

**Technical Report on a Microbial Ecology Approach
to Fecal Coliform Problems
at Field's Point Wastewater Treatment Facility,
Providence, RI under jurisdiction
of Narragansett Bay Commission**

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Abstract

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From 1995-1998, Field's Point Wastewater Treatment Facility (FPWWTF), under the Narragansett Bay Commission (NBC), has experienced seasonal problems with effluent fecal coliform bacteria exceedances. Decreased chlorine efficiency is hypothesized to be the primary source of this problem caused by physico-chemical characteristic changes in the wastewater that reduce the toxic ability of the chlorine. Bound legally by both fecal coliform level and chlorine residual limits for their final effluent, NBC has particular interest in both understanding the cause and potential predictors of this problem and has already done some research to investigate physical, chemical and operational factors presumed associated with the problem. This study takes a microbial ecology approach focusing specifically on the mixed liquor microscopic populations (diversity, density, dominance, and specific groups of protozoa, mesofauna, and bacteria) and associated ecological parameters (flow, rainfall, air temperature, MLDO, and chlorine dose). Regression analysis is used to compare the trends of these variables from 1995-1998 to effluent fecal coliform levels. Rainfall on the day of exceedances, organism dominance, and specific groups, flagellates, free swimming ciliates, and Nocardia show the strongest relationships to the quality of effluent measured by fecal coliform level. Multiple regression analyses are used for these and a few less strongly correlated variables (air temperature, minimum and total flow, mixed liquor dissolved oxygen, and rotifers). These analyses show the possibility of modeling the mixed liquor microbial system as the relationships between other parameters and fecal coliform levels come to be better understood and can be incorporated. Suggestions for operational control include closer monitoring of the relevant parameters identified in this study. Suggestions for further research include more thorough microscopic analysis of the mixed liquor and measurement of range and rate of some physico-chemical parameters already identified.

Introduction and Background

The Narragansett Bay Commission (NBC), a quasi-governmental agency, is the overseeing agency for the operation of Field's Point Wastewater Treatment Facility (FPWWTF) located in Providence, Rhode Island. FPWWTF treated an average dry weather volume of 44 million gallons per day (MGD) in 1997 with capacity to provide primary treatment for up to 200 MGD in wet weather conditions. Since 1994, the NBC has been concerned about periodic problems with fecal coliform levels in the FPWWTF's final effluent, which is released into the Providence River. This project reviews the work that NBC has already done in investigating the relationship between disinfection efficiency and bacterial kill, discusses some potential mechanisms of causation and explores the possibility of using microorganisms and other related parameters for early detection of disinfection and fecal coliform problems. The ultimate goal of this study is to develop microbial indicators of the fecal coliform problems at FPWWTF.

The FPWWTF is a secondary wastewater treatment facility involving a process called an activated sludge system¹ that recycles microorganisms as the wastewater progresses through a plant. Activated sludge systems were developed in the 1920s (Al-Shahwani and Horan 1991). The populations of "recycled" organisms provide a continuous inoculation that allows rapid breakdown of organic material and reduction of bacterial load (pathogenic and non-pathogenic) in the wastewater (Madoni 1986). Each stage prior to the chlorine disinfection is attempting to reduce the amount of organic matter in the water either by extracting solids from the water or by allowing microorganisms to consume the organic matter. (See Appendix 1 for partial diagram of secondary sewage treatment.)

After a cholera epidemic in the 1850's, the public health threat from raw sewage and poor drainage became a growing concern in Providence, the United States' seventh largest city at the time. The drainage system constructed in the 1870's was a combined system which collected both stormwater and sanitary sewage to flow together for release to local rivers and Narragansett Bay. Prior to 1900, wastewater was discharged into these water bodies untreated. The increased dilution from combining sanitary and wet weather flow was assumed to help reduce the risk from the waste contaminants (Narragansett Bay Commission). However, the present day consequence of the combined sewer system is that raw sewage overflow is unavoidable when there is greater than 0.25" of precipitation. Rainfall of 0.5" or greater instigates a closure of the Providence River and the Narragansett Bay to shellfishing. FPWWTF was built in 1901 (Narragansett Bay Commission).

Today, the wastewater is treated, and the NBC is governed by federal and state permits regarding the quality of the final effluent discharged to Narragansett Bay. These limits set by the Rhode Island Department of Environmental Management (RIDEM) pursuant to the Federal Clean Water Act (CWA) and subsequent Rhode Island Water Pollution Control Act are established to protect public health and aquatic life. See Table 1 for a list of daily and monthly final effluent permit levels (RIPDES permit #RI0100315)². Fecal coliform bacteria, found in the intestinal tracts of all warm-blooded animals, are used as indicators

of the presence of potentially pathogenic bacteria, viruses, and other parasites such as *Cryptosporidium*, *Giardia lamblia*, Norwalk virus, and *Vibrio cholerae* bacteria. The assumption is that fecal coliforms indicate the presence of untreated human sewage and, therefore, fecal coliform concentration in the final effluent is related to both disinfection efficiency and the possibility of waterborne bacteria and viruses being discharged to the Bay³. For both fecal coliform levels and Cl⁻ residual in the final effluent, the Rhode Island Department of Environmental Management (RIDEM) issues permits to the NBC.

Hypochlorite and other forms of chlorine (Cl⁻) are the most commonly used method of wastewater disinfection in the United States. The most direct method that FPWWTF has to reverse increasing levels of fecal coliforms is with chemical disinfection. This facility uses sodium hypochlorite. Because of recorded and suspected health threats that chlorine and chlorine by-products pose to both human populations and aquatic life, continually increasing the concentration of chlorine in the wastewater to compensate for elevated levels of fecal coliforms is a detrimental option. The safety and effectiveness of alternative disinfection methods are under investigation⁴. Field's Point WWTF discharges all of its effluent into the Providence River which flows into Narragansett Bay that is not only a focus of recreational and multi-million dollar commercial shellfish activity for the State of Rhode Island but also critical habitat for many aquatic species. Thus, both pathogenic risks and chemical contaminants are of particular relevance (Cabelli 1990).

Table 1. Permit #RI0100315 issued Sept. 30, 1992 by RIDEM, currently up for renewal.	Daily	Weekly	monthly
Fecal coliform (technology based)	400 MPN/100ml	400 MPN/100ml	200 MPN/100ml
Chlorine residual (modeling and dye study based)	65µg/L (interim 2mg/L)	-----	-----

Problem Description

For at least the last four years, NBC has experienced periodic exceedances of fecal coliform standards in final effluent between June and December which presents a potential public health problem. In order to reduce fecal coliform concentration, the NBC responds by increasing chlorine dose, resulting in periodic exceedances in Cl⁻ residual standards meant to protect aquatic life. This report summarizes the work that the NBC has already done in investigating the fecal coliform problems and then builds on that work through a series of investigations into various stages of the treatment process. There are many possible causes of fluctuations in the fecal coliform levels in the final effluent of FPWWTF. The parameters that affect the outcome can be divided into four categories: operational, chemical, environmental, and biological. (See Table 2 for an outline of the parameters affecting the treatment process.)

This project is designed assuming no single factor causes increases of fecal coliform levels in the final effluent. However, for the purposes of operational management of fecal coliform problems, it is important to note that there are two primary causal hypotheses. The first, a chemical hypothesis, credits the

mechanism of decreased chlorine efficiency to the increase of nitrite and nitrate compounds in the wastewater entering the chlorine contact chamber. These compounds, products of nitrification of ammonia, can couple with chlorine to form chloramines which are less effective disinfecting agents (Wanner 1998) and suspected “xenoestrogens,” compounds found to interfere with human and other animal endocrine systems (Colborn et al 1994). The second hypothesis offers a physical barrier explanation. This hypothesis proposes that the source of decreased efficiency is an increase in filamentous bacteria in the activated sludge. The filamentous bacteria can cause two very common treatment difficulties, bulking and reduced settleability, which can result in passage of a greater concentration of solids onto the chlorine contact chamber. These solids, which contain filamentous bacteria, other microorganisms, and organic matter, inhibit the chlorine from adequate disinfection of the wastewater.

The NBC has investigated these ten parameters: water temperature, flow, chlorine dose, chlorine use, effluent ammonia nitrogen, ammonia nitrogen removed, effluent TSS, MLSS, MLVSS, and MCRT. (See Glossary in Appendix I for definitions.) NBC used linear regression analyses to examine the correlation between these parameters (independent variables) and the final effluent fecal coliform levels (dependent variable). See Appendix 3 for summarized results of the NBC study. The data were analyzed in three different groupings: annual, January - June, and July- December. For the group of all months between 1995 and 1997, the strongest positive correlation to fecal coliform levels was water temperature ($R^2 = .12$). The second strongest positive correlation was ammonia removed ($R^2 = .06$). The correlation of fecal coliform and water temperature is consistent with the fecal coliform problems occurring in the warmer months of the year. This study emphasizes the multiple factors and annual trends associated with treatment quality and warmer months of 1995. Most importantly it supports the conclusion that there is not a single parameter that shows consistent correlation with the fecal coliform problems. This conclusion lends evidence to a multicausal hypothesis.

Table 2. Parameters possibly correlated with ineffective disinfection (see Glossary for explanation of abbreviations)			
Environmental	Operational	Chemical	Biological (activated sludge)
diurnal and seasonal fluctuations: flow (min, max, range)*, water* and air temperature**, rainfall**	Chlorine diffusion (east/west chlorine contact chamber differences)**	effluent NH ₃ *, NH ₃ removed*	dominant organism**
F/M ratio, composition of incoming waste	MCRT*	MLDO**	fluctuations of groups of microorganisms**
MLSS*, MLVSS*, TSS*, pH, SVI**	Chlorine dose*		diversity**
	MLDO**		density**

* parameters already investigated by NBC, **parameters investigated in this study

This Study

The scientific literature regarding performance of sewage treatment facilities cites many examples where microorganism populations have been used to measure efficiency of the treatment process. Curds and Cockburn (1970) established a relationship between the occurrence of certain protozoa and the biochemical oxygen demand (BOD) in the final effluent to predict monthly average of BOD. Al-Shahwani

and Horan (1991) also used protozoa and established a relationship between these organisms, sludge age and organic loading rate (food to mass ratio (F/M ratio)). Madoni (1994) developed a Sludge Biotic Index (SBI) to assess the performance of activated sludge treatment facilities. These studies establish reliable correlations between effluent quality, plant performance, and microorganisms in an activated sludge process. The focus of this project is to determine whether such a relationship can be established between microorganisms in the mixed liquor from the activated sludge process and the efficiency of chlorine disinfection. The relationship between microbial population characteristic and final effluent fecal coliform levels was examined because of published literature, recommendations from the NBC microbiologist, and the absence of any equivalent study conducted previously for or by NBC.

The focus of this study differs from the research already conducted by the NBC not only in the parameters on which it focuses as outlined in Table 2 but also in its intention. The final effluent fecal coliform problem has economic, operational, and environmental implications. While the specific cause or causes of such fluctuations in quality of the final effluent are important, operational solutions rely more on an understanding of when these problems will occur and the means to correct them. For these reasons, this study attempts to develop a means of predicting when these periods of fecal coliform problems are most likely to occur.

The central hypothesis of this study is that microorganisms and factors associated with the microbial ecology of the mixed liquor show predictable correlation with the effectiveness of chlorine disinfection. Two preliminary hypotheses were tested in order to localize the problem to the chlorine contact chamber and then to establish that the final clarifiers were treating the entire volume of water with consistency. (See Appendices 4 and 6.)

This project establishes a baseline of protozoan communities at the FPWWTF using samples taken from the mixed liquor, the solution of wastewater just after it leaves the aeration tanks. The intent is that, given a better understanding of the baseline populations, future fluctuations in populations can be used to predict periods of chlorine inefficiency. The theory is that with enough advance warning of potential disinfection inefficiency based on fluctuations in microbial populations, the plant operating conditions may be able to be altered to avoid both chlorine residual and fecal coliform permit violations.

The major stages of the sewage treatment process at FPWWTF are diagrammed in Appendix 1. The detention time, or the length of time in which the wastewater remains in each tank or chamber, is noted (based on plant average flow design of 65 MGD). The detention time generally decreases as flow increases. The water entering the plant is called influent. The treated wastewater released from the plant is called final effluent.

Despite this extensive study of 'fecal coliform problems' from sewage treatment at FPWWTF, this investigation is legally and ethically more preventative than reactionary. The fecal coliform exceedances discussed are daily exceedances of the monthly average permit limit (200 MPN). Daily exceedances of the legal daily limit which is higher (>400 MPN), occur less frequently, and the actual number of times the monthly average fecal coliform level exceeds 200 MPN are even more rare.

FPWWTF is a nationally recognized facility which takes extensive measures to prevent problems that represent threats to human health, recreation, and commerce before they manifest.

Materials and Methods

The central focus of this study was the analysis of the microbial populations in the mixed liquor and related ecological parameters. This section describes sampling location and sample times, microscopic and analytical techniques used, the monitoring of physical and chemical characteristics, and the statistical techniques used. The two preliminary studies conducted in conjunction with this study are detailed in Appendices 3 to 5. Historical data routinely collected from the period May 1995 - September 1998 were used for this study. All samples were collected and analyzed by NBC personnel.

Sampling

All samples were collected at FPWWTF (see Appendix 1 for plant diagram) in Providence, Rhode Island and analyzed at NBC's microbiological laboratory located at the Bucklin Point Wastewater Treatment Facility (BPWWTF) in East Providence, RI.

Grab samples for microscopic analysis are routinely taken in 250 mL plastic cups from the mixed liquor chamber daily at 7:30 a.m. The microscopic analyses are performed as soon as possible after transport to the laboratory and always within 2 hours of sample collection. The mixed liquor samples are thoroughly mixed prior to analysis.

Identification and enumeration of microscopic organisms

The multiple tube fermentation technique, as described in *Standard Methods* (APHA 1995), is used to generate a most probable number (MPN) of fecal coliform bacteria for final effluent samples. The multiple tube fermentation technique was run daily on the final effluent by NBC personnel.

The microscopic analysis of the mixed liquor used direct counting method with phase contrast illumination. Magnifications of 200x and 400x were used on the final clarifier samples and magnifications of 200x, 400x, and 1000x for speciation of filamentous bacteria, were used on the mixed liquor samples. An estimated .05 mL drop of sample was pipetted onto slides. One slide was examined for each mixed liquor sample. The microorganisms quantified were amoeba, flagellates, free swimming ciliates, stalked ciliates, rotifers, and nematodes. Filamentous bacteria, identified to the species level, were rated with a relative abundance on a scale of 0-6 (0-none, 1-few, 2-some, 3-common, 4-very common, 5-abundant, 6-excessive) (Jenkins et al 1993). The same examiner rated the abundance each time for the mixed liquor examination. Quantification was done in three linear passes over separate portions of the slide and the counts were compiled into a single quantity for each slide.

Physico-chemical monitoring

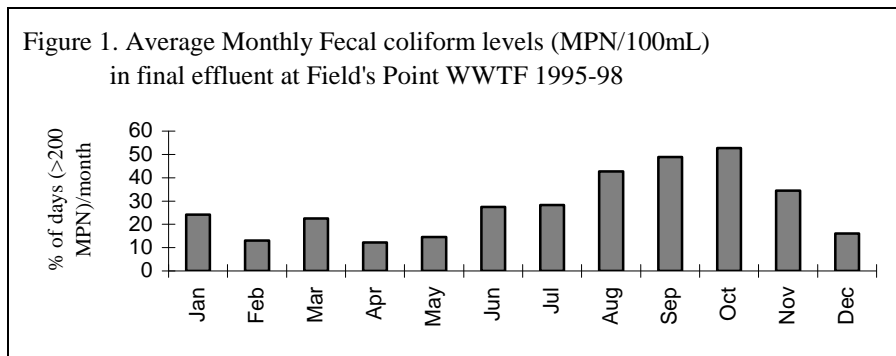
Ambient temperature at 6 AM (°C), daily rainfall (inches) from a NBC rain gauge, total plant flow in millions of gallons per day (mgd), individual clarifier flow (mgd), chlorine (Cl⁻) dose (lbs), and Cl⁻ residual (mg/L) (measurement made closest to time final effluent fecal coliform sample was taken) in the final effluent were also used in the data analysis.

Statistical Analysis

Excel version 5.0 and The SAS System release 6.12 were used for all statistical analysis. Pearson correlational analysis and single and multiple regression analyses were used for comparison of microorganisms and related ecological parameters with final effluent fecal coliform levels. Preliminary efforts at modeling assumed linear relationships. Relationships were assessed using scatterplots to determine linearity.

Results and Discussion

The motivation of this study was the periodic effluent fecal coliform problems that the FPWWTF experiences between June and December. The elevated fecal coliform levels are seasonal, increasing in late spring and persisting to early winter (See Figure 1). Plant operators and administrators hypothesize that the problem occurs primarily because of varying levels of efficiency by the disinfecting agent chlorine. This hypothesis was supported by preliminary studies that compared pre- and post-chlorination fecal coliform levels (Appendix 4 and 5) and the treatment efficiency of final clarifiers with respect to microbial population and secchi depth (Appendix 6). These studies supported the hypothesis that the entire volume of flow experiences consistent treatment between the aeration tanks and the chlorine contact chamber (See Appendices 1 and 4 -6 for details of both studies).



Environmental Parameters

The environmental parameters analyzed for this study were flow volume (daily minimum, maximum, total, and range in millions of gallons per day (MGD)), rainfall (inches), and morning (6 AM) air temperature. The parameter with the strongest correlation with effluent fecal coliform level is rainfall that occurred on the day of the fecal coliform measurement (1996-98 data). Rainfall was analyzed for day of and day before trends with effluent fecal coliform levels. Frozen precipitation was excluded from the analysis because of the uncertain time of entry into the wastewater system. The rainfall data from 1997 shows less correlation than when the combined data for 1996-98 are used. This discrepancy could be because of anomalous data from 1997 or due to an unknown trend of the 1996-98 data. The primary difference between the two groups is that most of the 1996-98 data are weekday measurements of fecal coliform levels while the 1997 data were collected daily. The results of the regression analysis for days of fecal coliform exceedances showed consistently weaker correlation for both day of and day before rainfall measurements.

All parameters of flow showed weak correlation with effluent fecal coliform results (log MPN), and morning air temperature showed less correlation with effluent fecal coliforms than all analyses of rainfall. See Table 2 for estimated slopes, standard errors, p-values and R^2 values for each of these parameters.

The finding that rainfall, but not flow, may relate strongly to chlorine efficiency has two possible explanations. The first possibility is that an inappropriate measure of flow has been used in this study. Perhaps mean or median flow would show stronger correlation and also allow for control of seasonal variation. Another approach could be analysis of the average deviation from the seasonal baseline. The first half of the year has a higher baseline flow due primarily to precipitation and an elevated groundwater table which results in infiltration to the wastewater pipe system (Camp Dresser and McKee). The second possibility is that the effect of rain is not on the influent itself but instead the action it has on the surface of the treatment tanks, specifically the final clarifier tanks. One explanation of the effect is that the rain could interfere with the settling and scum process in the clarifiers leading to the passage of more solids on to the disinfection stage. The first possibility, that flow does have a greater effect than observed at present, is hypothesized to be both more likely and, since it requires only a different reporting of a parameter already measured, it would be easier to study.

Table 2. Regression analysis for environmental parameters (independent variables) and effluent fecal coliform levels (log MPN) (dependent variables)	Estimated slope	Standard error	P-value	R ² value
Min. Flow 1997 (364 obs)	-.02	.004	.0001	.07
Max. Flow 1997 (364 obs)	-.001	.001	.46	.002
Total Flow 1997 (364 obs)	-.01	.004	.01	.02
Range Flow 1997 (364 obs)	.001	-	.36	-
Rainfall (day of) 1997 (364 obs)	.12	.15	.40	.002
Rainfall (day before) 1997 (364 obs)	.02	.15	.90	.000
Rainfall (day of) 1996-98 (375 obs.)	.46	.03	.0006	.03
Morning air temp. 1996-98 (375 obs.)	.01	.05	.0001	.05

Operational and chemical parameters

Chlorine dose (lbs.) and mixed liquor dissolved oxygen (MLDO) were analyzed⁵. Chlorine (Cl⁻) dose, also included in the preliminary NBC study, was re-analyzed to give consistency to statistical parameters (Table 3).

The observed weak negative correlation of the MLDO and fecal coliform levels is consistent with several ecological relationships that affect the quality of the treated wastewater. Low MLDO favors nitrifying bacteria, one hypothesized method of reduced Cl⁻ efficiency. High MLDO favors growth of organisms such as some species of stalked ciliates and free swimming ciliates that indicate favorable treatment conditions (Madoni 1986, 1993). Cl⁻ dose shows significantly less relationship with effluent fecal coliform levels than does the mixed liquor dissolved oxygen (MLDO).

Table 3. Regression analysis for operational and chemical parameters (independent variables) and effluent fecal coliform levels (log MPN) (dependent variables)	Estimated slope	Standard error	P-value	R ² value (adjusted)
MLDO 1997 (311 obs)	-.15	.05	.003	.02
Cl ⁻ dose 1997 (364 obs)	.0002	.00006	.0003	.03

Biological parameters

Three population-scale attributes of the microbial assemblage in the mixed liquor were examined – density, diversity, and dominance – as indicators of the “biological quality” of the mixed liquor (Madoni 1993). In addition, population attributes of the following groups were examined in detail: flagellates, free swimming ciliates, rotifers, and Nocardia. These factors were selected because of availability of data from historical analyses by NBC and published literature that stresses the of importance of protozoa, mesofauna, and actinomycetes (similar to filamentous bacteria) found in mixed liquor as indicators (Curds and Cockburn 1970, Al-Shahwani and Horan 1991, Madoni 1986, 1994)⁶ .

Dominance, particularly the ratio of flagellates to amoeba to free swimming ciliates, showed a striking pattern that overlapped the periods of effluent fecal coliform exceedances (Figure 2a). Nocardia are not included because they are enumerated on an abundance scale instead of by direct count method and

thus the measurements are not comparable. Flagellates, like fecal coliform exceedances, consistently have the greatest percentage of days of dominance for each month between late spring until fall. The Pearson correlation coefficient for flagellate dominance and days with fecal coliform exceedances is 0.1818. During the rest of the year, amoeba, free swimming ciliates, and stalked ciliates predominate.

Density is defined as the total number of organisms from all groups observed in daily microscopic analysis of the mixed liquor. Diversity is the number of different groups of organisms observed in the same analysis. Both parameters showed weak correlations with effluent fecal coliform levels. Density shows an annual pattern over the period analyzed (1995-98) having the highest monthly averages in late summer and lowest monthly averages in mid to late winter (See Figure 2b). Diversity is difficult to use as a parameter because the number of groups of microorganisms identified in the microscopic analysis is limited. Thus, the diversity measurement is usually fixed between 2 and 4.

Microorganism diversity and fecal coliform levels showed a negative correlation. The literature supports the finding that increased diversity is associated with better wastewater treatment, or, in this case, lower fecal coliform levels (Madoni 1994). However, until analysis includes speciation of at least some groups, measurement of diversity is not sensitive enough to be useful as an indicator. As with diversity, increased density in the mixed liquor is also associated with better operating conditions. The regression analysis did not support this association and the monthly average trend also paralleled fecal coliform exceedances, not supporting the predicted association. The difference can possibly be explained by the method of density measurement (direct organism count). Biomass measurement would be a more standardized and comparable measure. See Table 4 for slope estimates and p-values for all biological parameters.

In the individual group examination, the number of flagellates and the abundance of *Nocardia* filaments showed a positive correlation with fecal coliform levels. Free swimming ciliates showed the opposite effect. Flagellates and *Nocardia* appear to be an indicator of treatment conditions that inhibit effective chlorine disinfection, and the free swimming ciliates are indicative of conditions that favor effective disinfection. None of these groups of organisms seem to have a direct causal effect.

Flagellates stand out in both direct counting methods and dominance comparisons as one of the most useful indicators. Successional stage theory perhaps helps explain what is responsible for the correlation. Flagellates represent an early successional stage usually indicative of lower dissolved oxygen conditions whereas free swimming ciliates (and stalked ciliates) represent a later stage of succession (Madoni et al 1993). Madoni (1994) associates later stages of succession of mixed liquor populations with "higher quality effluent," i.e. lower BOD, lower TSS. The microorganisms of later successional stages are more efficient at processing or consuming organic matter and bacteria in the aeration stage of treatment. By the successional theory, the flagellates represent regression to, or stagnation in, an early stage. From the monthly average trend in dominance, we see flagellates, the representative organism for this stage, predominate.

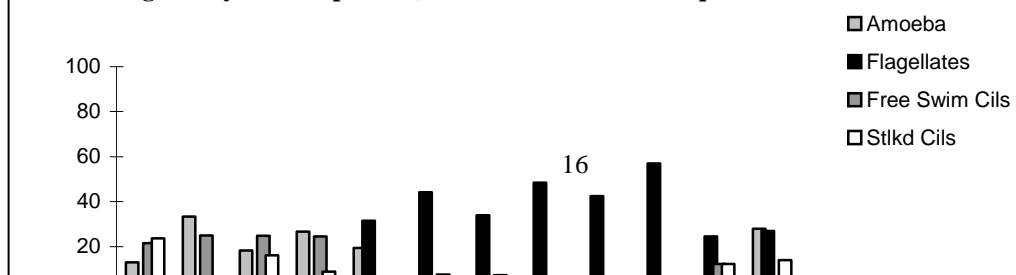
A plant condition that could cause regression to such trophic imbalance is the presence of toxic substances in the wastewater (Madoni 1986). Chlorination of the return activated sludge which is one source of toxicity is sometimes used in bulking conditions, most frequently occurring in the summer. However, the chlorination of RAS is used less frequently than the period of time in which flagellate dominance is excessive. Toxic chemicals can also enter the system with the influent. However, plant operators observe toxic influent effects less frequently than the observed period of flagellate dominance. Thus, perhaps the stagnation rather than regression explanation of remaining in an early successional stage is applicable.

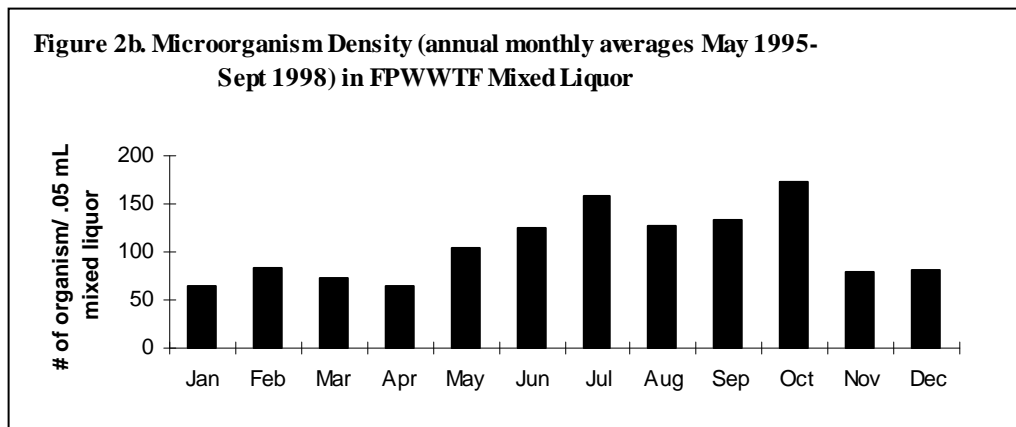
Flagellate dominance could be explained by conditions that favor organisms that are most mobile and reproduce rapidly (Madoni et al 1993). Flagellates have good mobility and reproduce faster than most of the other groups of organisms. Conditions that favor these conditions are an increased percentage of activated sludge wasting (decreased RAS:WAS) and lower food to mass (F:M) ratio. Other conditions that create limiting conditions responsible for trophic imbalance are lack of aeration and under- or overloading of the system with activated sludge. Free-swimming ciliates are also highly mobile but indicate slightly better performance conditions according to Madoni (1986).

Light availability at different depths in the various tanks and fluctuations in the photoperiod on a seasonal basis are also relevant to growth rate and organism dominance (Soeder et al 1986). Therefore, transparency (secchi depth) and transmissivity (turbidity) in the final clarifiers could theoretically influence species composition and abundance. For instance, light penetration and thermal gradients can be limiting factors in other enclosed bodies of water. However, whereas the period of time in which populations of organisms can dramatically increase their population is on the scale of days (Madoni et al 1993), the detention time in the final clarifiers is on the scale of hours. (In fact, the detention time for entry and exit from the entire plant averages 17 hours.) Thus, the residence time in the final clarifiers would appear to be insufficient to significantly affect population distributions arriving from the well-mixed aeration to the environmentally stratified clarifiers. This tends to support the use of mixed liquor samples to predict the nature of the water as it reaches the chlorination stage.

Table 4. Regression analysis for microorganism parameters (independent variables) and effluent fecal coliform levels (log MPN) (dependent variables)	Estimated slope	Standard error	P-value	R ² value (adjusted)
Density 1995-98 (699 obs)	.001	.0003	.0001	.02
Diversity 1995-98 (699 obs)	-.06	.043	.14	.002
Flagellates 1995-98 (699 obs)	.37	.05	.0001	.09
Free Swimming Ciliates 1995-98 (699 obs)	-.19	.00	.008	.04
Rotifers 1995-98 (699 obs)	.10	.15	.52	.001
Nocardia filaments 1997 (202 obs)	.19	.035	.0001	.12

Figure 2a. Number of days of organism dominance (annual monthly averages May 1995-Sept 1998) in FPWWTF Mixed Liquor





Multivariate analysis

Two multiple regression analyses were run to test plausible combinations of variables that might be more closely associated with final effluent fecal coliform exceedances than individual variables (Table 5). The first analysis combined flagellates (log flagellates +1), free swimming ciliates (log free swimmers + 1), morning air temperature, and rainfall (independent variables). The second analysis combined minimum daily flow, total daily flow, MLDO, Nocardia, Flagellates (log flagellates +1), Free Swimming Ciliates (log free swimmers + 1), and Rotifers (log rotifers +1) (independent variables). Both groups of variables were selected based on the strength of their relationship individually to effluent fecal coliform levels. Multivariate analysis showed some variables such as rainfall and free swimming ciliates (in the second analysis) to have stronger relationships to fecal coliform levels when the variation of other parameters is considered. However, flagellates, rotifers, air temperature, and MLDO showed weaker relationships. Overall, the multiple regression analysis explains a greater percentage of the variation (Analysis 1: $R^2 = .15$, Analysis 2: $R^2 = .22$) than any single parameter. This method of regression analysis is the preliminary step in developing a more comprehensive model for the mixed liquor ecology.

Table 5. Multiple regression analysis for microbial ecology parameters (independent variables) and effluent fecal coliform levels (log MPN) (dependent variable)

	estimated slope	Standard error	P-value	R ² value (adjusted)
<u>Analysis 1</u>				
Flagellates	.34	.08	.0001	.15
Free Swim. Cils.	-.18	.09	.06	
Air Temp	.005	.003	.14	
Rainfall	.55	.13	.0001	
<u>Analysis 2</u>				
Min. Flow	-.001	.01	.91	.22
Total Flow	.02	.01	.05	
MLDO	-.06	.07	.39	
Nocardia	.13	.05	.01	
Flagellates	.32	.14	.03	
Free Swim. Cils.	-.30	.14	.03	
Rotifers	-.02	.31	.95	

Conclusions and Suggestions

The parameters which show the strongest relationship with effluent fecal coliform levels are same day rainfall, flagellates, free swimming ciliates, and Nocardia filaments in the mixed liquor, and MLDO. Since all populations of organisms react to external environmental conditions, availability of nutrients and oxygen and interspecies niche competition probably also serve as indicators. The interconnectedness of all the identified parameters is important. An advantage of using these parameters, the specific groups of organisms and the characteristics of their environment, as indicators is their ease of measurement. Given the appropriate equipment, all of these parameters can be measured rapidly, either on-site or in the treatment facility's laboratories.

Methods of Control

Several methods of operational control can be applied to the treatment process when treatment problems identified become apparent. For example, flow rate to the chlorination system can be adjusted by changing the number of clarifier tanks on-line when a shorter detention time would reduce the growth of undesirable organisms. Others include increasing mixed liquor dissolved oxygen, chlorinating the RAS, or increasing the chlorine dosage in the disinfection. Another type of intervention involves the adjustment of the quantity of activated sludge returned to the aeration tanks through increasing or decreasing the RAS:WAS ratio, also called increasing/decreasing the mixed liquor (The Microlife 1994).

FPWWTF has plans to implement four additional aspects of treatment to improve their ability to produce high quality treated wastewater. They will include adding an Oxidation-Reduction Potential (ORP) monitor between final clarification and chlorination; adding an improved chlorine diffuser unit called a Water Champ; adding a dechlorination stage; and eventually adding nitrification and denitrification stages to the aeration system to reduce the amount of nitrogen that enters the Providence River with the treated wastewater.

Currently, Cl^- dose is based on flow volume, most recent Cl^- residual, and calculated Cl^- demand. The dosage is insensitive to characteristics of the water that might reduce efficiency (e.g. variations in chemical composition, solids concentration, and turbidity). For Cl^- residual, the lack of a strong correlation is expected and important. A strong positive correlation would indicate reduced efficiency. The ORP monitor aimed particularly at providing more sensitivity to Cl^- dosage is scheduled to be added to FPWWTF before the end of 1999.

The data collected during this study suggest that more attention should be paid to development of microbial indicators, particularly conditions affecting flagellate abundance. Many of the following research suggestions could easily be incorporated into the routine data collection that occurs daily at the treatment facilities and laboratories.

Research suggestions

Additional, potentially important parameters which are not measured routinely at present but could be collected are daily air temperature range, rate of rainfall, density based on biomass rather than number of organisms, counting of crawling (or sessile) ciliates, and presence and speciation of stalked ciliate colonies in the mixed liquor. The suggestion for daily temperature range is based on diurnal fluctuations of many of the physico-chemical parameters affecting the mixed liquor (Soeder et al 1986). The rate of rainfall would be relevant in trying to distinguish or incorporate the different effects of rainfall and flow volume fluctuations. Density based on biomass would be a more standardized measurement than the direct counting method (number of organisms) currently used. And lastly, the recommendations for changes in the microscopic analysis are based on studies by Curds and Cockburn (1970), Madoni (1986), and Salvadó et al (1995) that emphasize the importance of distinguishing three groups of ciliates (free swimming, sessile, and stalked) as well as several key species within each of these groups. All of these suggested parameters are adjustments to the data collection already performed routinely. "Food to mass ratio" is another variable already collected but not included in this study which could be incorporated in further analysis. These factors might help explain both the consistencies and inconsistencies in the microbial populations and effluent fecal coliform level results and also improve the predictive power of other parameters already monitored, such as amoeba population fluctuation.

In further efforts to model the mixed liquor ecological system, non-linear models could be considered as many parameters, particularly related to physico-chemical factors, do not have linear relationships (Salvadó et al 1995).

¹ The first full scale activated sludge treatment facility was installed 1922 (Al-Shahwani and Horan 1991).

² Violations of fecal coliform and chlorine residual permit limits, called total residual chlorine (TRC), present potential public health and environmental problems. U.S. Environmental Protection

Agency (EPA) established water quality criteria pursuant to Federal Water Pollution Control Act, or the Clean Water Act, in 1972 (40 CFR 120 - 140). NBC is authorized to discharge treated wastewater into Narragansett Bay and local rivers under Chapter 46-12 of the RI General Laws. Following implementation of the Clean Water Act, states were given a choice either to adopt the water quality management criteria and set permit levels accordingly or justify why they chose to do otherwise. Rhode Island adopted the EPA's criteria. The subsequent permit limits pursuant to Rhode Island Discharge Elimination System (RIDES) are based on both available technology and the water flow patterns surrounding the treatment facilities. Fecal coliform limits are technology-based standards. The permitted levels, 200 MPN/100ml (monthly) and 400 MPN/100mL (daily), are based on standards proven attainable by accepted disinfection techniques. In contrast, the TRC level is set based on dilution rates and flow patterns attained using the EPA's UPLUME modeling system and also a dye study conducted by the NBC. The TRC permit level was set at 65 µg/L of final effluent based on the more conservative modeling technique and the field-based dye study. However, because Field's Point is in the process of upgrading their system to include a dechlorination stage, an interim level of 2 mg/L was established to enable FPWWTF to meet the criteria until their upgrade is complete (Liberti).

³ While fecal coliform bacteria are used worldwide to indicate the presence of human sewage, some scientists argue that they underestimate the actual pathogenic threat of sanitary wastewater. Some pathogenic organisms such as *Cryptosporidium*, *Giardia* cysts, and viruses show greater resistance to disinfection than the indicator bacteria (States et al 1998, Tyrrell et al 1995). The primary impediments to switching the indicator organism are 1) the danger of enumerating species which are more pathogenic on a daily basis in a wastewater treatment facility laboratory, 2) difficulty and reproducibility of cultivation techniques, 3) turn around time for results, and 4) reliability of alternate indicators regarding indicating human sewage and/or human pathogens. Sosbey et al (1998) and Tree et al (1997) suggest spores of the anaerobic bacteria *Clostridium perfringens* and F+ bacteriophage as alternative bacterial and viral indicators. Yet the enumeration techniques for these alternative indicators are not yet developed enough to be used consistently in sewage treatment plants.

⁴ The two primary disinfection alternatives to chlorine are ultraviolet disinfection and ozonation. Bucklin Point WWTF, NBC's second sewage treatment plant located in East Providence, RI has a contract to replace their current chlorine disinfection system with an ultraviolet (UV) system. Some questions are raised as to the effectiveness of UV systems because of chemical build-up that occurs on the light casing inhibiting their effectiveness. However, the technology continues to improve and the greatest benefit of UV systems is that they eliminate the need to transport and store highly toxic chlorine liquid and gas compounds. The use of chlorine as a disinfecting agent, the primary method of disinfection in the US since the beginning of the century, is known to create chemical by-products, such as trihalomethane and haloacetic acid, that are reported to have toxic effects on human and non-human life forms (Rebhun et al 1997). Farther-reaching effects of these organochlorine compounds are still being discovered. Colborn et al (1994) have done extensive research on the endocrine disrupting effects of these chemicals and their possible reproductive consequences. Concerns associated with chlorine-containing chemicals as disinfection agents have driven recent efforts to find alternative disinfection sources or means of reducing the hazard of chlorination. These methods involve either adding a dechlorination stage to a plant's existing system or implementing ultra-violet (UV) irradiation or infiltration of ozone gas (Isaac 1998, Linden et al 1998, MacCrehan et al 1998). The Field's Point facility is implementing a dechlorination stage to its system which is scheduled to be operational in January 1999.

⁵ The analysis of differences of Cl⁻ residual between the east and west sides of the chlorine contact chamber was qualitative. Fecal coliform MPN results showed the east track as consistently 10-100 times higher than the west. The difference was credited to hydraulic imbalance in the mixing process where the disinfecting agent hypochlorite was added. Addition of a new diffuser pipe reduced the difference in the fecal coliform levels on east and west sides.

⁶ Other groups of microorganisms were excluded either because of lack of data, such as crawling ciliates, or, in the case of amoeba, stalked ciliates, and nematodes, because of weaker correlations with

effluent fecal coliforms based on preliminary analyses and literature research. Algae are not a prevalent group in mixed liquor because the turbidity prevents light penetration (Madoni 1986). Rotifers are included because of postulations by the NBC microbiologist of an inverse relationship with flagellates and fecal coliform levels from a qualitative assessment specific to FPWWTF mixed liquor. Evidence thus far suggests little relationship between rotifers and both flagellates and fecal coliforms. However, as outside research also emphasizes the important role of rotifers, a more detailed analytical approach such as the inclusion of other groups might reveal rotifers as better predictors of more specific conditions (Soeder et al 1986).