
				11,000,000	2,400,000	----	----	----	2,111		759		257		2,870		400	580			----
				>24,000,000	930,000	----	----	----	----		----		----		----		----	----			----
			10/20/06	1,500,000	230,000	760	12	<9.2	----	EV	----	EV, REO	----	EV, AdV	----	EV	----	----	76	73.7	----
				930,000	230,000	----	----	----	775		412		1,677		108		360	560			----
				230,000	150,000	----	----	----	----		----		----		----		----	----			----
			11/8/06	4,600,000	230,000	----	----	----	----	----	----	----	----	----	----	----	----	----	72	43	----
				4,600,000	930,000	398	462	<9.2	----	----	----	----	----	----	----	----	----	----			----
				2,400,000	930,000	----	----	----	----	----	----	----	----	----	----	----	----	----			----
			3/2/07	430,000	390,000	----	----	----	----	----	----	----	----	----	----	----	----	----	33.9	58.4	----
				930,000	210,000	----	----	----	----	----	----	----	----	----	----	----	----	----			----
				430,000	230,000	----	----	----	----	----	----	----	----	----	----	----	----	----			----
			4/27/07	2,400,000	430,000	----	----	----	----	EV, REO	----	EV, REO	----	AdV	----	EV	----	----	55	74.6	----
				2,400,000	2,400,000	2,200	30	<2.4	143		429		1,424		186		130	ND*			----
				930,000	2,400,000	----	----	----	----		----		----		----		----	----			----
		SE (pre-chlor)	9/14/06	1,100,000	150,000	----	----	----	59	EV	48	REO	12	AdV	8	EV	6.5	0.4	----	----	----
				>2,400,000	240,000	----	----	----	68		151		8		3		5.7	0.6			----
				>2,400,000	240,000	----	----	----	115		216		23		5		6.7	0.91			----
			10/20/06	930,000	----	320	14	<9.2	40	EV	236	EV, REO	66	AdV	72	---	10.4	15	----	----	----
				390,000	23,000	100	30	<9.2	35		44		22		127		11.4	16.1			----
				930,000	43,000	30	22	9.2 (1.3-68)	57		205		57		36		12.5	14.71			----
			11/8/06	2,400,000	43,000	61	63	<1.8	----	----	----	----	----	----	----	----	----	----	----	----	----
				930,000	43,000	70	66	1.8 (0.3-13.6)	----	----	----	----	----	----	----	----	----	----	----	----	----
				2,400,000	93,000	80	78	<1.8	----	----	----	----	----	----	----	----	----	----			----
			3/2/07	930,000	230,000	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----
				150,000	93,000	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----
				230,000	930,000	----	----	----	----	----	----	----	----	----	----	----	----	----			----
			4/27/07	2,400,000	430,000	220	<0.2	<0.48	2	EV	2	REO	23	AdV	19	EV	5.8	ND*	----	----	0.03
				2,400,000	430,000	170	<0.2	<0.48	4		102		51		24		5.4	ND*			0.03
				2,400,000	430,000	250	<0.2	<0.48	<1.9		2		98		6		1.7	ND*			0.04
		Effluent	9/14/06	930	11,000	64	36	0.3 (0.04-2.1)	76	EV, REO	58	REO	15	AdV	2	---	1.6	2.2	37	33.3	0.26
				>24,000	>24,000	56	42	<0.3	143		393		9		1		2.8	4.2			0.16
				>24,000	>24,000	58	34	<0.3	55		18		20		1		1	1.5			0.13
			10/20/06	1,500	9,300	320	8	<9.2	6	EV	19	REO	19	AdV	19	---	1.8	2.5	19	21.8	0.49
				4,300	>240,000	270	2	<9.2	5		20		20		21		2.5	3.1			0.64
				930	2,000	220	8	<9.2	18		40		32		34		3.9	7			0.61
			11/8/06	>240,000	>240,000	41	43	<1.8	----	----	----	----	----	----	----	----	----	----	17	30	1.18
				930	46,000	40	46	<1.8	----	----	----	----	----	----	----	----	----	----			1.43
				930	24,000	60	52	1.8 (0.3-13.6)	----	----	----	----	----	----	----	----	----	----			1.33
			3/2/07	930	230	----	----	----	----	----	----	----	----	----	----	----	----	----	9.5	25.9	0.3
				1,500	24,000	----	----	----	----	----	----	----	----	----	----	----	----	----			0.94
				24,000	9,300	----	----	----	----	----	----	----	----	----	----	----	----	----			1.05
			4/27/07	9,300	5,500	690	<0.2	<0.48	4	---	2	---	25	AdV	2	---	3.6	ND*	35.1	31.8	0.5
				15,000	24,000	720	2	<0.48	5		<1.8		23		2		2.7	ND*			0.7
				7,500	46,000	590	<0.2	<0.48	3		3		10		1		4.8	ND*			0.57

PE - Primary effluent SE (pre-chlor) - Secondary effluent prior to chlorination

EV = Enterovirus AdV = Adenovirus RV = Rotovirus Av = Astrovirus REO = Reovirus

*ND - Not done due to the high background concentration of indigenous bacteria

Plant Operating Mode	Plant	Location	Date	Fecal Coliform mpn/100 ml	*Entero* mpn/100 ml	*Giardia* cysts/ L	*Crypto* oocysts/ L	MPN/ L Infectious *Crypto*	BGM		MA-104		PLC/PRF/5		CaCo-2 cells		Famp bacteriophage / ml	C3000 bacteriophage / ml	BOD (composite) mg/l	TSS (composite) mg/l	Residual Chlorine mg/l
									Infectious Units/L	Virus Detected	Infectious Units/L	Virus Detected	Infectious Units/L	Virus Detected	Infectious Units/L	Virus Detected					
Wet	Plant 2	Influent	11/8/06	430,000	120,000	----	----	----	----	----	----	----	----	----	----	----	----	----	58	136	----
				430,000	230,000	----	----	----	----	----	----	----	----	----	----	----	----	----			----
				2,400,000	210,000	----	----	----	----	----	----	----	----	----	----	----	----	----			----
			1/8/07	2,400,000	930,000	30	<1.0	<2.4	297	EV, REO	190	EV,RV, REO	72	EV, AdV	216	EV	200	400	----	----	----
				4,600,000	430,000	150	<1.0	<2.4	914		914		914		229		370	580			----
			4/12/07	2,400,000	230,000	140	<1.0	<2.4	4,068	EV, REO	4,068	EV, RV, REO	6,576	EV, AdV	1,140	EV	100	----	53	67	----
				1,500,000	430,000	2,200	40	<2.4	54		110		581		309		120	----	152.8	368.3	----
				430,000	430,000	890	<1.0	<2.4	429		222		7,398		1,101		90	----			----
			7/18/07	1,500,000	93,000	60	<1.0	<2.4	1,520	EV, REO	1,520	EV, RV, REO	914	EV, AdV	360	EV	244	ND*	201	132.8	----
				230,000	150,000	70	<1.0	<2.4	5,420		5,420		1,898		220		188	ND*			----
		PE	11/8/06	2,400,000	430,000	----	----	----	----	----	----	----	----	----	----	----	----	----	60	61	----
				930,000	430,000	----	----	----	----	----	----	----	----	----	----	----	----	----			----
				430,000	230,000	----	----	----	----	----	----	----	----	----	----	----	----	----			----
			1/8/07	930,000	750,000	90	<1.0	<2.4	190	EV	190	EV, RV	225	EV, AdV	190	EV	220	230	44	50	----
				2,400,000	1,200,000	----	----	----	----		----		----		----		----	----			----
			4/12/07	2,400,000	4,600,000	----	----	----	----	EV, REO	----	EV, RV	----	EV, AdV	----	EV	----	----	86	140	----
				11,000,000	930,000	<1.0	10	<2.4	686		2,847		1,140		2,847		130				----
				>24,000000	>24,000000	----	----	----	----		----		----		----		----	----			----
			7/18/07	2,100,000	430,000	----	----	----	----	EV, REO	----	EV, RV, REO	----	EV, AdV	----	EV	----	----	165	105.1	----
				930,000	210,000	20	<1.0	<2.4	914		914		3,800		810		258	ND*			----
		SE (pre-chlor)	11/8/06	430,000	430,000	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----
				430,000	75,000	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----
				430,000	430,000	----	----	----	----	----	----	----	----	----	----	----	----	----			----
			1/8/07	430,000	43,000	3	3	<2.4	48	EV	48	RV, REO	15	AdV	29	EV	11.1	15.9	----	----	----
				750,000	93,000	3	1	<2.4	52		32		32		27		3.4	5.1			----
			4/12/07	930,000	43,000	52	<0.2	<0.5	7	EV, REO	7	RV	21	AdV	26	EV	5.7	----	----	----	----
				930,000	23,000	<0.2	<0.2	<0.5	3		6		17		4		5.7	----			----
				430,000	43,000	42	<0.2	<0.5	<2		3		54		7		7.4	----	----	----	----
			7/18/07	430,000	93,000	16	<0.2	<0.5	43	EV	17	REO	69	EV, AdV	14	---	16.5	ND*			----
				230,000	93,000	40	<0.2	<0.5	10		5		44		9		32.4	ND*	----	----	----
		Effluent	11/8/06	>240,000	46,000	----	----	----	----	----	----	----	----	----	----	----	----	----	9	23	0.19
				>240,000	24,000	----	----	----	----	----	----	----	----	----	----	----	----	----			0.26
				>240,000	24,000	----	----	----	----	----	----	----	----	----	----	----	----	----			0.46
			1/8/07	2,300	4,300	8	2	<2.4	25	EV	5	RV, REO	16	----	21	----	6.9	1.4	21	13	0.48
				90	930	7	1	<2.4	6		6		8		4		1.7	3.4			0.87
			4/12/07	46,000	15,000	210	<0.2	<0.5	2	----	<2	----	6	----	<2	----	4.7	----	28.4	26.25	0.3
				4,300	>240,000	580	<0.2	<0.5	<2		<2		6		<2		6.4	----			0.52
				1,500	930	710	<0.2	<0.5	<1		<1		<1		<1		2.2	----			0.65
			7/18/07	>240,000	9,300	720	<0.2	<0.5	14	EV	12	REO	104	AdV	9	----	9.6	ND*	30	17.2	0.52
				24,000	110,000	40	<0.2	<0.5	13		4		41		9		12.2	ND*			0.71

PE - Primary effluent SE (pre-chlor) - Secondary effluent prior to chlorination
EV = Enterovirus AdV = Adenovirus RV = Rotovirus Av = Astrovirus REO = Reovirus
*ND - Not done due to the high background concentration of indigenous bacteria

Plant Operating Mode	WWPT	Location	Date	Fecal Coliform mpn/100 ml	*Entero* mpn/100 ml	BOD (composite) mg/l	TSS (composite) mg/l	Residual Chlorine mg/l
Wet	3	Influent	11/8/06	430,000	1,200,000	76	83	----
				2,100,000	230,000			----
				>24,000,000	230,000			----
			1/8/07	4,600,000	230,000	40	41.6	----
				2,400,000	930,000			----
				2,400,000	930,000			----
			3/2/07	430,000	430,000	65	76.2	----
				43,000	93,000			----
				>24,000,000	93,000			----
			4/4/07	2,400,000	430,000	92.4	69.6	----
				930,000	150,000			----
				430,000	93,000			----
		PE	11/8/06	>24,000,000	230,000	225	212	----
				2,500,000	230,000			----
				2,400,000	2,400,000			----
			1/8/07	23,000	23,000	137.6	138.9	----
				4,600,000	750,000			----
				11,000,000	4,600,000			----
			3/2/07	2,400,000	93,000	99.7	212.2	----
				430,000	93,000			----
				930,000	430,000			----
			4/4/07	1,500,000	230,000	141.2	26.1	----
				2,400,000	930,000			----
				930,000	230,000			----
		SE (pre-chlor)	11/8/06	7,000	<3000	----	----	----
				19,000	<3000			----
				4,000	<3000			----
			1/8/07	23,000	150,000	----	----	----
				9,000	<3000			----
				<3000	<3000			----
			3/2/07	<3000	<3000	----	----	----
				<3000	<3000			----
				58,000	<3000			----
			4/4/07	23,000	210,000	----	----	----
				930,000	93,000			----
				23,000	230,000			----
		Effluent	11/8/06	930	2,300	40	59	0.5
				2,300	430			0.62
				4,300	210			1
			1/8/07	<30	230	32	52.7	0.75
				90	90			1.2
				40	430			0.85
			3/2/07	230	340	46	66.1	1.39
				930	930			1.19
				4,300	340			1.3
			4/4/07	210	46,000	31.1	45.9	1.08
				210	430			1.12
				9,300	46,000			1.22

PE - Primary effluent
SE (pre-chlor) - Secondary effluent prior to chlorination

Appendix B

Maceration Results

Location	Plant Operating Mode	Plant	Date	Fecal Coliform		Enterococcus	
				Macerated mpn/100 ml	Unmacerated mpn/100 ml	Macerated mpn/100 ml	Unmacerated mpn/100 ml
Final Effluent	Dry	Plant 1	7/17/06	2,400	390	230	4
				4,600	1,200	430	93
				11,000	1,500	430	23
		Plant 2	8/14/06	15	<3	<3	<3
				93	15	9	<3
		Plant 3	8/23/06	4	4	<3	<3
			4/23/07	90	230	4,300	4,300
				230	150	9,300	2,100
	Wet	Plant 1	9/14/06	11,000	930	>24,000	11,000
				>24,000	>24,000	>24,000	>24,000
				>24,000	>24,000	>24,000	>24,000
			11/8/06	4,300	>240,000	24,000	>240,000
				7,500	930	24,000	46,000
			3/2/07	9,300	930	9,300	230
				9,300	1,500	2,500	24,000
			4/27/07	24,000	9,300	110,000	5,500
				4,300	1,500	46,000	24,000
		Plant 2	11/8/06	>240,000	>240,000	110,000	24,000
				24,000	>240,000	9,300	24,000
			1/8/07	40	90	430	930
			4/12/07	110,000	4,300	24,000	>240,000
				9,300	1,500	4,300	930
			7/18/07	>240,000	>240,000	15,000	9,300
				>240,000	24,000	5,000	110,000
		Plant 3	11/8/06	24,000	2,300	750	430
				9,300	4,300	230	210
			1/8/07	430	90	2,300	90
				430	40	430	430
			3/2/07	230	230	12,000	340
				2,300	930	1,500	930
			4/4/07	2,800	210	46,000	46,000
				930	210	230	430

Appendix C

Flow and
Estimated Chlorine Contact Time Data

FLOW RATE DATA

Wet-Weather Blending Runs

Date	WWTP	LOCATION	TIME	WWTP INFLUENT (MGD)	SECONDARY INFLUENT	BYPASSED FLOW	BYPASSED (%)	Estimated Chlorine Contact Time (min)
9/14/2006	1	SP1-1	8:05	199	181	18	9	17.9
		SP1-2	8:50	248	181	67	27	14.4
		SP1-3	9:35	249	182	67	27	14.3
10/20/2006	1	SP1-1	9:30	248	183	65	26	14.4
		SP1-2	10:15	242	182	60	25	14.7
		SP1-3	11:00	171	172			20.8
11/8/2006	1	SP1-1	10:15	247	200	47	19	14.4
		SP1-2	11:03	245	200	45	18	14.5
		SP1-3	11:48	245	200	45	18	14.5
3/2/2007	1	SP1-1	7:55	249	191	58	23	14.3
		SP1-2	8:40	248	188	60	24	14.4
		SP1-3	9:25	248	188	60	24	14.4
4/27/2007	1	SP1-1	8:00	248	194	54	22	14.4
		SP1-2	8:45	248	189	59	24	14.4
		SP1-3	9:32	249	178	71	29	14.3
11/8/2006	2	SP1-1	10:15	123	-	-	-	-
		SP1-2	11:00	127.2	-	-	-	-
		SP1-3	11:45	127.8	-	-	-	-
1/8/2007	2	SP1-1	8:55	124	-	-	-	-
		SP1-2	9:40	90	-	-	-	-
		SP1-3	ND	ND	-	-	-	-
4/12/2007	2	SP1-1	7:55	137.4	-	-	-	-
		SP1-2	8:40	136	-	-	-	-
		SP1-3	9:25	137	-	-	-	-
7/18/07	2	SP1-1	7:50	124	-	-	-	-
		SP1-2	8:35	121	-	-	-	-

FLOW RATE DATA Cont'd

Wet-Weather Blending Runs

Date	WWTP	LOCATION	TIME	WWTP INFLUENT (MGD)	SECONDARY INFLUENT	BYPASSED FLOW	BYPASSED (%)	Estimated Chlorine Contact Time (min)
11/8/2006	3	SP1-1	11:25	566	-	-		-
		SP1-2	12:05	574	-	-		-
		SP1-3	12:50	580	-	-		-
1/8/2007	3	SP1-1	9:10	459	-	-		-
		SP1-2	9:50	400	-	-		-
		SP1-3	10:35	401	-	-		-
3/2/2007	3	SP1-1	7:45	406	-	-		-
		SP1-2	8:30	419	-	-		-
		SP1-3	9:15	500	-	-		-
4/4/2007	3	SP1-1	13:50	439	-	-		-
		SP1-2	14:34	436	-	-		-
		SP1-3	15:20	445	-	-		-

Dry-Weather Blending Runs

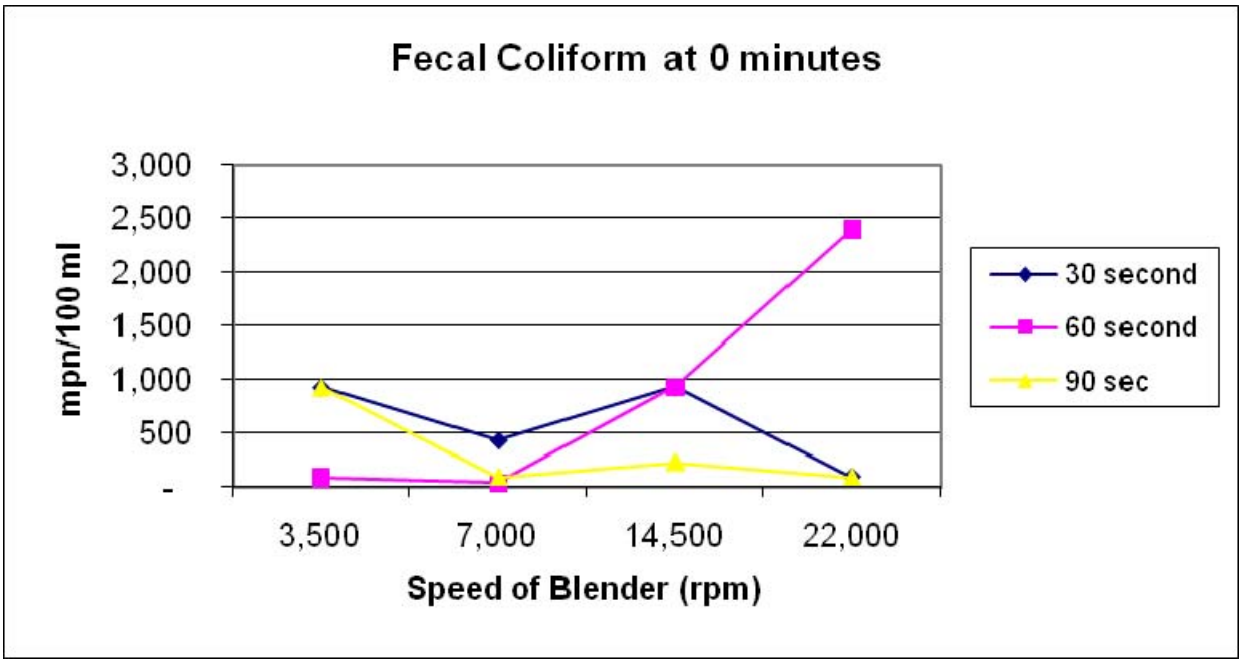
Date	WWTP	LOCATION	TIME	WWTP INFLUENT (MGD)	SECONDARY INFLUENT	BYPASSED FLOW	BYPASSED(%)	Estimated Chlorine Contact Time (min)
7/17/2006	1	SP1-1	9:30	138	-	-	-	25.8
		SP1-2	10:15	128				27.8
		SP1-3	11:00	130				27.4
8/14/06	1	SP1-1	8:30	111	-	-	-	32.1
		SP1-2	9:15	112	-	-	-	31.8
		SP1-3	10:00	113	-	-	-	31.5
8/14/2006	2	SP1-1	8:30	29.9	-	-	-	-
		SP1-2	9:15	30.9				-
		SP1-3	10:00	33.1				-
8/23/2006	3	SP1-1	9:08	216-	-	-	-	-
		SP1-2	9:55	236				-
		SP1-3	10:40	234				-
4/24/2007	3	SP1-1	9:15	262				-
		SP1-2	10:00	250				-
		SP1-3	10:45	233				-

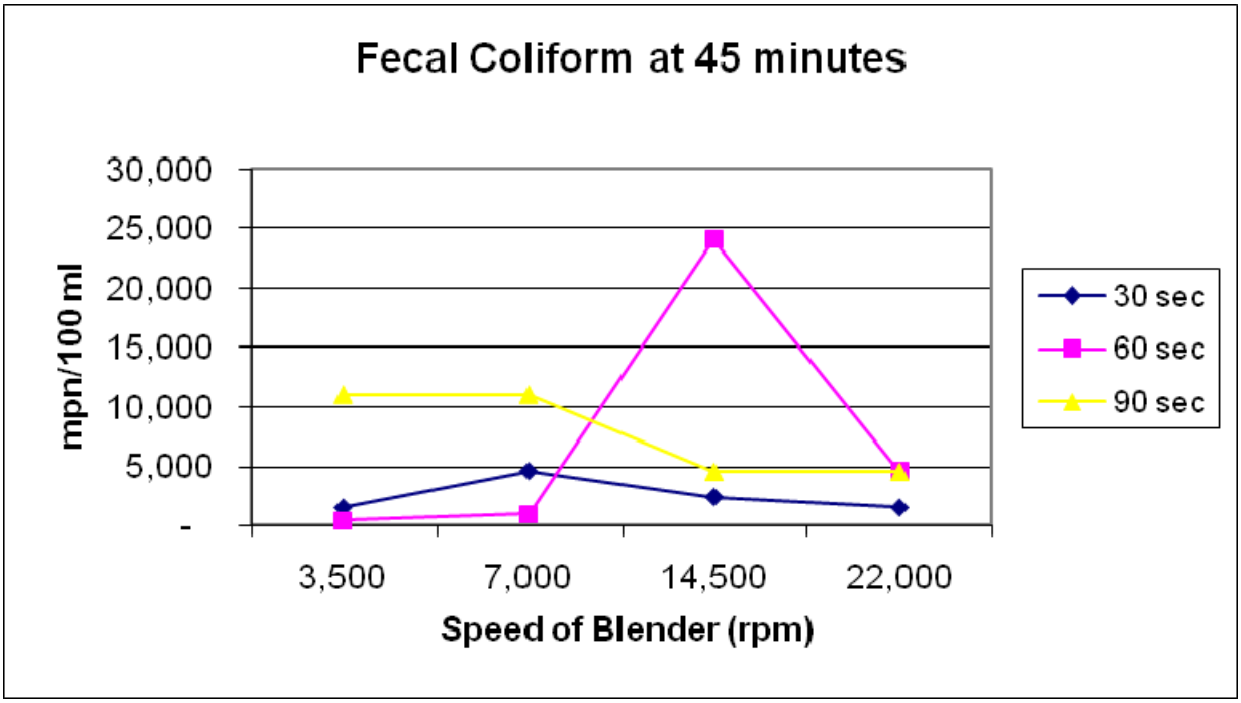
Appendix D

Maceration Optimization Analyses

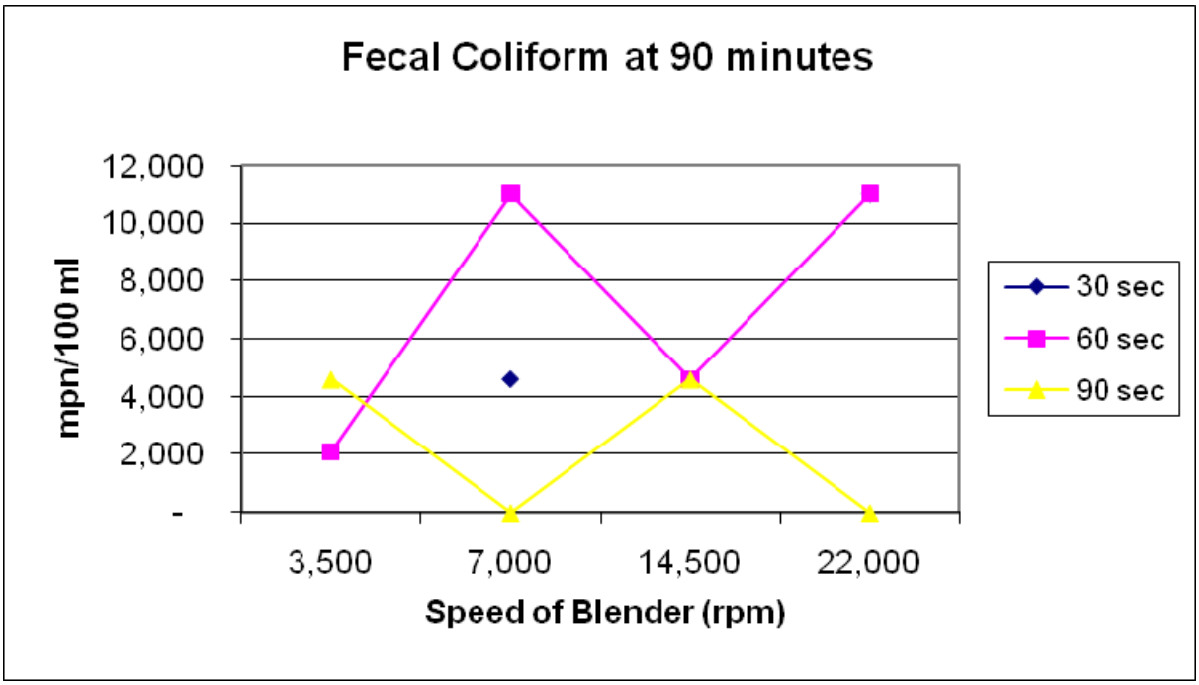
Charts for Maceration Optimization Analyses

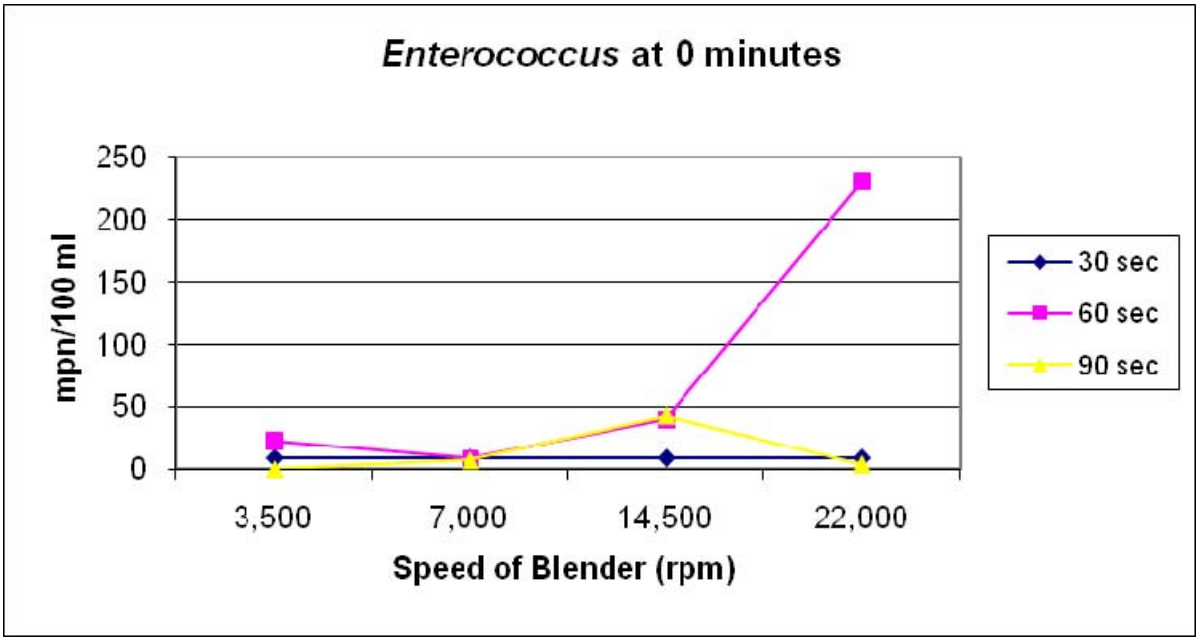
WWTP 1 – Dry Run - July 17, 2006



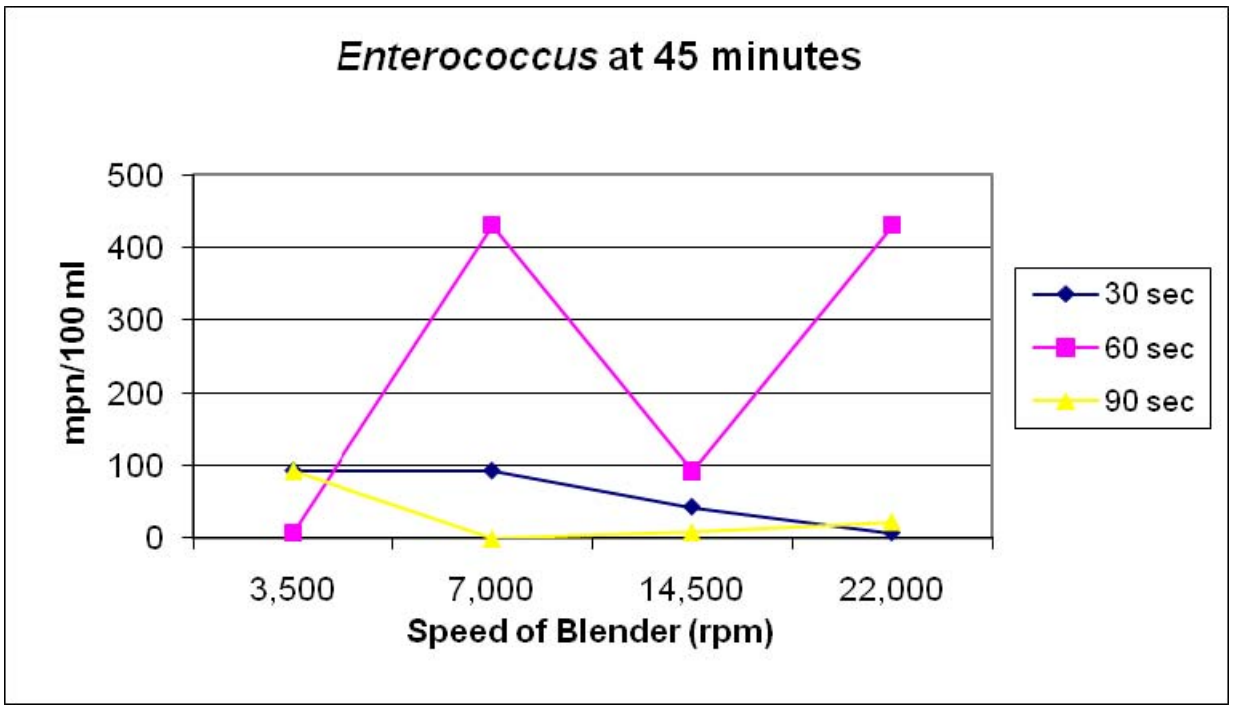


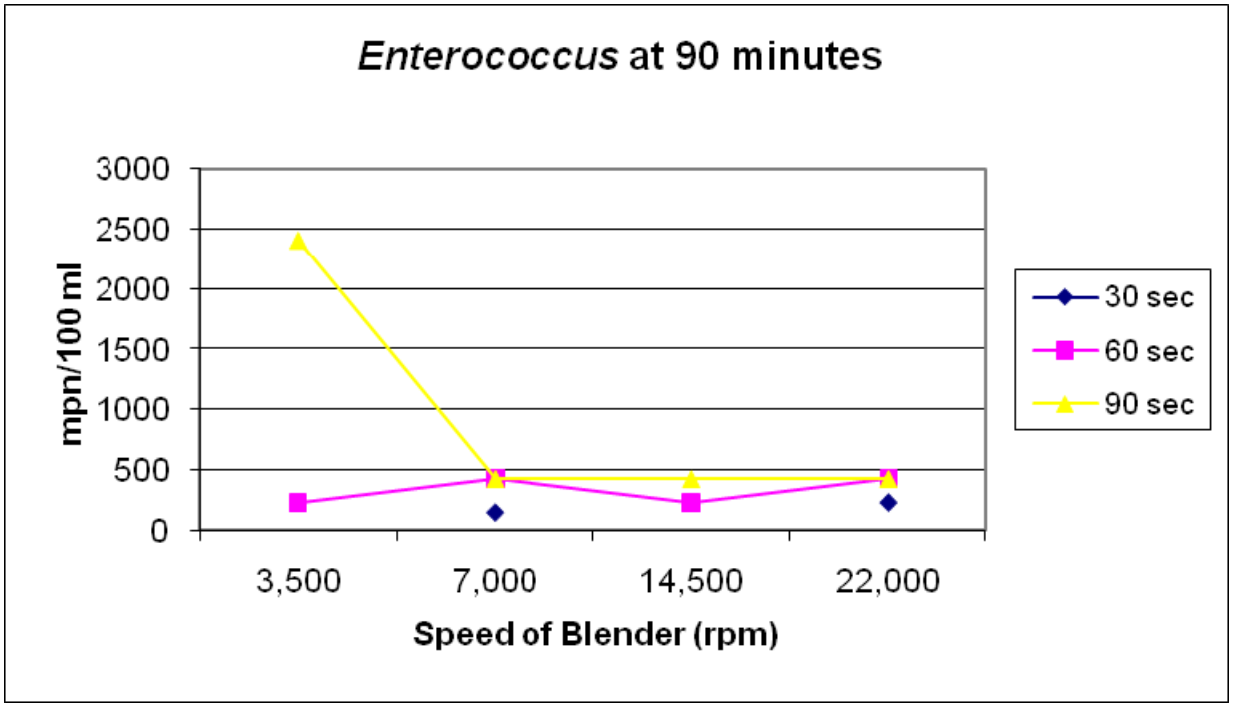
WWTP 1 – Dry Run - July 17, 2006



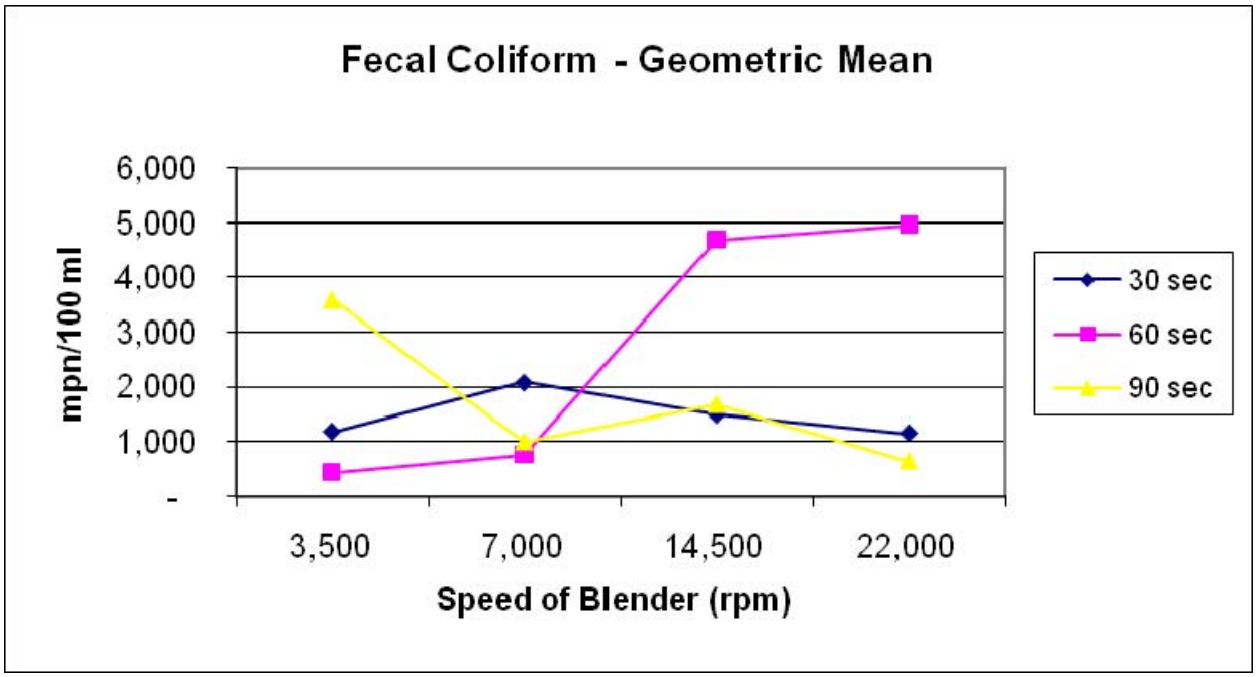


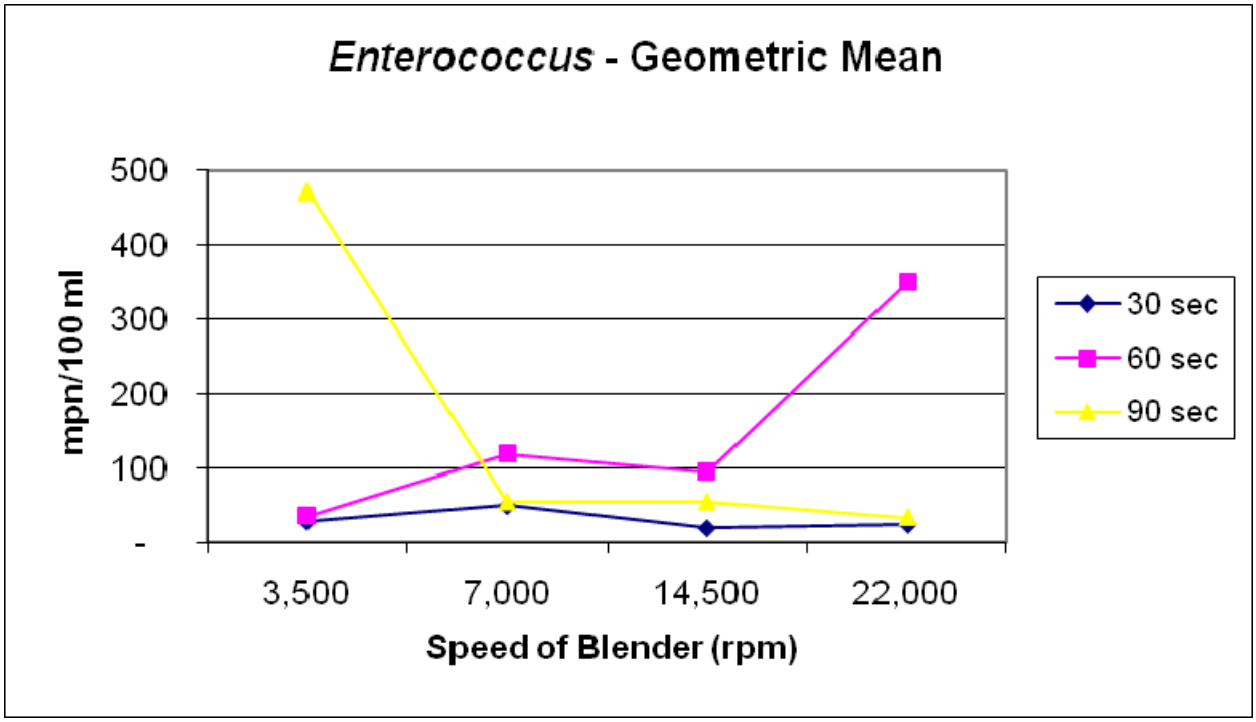
WWTP 1 – Dry Run - July 17, 2006



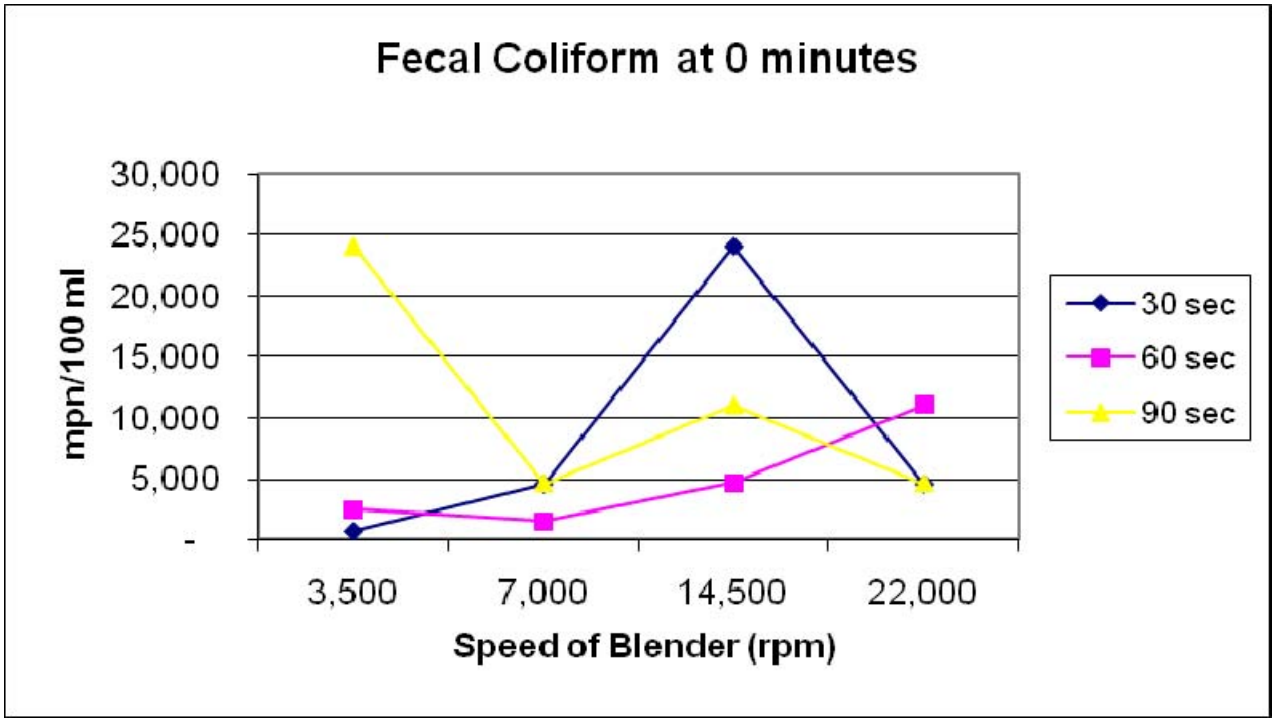


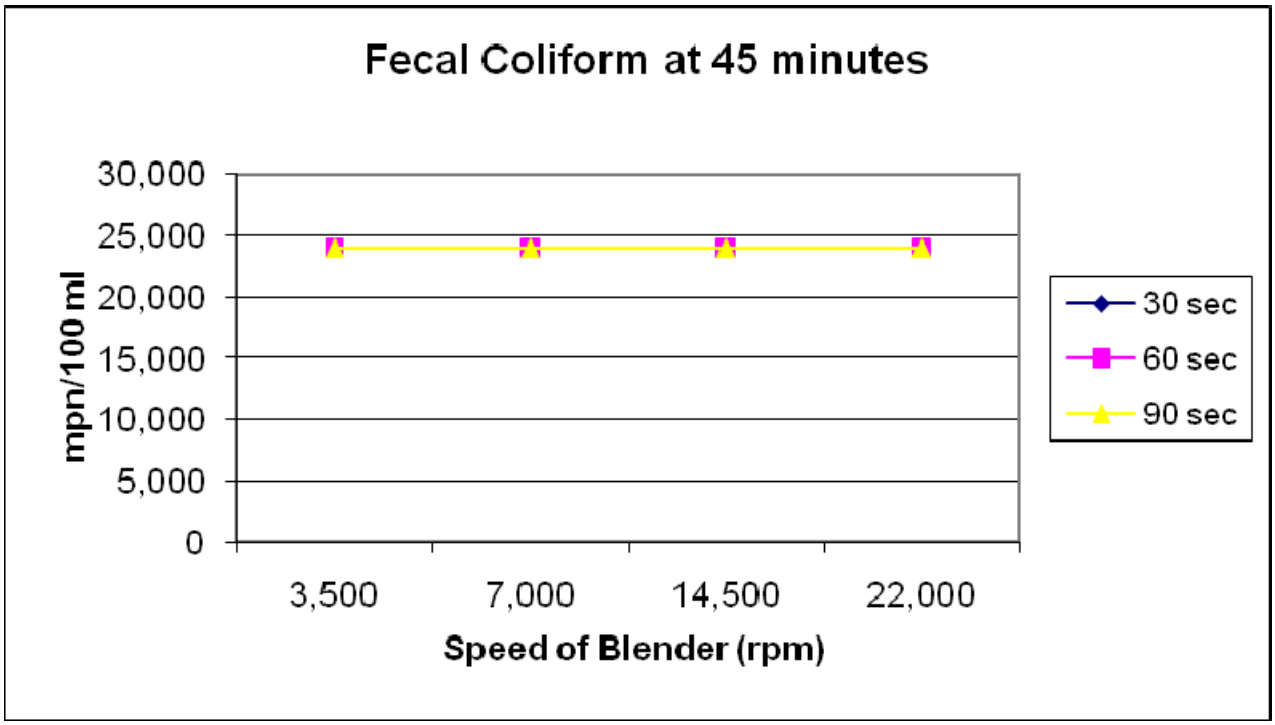
WWTP 1 – Geometric Means for Dry Run on July 17, 2006



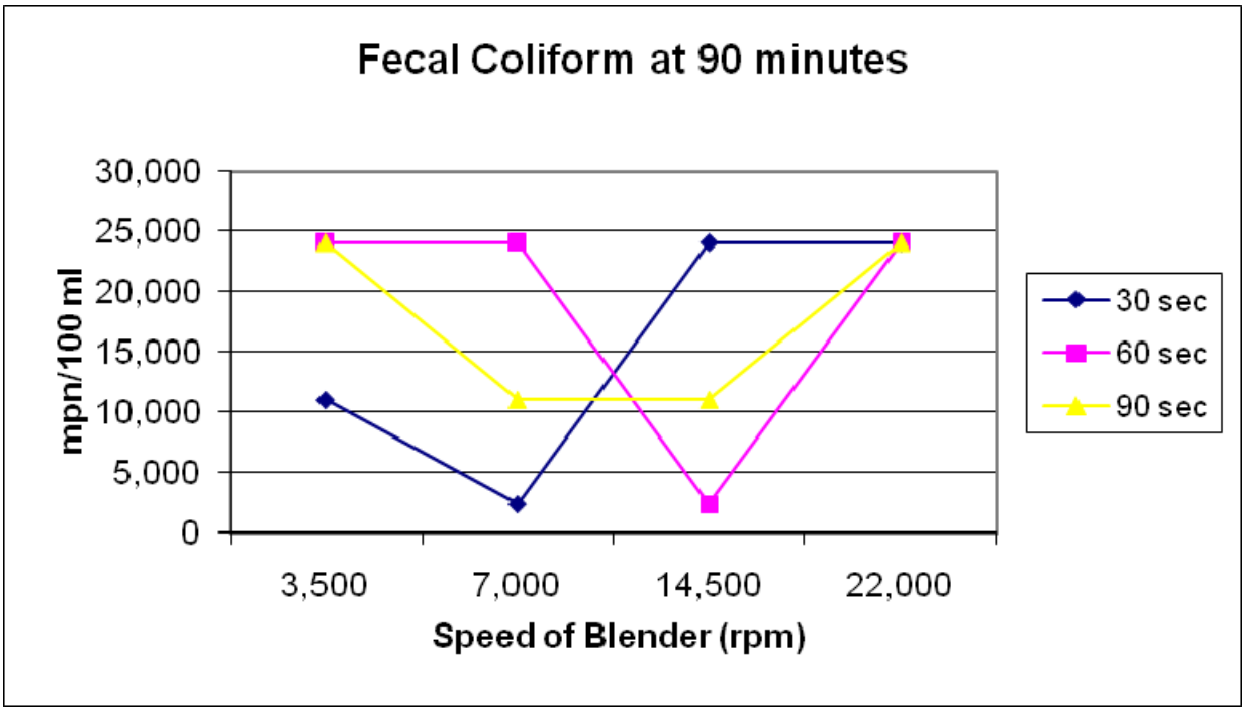


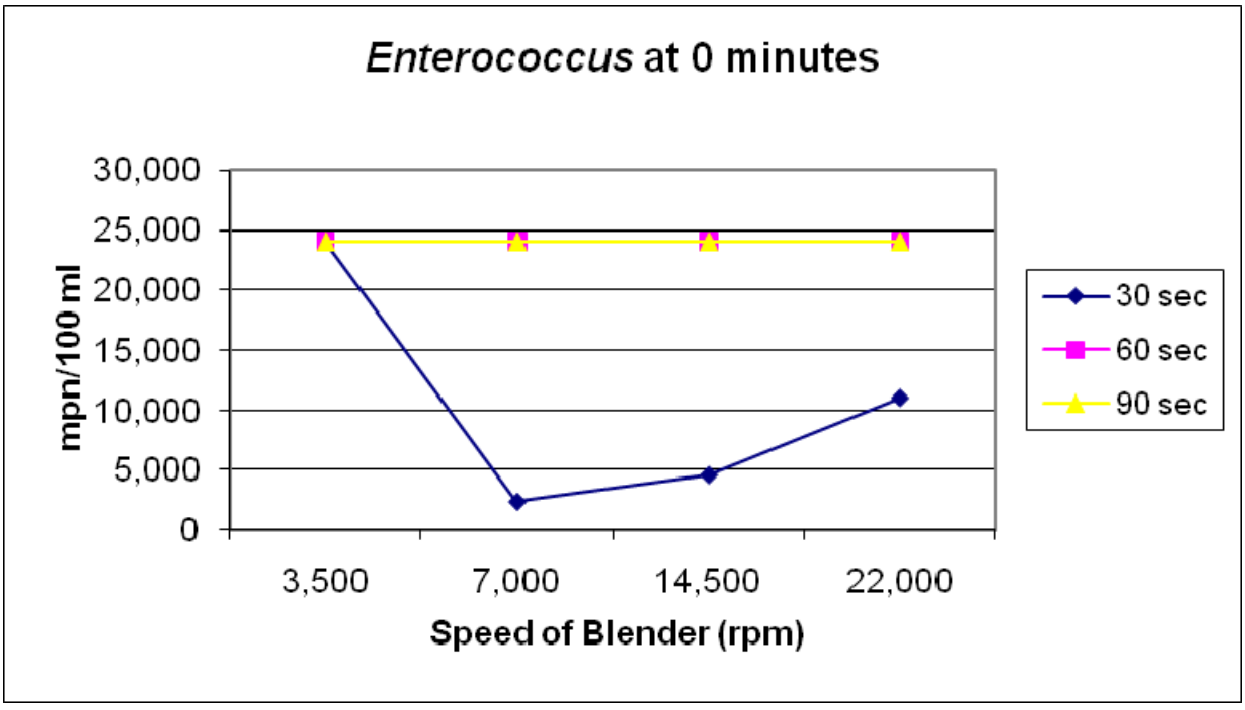
WWTP 1 - Wet Run - September 14, 2006



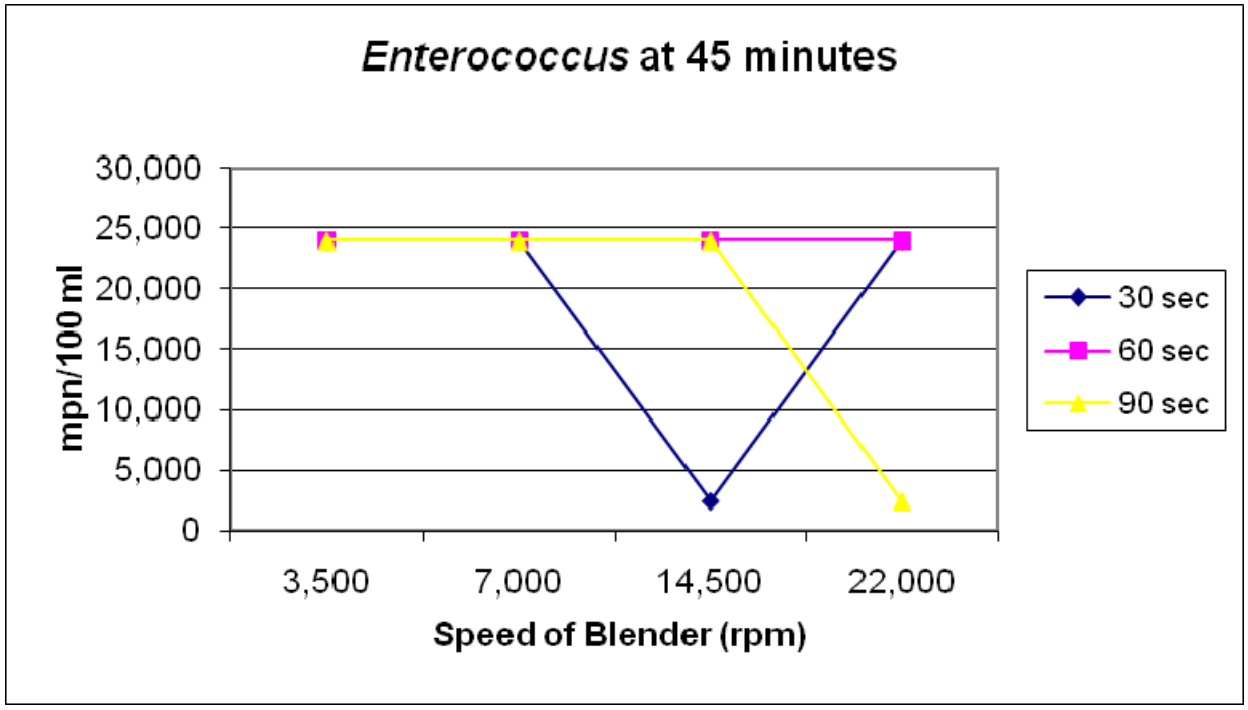


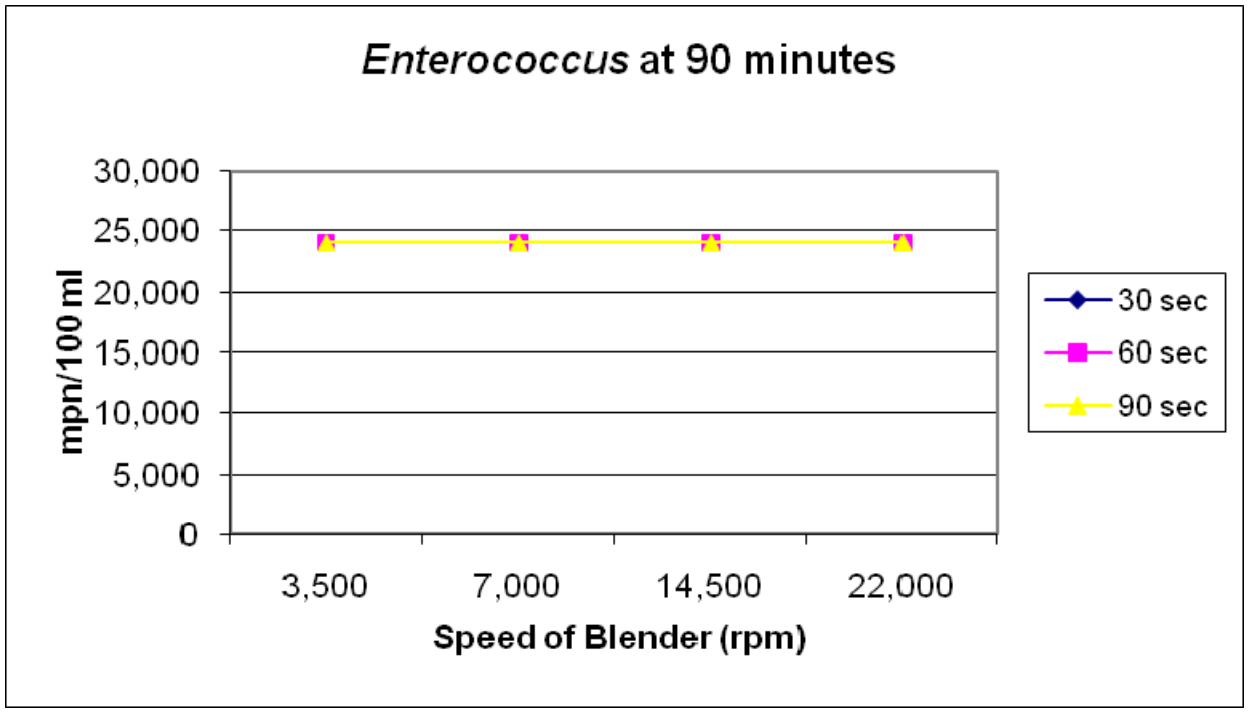
WWTP 1 - Wet Run - September 14, 2006



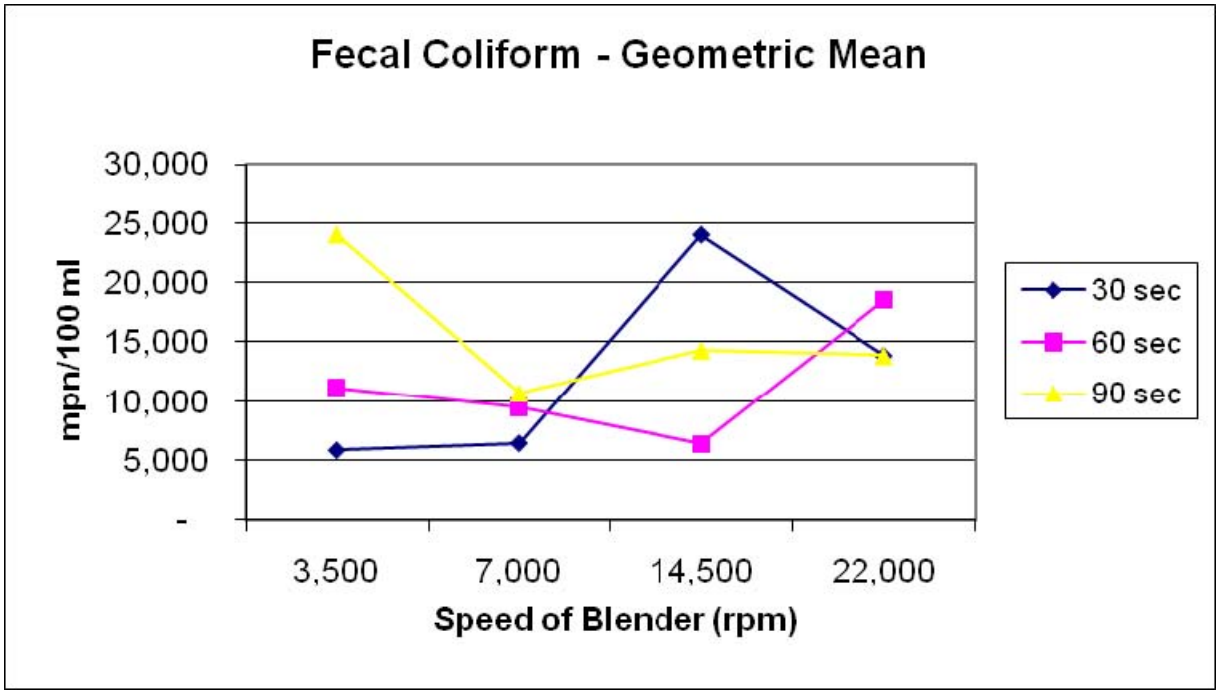


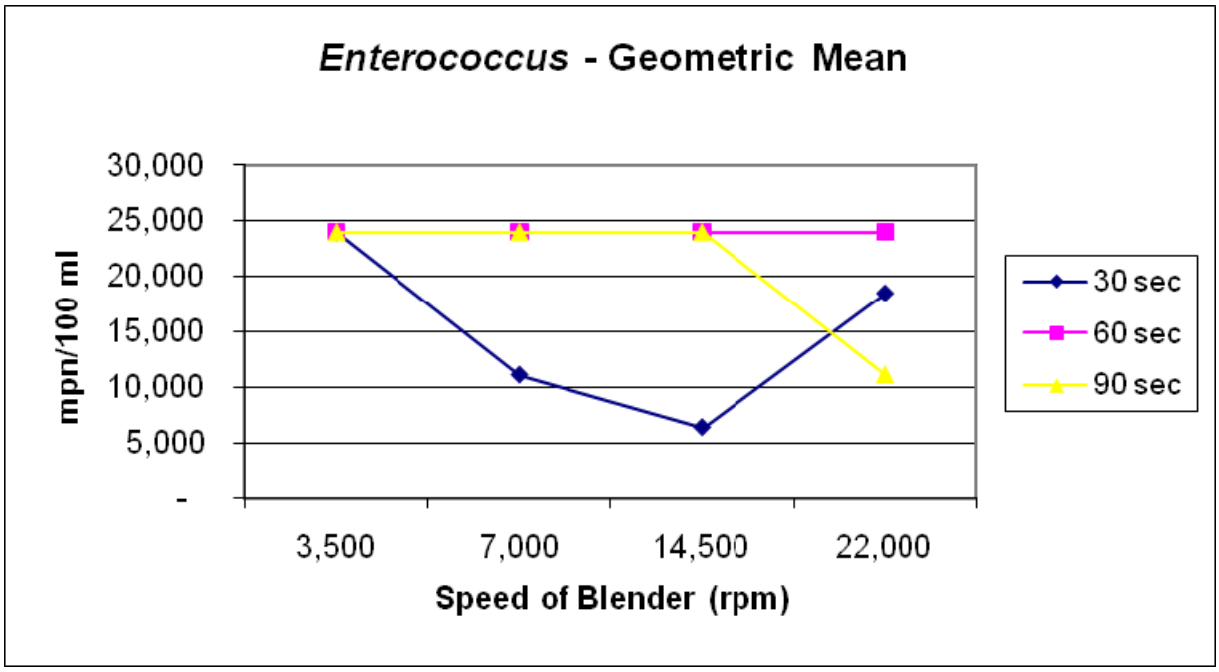
WWTP 1 - Wet Run - September 14, 2006





WWTP 1 – Geometric Means for Wet Run on September 14, 2006





Appendix E

Analytical Methods Used for Infectious *Cryptosporidium* and Virus Analyses

Summary of Methods used by Biological Consulting Services Laboratories

for

Infectious *Cryptosporidium* and Virus Analyses

***Cryptosporidium* viability assay**.
Concentrates from the IMS procedure were delivered to BCS Laboratories in Miami, Florida, following sample collection and analysis of samples by EPA Method 1623 by the EPA contract laboratory. Concentrates were analyzed for infectious *Cryptosporidium* as described by Slifko et al. (1997, 1999). Briefly, IMS concentrates (50 µl) were pretreated (8 min at room temperature) with a 10.5% (vol/vol) sodium hypochlorite (Sigma-Aldrich, St. Louis, Mo.) solution in Phosphate Buffered Saline (pH 7.2) to enhance excystation. The samples were washed once by centrifugation and were suspended in 1 ml cell culture medium supplemented with 10% fetal bovine serum and other additives (2% 1 M HEPES and 2 mM L-glutamine). Aliquots of this suspension were inoculated onto human ileocecal adenocarcinoma cell (HCT-8) monolayers cultivated in eight-well chamber slides (LabTech II; Nalgene Nunc, Naperville, Ill.). Slides were incubated in a 5% CO2 atmosphere at 37°C for 72 h. After incubation, slides were fixed with 100% methanol for 8 min and labeled by direct immunofluorescence with rat anti-*C.parvum* sporozoite-FITC (Waterborne Inc.) Slides were examined under epifluorescence and DIC microscopy, and each well was scored as positive or negative for infection. The results were entered into a most probable number (MPN) program and results were expressed as the number of infectious oocysts per 100 liters on the basis of the equivalent volume examined.

***Cryptosporidium* genotyping.**
Positive slides were marked with a permanent marker, and DNA was extracted directly from the slides following microscopic analysis. Cover slips were removed with a razor blade and cell monolayers were scraped with a sterile scalpel and resuspended in 50 µl of molecular-grade water. DNA was purified using a Qiagen DNA extraction kit (Qiagen, Inc.). Molecular characterization of *Cryptosporidium* species and genotypes were determined using a nested PCR-restriction fragment length polymorphism assay of the 18S small-subunit rRNA gene fragment (Xiao et al., 1999, 2000). For restriction fragment analysis, 20 µl of the secondary PCR product was digested in a 25 -µl (total volume) reaction mixture containing 20 U of SspI (New England BioLabs, Beverly, Mass.) for species diagnosis or 20 U of VspI (MBI Fermentas Inc., Hanover, Md.) for genotyping of *C. parvum*. Digested products were fractioned on a 2.0% agarose gel and visualized by ethidium bromide staining. The patterns of DNA bands were used to differentiate the species and genotypes of *Cryptosporidium* parasites according to methodology described by Xiao et al. (1998, 1999).

Enteric virus assay.
1MDS virus filters were shipped to BCS Laboratories, Inc. in Gainesville, Florida, and were processed immediately upon receipt. Filters were eluted with 1 L of 3% BBL V beef extract/Glycine (pH 9.2, 25°C), concentrated by organic flocculation, and assayed for viable enteric viruses by the observation of cytopathic effects (CPE) on recently passed (<4 days) Buffalo Green Monkey (BGM), Rhabdosarcoma (RD), and MA-104 cells. Positive controls were performed in a designated area using Poliovirus I. The most probable number (MPN)

determinations were calculated using EPA software. To increase sensitivity, samples were split for assay by cytopathology and ICC/PCR. Cell extracts were then pooled for PCR analysis.

Integrated Cell Culture PCR (ICC-PCR).
An integrated cell culture RTPCR method was used to detect viruses that do not cause cytopathic effects (CPE) in cell culture. Non-CPE viruses (e.g Noroviruses) can be present in treated waters and many viral pathogens can infect cell cultures without causing CPE. These viruses can subsequently be detected by PCR or RT-PCR. RT-PCR was performed on all viruses with the exception of Adenoviruses (DNA genome) for which standard PCR analysis were performed (Grimm et al., 2004; Oberste et al., 2006; Reynolds, 2004; Spinner and DiGiovanni, 2001; van Heerden et al., 2005).

Coliphage analyses.
Somatic and male-specific coliphages were analyzed by two methods: a modified version of the agar overlay method (EPA Method 1602) using E. coli (host strain F+amp for male-specific and host strain C3000 ATCC 15597 for both male-specific and somatic) and a version of the large volume (1L) presence/absence assay of (EPA Method 1601) for treated effluent.

Bacteriophages were enumerated as plaque forming units (PFU) per 100 mL or by MPN using the presence/absence assay.