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Assessing the role of protists in removing E coli in slow sand filters

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ASSESSING THE ROLE OF PROTISTS IN REMOVING *E.COLI* IN SLOW SAND FILTERS

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ABSTRACT

ASSESSING THE ROLE OF PROTISTS IN REMOVING *E.COLI* IN SLOW SAND FILTERS

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The organic layer that forms on top of the sand bed in slow sand filters, known as the *schmutzdecke*, is vital for bacterial removal. The *schmutzdecke* consists of abundant bacteria and protists, and is where suspended particles can be strained, organic matter compounds broken down, and microorganisms are entrapped.

Some varieties of protists prey upon bacteria. Their role in bacterial removal is not well quantified. The goal of this study was to confirm the relationship between filter run time and protistan abundance, to determine the significance of protistan predation on *E. coli*, and whether protists can be "seeded" onto filters to improve SSF startup times.

Results from a series of bench- and full-scale experiments confirmed a relationship between increased ripening time and increased biomass, protistan abundance, and *E.coli* removals. The "seeding" studies showed increased protistan populations in some filters, and a strong correlation between protistan abundance and CO_2 respiration.

CHAPTER 1

INTRODUCTION

1.1 Background

Slow sand filters (SSF) are a water treatment technology in which water is allowed to percolate through a layer of sand by gravity and is collected in an underlying piping system. They are used around the world, in rural areas of developing nations as well as major industrialized cities, and vary in size and sophistication from simple homemade systems designed for single residential use to the massive water supplies for London and Amsterdam.

Slow sand filtration is one of the oldest recorded engineered water treatment methods, and was superseded in many water treatment facilities during the twentieth century by newer technologies such as rapid filtration. However, it has several advantages over other filtration systems and remains a superior option under certain conditions (MWH, 2005). Today, it is considered a top choice for small systems, whether in rural communities or the developing world. Distinguishing characteristics of slow sand filtration, besides its slow filtration rate, are the cleaning of the sand bed by surface scraping and sand removal, the absence of a need for chemical pretreatment, lack of backwashing, and relatively long run times between cleanings (AWWARF, 1991).

Modern SSFs typically operate at hydraulic loading rates of 0.1 – 0.3 $m^3/m^2 \cdot h$ or m/h, much less than other modern filtration systems such as rapid filters, which typically operate at up to 100 times that level. For this reason, SSFs require more land area than

other contemporary systems to treat a given flow. However, they are relatively simple from an operations standpoint, and also require no chemical addition other than for disinfection purposes. These factors result in less operator training and lower operations costs (Hendricks, 2006).

SSFs offer water treatment via several mechanisms, including straining, adsorption, and biological degradation including predation (Hammer, 2001). The biological removal mechanism is perhaps the one that most sets SSFs apart from other filtration technologies. Biological activity on the interface between the supernatant water and sand increases with time ("ripening"), forming a layer of organic material called a schmutzdecke. A filter can be described as "ripened" when concentrations of effluent bacteria are less than influent bacteria. Eventually the schmutzdecke becomes too thick and begins to increase headloss, at which time removal of that layer must take place for continued optimal operation (defined hereafter as "cleaning"). A period of re-ripening then follows before the filter again attains an acceptable level of performance (AWWARF, 1991).

Biological degradation, including predation, occurs in slow sand filtration. Microscopic organisms called protists have been observed in schmutzdecke samples. Protists, which are introduced to the filter in the influent water and are incorporated into the schmutzdecke, are known to actively prey upon bacteria (Purves et al. 2001). While predation is well studied, its contribution to pathogen removals in SSFs is not well understood. A better understanding of the role protistan predation plays in slow sand filtration may provide means to improve performance of existing SSFs and help encourage consideration of them as a treatment option in new facilities.

1.2 Protists

Protists are eukaryotic organisms, often microscopic, that are not plants, animals, or fungi. This group is sometimes referred to as Kingdom Protista to distinguish it from Kingdoms Plantae, Animalia, Monera, Archea and Fungi (in the six-kingdom system). Technically the term " protist" is a taxonomic "catch-all" rather than a separate evolutionary grouping. Protists are a widely varied group: some are stationary, others motile; some are heterotrophic, and others photosynthetic. Most protists are unicellular, however there are many multi-cellular exceptions (Purves et al, 2001). Protists were first observed in SSF media in 1973 (Lloyd). The types of protists most often found in SSFs include amoebae, flagella, and ciliates, all of which are heterotrophic protozoa and known to prey upon other microscopic organisms such as bacteria (Lilley, 2008).

1.3 Problem Statement and Research Objectives

The role protists play in SSFs remains poorly quantified. The first intention of this study is to gain a better understanding of whether protists contribute significantly to microbial removals in SSFs, and if so, how much. The second intention is to assess the feasibility of improving SSF performance and decreasing post-cleaning ripening delays by increasing protistan abundance.

The specific research goals of this study included:

1) Assessing the inter-relationship between SSF ripening time, schmutzdecke biomass accumulation, protistan abundance, and *E.coli* removals;
2) Quantifying the *E.coli* removal contribution of protists;

3) Assessing protist seeding to enhance *E.coli* removals.

First, a bench-scale ripening study was conducted to confirm a known relationship between ripening time, schmutzdecke biomass, protistan abundance, and microbial removals. Numerous characteristics of filter columns ripened over 3, 7, 14, 28 and 42 days were assessed and compared. The filters were challenged with *E.coli* and synthetic microspheres to test removals. The same characteristics (except the microbial challenge, which was excluded for public health reasons) were also studied in real-world SSF samples: one filter that had been operating for 30 days and another than had been ripening for a year.

Spun-fiber pre-filters were used to exclude particles of protist size or larger from an experimental bench-scale SSF column. The characteristics of the experimental column were compared to a control with a much looser pre-filter. The aim was to quantify the microbial removals of a filter which theoretically had lower protistan abundance than the control and thereby quantify the link between protists and microbial removals.

Finally, the hypothesis that high protistan abundances improves SSF microbial removal performance was put to the test on the bench-scale. Two SSFs ripened in parallel under identical conditions were compared: one which had been amended 48 hours earlier with a protist-rich "seed" from a third, fully ripened schmutzdecke; and the other which had been left alone as a control.

CHAPTER 2

LITERATURE REVIEW

2.1 History of Slow Sand Filtration

The first mention of filtration of drinking water was in ancient Sanskrit writings around 4000 B.C., which mention that impure water "may be purified by filtration through sand and coarse gravel...." (MWH, 2005). While it has long been known that water can be cleaned by passage through sand, the first SSF in modern history was built in Paisley, Scotland in 1804 by John Gibb. The filter provided clean water for Gibb's bleaching business, as well as the local public (AWWARF, 1991).

By 1800 the idea of purifying water through filtration had caught on in Europe. Early engineered designs had failed, since they had no means for cleaning and removing material that built up and clogged them over time. In 1791 the first filter with backwash capability had been designed, followed soon after by filter designs that allowed scraping of surface buildups. Filters that included backwash capability were then known as "mechanical filters" and would become today's rapid filters, and those that were cleaned via scraping eventually became known as SSFs (Hendricks, 2006).

In 1829, James Simpson designed a SSF for the Chelsea Water Works Company on the north bank of the Thames River. His design was a filter one-acre in area with a daily flow of 2.25 to 3 million gallons per day (MGD). Simpson's system was the first use of a water treatment process for a piped public water supply. His Chelsea filter's

other parameters, such as hydraulic loading rate, sand size, sand bed depth, and water depth, became common standards for SSF design (AWWARF, 1991).

Simpson noted that "the bed generally produces better water when it is pretty well covered with silt than at any other time," and also observed that there was more taking place than simple straining of particles, an unidentified process he referred to as 'fermentation' (Hendricks, 2006).

The success of the Chelsea filter was followed by the implementation of similar designs throughout Britain and continental Europe. Facilities were installed in Berlin (1856), Zurich (1884), Hamburg (1893), and Budapest (1894). By 1859, drinking water filtration was required by law in England (AWWARF, 1991). The first filter in the United States was constructed in Poughkeepsie, New York in 1870, to filter water from the Hudson River. Slow sand filtration was put to the test in the Hamburg cholera epidemic of 1892. Over 8,500 deaths were recorded in Hamburg, while the waterborne cholera killed very few in neighboring Altona. Altona was adjacent to Hamburg and even downstream of its sewage discharge, but unlike Hamburg, Altona operated SSFs (AWWARF, 1991).

By 1920 there were 20 SSFs operating in the United States, and hundreds more in Europe. The U.S. number had increased to 100 by 1940 and 225 by 1994. Despite this growth, numbers of SSFs were, and remain, a fraction of the numbers of rapid filters in operation in the U.S (Hendricks, 2006).

Around 1980, a reinvestigation of slow sand filtration for use in small communities that utilize surface waters began in the U.S. This reinvestigation was based in part on the USEPA's research into low-cost, simple systems to help small communities

comply with the Surface Water Treatment Rule of 1989. Something of a revival of the technology has taken place in the U.S.. Between 1990 and 2005, over 20 new SSFs were constructed in New England (Unger, 2006).

2.2 Design and Operation

SSFs are in essence a bed of sand contained in a boxed structure with apparatus for influent and effluent flow control. Typical design criteria described in the Ten State Standards as well as the International Water and Sanitation Centre (IRC) are often cited. Water is dispersed over the top of the sand bed in such a way that the surface of the bed is not agitated. Supernatant water sits on top of the sand layer and percolates through by gravity. The available head, the hydraulic conductivity of the media used, as well as the amount of schmutzdecke growth, determines the filtration rates. The four components of SSF design are:

1. Supernatant storage capacity
2. Filter bed
3. Under-drain system
4. Flow control apparatus (AWWARF, 1991).

The supernatant storage capacity should be designed to hold 3 to 24 hours of water for the filtration system. This provides for some equalization of influent water quality, sedimentation of heavy particulates, and time for biological action to take place. However, the primary purpose of the supernatant water is to provide the driving head to push the water through the sand bed and underdrain systems (AWWARF, 1991).

The filter bed itself is designed as follows. The first stage involves sizing of the sand bed. The bed area is typically determined by the hydraulic loading rate (HLR) used.

The bed area can be calculated simply using the influent flow and the normal range for the HLR in SSFs (typically 0.1 – 0.3 $m^3/m^2 \cdot h$, or m/h). A wider range of acceptable values is sometimes cited as 0.04 – 0.4 m/hr (AWWARF, 1991).

The depth of the sand bed is determined by a function of the desired period of operation before sand replacement, the frequency of cleaning, and the sand depth removal per scraping. Typically SSF bed depths range from 0.5m to 1m. Most of the biological activity and removals occur in the top few centimeters of the bed, and therefore extra depth provides longer time periods and more scrapings between sand replacements, rather than increased performance (AWWARF, 1991).

The third major design step is the sizing of the sand media. Native sand should be utilized where possible (AWWARF, 1991). Sand sizes are based on d_{10} and UC values. Typical values for these parameters are d_{10} = 0.2 – 0.4 mm. Uniformity coefficients (UC) may be as low as 1.5 but sometimes are higher than 3.0 (AWWARF, 1991).

Table 2.1 Design standards and typical values for SSFs (adapted from Unger, 2006).

Design Parameter	IRC Manual Standards[1]	Typical Design Values[2]
Design Life	10 – 15 years	> 100 years[3]
Period of Operation	24 hours / day	24 hours / day
Hydraulic Loading Rate	0.1 – 0.2 m / h (0.04 – 0.08 gpm / ft^2)	0.06 – 0.4 m / h
Filter Bed Area	10 – 200 m^2	varies
Height of Filter Bed		
Initial	0.8 – 0.9 m	0.6 – 1.2 m
Minimum	0.5 – 0.6 m	0.5 m
Specification of Sand		
Effective Size (d_{10})	0.15 – 0.3 mm	0.18 – 0.44 mm
Uniformity Coefficient	< 3 – 5	1.5 – 4.7
Underdrain Height (Including Gravel Layer)	0.3 – 0.5 m	0.6 m
Supernatant Water Height	1 m	0.9 – 1.3 m

[1]IRC (1989)
[2]AWWARF (1991)
[3]City of Rutland, VT

The under-drain system in a SSF must be designed to support the weight of the filter media, to facilitate uniform flow across the entire surface area of the filter bed, and to provide a means of draining the filtered water from the sand bed. Consideration must be made for the cleaning equipment's weight on the under-drain system, especially in larger filters where tractors or trucks may be driven onto the sand bed. Improper under-drain design may result in short-circuiting of the sand bed (AWWARF, 1991).

Finally, flow control systems are used to restrict the water flow through the sand bed and maintain submergence of the media under all conditions. They typically involve control valves, an adjustable weir, vent system, and an effluent line to a clear-well (AWWARF, 1991). An elevation view of a typical SSF configuration is depicted in Figure 2.1.

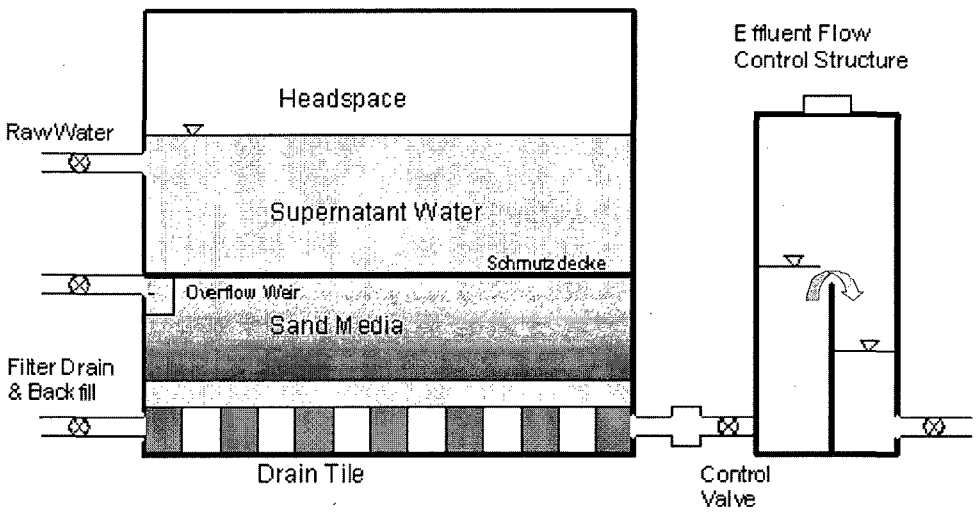


Figure 2.1 SSF schematic. (adapted from Partinoudi et al., 2006 and Unger, 2006)

Operation of SSFs consists of two stages: filtration and regeneration. Head loss increases slowly as a biofilm (schmutzdecke) forms on the filter surface over the period of a filter run which may last weeks or months depending on the design and influent water characteristics. The filtration stage is ended and cleaning takes place when the head loss reaches the available head in the system. Cleaning usually involves the scraping and removal of the top 1 to 2 cm of media along with the schmutzdecke. Some facilities harrow the top of the media bed to mix it in rather than scraping. The cleaning cycle can be repeated many times, often for several years, before the sand must be entirely replaced ("re-sanding"). Scraping removes most of the biologically active schmutzdecke and regeneration of this layer may take several days or weeks. Ripening after complete re-sanding may involve even more time. During this regeneration time, effluent water is not well filtered and therefore is wasted or rerouted back through the system until the filters are fully ripened and at optimal performance (MWH, 2005).

2.3 Advantages and Disadvantages

The most obvious advantage of slow sand filtration is its simplicity of operation and maintenance. There are typically no mechanical elements involved, and usually no chemical addition. Additionally the technology is able to meet several treatment objectives simultaneously (e.g. microbial removal and disinfection byproduct precursor reduction). However, it requires a relatively large amount of land, requires a source water supply that is low in algae and turbidity, and has long restart times after maintenance (Hendricks, 2006). Advantages and disadvantages of SSF are outlined in Table 2.3.

Table 2.2 Advantages and Disadvantages of SSF.

Advantages	Disadvantages
1) Infrequent maintenance requirements. 2) Usually requires no mechanical elements. 3) Usually requires no chemical additives. 4) Achieves multiple treatment goals (e.g. microbial removals and DBP-precursor reduction).	1) Large land requirements. 2) Requires low turbidity and low algae source water. 3) Long post-maintenance restart times.

2.4 Removal Mechanisms

The purification of water in SSFs is the result of physical straining through the developing schmutzdecke and the top few millimeters of sand, together with schmutzdecke biological activity. Therefore, both physical and biological mechanisms are important in removal of impurities via SSF (Haarhoff, 1991).

Major biological removal mechanisms are direct predation (such as by protists), scavenging, natural death and inactivation, and metabolic breakdown (Haarhoff, 1991). As the schmutzdecke ripens, the surfaces of the sand grains develop a sticky layer of organic material that absorbs to the particles by various attachment mechanisms. Since the schmutzdecke is biologically active, organic impurities are metabolized and converted to water, carbon dioxide and harmless salts. The biologically active section of the entire filter bed may extend as deep as 0.4-0.5 m down from the filter surface (Van Duk, 1978).

The physical mechanisms contributing to particle removal in SSF are surface straining, interception, transport mechanisms such as sedimentation and diffusion, and attachment mechanisms (Weber-Shirk and Dick, 1997a). These physical processes are important in removing particles from influent water, as well as inorganic constituents that cannot be broken down through biological means. Certain constituents are degraded by multiple mechanisms. For example, adsorption and biodegradation are considered together to be the primary natural organic matter removal mechanisms (Collins, 1992).

Questions remain as to whether it is the physical formation of the sticky schmutzdecke mat and associated adsorption, or the activity of the schmutzdecke biota (including predation and non-specific degradation), that is of more importance in microbial removals.

One study assessed five possible microbial removal mechanisms in the schmutzdecke, and concluded that only physical/chemical adsorption was confirmably significant. It suggested that both straining and predation appeared to contribute but were not precisely quantified, and that biologically mediated adsorption likely occurred, but was not identified or isolated from other removal mechanisms. Cell death/inactivation was confirmed to be an insignificant (Unger, 2006).

Another study examined biological removal mechanisms by using azide to inhibit biological growth in ripened bench-scale filters while leaving the physical structure of the biofilm intact (Weber-Shirk and Dick, 1997b). The application of azide caused an immediate reduction in log *E.coli* removals. The authors suggested that removals by adsorption caused by physical straining and sticking in the biofilm should have continued to contribute to log removals even after the application of azide. This was not the case.

The reduction in log removals was immediately reversed when the azide application was stopped. This suggested that active biota—whether through direct predation or non-specific degradation from antagonistic bacteria—are more important to microbial removals than physical adsorption in the schmutzdecke.

A second Weber-Shirt and Dick study (1997a) concluded that biological mechanisms combined with physical/chemical mechanisms accounted for removal of influent particles less than about 2um in diameter, whereas physical/chemical mechanisms alone accounted for removal of influent particles greater than 2um in size.

2.5 Removal Capabilities

"*As the technology of treating drinking water evolves, slow sand filtration is a low-tech process that continues to be effective in a high-tech world*" (Tanner, 1997).

SSFs have a proven ability to remove pathogenic microorganisms as well as reduce turbidity and remove other consituents. Well-ripened SSFs can achieve 3- to 4-log removal of *Giardia* cysts. Log removal of coliforms can exceed 4-log, of which 1- to 3-log removal is attributed to the schmutzdecke (Bellamy et al., 1985). Additionally, SSFs can reduce iron and manganese, THM precursors, and dissolved organic carbon (AWWARF, 1991). Table 2.3 summarizes the typical performance of SSF for various pathogens and constituents.

Table 2.3 Approximate performance predictions for a selection of water quality constituents treated by SSF. (adapted from AWWARF, 1991; Amy et al., 2006; and Rachwal et al., 1996)

Constituent	Approximate Expected Removal
TOC	25%
Turbidity	25 - 40% achieve < 1 NTU in effluent
Coliform bacteria	2-log to 4-log
Giardia cysts	3-log to 4+-log
Enteric viruses	2-log to 4-log
Cryptosporidium oocysts	> 4 log units
Dissolved organic carbon (DOC)	< 15 – 30 %
Biodegradable organic carbon (BDOC)	< 80 %
THM precursors	< 20 – 35 %
Particles (2-10 um)	1- 3-log
Iron / Manganese	> 67 %

2.6 United States SSF Regulations

The most recent regulation in the United States affecting SSF systems is the Long Term 2 Enhanced Surface Water Treatment Rule (LT2), enacted by the USEPA in 2006. LT2 amends the Surface Water Treatment Rule, Interim Enhanced Surface Water Treatment Rule, and Long Term 1 Enhanced Surface Water Treatment Rule, which collectively mandate a 4-log removal or inactivation of viruses, a 3-log removal or inactivation of *Giardia* cysts, and a 2-log removal or inactivation of *Cryptosporidium* oocysts for municipal drinking water treatment systems using surface water as a source (USEPA, 2006).

Additionally, for municipal drinking water treatment systems that treat water at high risk for *Cryptosporidium* outbreaks, LT2 mandates improved removal of *Cryptosporidium*. High risk systems include all unfiltered systems and filtered systems with a significant occurrence of *Cryptosporidium* in the source water. Systems are placed into one of four groups depending on the occurrence of *Cryptosporidium* in their source

water. A predetermined number of log removal credits are required for each group (USEPA, 2006). SSF technology is automatically awarded 2.5 log removal credits, thereby satisfying the requirements for all but the highest risk group (Dowbiggin et al., 2006).

The USEPA Stage 1 and Stage 2 Disinfection / Disinfection By-Product (D/DPB) Rules set maximum contaminant levels (MCLs) for disinfection by-products and limit the amount of disinfectant that can be used by operators. SSFs help meet the D/DPB Rules by decreasing the natural organic precursors that are transformed by disinfectants such as chlorine or ozone into the regulated disinfection by-products. However, they are often not as effective at removing the organic DPB precursors as other treatment methods, so ozone pretreatment or a GAC sandwich layer as described above may be added to the basic SSF bed to improve such removals (Collins et al. 1996).

The USEPA Total Coliform Rule (TCR) also has implications for slow sand filtration. Under the TCR, no more than five percent of all water samples in a given month may be positive for total coliforms. Positive results require a follow-up repeat sampling. A repeat sample positive for fecal coliforms or *E. coli* constitutes a violation (MWH, 2005).

2.7 Schmutzdecke Biomass and Ripening

The schmutzdecke, the biofilm that forms on the surface of a SSF as it ripens, was described by Huisman and Wood as "a teeming mass of microorganisms, bacteria, bacteriophages, [and] predatory organisms such as rotifers and protozoa, all feeding on the adsorbed impurities and upon each other" (1974). The schmutzdecke is known as an "intense treatment zone," (AWWARF, 1991). Research as early as 1899 and 1902

attributed the performance of SSFs to biological processes at the sand-water interface (Kemna, 1899; Rideal, 1902), and recent research has further quantified the removal capabilities offered by the ripened schmutzdecke (Unger, 2006).

Varying definitions of how to define the schmutzdecke in terms of filter depth exist. Often a clearly visible filter "cake" forms on top of the sand bed, but increased biological activity can extend further down into the sand. Schmutzdecke depth and characteristics vary depending on sand size, ripening time, and influent water conditions. Its depth is often defined in terms of empty bed contact time (EBCT) (Unger, 2008). EBCT is a function of filter depth (L) and hydraulic loading rate (HLR). EBCT = L / HLR. For example, a filter with a ripened schmutzdecke 1.5 cm in depth and a hydraulic loading rate of 0.3 m^3/m^2*hour would have a schmutzdecke EBCT of 3 minutes.

Early research on protists within SSFs found that a ripened schmutzdecke may contain up to 8.5 x 10^4 protists per cubic centimeter of material (Richards, 1974). Research in the mid 1990s more closely examined the bacterial removal potential of the protozoa in the laboratory (Lloyd, 1996).

Hence, it has been well established that the schmutzdecke is the most intense treatment zone of a SSF, and that protists are abundant within the schmutzdecke and are capable of removing bacteria. However, as the following section details, aside from work by Lloyd, relatively little research has quantified the role these protists play in bacterial removal performance (Weber-Shirk and Dick, 1997b).

2.8 Protists in SSFs

The terminology and classification system having to do with protists has changed several times in recent decades. The term "protist" refers to unicellular eukaryotes that

are not parts of Kingdoms Animalia, Plantae or Fungi. In some classification schemes, protists are given their own Kingdom Protista (Purves, et al., 2001). However they are classified, the two most common subcategories of protists are "animal-like" protists (also called protozoa), and "plant-like" protists (algae). Protists, usually 2 – 200 um in size, are the emphasis of this research. For clarity, 'protozoan' hereafter is to be considered synonymous with 'protist'. Protists exhibit a variety of mechanisms to capture their prey, which has led to a considerable diversification of morphologies. However, in basic terms they can be split into three main categories: amoebae, flagellates, and ciliates (Parry, 2004). See Table 2.4 for a summary of protistan categories and subcategories. Note that this research focuses on heterotrophic protists, and does not include photosynthetic protists such as algae.

Table 2.4 Major groups within Kingdom Protista. (adapted from Unger, 2006 and Raven and Johnson, 1999)

Group	Subdivision	Typical Example
Sarcodina: Heterotrophs with no permanent locomotor apparatus		
	Amoebae	*Amoeba*
	Forams	Forams
	Radiolarians	Radiolarians
Algae: Photosynthetic protists that are multicellular or largely multicellular		
	Red	Coralline algae
	Brown	Kelp
	Green	*Chlamydomonas*
Diatoms: Photosynthetic protists that are unicellular, many with a double shell of silica		
		Diatoma
		Golden algae
Flagellates: Protists with locomotor flagella		
	Dinoflagellates	Red tides
	Euglenoids	*Euglena*
	Zoomastigotes	Trypanosomes
Sporozoa: Nonmotile, spore-forming unicellular parasites		
		Plasmodium
Ciliates: Heterotrophic unicellular protists with cells of fixed shape possessing two nuclei and many cilia		
		Paramecium
Molds: Hetertrophs with restricted mobility and cell walls made of carbohydrate		
	Cellular Slime Molds	*Dictyostelium*
	Plasmodial Slime Molds	*Fuligo*
	Water Molds	Water molds, rusts, mildew

As mentioned previously, protists are abundant in the ripened schmutzdecke. Many such protists are bacterivores, meaning they prey upon active bacteria as a food source (Hahn and Hofle, 2000). Some biofilm-associated species have been observed consuming up to 60 bacteria and scouring a biofilm area of up to $7x10^4$ μm^2/hr. Protists observed in a river environment have been shown to consume bacteria at a rate between 1.1 and 90.4 bacteria per protist per hour (Kinner et al, 1998).

Historically, research on protists associated with biofilms such as the schmutzdecke was difficult due to methodological issues. Removing protists from a biofilm changes the ambient environmental conditions, which may damage delicate protistan membranes (Arndt, et al., 2003). However, in studies that did successfully observe their behavior, protists have been observed removing between 30-100% of new bacterial production per day and maintain their prey in a 'physiological state of youth'. They also play a role in cycling of carbon, nitrogen, and phosphorus (Parry, 2004). Other research has suggested that selective predation by protists may result in selection for better adapted organisms and may strengthen the surviving bacterial community and increase available substrate (Kinner et al, 2002).

Additionally, freshwater protists are known to be very rapid colonizers given an appropriate surface. Arndt (2003) observed flagellates attaching to glass slides placed into river water after as little as 10 minutes. Lloyd (1996) stated that bacterivores colonized the first 5 to 10 cm of a resanded SSF within the first few days and persisted throughout the filtration cycle.

Two pieces of early research into protists in SSFs specifically reported on methods of retrieving protists from SSFs and gave preliminary results on the types present and estimates of abundance, but did not quantify the role protists play in filter removal performance. One of the two studies concluded that the number of *E.coli* in the effluent of a SSF varied inversely with the abundance of flagellates and ciliates in the filter itself (Lloyd, 1973; Richards, 1974).

Lloyd's research found that *Vorticella*, a type of peritrich, were the protists most commonly feeding on bacteria suspended in influent water (1974). His later research

showed a bench-scale SSF ripened for five days and inoculated with *Vorticella* removing 10-30% more *E.coli* than a control cell. These improvements were not as dramatic when the experiment was repeated after six days of ripening, indicating that by six days, non-protist related removal mechanisms had matured enough to outweigh performance improvements offered by inoculation. When the protistan inoculant was switched to *T. pellionella*, a grazer rather than suspension-feeder, there were no significant improvements in bacterial removals. *T. pellionella* grazes bacteria from sand grain surfaces rather than preying upon suspended bacteria, which may explain why removal improvements were not significant compared to *Vorticella.* Lloyd concluded, "suspension feeding by peritrichs seems to be a powerful mechanism for removing large populations of bacteria during slow sand filtration" (1996).

More recently, Unger (2006) concluded, "protistan predation may play a critical role in *E. coli* removal in SSF…either by grazing of surface-associated bacteria to limit detachment and open up adsorption sites or by intercepting bacteria in pore water, but neither mechanism was confirmed." Unger successfully seeded protists onto operating filters, but observed no subsequent increase in *E. coli* removal, and recommended further research. He did, however, observe a correlation between increased *E.coli* removal with SSF ripening time and protistan abundance in the top 5mm of the filter column (see Figure 2.2.).

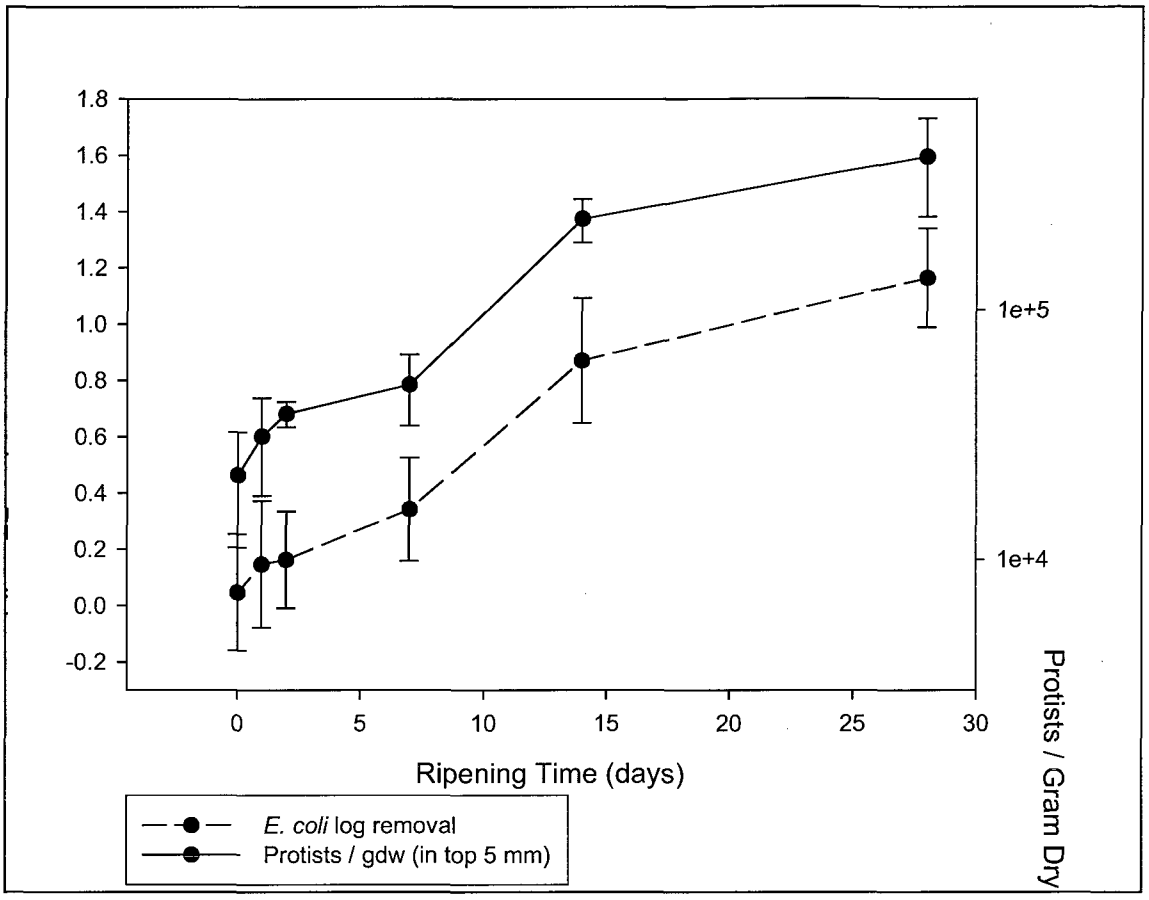


Figure 2.2 Protistan abundance and E. coli removal in sand columns after various ripening times (Adapted from Unger, 2006).

CHAPTER 3

EXPERIMENTAL APPROACH AND METHODOLOGY

The overall goal of this research is to quantify the role of protists in SSFs and their contribution to bacteria removals. Several experimental phases were designed to meet the three objectives previously outlined (i: assess the inter-relationship between SSF ripening time, schmutzdecke biomass accumulation, protistan abundance, and *E.coli* removals; ii: quantify the *E.coli* removal contribution of protists; and iii: assess protist seeding to enhance *E.coli* removals). Table 3.1 depicts the schedule of completion for each of these phases and their associated experiments. Section 3.1 presents the experimental setup of these various experimental phases within the framework of the three objectives. Sections 3.2 and 3.3 explain the details of the materials used and the lab analyses conducted. Finally, the quality control (QC) measures are presented in Section 3.4.

Table 3.1 Experimental Approach

Assess the inter-relationship between SSF ripening time, schmutzdecke biomass accumulation, protistan abundance, and *E.coli* removals	
Staggered ripening time study	June - August, 2007
Summer field sampling	August, 2007
Winter field sampling	February, 2008
Quantify the *E.coli* removal contribution of protists	
Pre-filter study	December, 2007 - January, 2008
Assess protistan seeding to enhance *E.coli* removals	
Bench-scale seeding study	February - May, 2008

3.1 Assessing the Inter-relationship Between SSF Ripening Time, Schmutzdecke Biomass Accumulation, Protistan Abundance, and *E.coli* Removals

Bench-Scale Ripening Study

Previous research has revealed a significant relationship between SSF ripening time, active biomass, and microbial removal (Unger, 2006). To confirm and further explore this relationship as well as the additional variable of protistan abundance, two bench-scale challenges were completed using a series of filter columns that were set up in parallel and allowed to ripen in a staggered fashion (See Figure 3.1). The specific equipment used is described in Section 3.4. In the first challenge, one 4.8cm inside-diameter bench-scale column was allowed to ripen for 42 days, the second for 21 days, the third for 14 days, the fourth for seven days and the fifth for 3 days. The ripening scheme for the second challenge was only slightly different (44, 30, 16, 5, and 2 days, respectively). This slight change in ripening scheme from the first bench-scale study was of a logistical nature rather than a scientific one. The bench-scale and ripening setup was otherwise identical to the first ripening experiment, except for the time of year. The second challenge was conducted in May 2008 versus August 2007 for the first challenge.

The only pre-treatment for influent water was a 55 gallon plastic settling tank in which the raw river water settled before being drawn into the bench-scale columns via peristaltic pump. Each column was fed 10 mL/min raw water – corresponding to a hydraulic loading rate of 0.33 m^3/m^2 per hour (m/h) – from the Oyster River in Durham, NH at the UNH/Durham Water Treatment Plant for the duration of its ripening and checked five times per week to ensure proper flow rate. Plant operators were consulted periodically during ripening to relay information on any changes concerning the quality

of the raw influent water, such as a heavy rain event which would increase turbidity and organic loads in the source water.

The average influent water temperature during filter ripening was 12°C for the first challenge and 10°C for the second. The columns were kept dark to prevent algal growth.

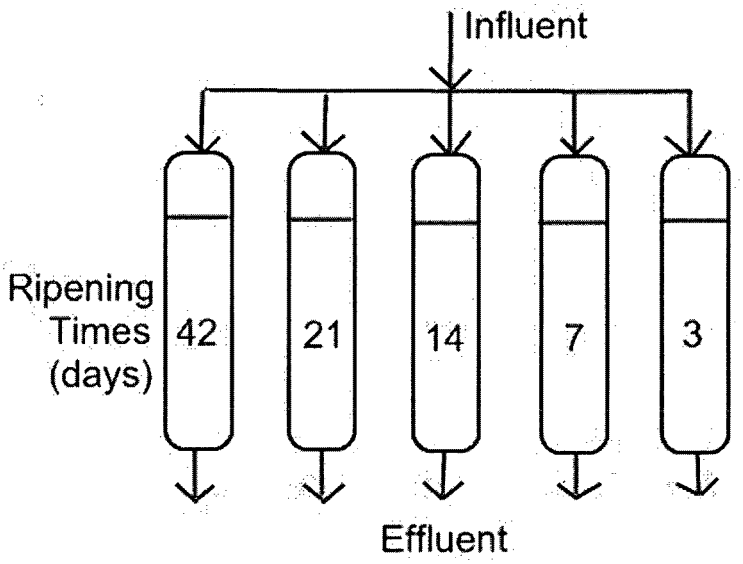


Figure 3.1 Diagram of bench-scale ripening study filter arrangement.

At the end of the ripening period of both challenges, all filters were challenged with a raw water solution that had been amended with f-amp *E.coli* cultured in the lab to a predicted concentration of 10^4MPN/100mL, and in the first challenge a concentration of 10^4#/mL 5um synthetic fluorescing microspheres (Duke Scientific, Palo Alto, CA). *E.coli* was used as a readily available and easily quantifiable surrogate for the assessment of filter bacterial removal performances. The 5um microspheres were chosen because they are comparably larger than *E.coli*, and spherical instead of rod shape, therefore allowing

assessment of physical removals for an alternative particle shape. Additionally, this particle shape and size roughly resembles *Cryptosprodium,* an organism of concern in treatment of surface waters.

After collecting a sample for later confirmation of bacterial concentration, the challenge solution was fed to the filter columns at 10 mL/min per filter (corresponding to 0.33 m/h) and allowed sufficient time for three bed volumes to pass through the filters (approximately 90 minutes given the column volume and flow rate).

After the initial flow-through time, samples of influent and effluent water were collected in 250mL amber jars and stored in a cooler and transported to the lab for analysis of *E.coli* and microsphere removals and other characteristics. These influent samples were drawn from the end of influent tubing rather than directly from the challenge solution container to ensure that loss of *E.coli* in the tube was not occurring through attachment to tube walls.

Additionally, cores of the top (and in the first challenge, the bottom) 5mm of media in each filter column were collected and transferred to Whirl-Pak bags (Nasco, Inc., Loves Park, IL) for general biological analysis (phospholipid analysis for phosphate biomass and CO_2 respiration for biological activity) and protist counts. The 5mm depth corresponds to an EBCT of approximately 1 minute. Approximately 10 grams of media from the top of the filter and 10 grams from the bottom of the filter were cored for this purpose using a modified pipette tip. The use of challenge microspheres and collection of bottom media cores were omitted from the second bench-scale challenge as it was intended to focus specifically on the biological characteristics of the upper layer of filter media (the schmutzdecke), and *E.coli* removals in relation to ripening time.

Field Sampling

Sampling of a full-scale SSF was considered important to ensure that the relationships observed at the bench-scale held true in real-world conditions. A true comparison to the bench-scale studies, with five ripening time-staggered filters, is not feasible on the full-scale. However, staff at the Springfield, MA slow sand filtration facility (located in the adjacent community of Westfield, MA) allowed access to two of their filters on August 13th, 2007. The Westfield facility was built in the early 1900s. It currently serves approximately 200,000 customers. Maintenance procedures have changed little over its century of operation. The accumulated schmutzdecke is scraped clean annually by laborers and the sand bed is completely removed and refreshed with new sand approximately every decade. Continuous operation of the plant during maintenance is made possible by the fact that there are many other filters available to share the load while one is off-line for maintenance.

One such filter had been recently scraped and had been ripening for 46 days as of August 13th, 2007. The other was due for scraping and had been ripening for 367 days. These filters were chosen by the Springfield Water and Sewer staff based on their maintenance schedule with the author's goals in mind. Thus, these two filters essentially allowed a comparison between a well-ripened filter with a developed schmutzdecke, and a relatively fresh filter with a developing, thinner schmutzdecke layer. Under normal operating conditions for the filters, two or more meters of supernatant water are present above the filter sand. Operators closed the influent valves prior to the author's arrival, allowing the supernatant water to drain to just below the filter surface of the schmutzdecke thereby allowing easy access for taking cores.

The influent water temperature was 12.5°C on the date of sampling. Three cores were taken from each filter using a simple 2.5cm inside diameter corer, essentially a hollow metal tube with a removable plug on one end. Care was taken to choose three coring locations that were far apart, and in areas where the schmutzdecke remained moist. As the filter beds were almost half a hectare in area, the surface is not necessarily uniformly even. This resulted in several areas where the sand bed was slightly higher in elevation than the rest of the bed. When the filters were drained by operators, these areas of the schmutzdecke became dry, and were avoided when choosing core locations. The top 15cm of media were cored. This was accomplished by inserting the corer into the media with force to the proper depth, the rubber plug was then tightly inserted in the top end of the tube, and the core, including filter media, was then pulled slowly upward. The top 5mm and bottom 5mm of each core were transferred to Whirlpack bags (Nasco, Modesto, CA) and transported to the lab in coolers for analysis. Operations staff provided data on their periodic checks of total coliform removals, ambient water conditions, and filter design specifications.

As a contrast to samples taken under summer conditions, winter samples from the same facility were desired for a seasonal comparison. The Springfield SSF staff made two more filters available on February 28th, 2008, with a maintenance regime similar to the summer sampling. One filter had been online for 19 days, and the other for 356 days. The influent water temperature was 2°C as opposed to 12.5°C for the summer sampling.

It should be noted that there was a significant logistical difference in accessing one of the filters for this sampling round. Operations staff did not close off influent water as quickly as in the summer visit, therefore upon the author's arrival one of the two

winter filters had not fully drained. Approximately 30cm of supernatant water remained over the surface of the filter bed of the 19 day ripened filter. Cores were collected as usual with the addition of the use of another end-plug to seal the lower end of the core as soon as possible to prevent supernatant water from infiltrating the bottom of the core and skewing biological results. Cores from the drained 356 day filter were collected normally.

3.2 Attempt to Quantify the *E.coli* Removal Contribution of Protists

After confirming the relationship between ripening time, active biomass, microbial removals, and protistan abundance, the next goal was to isolate the removal contributions provided by the protistan abundance (predation) from the removals provided by the growth of the schmutzdecke biomass in general (attachment and straining).

An estimate of bacterial consumption rates by protists in slow sand filters was calculated based on research that observed a uptake rates ranging from 1.1 – 90.4 bacteria per protist per hour in river water (Kinner et al, 1998). The conservative end of the range was used (1.1 bacteria per protist per hour), along with a protistan abundance of $2x10^3$ protist per mm^2 of filter surface area observed in minimally ripened filters in preliminary SSF assessments in this study. In the 4.8cm diameter filters used in this study, this resulted in a potential total bacterial consumption capacity of $5.9x10^6$ bacteria per hour (see Appendix C for calculations). Therefore a challenge application of 10^4MPN/100mL applied at a rate of 10mL/min over approximately 90 minutes should be well within the uptake capacity of the filter protistan population.

To help test this calculation and quantify the protistan population's bacterial removal capabilities, two bench-scale filters were ripened in parallel using the same raw

water source and the same flow rate. Both filters' influent was first pumped through a 5 um wound polypropylene filter cartridge (Penteck, Sheboygan, WI) to remove large particles prior to entering the SSF column. Additionally, one filter was fitted with a 0.5um filter cartridge (Pentek, Sheboygan, WI) between the 5 um filter and the SSF column, while the other had no further pre-filtration (See Figure 3.2). The 0.5um filter was chosen based on its hypothetical ability to remove the average sized protist observed in SSFs (1 to 2um). The goal was to ripen one filter with greatly reduced protistan abundance in the influent, and to ripen the other with normal protistan abundance (or only slightly reduced, taking the common 5um pre-filter into account). Thus, it was predicted, one filter would develop a higher protistan population in the schmutzdecke than the other. The filters were ripened for four weeks at 10 mL/min (0.33 m/h) and then challenged with a 10^4MPN/100mL f-amp *E.coli* amended raw water solution. The setup was checked daily due to potential for clogging problems with the 5um pre-filter. Post-challenge, effluent samples were taken as well as media samples of the top 1 cm of the filters.

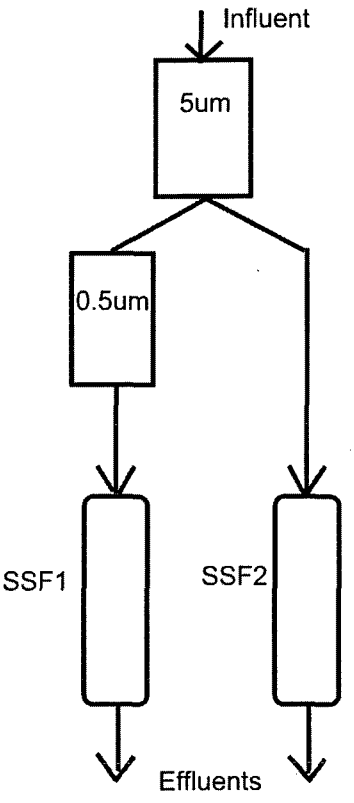


Figure 3.2 Pre-filter experiment design.

3.3 Assessing Protistan Seeding to Enhance *E.coli* Removals

To help quantify the role protists play in removing pathogens, and to possibly reveal a method of improving SSF startup performance, a seeding approach was assessed. Protists associated with filter material cored from a fully-ripened bench-scale SSF were extracted with a phosphate buffer solution and introduced to relatively unripened bench-scale filters as a liquid "seed". These seeded columns, as well as several control columns,

were challenged with a 10^4MPN/100mL F-amp *E.coli* solution. Log *E.coli* removals, as well as filter biological parameters and protistan abundance levels were assessed.

To create the seed that was added to each of the three columns, approximately 50 grams of schmutzdecke material (taken to a depth of 1 cm) from a bench-scale SSF that had been ripening for ten weeks were removed and placed into an amber jar. A phosphate buffer solution was gently swirled into the jar remove attached protists from particles, as per method outlined by Hines (1998). This process was repeated several times decanted each time into another jar to a volume of 600mL. A 10mL sample of this seed solution was used to quantify the protist abundance therein, revealing a concentration of approximately 10^4 protists #/mL. The remaining solution was split evenly and run through the three designated seed filters columns (~197 mL each). The columns were then allowed to run normally filtering raw water for 48 hours before the microbial challenge of 10^4MPN/100mL F-amp *E.coli* was conducted. This time frame was intended to allow extracted protists time to settle onto filter surface, acclimate, and resume feeding prior to the challenge.

The filters chosen for seeding had been allowed to ripen for two days, five days, and 16 days. Raw water was from the Oyster River at the UNH/Durham Water Treatment Plant, with an average influent water temperature of 11°C over the ripening period. The seeded filters were challenged in parallel with unseeded control filters that had been on the same pre-ripening schedule, and protistan abundance, biological characteristics, and *E.coli* removals were then compared.

3.4 Bench-Scale Apparatus

Each sand filter column was constructed using a 4.8cm inside diameter, 30cm length glass chromatography column (Kontes, Vineyard, NJ) with TFE adapters on each end. Stainless steel mesh screens fitted into the bottom adapter supported the sand in the columns and prevented sand from entering and blocking the effluent tube. Columns were packed with sand under saturated conditions by adding several centimeters of water to the column with the bottom end cap screwed on and sealed. Approximately 50g sand at a time was added followed by tapping the column lightly three times between increments to release air bubbles until approximately 23cm of sand depth was reached. Raw water was then slowly added until the column was completely filled. The upper TFE adapter was then screwed on. Black Norprene tubing connected a constant flow raw water storage tank to the top of each column via peristaltic pumps (Masterflex). A custom-made wooden holding rack was used to securely hold up to eight columns at a time.

The sand media used was designated "O" sand supplied by Holliston Sand Co. (Slatersville, RI). This type of sand is used in several SSF plants in the New England region. The Holliston sand was rinsed with tap water in a five gallon pail using a hose nozzle to fluidize fine particles. The turbid water was then decanted, and this was repeated until the decanted rinse water appeared clear. The final rinsed sand had an effective size (ES) of 0.39 mm and a uniformity coefficient (UC) of 2.2.

3.5 Raw and Challenge Water Quality

Raw Water Quality

Raw water from the Durham, NH/UNH water treatment plant, which is drawn primarily from the adjacent Oyster River, was used to ripen the filters. The river is dammed into a small reservoir adjacent to the treatment plant. The only treatment the raw water received prior to reaching the bench-scale SSFs was screening to remove sticks and large debris, and settling in a 55 gallon plastic drum prior to remove clumps of organic material that might clog the Norprene tubing. This raw water was also used as the base for the creation of experimental challenge solutions. The temperature and turbidity of the raw water varies seasonally and with weather events. A summary table of raw water quality during the experimental phase of this study is shown below in Table 3.2. Quality characteristics raw data are compiled and provided in the Appendix B.

Table 3.2 Durham/UNH Water Treatment Plant - Average Raw Water Quality Data Over 12 Month Period

	Conductivity uMHOs	Alkalinity mg/L $CaCO_3$	Fe mg/L	Total Mn Mg/L	Hardness (mg/L $CaCO_3$)	D.O. (mg/L)	UV-254 Abs.	Temp. (°C)	pH Units	Turbidity (NTU)	Total # of Samples
Average	153	28	0.524	0.118	18.9	9.7	0.305	11.9	6.7	6.4	242
Range	41 to 293	10 to 55	0.247 to 0.912	0.028 to 0.496	6 to 48	4.2 to 14.8	0.139 to 0.709	1.4 to 22	5.5 to 7.5	1.81 to 41.6	

UNH/Durham Water Treatment Plant Raw Water Quality

Challenge Microbe

The primary challenge organism for assessment of microbial removal performance was "f-amp" *E.coli*. *E.coli* cells are rod-shaped, approximately 2um in

length, and 0.8um in diameter. They are one of a group of bacteria known as coliforms, which are of concern in drinking water treatment. Fecal coliform bacteria are associated with the intestinal tract of mammals, and though most strains are not directly harmful to humans, their presence is considered an indicator of fecal contamination in surface water. *E.coli* in particular are often used in laboratory settings because they are easily cultured and their genetics are relatively simple and easily manipulated. F-amp is a strain that has been modified to be resistant to streptomycin and amoxicillin and cultured in tryptic soy broth in the lab. All F-amp *E.coli* for this research was acquired from the University of New Hampshire's Department of Microbiology. The goal concentration for F-amp E.coli in the challenge water solution was 10^4MPN/100mL.

Additionally, synthetic green-fluorescing polymer microspheres (5 um diameter) were used in one experimental challenge to assess removals of larger (non-biological) particles of a different shape (Duke Scientific, Palo Alto, CA). The goal concentration for microspheres in the challenge water solution was typically 10^4#/mL.

3.6 Analytical Techniques and Methods

Descriptions of lab and field methods used in this research are summarized as follows. Detailed standard operating procedures for each method may be found in Appendix A.

E.coli Enumeration

F-amp *E.coli* was enumerated using the IDEXX Quanti-Tray® system (IDEXX, Westbrook, ME) and the associated ultraviolet light cell-counting technique. Specifically, Colilert® powder was added to a 100mL water sample, or a dilution of it

based on the roughly expected concentration, and shaken until fully dissolved. This solution was then decanted into an IDEXX Quanti-Tray® and sealed using a Quanti-Tray® Sealer. After sealing the trays were labeled and placed in a 37°C incubator for 24 hours. Cells on the trays' grid were then counted based on color change to ultraviolet blue. These tray cell counts were then located on an IDEXX MPN table which yielded *E.coli* count estimates. Duplicate trays were performed as a quality control measure. Original samples were stored in a refrigerator for at least 24 hours in the case that an IDEXX tray was loaded with too-great a concentration and resulted in a try on which the entire grid turned ultraviolet blue. In that case, the process would be repeated after a 100 fold dilution of the original sample.

Shmutzdecke Biological Characteristics

Shmutzdecke biological characteristics were quantified using phospholipid and CO_2 respiration analyses.

Phospholipid Analysis. All cells contain phospholipids, which are turned over quickly during cell metabolism and therefore can be an indicator of viable biomass (Vestal and White, 1989). Biomass in the shmutzdecke and sand media was analyzed using the phospholipid extraction introduced by White (1979) and later refined by Findlay et al. (1989). Phospholipids were extracted by adding chloroform and methanol to the media sample and letting it stand for 6 hours. Dilute sulfuric acid and more chloroform was then added to separate the chloroform into a second layer. The samples were then allowed to stand overnight as the phospholipids were partitioned into the chloroform. Some of the chloroform layer was then extracted the next morning with a syringe and moved into

fresh vials. The chloroform extracts were then dried under a nitrogen stream, and oxidized with potassium persulfate in a 103^0C oven overnight to free the phosphate. The freed phosphate was then dyed with ammonium molybdate and malachite green to form a green compound that was measured colorimetrically with a spectrophotometer (Hitachi U-2000, New York, NY) at 610nm. The absorbance was recorded and compared to a phosphate standard curve. Concentrations yielded were in units of nmol phosphate per gram dry weight sand. This concentration is referred to as "biomass" or simply "phosphate" in this report. See Appendix A for a detailed standard operating procedure for phospholipid analysis.

CO_2 Respiration. Organisms that are aerobic respirators, including many bacteria and heterotrophic protists, release carbon dioxide as part of their metabolism. This carbon dioxide can be measured over time as an indicator of the quantity of biological activity taking place in a given sample. In this research, a known mass of sand was incubated for 24 hours at 25^0C in a sealed vial of known volume. The total CO_2 respired was measured on a Licor 6252 CO_2 analyzer (IRGA) (Lincoln, NE) and compared to a standard curve of pure CO_2 analyzed on the same instrument. Respiration was then normalized to ug C released per gram dry weight sand per day (Knorr, M., 2007).

Water Quality Analyses

Several other analyses were used to obtain data on influent and effluent water quality for each experiment.

Turbidity and pH. Turbidity is a measure of the amount of suspended solids in water. Water with high turbidity can appear cloudy or even muddy. pH is a measure of the hydrogen ion concentration of water, typically ranging from 0 to 14. A low pH indicates acidic water, whereas a high pH indicates basic water. A pH of 7 is considered neutral. Turbidity and pH can be measured with simple instruments, and were monitored in influent water and recorded daily by the staff of the water treatment plants that cooperated with this research.

UV Absorbance. The turbidity of a sample of water has an effect on the amount of light that is absorbed or passes through the sample. Influent and effluent samples were measured at 254nm using a spectrophotometer (Hitachi U-2000, New York, NY). Beers Law states that absorbance is proportional to the concentration of the analyte for a given adsorption pathlength at a given wavelength. UV absorbance at 254nm is a useful surrogate parameter for estimating the raw water concentrations of organic carbon and trihalomethane precursors (Standard Methods, 2006). UV absorbance results were obtained for influent and effluent water in bench-scale experiments.

Total Organic Carbon. Total Organic Carbon (TOC) analysis involves the oxidation of organic carbon to carbon dioxide in the presence of ultraviolet light. The carbon dioxide is measured by a non-dispersive infrared analyzer (Sievers Model 800 TOC Analyzer with Autosampler). These readings are then compared to readings of known standards and converted to concentrations of organic carbon. TOC results were obtained for

influent and effluent water in bench-scale experiments. For the purpose of this research, TOC analysis provides an additional measure of the level of filter ripening.

Protistan Enumeration

Protistan enumeration was achieved by filtration with primulin stain followed by manual microscope counts. Water samples were filtered through nitrate cellulose backing filters and a 0.8um protist filter. The filters were then stained using Tris-HCl and primulin. The filters were allowed to set in dark conditions until dry the mounted on microscope slides for counts. The same process was used for counts of protists attached to sand and shmutzdecke media, however an initial shaking extraction using phosphate buffer was used to move the attached protists into a liquid phase for filtering. Protistan abundances in this study were calculated as both protist number per gram dry weight of sample (# protists/gdw) and protist number per mm^2 of SSF surface area sampled. The latter was emphasized rather than gram dry weight measurements in reporting results. Measurements of protistan abundance per gram dry weight may be influenced by denser particles of filter media (sand grains) that happen to be in the sample thereby increasing dry weights when compared to samples that did not contain such particles. Expressing results in terms of abundance per sampled surface area eliminated this concern, given that all samples were cored to the same depth. Most of the counts were referenced from Lilley (2008).

3.7 Quality Control / Quality Assurance

Quality Control (QC) procedures were developed for each method to maintain the rigor of data. A record of QC measures, any problems identified, and actions taken in response were written in lab notebooks along with recorded data.

Laboratory Method Quality Control Measures

Quality control measures used in the laboratory included use of controls and replicates, observing maximum allowable holding times for samples, and understanding the limits of quantification for a given analysis. The quality control procedures (QC) for laboratory methods are outlined in Table 3.3. Sample collection and preservation procedures are outlined in Table 3.4. More detail on QC measures for specific methods are provided in the SOP for each method in Appendix A.

Table 3.3 Quality control measures for analytical methods.

Analysis	Replicates	Quality Control (QC) Measures	Limit of Quantification
F-amp *E. coli* Enumeration with IDEXX	1	• Positive and negative controls	• 1 MPN / 100 mL
Phospholipid Analysis (Biomass)	3	• 6 standard calibration curve • Read blank after every 10 samples	• 1 nmol PO_4 / gram dry weight
CO_2 Respiration (Biological Activity)	2	• 5 standard calibration curve	• Not determined

*During each sampling event, at least one sample was analyzed in duplicate to assess method variability.

Table 3.4 Summary of preservation, containers, and holding times.

Microbial Analyses	Min. Volume	Preservation	Container Type[a]	Holding Time
E. coli	100 mL	Refrigerate	P	0-24 hrs.
Protists	100 mL	Refrigerate	P	0-6 hrs.
Water Quality	**Min. Volume**	**Preservation**	**Container Type**	**Holding Time**
pH	100 mL	N/A	P	immediate
Temperature	100 mL	N/A	N/A	immediate
TOC	250 mL	H_3PO_4	P or G	14 days
Sand and Schmutzdecke	**Min. Volume**	**Preservation**	**Container Type**	**Holding Time**
Biomass (Phospholipids)	5 g	Refrigerate	G	0-24 hrs.
Bio. Activity (Respiration)	5 g	Refrigerate	G	0-24 hrs.

[a] P = Plastic (HDPE), G = Glass

Field Quality Control Measures

Bench-Scale Experiments

During bench-scale challenges involving *E.coli*, one preliminary issue needed to be resolved before analysis. Several meters of rubber tubing were often involved in moving raw or challenge water to the tops of the filter columns. Therefore the losses of *E.coli* from attachment to the inside of the tubes were unknown. These losses were assessed by sampling challenge influent from the filter end of the tube rather than from the storage container.

Another decision made concerning the *E.coli* challenges was the amount of challenge solution to filter before sampling effluent. Work by Unger (2006) showed that after one bed volume had passed, *E.coli* concentrations in the effluent plateaued. As a conservative measure of ensuring that steady-state effluent concentrations had been reached, three bed volumes were filtered before sampling in all experiments for this research. Figure 3.3 shows the results of Unger's study on filter throughput.

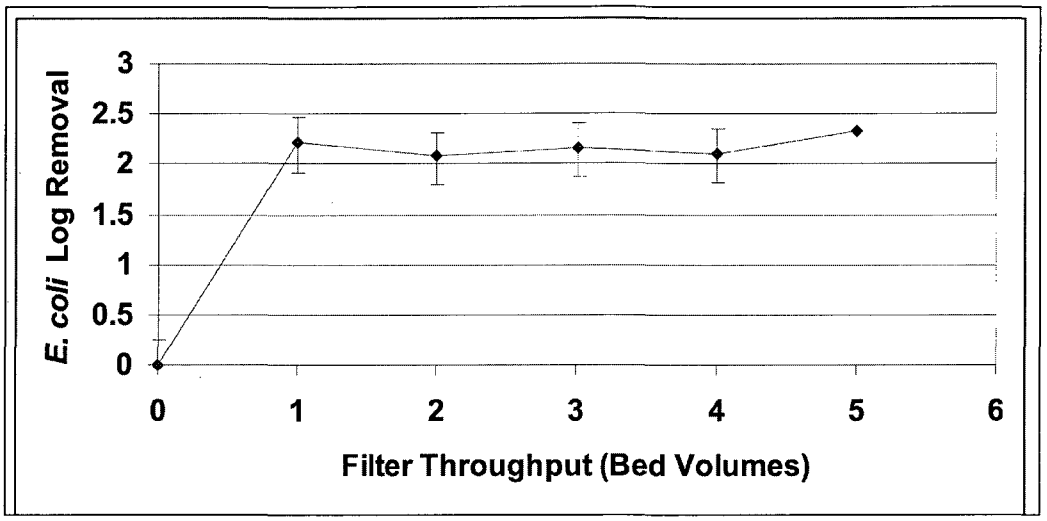


Figure 3.3 *E. coli* removal over time in a lab-scale experimental sand filter during continuous *E. coli* challenge at a concentration of 1.33 x 10^5 / 100 mL. Steady state is reached before one bed volume (22.5 minutes) has been introduced (Unger, 2006).

Field Sampling

Field sampling of full-scale operating SSFs presented a different challenge for QA/QC than did lab analysis or bench-scale experiments. The sampling timing was at the discretion of facility personnel. Because of this, at the time of each sampling different levels of supernatant water were present on the surface of the filter. In one case, several inches of water remained. In another, water had been fully drained for several days. To best ensure quality of data, triplicate cores were taken from each filter. In filters that had been allowed to fully drain, samples were taken from the dampest areas possible to avoid sampling an area in which shmutzdecke microbes had been dried out.

CHAPTER 4

RESULTS AND DISCUSSION

The results and discussion of this research are organized according to the specific objective they targeted. The objectives of this research were as follows:

1) Assess the inter-relationship between SSF ripening time, schmutzdecke biomass accumulation, protistan abundance, and *E.coli* removals.
2) Quantify the *E.coli* removal contribution of protists.
3) Assess protist 'seeding' to enhance *E.coli* removals.

It should be noted that all data relating to protistan abundance were obtained via Lilley's (2008) partner study to this research.

4.1 Assessing the Inter-relationship Between SSF Ripening Time, Schmutzdecke Biomass Accumulation, Protistan Abundance, and *E.coli* Removals

As described in the literature review, several studies have shown an increasing relationship between SSF ripening time, the biological growth of the schmutzdecke, and microbial removals (most recently: Unger, 2006). The following experiments sought to further explore and quantify that relationship in bench- and full-scale filters. The first two examined series of bench-scale SSFs ripened in a staggered fashion and then challenged with *E.coli* simultaneously. Biomass characteristics, protistan abundances, and *E.coli*

removals were then analyzed. The third and fourth studies were conducted at a full-scale SSF facility in Westfield, MA which served greater Springfield, MA. The same characteristics were examined in the full-scale as in the bench-scale (with the exception of the microbial challenge due to safety constraints) in the winter and summer seasons.

Bench-Scale Ripening Studies

In this study, the relationships between a SSF's ripening time, protist abundance, and *E.coli* removal performance as explored by Unger (2006) were reexamined and expanded to include phospholipid biomass and CO_2 respiration activity in the filter media. Two bench-scale experiments were conducted; the first in June 2007 and the second in May 2008. In each, five bench-scale SSF columns were set up to ripen in parallel in a time-staggered fashion.

Phospholipid and CO_2 respiration analyses showed upward trends with filter ripening time in the top 5mm of filter media. The same analyses performed on media from the bottom 5mm of filter media showed a weaker upward trend. Averages of top and bottom activity and biomass results are compiled in Tables 4.1 and 4.2. Protistan abundance and *E.coli* removal results are compiled in Table 4.3. The results from the top samples are depicted in Figure 4.1. The strong upward trend observed in the top 5mm samples, indicating a relationship between ripening time and biomass formation, is consistent with results of previous research on the schmutzdecke (Campos et al., 2002; Weber-Shirk and Dick, 1997b; Unger, 2008).

Table 4.1 Top 5mm biomass and activity results from first ripening study.

Ripening Study #1 - Top 5mm Results Averages				
Filter Ripening Time (days)	Top or Bottom	CO2 Respiration (ug C respired / gdw · day)	Biomass (nmols PO_4 / gdw)	Activity / Biomass (ug C / nmol PO_4 * day)
3	**T**	22.3 ± 3.9	25.5 ± 2.3	0.87
7	**T**	23.3 ± 3.4	24.0 ± 0.8	0.97
14	**T**	34 ± 0.1	44.5 ± 4.4	0.76
21	**T**	51.8 ± 7.4	61.9 ± 7.1	0.84
42	**T**	91.5 ± 2.3	161.3 ± 11.3	0.57

Table 4.2 Bottom 5mm biomass and activity results from first ripening study.

Ripening Study #1 - Bottom 5mm Results Averages				
Filter Ripening Time (days)	Top or Bottom	CO2 Respiration (ug C respired / gdw · day)	Biomass (nmols PO_4 / gdw)	Activity / Biomass (ug C / nmol PO_4 * day)
3	**B**	4.4 ± 0.2	6.1 ± 2.2	0.72
7	**B**	5.6 ± 0.2	7.6 ± 1.2	0.74
14	**B**	6.2 ± 0.8	9.7 ± 0.2	0.64
21	**B**	7.3 ± 0.2	12.6 ± 0.8	0.58
42	**B**	11.4 ± 1.0	11.5 ± 1.0	0.99

Table 4.3 Protist and *E.coli* removal results from first ripening study.

Ripening Study #1- Protist and E.Coli Data			
Filter Ripening Time (days)	Schmutzdecke Protist # / gdw	Schmutzdecke Protist # / mm^2	Log E.coli Removals
3	$2.59 \pm 1.0 \times 10^5$	$3.5 \pm 1.4 \times 10^3$	0.51 ± 0.03
7	$1.14 \pm 0.15 \times 10^6$	$1.88 \pm 0.27 \times 10^4$	1.12 ± 0.01
14	$9.83 \pm 2.1 \times 10^5$	$1.28 \pm 0.24 \times 10^4$	2.15 ± 0.02
21	$8.06 \pm 0.66 \times 10^5$	$1.0 \pm 0.09 \times 10^4$	2.60 ± 0.08
42	$2.81 \pm 0.8 \times 10^6$	$1.4 \pm 0.14 \times 10^4$	1.89 ± 0.02

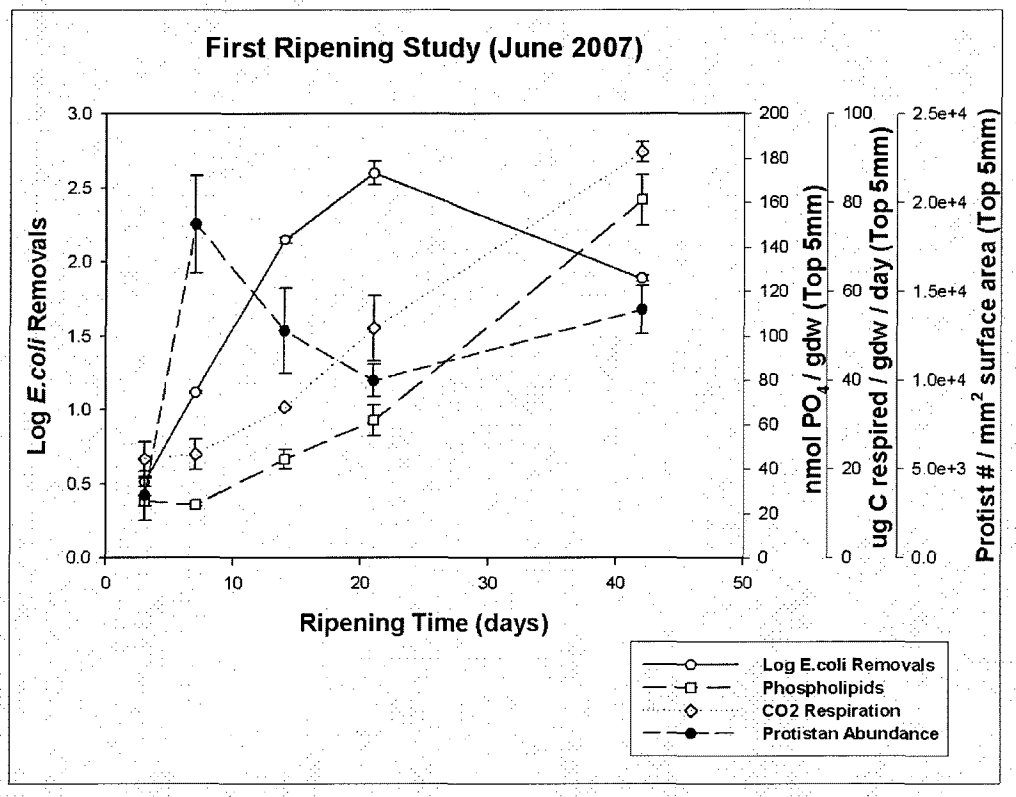


Figure 4.1 Ripening study: Ripening time versus biological parameters in top 5mm of filter media.

Results for phospholipid and CO_2 respiration analyses were as predicted, showing a steady increasing trend with added ripening time. Top sample biomass as measured by phospholipids ranged from 25.5 nmols of PO_4 per gram dry weight in the three day ripened filter to 161.3 nmols of PO_4 per gram dry weight in the 42 day ripened filter. CO_2 respiration increased from 22.3 ug carbon respired per gram dry weight per day to 91.5 ug carbon respired per gram dry weight per day, respectively. Bottom sample biomass as measured by phospholipids and CO_2 respiration numbers were much lower, as expected due to the fact that most of the biological activity occurs in the schmutzdecke layer. However, the bottom layer showed steady increases as well, with biomass as measured by phospholipids ranging from 4.4 to 11.4 nmol of PO_4 per gram dry weight, and CO_2

respiration ranging from 6.1 to 11.5 ug C carbon respired per gram dry weight per day, indicating that with time biological growth did penetrate to the deepest part of the column. Activity per biomass results (calculated by dividing CO_2 respiration results by biomass as measured by phospholipid results) did not reveal any noticeable trend.

Log *E.coli* removals increased steadily as predicted, peaking at 2.6 log after 21 days ripening, but then decreasing sharply. This drop was unexpected. However, it may be explained by the fact that cores were taken from the 42 day filter the day before the challenge for a "practice run" on quantifying protistan abundance. In retrospect, these cores must have created a hole in the ripened schmutzdecke and could have allowed greater passage of *E.coli* during the challenge, hence creating the sharp decrease in removal after the 21 day ripened filter. This problem prompted a second ripening study, explained later in this section.

The predicted behavior for protistan abundance would be a continual increase, or a sharp increase followed by a leveling off as a steady population was reached. In this experiment, however, protistan abundance (expressed as number of protists per mm^2 of filter surface area) peaked in the seven day ripened filter and then decreased, only to increase again slightly after 21 days.

Several other secondary parameters were studied in the analysis of this challenge and are outlined in Table 4.4. Results of Total Organic Carbon (TOC) analysis, UV absorbance tests, and microsphere removals are included. TOC showed a generally improving trend in removals with filter ripening, with a reduction in the seven day filter. The removal percentages ranged from 1.9% to 8.8%. UV absorbance (254nm) showed a decrease with filter ripening, indicating the presence of less adsorbent material in effluent

samples with ripening time. However, the 42 day filter showed a slight increase, possibly due to the aforementioned early coring of that filter.

Microsphere influent levels averaged $1.7x10^4$/mL. Log removals ranged from 3.2 log in the 3 day filter to 3.9 log in the 21 day filter, and in the 42 day filter no microspheres at all were detected in the effluent samples, suggesting complete removal. These values indicate superior removal performance for 5um spherical non-biological particles than for the smaller 2um rod-shaped *E.coli* cells.

Table 4.4 First ripening study: natural organic matter and microsphere reductions.

	Average TOC (ppm)	Standard Error	TOC (% reduction)	UV Abosrbance (254nm)	Standard Error	Log Microsphere Removal	Microspheres (% reduction)
Average Influent:	6.90	0.05	0.0	0.324	0.003	0.0	0.0
Average Effluents:							
3 day	6.77	0.02	1.9	0.291	0.005	3.2	99.94
7 day	6.79	0.04	1.6	0.279	0.009	3.6	99.97
14 day	6.69	0.11	3.0	0.255	0.004	3.6	99.97
21 day	6.57	0.04	4.8	0.254	0.003	3.9	99.99
42 day	6.29	0.00	8.8	0.269	0.002	None present	100.00

The second ripening study was a more focused effort, examining only the top 5mm of each filter. Results confirmed that increased ripening time roughly corresponded with increased biological activity, biomass, protistan abundance, and log *E.coli* removals. Summaries and a depiction of these results are presented in Table 4.5, Table 4.6, and Figure 4.2. As opposed to the first ripening experiment, the results were generally as predicted, confirming the positive relationship between the aforementioned parameters.

Protistan abundance was comparable to an earlier study conducted by Unger (2006), with numbers of protists ranging from 2.09×10^3 to 4.51×10^3 per mm^2 filter surface area. By comparison, Unger reported a range from 5.9×10^2 to 5.0×10^3 using a similar ripening scheme. Other studies have also observed this relationship between ripening time and protistan abundance in the schmutzdecke (Lloyd, 1974; Richards, 1974; Mauclaire et al., 2006).

The increasing trend in protistan abundance over time is only present when calculated as protist number per mm^2 of filter surface area, and not present when calculated as protist number per gram dry weight. It is likely that gram dry weight measurements for schmutzdecke parameters are easily skewed by stray sand particles included by chance in the core of otherwise much lighter cake material. Therefore, in schmutzdecke measurements units of surface area are more appropriate. Gram dry weight units are likely more appropriate for depth samples where the majority of the sample is made up of sand grains.

Log *E.coli* removals also increased steadily with ripening time, from 0.31 log in the two day ripened filter to 1.6 log in the 44 day ripened filter. This compares favorably with Unger's work, in which he observed log removals of 0.1 in a two day ripened filter increasing to 1.2 in a 28 day ripened filter (2008).

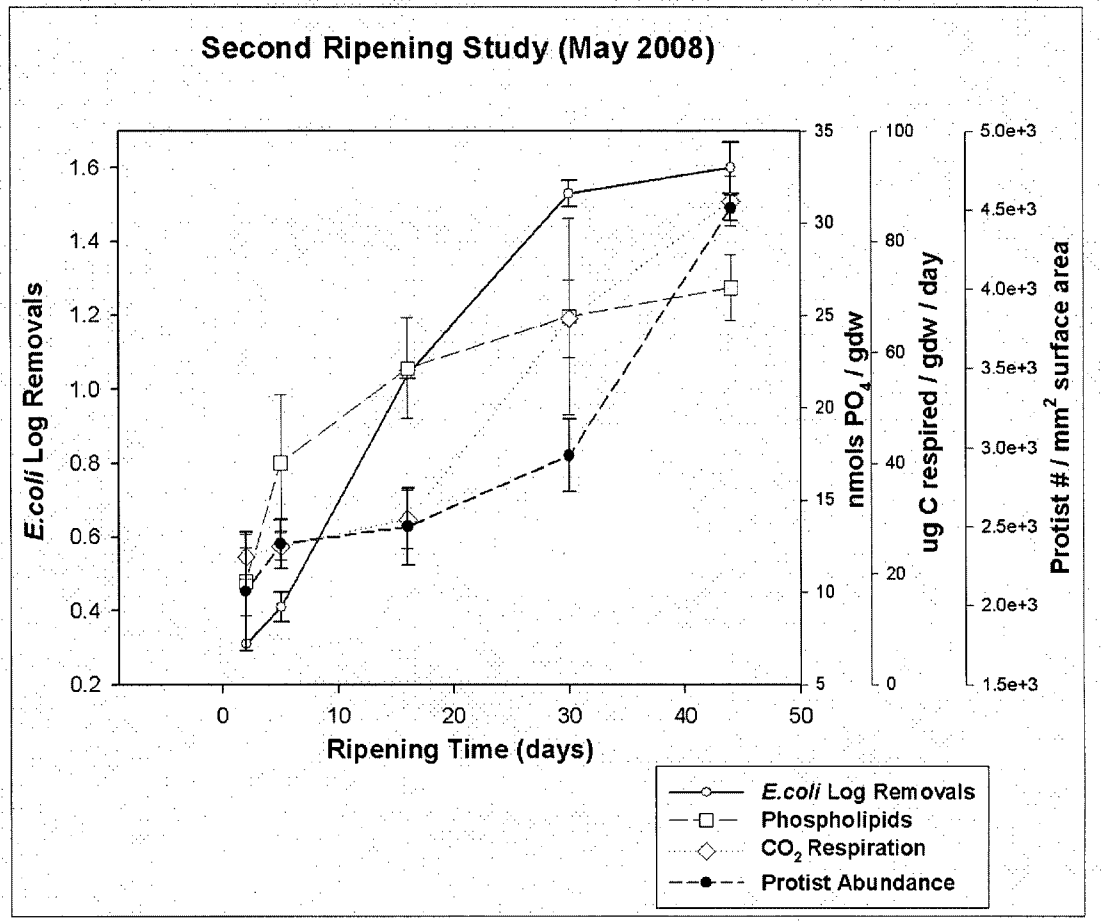
Table 4.5 Biomass and activity results from second ripening study.

Ripening Study - Results Averages			
Filter Ripening Time (days)	CO_2 Respiration (ug C respired / gdw · day)	Biomass (nmol PO_4 / gdw)	Activity / Biomass (ug C / nmol PO_4 * day)
2	23.1 ± 4.0	10.6 ± 1.9	2.18
5	25.0 ± 2.5	17 ± 3.7	1.47
16	29.9 ± 5.3	24.9 ± 2.7	1.20
30	66 ± 6.9	26.5 ± 5.3	2.49
44	87.3 ± 4.6	22.1 ± 1.8	3.95

Table 4.6 Protist and *E.coli* removal results from second ripening study.

Ripening Study - Results Averages			
Filter Ripening Time (days)	Protist # / gdw	Protist # / mm^2	Log *E.coli* Removals
2	$2.16 \pm .186 \times 10^5$	$2.09 \pm .081 \times 10^3$	0.31
5	$2.54 \pm .480 \times 10^5$	$2.39 \pm .229 \times 10^3$	0.41
16	$4.55 \pm 1.41 \times 10^5$	$2.49 \pm .245 \times 10^3$	1.04
30	$2.96 \pm .157 \times 10^5$	$3.12 \pm .148 \times 10^3$	1.53
44	$4.20 \pm .544 \times 10^5$	$4.51 \pm .374 \times 10^3$	1.6

Figure 4.2 Ripening time versus *E.coli* log removals, phosphate concentration in top 5mm, and CO_2 respiration in top 5mm.

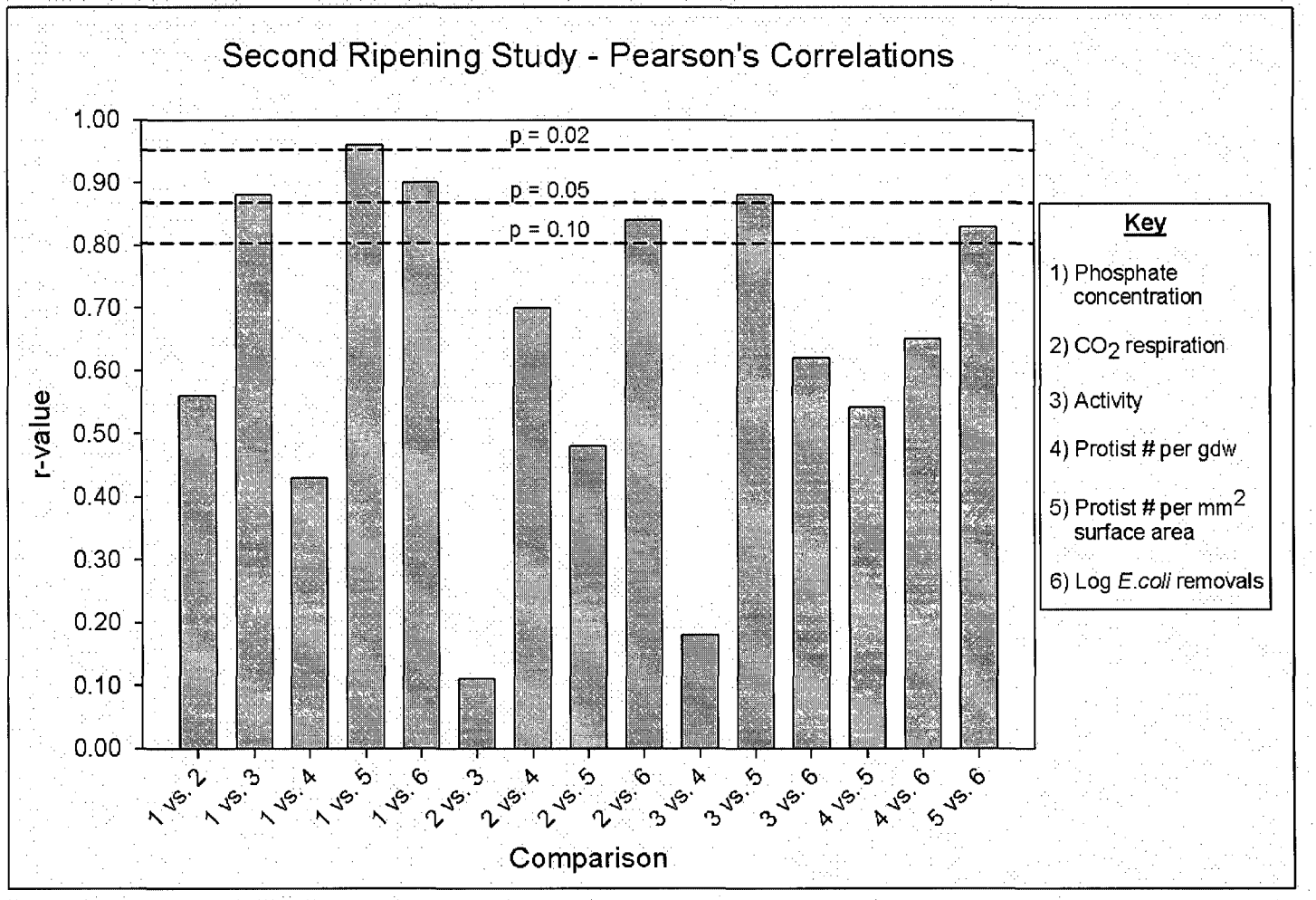


Possible correlations among results averages for biomass as measured by phospholipids, CO_2 respiration, activity per biomass, protistan abundance, and log *E.coli* removals were explored. Pearson's correlations indicated a positive correlation between several of the parameters. Both biomass as measured by phospholipids and CO_2 respiration correlated positively with log removals (p=0.05 and 0.10, respectively). Protistan abundance per mm^2 surface area correlated positively with log removals (p=0.10), and with activity (p=0.05). Biomass as measured by phospholipids correlated positively with both activity and log removals (p=0.05). Phospholipid concentration had an especially strong correlation with protistan number per mm^2 surface area (p=0.02). See Table 4.7 for a complete correlation matrix showing r-values for the correlations between each parameter. See Figure 4.3 for a graphical representation of the correlations and their levels of significance.

Table 4.7 Second bench-scale ripening study – correlation matrix including biomass as measured by phospholipids (PO_4 Conc.), CO_2 respiration, activity/biomass, protistan abundance, and log *E.coli* removals.

Second Ripening Study - Filter Parameters Correlation Matrix						
df = 3	PO_4 Conc.	CO_2 Resp.	Activity/ Biomass	Protist # / gdw	Protist # / mm^2 SA	Log *E.coli* Removals
PO_4 Conc.	1	-	-	-	-	-
CO_2 Resp.	0.56	1	-	-	-	-
Activity/Biomass	0.88	0.11	1	-	-	-
Protist #/gdw	0.43	0.70	0.18	1	-	-
Protist #/mm^2 SA	0.96	0.48	0.88	0.54	1	-
Log *E.coli* Removals	0.90	0.84	0.62	0.65	0.83	1
Critical values: 0.81 for p=0.10, 0.88 for p=0.05, 0.93 for p=0.02						

Figure 4.3 Second bench-scale ripening study – results of correlations of phosphate concentration, CO_2 respiration, activity, protistan abundance, and log *E.coli* removals.



CO_2 respiration, and especially biomass as measured by phospholipids, both measures of schmutzdecke biological maturity, correlated positively with log *E.coli* removals. This is consistent with established research indicating the link between level of schmutzdecke ripening and log *E.coli* removals (Unger, 2008; Partinoudi et al., 2006). Unger, however, observed a stronger correlation between microbial removals and biological activity as measured by CO_2 respiration, than between microbial removals and biomass (2008).

Biomass as measured by phospholipids showed a positive correlation to protistan abundance with a level of significance superior to the other parameters' correlations ($p=0.02$). Protists themselves are, of course, part of overall biomass measures. Additionally, as heterotrophic protists require biomass to prey upon, increases in biomass may lead to an increased food source, thereby allowing growth in protistan abundance.

The positive correlation between protist number per mm^2 surface area and log removals is of central importance in this research. Correlation analysis does not indicate whether such relationships are causal or simply associative. Since biomass as measured by phospholipids and CO_2 respiration each correlated with log removals as well, and biomass as measured by phospholipids correlated with protistan abundance, it is impossible to say with certainty whether increased biomass and activity in general, or protistan predation, were responsible for improved removals.

Full-Scale Ripening Study

Full-scale SSFs – part of a treatment facility serving over 200,000 people – were examined in order to confirm the findings of the bench-scale studies in the real world. Cores and influent/effluent samples were taken from the Springfield, MA SSFs in August 2007 and February 2008. During the summer and winter, two filters were made available by facility staff at each sampling event. General biological data on the filters were desired. Given the timeframe of the two sampling events, a seasonal comparison was also made. This allowed an assessment of the effects of colder versus warmer influent water.

At the time of each sampling event, two of the treatment facility's SSFs were made available. In both the summer and winter events, one filter had been ripened for approximately one year and was due for cleaning by scraping, and the other had been cleaned and put online less than two months prior to sampling. This operational difference allowed for the comparison between ripening time of the schmutzdecke layer during both warm and cold temperatures.

As expected, increased ripening time of full-scale filters corresponded with increased CO_2 respiration, biomass as measured by phospholipids, and protistan abundance in the schmutzdecke. The full-scale SSF data are summarized in Tables 4.8 and 4.9.

In the summer event, samples from the schmutzdecke of the fully ripened filter respired at a rate of 24 ug C / gdw * day versus 19 ug C / gdw * day for the recently-scraped filter. Biomass as measured by phospholipids was observed in the fully ripened filter at a level of 24 nmol PO4 / gdw versus 11 nmol PO4 / gdw in the recently-scraped filter.

In the winter event, samples from the fully ripened filter respired at a rate of 57 ug C / gdw * day versus 14 ug C / gdw * day for the recently-scraped filter. Biomass as measured by phospholipids was observed in the fully ripened filter at a level of 105 nmol / gdw versus 17 nmol / gdw for the recently-scraped filter.

Biomass as measured by phospholipids and CO_2 respiration were higher in the fully-ripened filters, which is consistent with the bench-scale findings. Of note is the much greater biomass result for the fully-ripened filter in the winter sampling. This difference in biomass as measured by phospholipids may be due to the greater oxygen penetration allowed by colder water temperatures, thereby increasing biological phosphate production over the entire filter depth (Kinner, 2008).

The data obtained by CO_2 respiration analysis and the biomass as measured by phospholipids data obtained were divided to yield an "activity per unit of biomass". Activity per unit of biomass was higher in the summer than in winter (1.61 and 1.00 in summer versus 0.79 and 0.54 in the winter), which is an expected effect of temperature on microbial metabolism. Warmer water temperatures are conducive to increased activity per biomass (Kinner, 2008). This is consistent with pilot scale work conducted by Partinoudi et al. (2006).

Protistan abundance results were not consistent with those observed in the bench-scale ripening studies. When calculated as protist number per mm^2 of filter surface area, protistan abundances of the ripened filter were lower than those of the unripened filter in both seasons (4.95×10^3 versus 1.44×10^4 protist # / mm^2 in summer; 2.97×10^3 versus 6.62×10^3 protist # / mm^2 in winter). However, unlike in the bench-scale experiment, protistan abundance was greater in both the summer and winter fully-ripened filter when

calculated as protist number per gram dry weight. This difference in results depending on units used could be explained by the fact that both sampled filters, in both seasons, had noticeably thicker schmutzdecke accumulation than did the filters in the bench-scale experiment. Therefore, a core taken to a depth of 5mm on the full-scale filters contained almost entirely schmutzdecke material and minimal sand grains. The same core taken on the bench-scale filters with less schmutzdecke material contained many sand grains. The heavy sand grains can throw off protistan abundance results when calculated as number per gram dry weight (hence the use of the 'protist number per mm^2 surface area' measure used in the bench-scale study). The gram dry weight measure becomes more useful in a situation without large sand grains adding the potential for large sample weight differences.

Log removals of total coliforms by the full-scale SSFs were based on sampling results recorded over 30 day periods by facility staff (except in the case of the recently-scraped filter in the winter event that had only been online for 19 days, from which a 19 day average was calculated). Log removals were slightly improved in the summer months. The fully-ripened filter sampled in the summer season (11°C influent water at time of sampling) had most recently removed 2.82 log total coliforms. A similarly ripened filter in the winter season (2.5°C influent water at time of sampling) had most recently removed 1.77 log total coliforms. This is consistent with findings by Bellamy (1985), in which filters with cold influent water (2 – 5°C) removed fewer coliforms than warmer filters (17°C).

As depicted in Tables 4.8 and 4.9, the fully-ripened filters had greater total coliform log removals than the less-ripened filters in both seasons (2.82 versus 1.68 in

summer; 1.77 versus 1.29 in winter), which is consistent with the bench-scale observations.

Table 4.8 Biomass and Activity results from full-scale sampling (August, 2007 and February, 2008).

Full-Scale Sampling - Biomass and Activity Results			
Ripening Time (days)	**CO2 Respiration (ug C respired / gdw · day)**	**Biomass (nmol PO_4 / gdw)**	**Activity per Unit of Biomass (ug C respired / nmol PO_4 * day)**
	Summer (Influent Temperature = 11°C)		
45	19.19 ± 1.66	11.91 ± 2.50	1.61
362	24.02 ± 4.41	23.96 ± 1.70	1.00
	Winter (Influent Temperature = 2.5°C)		
19	13.58 ± 1.42	17.23 ± 1.42	0.79
356	56.65 ± 3.58	104.92 ±3.58	0.54

Table 4.9 Protist, coliform, and turbidity results from full-scale sampling (August, 2007 and February, 2008).

Full-Scale Sampling - Protist, Coliform, and Turbidity Results				
Ripening Time (days)	**Protist # · gdw^{-1}**	**Protist # * $(mm^2)^{-1}$**	**Coliform Log Removal (30 day average)**	**Turbidity Removal (%)**
	Summer (Influent Temperature = 11°C)			
45	$4.30 \pm 1.55 \times 10^5$	$4.95 \pm 1.45 \times 10^3$	1.68	82.4
362	$1.15 \pm .161 \times 10^6$	$1.44 \pm .199 \times 10^4$	2.82	92.2
	Winter (Influent Temperature = 2.5°C)			
19	$1.76 \pm .411 \times 10^5$	$2.97 \pm .573 \times 10^3$	1.29	80.3
356	$1.28 \pm .132 \times 10^6$	$6.62 \pm .494 \times 10^3$	1.77	91.8

These results indicate that the trends observed in the bench-scale experiment in CO_2 respiration, biomass as measured by phospholipids, and log removals generally correspond with those found in full-scale filters. Protistan abundances did not compared well to the bench-scale ripening study based on units of protists per mm^2.

While there were not enough available full-scale filters to have five-sample time series as in the bench-scale study, therefore making a better comparison possible, it is reasonable to expect that full-scale filters behave in a similar fashion biologically during ripening as bench-scale filters with similar raw waters and operating conditions. However, the protistan abundance discrepancy deserves further examination.

4.2 Attempt to Quantify the *E.coli* Removal Contribution of Protists

Two filter columns, one with a mesh 5um pre-filter, the other with a 0.5um mesh pre-filter, were assessed for biological parameters, protistan abundance, and log *E.coli* removals. This 0.5um pre-filter was intended to prohibit more protists in influent water from reaching the SSF when compared to the parallel 5um pre-filter configuration.

Results show a greater *E.coli* log removal in the filter column that had been allowed to ripen with only the 5um pre-filter than the filter that had ripened with the 5um and 0.5 um pre-filters (see Table 4.10). However, protistan abundance results for both filters are within the margin of standard error.

Table 4.10 Protist exclusion study results summary.

Protist Exclusion Study - Results Averages			
Filter	**nmol PO_4 / gdw**	**Protist # / gdw**	***E.coli* Log Removal**
5 um pre-filter	18.7 ± 1.1	110.9 ± 3.8	2.00
0.5 um pre-filter	16.6 ± 2.2	117.4 ± 40.5	1.60

At first, it was assumed that the pre-filters did not serve the intended purpose of limiting the number of protists that would enter the SSF column. However, the protistan

abundance numbers in both filters were an order of magnitude or more below the abundance numbers observed in ripened filters in other studies for this research. This indicated that the 5um pre-filter that was common to both SSF columns removed the majority of the protists in the influent. It had been predicted that most protists, being less than 5um in size, would pass this pre-filter and then would be either blocked by the 0.5 um pre-filter on its way to one SSF, and in the other case travel onto the other SSF. However, it is likely that as time passed the 5um pre-filter become clogged with larger particles, creating a mat of material that could trap particles much smaller than the filter was designed to catch. Future studies approached in this fashion should use a much larger filter weave to block large particles to be sure that protists can pass on before meeting finer pre-filters, or should replace the filters more frequently.

4.3 Assessing Protist Seeding to Enhance *E.coli* Removals

The effects of applying a "seed" solution containing approximately 10^4 protists per mL in phosphate buffer to bench-scale SSF columns were examined. Laboratory analyses performed included general biological analysis (biomass as measured by phospholipids, and CO_2 respiration) and protist counts of cores of the top 5mm of filter media, and *E.coli* counts for determination of log removals. Results are displayed in Table 4.11 and Figures 4.4 and 4.5.

When calculated as protists per mm^2 of filter surface area, higher protistan abundances were observed in the seeded two day and 16 day ripened filters when compared to controls, but not in the seeded five day ripened filter (Figure 4.5). The two day and 16 day seeded filters showed protistan abundances approximately 50% and 30%

higher than their respective controls. The five day filter's protistan abundance was roughly the same as its respective control.

No significant log *E.coli* removal improvements were observed in any of the seeded filters – increased protistan abundance or not (Figure 4.4). A recent trial of protist seeding by Unger (2006), using a different seed solution preparation method, likewise did not observe improvements in log *E.coli* removals. Unger's study challenged seeded filters with *E.coli* immediately after application of seed. It was later hypothesized that allowing an acclimation period, as in the 48 hour delay used in this study, would allow the protists to settle and begin feeding. Though the 48 hour delay may have allowed feeding to commence, it was not on a scale large enough to significantly affect log *E.coli* removals.

Unger's seeding method in part used a centrifuge to separate protists out of an extraction solution into a pellet, and then later re-suspended them in seed solutions of varying concentration based on volume of centrifuged pellet material used. The seeding method in this research used a phosphate buffer solution for protistan extraction which was later directly seeded at a concentration of approximately 10^4 protist #/mL without the centrifuge step. Unger was able to obtain a seed solution of approximately 10^5 protist #/mL using the additional centrifuge step. Both seeding methods resulted in improved protistan abundances in seeded filters, though the centrifuge method resulted in an order of magnitude improvement in abundance versus controls, whereas the non-centrifuge seed method used in this study resulted in more modest improvements of 57% in the best case (two day ripened seeded filter versus two day ripened control).

However, the simplicity offered by the non-centrifuge method is more in line with the intention of exploring a technique that could be usefully applied to full-scale

operating facilities. The use of the centrifuge technique on such a scale would be impractical. Further comparison between the two studies is not highly useful, however, as the Unger study used a different ripening scheme using pre-filters, in addition to use of a different seeding technique.

It is also likely that, despite the estimated protistan uptake capacity described in Section 3.2 being much greater than the applied challenge bacteria, there is a major confounding factor: background bacterial concentrations in the influent stream. Research of surface waters have shown total bacterial counts in concentrations as high as $1x10^{10}$MPN/100mL, dwarfing the 10^{4}MPN/100mL challenge concentration used in this research (Malley, 2009). If the protistan population's total uptake capacity was, in essence, "filled" by influent bacteria from the river, then the spike of *E.coli* during the challenge may have been too small to have made an observable impact.

Despite lack of improvement when comparing seeded filters versus controls, the log removal data in this study again confirm a generally increasing removal trend with ripening time, especially in the span from five days of ripening to sixteen. During that eleven day period, log removals jumped from 0.47 and 0.41 in the seeded filter and control, respectively, to 1.05 and 1.04.

A close relationship between protistan abundance per mm^2 of surface area and CO_2 respiration was observed when data sets included results from both seeded and control filters (Figure 4.5). The protistan data set and the CO_2 data set showed a strong correlation ($r = 0.95$, critical value = 0.87, $p = 0.01$). This close relationship was not observed between protistan abundance and biomass as measured by phospholipids, despite the fact that a prior bench-scale ripening experiment in this research did reveal

such a correlation. The prior experiment with normally ripened columns showed a correlation between biomass as measured by phospholipids and protistan abundance, while the experiment with both seeded and naturally ripening columns (controls) instead showed a strong correlation between CO_2 respiration and protistan abundance.

The reason for this change in correlation is unclear. It is possible that the phosphate buffer solution used in the creation of the seed solution affected phospholipid levels in the schmutzdecke as it filtered through, thus affecting potential correlations. This effect would not have been present in the prior study since it did not involve seeding. Logically, such an increase in phosphate levels would increase phospholipid concentrations. This seems unlikely, however, given that biomass as measured by phospholipids were not uniformly greater in the seeded filters than in control filters. CO_2 respiration levels were not significantly higher in seeded columns when compared to controls, either.

A second possibility may involve the range of ripening times examined in each study. The bench-scale ripening study with normally ripened filters contained five sets of filters, the longest of which was ripened to 44 days. In the study with seeded filters, the longest ripening time was only sixteen days. The set of filters with the longer range of ripening time showed the correlation between protistan abundance and biomass as measured by phospholipids, whereas the set of filters with the shorter range of ripening time showed the protistan correlation to CO_2 respiration. This could be an indication that in the early stages of filter ripening, protistan abundance closely relates to CO_2 respiration, while over the longer term, in data sets that include filters with more fully

developed schmutzdecke, it more closely relates to biomass as measured by phospholipids.

Table 4.11 Seeding study results table – CO_2 respiration, phosphate concentration, activity/biomass, protist abundance, and log *E.coli* removals.

Seeding Study - Results Averages						
Ripening Time (days)	CO_2 Respiration (ug C respired / gdw · day)		Biomass (nmol PO_4 / gdw)		Activity / Biomass (ug C / nmol PO_4 * day)	
	Seeded	Control	Seeded	Control	Seeded	Control
2 day	37.3 ± 3.6	23.1 ± 4.0	18.8 ± 1.3	10.6 ± 1.9	1.98	2.18
5 day	24.8 ± 3.8	25.0 ± 2.5	8.4 ± 1.3	17 ± 3.7	2.95	1.47
16 day	42.9 ± 7.4	29.9 ± 5.3	32.9 ± 6.3	24.9 ± 2.7	1.30	1.20

Ripening Time (days)	Protist # / gdw		Protist # / mm^2 surface area		Log *E.coli* Removals	
	Seeded	Control	Seeded	Control	Seeded	Control
2 day	$2.28 \pm .27 \times 10^5$	$2.16 \pm .19 \times 10^5$	$3.28 \pm .20 \times 10^3$	$2.09 \pm .08 \times 10^3$	0.37	0.31
5 day	$2.92 \pm .27 \times 10^5$	$2.54 \pm .48 \times 10^5$	$2.33 \pm .03 \times 10^3$	$2.39 \pm .22 \times 10^3$	0.47	0.41
16 day	$4.06 \pm .15 \times 10^5$	$4.55 \pm 1.4 \times 10^5$	$3.23 \pm .12 \times 10^3$	$2.49 \pm .24 \times 10^3$	1.05	1.04

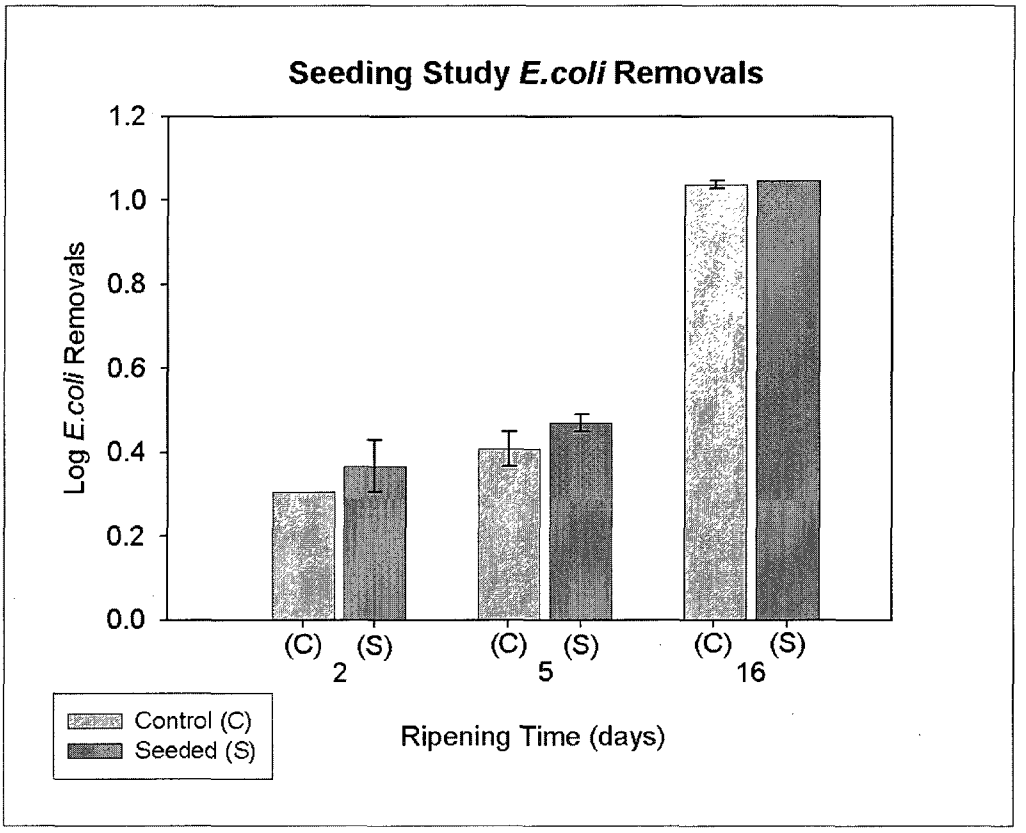


Figure 4.4 Seeding study log *E.coli* removals versus ripening time and seeded/control status.

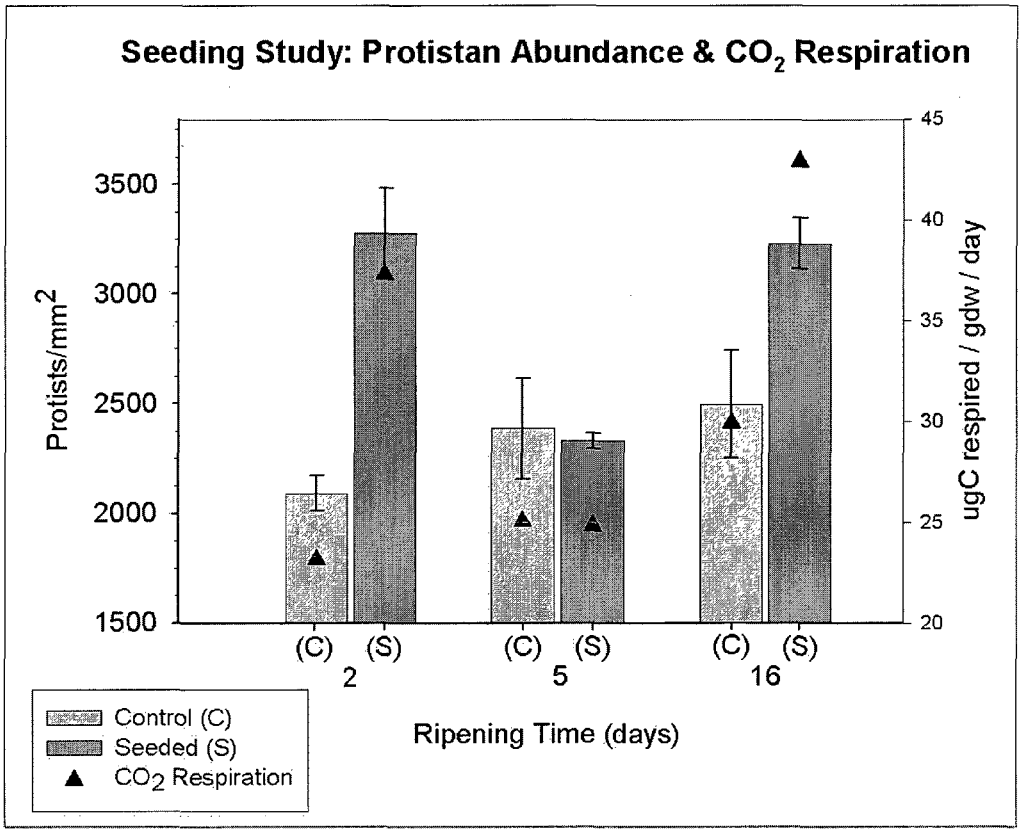


Figure 4.5 Protist # per mm^2 of filter media and CO_2 respiration versus ripening time and seeded/control status.

CHAPTER 5

CONCLUSIONS

The bench-scale ripening studies confirmed that increased ripening time corresponded with increased schmutzdecke biomass, increased number of protists, and increased *E.coli* removals. Positive correlations were observed between biomass as measured by phospholipids and log *E.coli* removals, and CO_2 respiration and log removals. Protistan abundance also showed a positive correlation with log *E.coli* removals. Additionally, biomass as measured by phospholipids correlated positively with protistan abundance.

Full-scale SSFs showed a similar correspondence between ripening time, schmutzdecke biomass, and protist numbers. Activity per unit of biomass was higher in the summer due to warmer influent water, as expected. However, it was discovered that biomass as measured by phospholipids was greater during the winter than the summer. It is possible that this increased level is due to greater oxygen penetration in winter water temperatures, and subsequent increased phospholipid formation (Kinner, 2008).

Attempts to quantify the level of *E.coli* removal caused by protistan activity using wound-fiber pre-filters led to inconclusive results. The configuration of the pre-filters (a common 5um pre-filter followed by a 0.5um pre-filter for one SSF column and a control SSF column with no 0.5um pre-filter) may have caused a higher-than-anticipated removal of influent protists. It is possible that with the amount of organic material removed by the common 5um pre-filter, it became slightly clogged, therefore straining out particles

smaller than 5um, including protists. Such removals would not have allowed protists to colonize the control SSF as designed.

Application of a "seed" solution containing 10^4 # protists/mL extracted from a ripened schmutzdecke to bench-scale filters that were relatively unripened offered mixed results. Two of the three seeded filters showed improvements in protistan abundance 48 hours after seeding, on the order of 50 percent increases. No significant improvements in log *E.coli* removals were observed. The lack of removal improvements may be indicative of an inability on the part of protists to begin feeding after being introduced to a new filter, the need for a greater seed volume or concentration. It may also suggest that protistan predation is not significant enough to be observed independently of other removal factors.

Additionally, a significant positive correlation between protistan abundance and CO_2 respiration results ($p = 0.01$) was observed in the seeding study. No correlation was observed between protistan abundance and biomass as measured by phospholipids, as was observed in the prior ripening study. It is possible that this is due to the difference in the ripening schemes of the two experiments. In the prior ripening study, biomass as measured by phospholipids correlated positively with protistan abundance in a set of filters the longest of which was ripened to 44 days. In the seeding study, the longest ripened filter was ripened to only 16 days. This could suggest that protistan abundance correlates positively with CO_2 respiration over short term ripening periods, and with biomass as measured by phospholipids over longer-term ripening periods.

CHAPTER 6

RECOMMENDATIONS

The relationship between CO_2 respiration, biomass as measured by phospholipids, and protistan abundance should be further explored. Pre-filter studies of the type conducted in this research should be reworked to prevent pre-filter clogging and straining of protists. Also, further research into the potential of seeding is necessary before conclusions can be drawn in regard to its feasibility. A comparison of extraction and seeding techniques and the subsequent improvements (or lack thereof) in protistan abundance they offer should be conducted independently of *E.coli* challenge experiments. Survivability of transplanted protistan populations should also be assessed. When a method of seeding that offers consistently improved subsequent protistan abundances in the schmutzdecke is confirmed, microbial challenges of varying concentration should be performed. Additionally, similar bench-scale experiments should be performed with other challenge microbes to assess the potential for their removal via predation.

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APPENDIX A – STANDARD OPERATING PROCEDURES

- CO_2 RESPIRATION
- MICROBIAL BIOMASS MEASUREMENT BY PHOSPHOLIPID EXTRACTION
- DETECTION OF TOTAL COLIFORMS / *E. COLI*
- ULTRAVIOLET ABSORBANCE (UV_{254})
- TOTAL ORGANIC CARBON
- ENUMERATION OF PROTISTS BY PRIUMLIN STAINING FROM WATER SAMPLES
- ENUMERATION OF PROTISTS ON SAND AND SCHMUTZDECKEN BY PRIMULIN STAINING
- CHROMIC ACID WASH STATIONS

Standard Operating Procedures
CO_2 RESPIRATION

Principle

Aerobes convert oxygen to carbon dioxide. Wet sand is sealed in a jar with a rubber septum, and measuring the CO_2 concentration of the air after a specified amount of time (24 hours) provides an indication of microbial activity.

Sample Collection and Storage

Sand samples are stored at 4^0C in chromic-acid washed glass jars with screw caps completely submerged in water from their environment.

Retain some influent challenge water in a separate container for blank analysis and refrigerate.

Maximum holding time: 24 hours.

Equipment

a. 20 mL vials with rubber septa
b. Licor 6252 CO_2 analyzer (IRGA)
c. Incubator adjusted to 25°C
d. Syringes of 5 and 50 mL

Reagents

a. RO water
b. Carbon dioxide standard (gas cylinder)
c. Compressed air zero grade (gas cylinder)

Method

Incubation

a. Weigh empty 20 mL vial w/o cap. Tare empty vial. Add 1 g wet sand.
b. Add 0.1 mL of appropriate water. For SSF, use feed water from the corresponding filter.
c. Prepare duplicate water blanks from each filter's influent: Add 0.1 mL of appropriate water and no sand.
d. Cap each vial with rubber septum and seal with metal ring.
e. Incubate at 25^0C for 24 hours.

CO_2 Analysis

a. Take all jars out of the incubator.
b. Set up the Licor 6252 CO_2 analyzer according to posted instructions.
c. Establish a standard curve before analyzing samples. Make sure that the standard curve brackets all samples. Standards used were: 0.5 mL, 2.0 mL, 5.0 mL, 10.0 mL, and 20.0 mL.
d. Pump syringe with ambient air 3 times to rinse.
e. Plunge syringe in sample jar or vial and pump syringe (valve open) 3 times with 4-5 mL of sample, then take a 4 mL sample and close valve.
f. Open syringe valve, bring sample volume to down to 3 mL. Inject the 3 mL sample in the CO_2 analyzer and wait for reading (integration value) to stabilize. The integration value should fall below 1 µmol/mol, preferably < 0.5 µmol/mol.
g. Record value.
h. Repeat all steps for each sample.

Calculations

1. Convert mL of CO_2 standard to µmol using the ideal gas law, assuming a pressure of 1 atm and a temperature of 22.5^0C:

$$\text{ìmol } CO_2 = \frac{\text{mL } CO_2}{RT} = \frac{\text{mL } CO_2}{0.082 * 295.5}$$

2. Convert µmol CO_2 to µg carbon:

$$\text{ìg C} = \text{ìmol } CO_2 * \frac{12.012\,\text{gC}}{\text{mol } CO_2}$$

3. Generate a calibration curve of IRGA reading vs. µg C.

3. Convert µg C from calibration curve to CO_2 respired as µg C per gram dry weight sand and time:

$$\frac{\text{ìg C}}{\text{gdw} \cdot \text{day}} = \text{ìg C} * \frac{22.66\,\text{mL in jar}}{3\,\text{mL analyzed}} * \frac{1}{\text{grams sand dry weight}} * \frac{1}{1\,\text{day}}$$

Quality Control

a. Replicates: analyze each sample at least in duplicate.
b. Blanks: use water blanks to correct for contributions from microorganisms in water.

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Standard Operating Procedures
MICROBIAL BIOMASS MEASUREMENT
BY PHOSPHOLIPID EXTRACTION

Principle

Phospholipids are ubiquitous in cell membranes and are turned over relatively quickly during metabolism. They are therefore a good indicator of viable biomass. This method eliminates some of the difficulties of other biomass measurements because it is performed in situ, thus avoiding the use of surfactants and their associated variability in recovery.

Lipids are extracted in a mixture of methanol, chloroform and water. After the extraction is completed, more chloroform and dilute sulfuric acid are added to separate the solvents, causing the lipids to settle in the chloroform phase below the water and methanol. Lipid-containing chloroform is withdrawn and dried under nitrogen. Phosphate is then liberated by oxidation with potassium persulfate and colored by reaction with ammonium molybdate and malachite green to form a lime green solution that is analyzed colorimetrically.

Sample Collection and Storage

Sand samples are stored at 4^0C in chromic-acid washed glass jars with screw caps completely submerged in water from their environment.

Maximum holding time: 24 hours. An analysis by Page (1997) found no significant difference in phospholipids in split samples analyzed after 1 hour or 24 hours holding time.

Equipment

a. 103^0C oven
b. Spectrophotometer
c. Matched quartz cuvettes, 1.0 cm pathlength
d. 20 mL and 10 mL vials with TFE-lined screw caps
e. Syringes
f. Pipetters
g. Compressed nitrogen tank and manifold

Reagents

Concentrated Sulfuric Acid (36 N) H_2SO_4 and Dilutions (6 month storage)

0.36 N H_2SO_4:

Dilute 10 mL stock to 1 L with distilled RO water

5.72 N H_2SO_4

Dilute 159 mL stock to 1 L with distilled RO water

Chloroform, pesticide grade

Methanol, pesticide grade

Nitrogen Gas, pre-purified

Acidified Water (6 month storage refrigerated)

Dilute 4.0 mL H_2SO_4 stock (36 N) to 500 mL with distilled RO water

Potassium Persulfate (6 month storage)

5% $K_2S_2SO_4$ in 0.36 N H_2SO_4:

Dissolve 5 g $K_2S_2SO_4$ with 0.36 N H_2SO_4 to 100 mL

Ammonium Molybdate (6 month storage)

2.5% $(NH_4)_6Mo_7O_{24}•4H_2O$ in 5.72 N H_2SO_4:

Dissolve 2.5 g $(NH_4)_6Mo_7O_{24}•4H_2O$ with 5.72 N H_2SO_4 to 100 mL

Malachite Green (6 month storage in the dark)

0.111% polyvinyl alcohol and 0.111% malachite green in water:

Dissolve 0.555 g polyvinyl alcohol (100% hydrolyzed) in 500 mL distilled RO water at 80^0C, cool, then add 0.555 g malachite green. Ensure thoroughly mixed (no precipitate) before each use.

Potassium Phosphate Standard 0.2 mM (prepare fresh daily)

KH_2PO_4 Molecular Weight = 136 g/mol

1) Make 2 M stock: Dissolve 0.272 g KH_2PO_4 in 1 L distilled RO water

2) Dilute 20 mL stock to 200 mL in distilled RO water to make 0.2 mM standard

Method

Day 1

Extraction

a. Weigh empty 20 mL vial w/o cap. Tare empty vial. Add 1 g wet sand.
b. Under the fume hood: Add 2.5 mL chloroform and 5.0 mL methanol, cap tightly, swirl by hand for 10 sec.
c. Let vials stand 2 – 24 hours (6 hours used in this study) to allow biomass to be extracted by solvent. Record standing time allowed.
d. Add 2.5 mL chloroform and 4.0 mL acidified water, tighten cap, and swirl for 10 sec. The chloroform phase is now below the water and methanol.
e. Let vials stand overnight (16 hours used in this study).

Day 2

Extraction (cont'd)

a. Using a needle-tipped syringe, transfer 2 mL of chloroform extract to 10 mL vials. Rinse syringe 2x with chloroform and 1x with the next sample before extracting the next sample.
b. Create standards. Add 0, 10, 25, 50, 75, and 100 uL potassium phosphate standard solution to 10 mL vials in triplicate.
c. Dry down chloroform extracts and standards under nitrogen stream at 15 psi in a 50^0C water bath. Use a test tube rack and manifold to dry many samples at once.

Digestion

a. Add 0.9 mL potassium persulfate reagent to each dried standard and sample. Tighten caps.
b. Place in 103^0C oven overnight.

Day 3

Color Change

a. Allow vials to cool to room temperature.
b. Add 0.2 mL ammonium molybdate, mix by hand, let stand for 10 min.
c. Add 0.9 mL malachite green, mix by hand, let stand 30 min. N.B.: Malachite green must be thoroughly stirred before use.
d. N.B.: If a yellow color develops instead of a green color, potassium persulfate may be expired, and organic carbon is interfering with the color change. Make fresh potassium persulfate reagent.

Measurement

a. Measure absorbance with spectrophotometer at 610 nm against RO water. Zero on RO water.
b. Between samples, rinse cuvette 1x with methanol, 1x with RO water, and 1x with a small volume of the next sample.

Dry Weight Determination

a. Decant excess liquid from 20 mL vials into hazardous waste container.
b. Dry at 60^0C for at least 48 hours. Studies found no significant reduction in weight drying at 103^0C vs. 60^0C or when drying longer than 48 hours.
c. Weigh sample and vial and subtract vial weight to determine dry sample weight.

Calculations

1. Calculate final PO_4^{3-} concentration in 10 mL vials for calibration curve:

Let V be the volume of standard added to the vial before drying.

1) Moles KH_2PO_4 in vial = $0.2\times10^{-3}\ \frac{\text{mol}\,KH_2PO_4}{\text{L}} * V$.
2) KH_2PO_4 dissociates according to the equation

$$KH_2PO_4 \rightarrow K^+ + 2H^+ + PO_4^{3-},$$

so 1 mole KH_2PO_4 corresponds to 1 mole PO_4^{3-}.

3) Phosphate is digested by addition of 0.9 mL potassium persulfate and then reacted with 0.2 mL ammonium molybdate and 0.9 mL malachite green for a final volume of 2.0 mL.
4) Let V_a = 0.002 L represent this final volume.
5) Then, the final concentration, C_f, of phosphate before spectrophotometry is given by:

$$C_f = \frac{0.2\times10^{-3}\ \frac{\text{mol}\,KH_2PO_4}{\text{L}}}{0.002\,\text{L}} * V = 0.1V$$

Volume Phosphate Standard Dried, V (uL)	Final Concentration of Phosphate in 2 mL Reagents Analyzed, C_f (umol/L)
0	0
10	1.0
25	2.5
50	5.0
75	7.5
100	10

2. Determine phosphate concentration, C_f, from dried sample extracts using calibration curve.

3. Calculate moles PO_4^{3-} per gram dry weight of original sample:

$$\frac{\text{nmol}\,PO_4^{3-}}{\text{gdw}} = \frac{C_f * V_a * 5\,\text{mL chloroform total} * 1000 \frac{\text{nmol}}{\mu\text{mol}}}{(2\,\text{mL chloroform extracted})(\text{g dry sand})}$$

$$= \frac{5 * C_f}{\text{g dry sand}}$$

Quality Control

a. Blanks: zero spectrophotometer with RO water blank. Readback blank every 10 samples and at end of run to monitor for drift.
b. Replication: analyze every sample at least in duplicate and preferably in triplicate.
c. LOD/MDL/LOQ: Estimated LOD was 1 nmol PO_4 for a 2 mL volume of chloroform extracted (Wang 1995).
d. Avoid phosphorus contamination

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Standard Operating Procedures
DETECTION OF TOTAL COLIFORMS / *E. COLI*

Principle

The IDEXX Quanti-Tray/2000 provides an easy, rapid and accurate count of coliform bacteria and *E.coli*. The IDEXX Quanti-Tray/2000 is a semi-automated quantification method based on the Standard Methods Most Probable Number (MPN) model. The Quanti-Tray® Sealer automatically distributes the sample/reagent mixture into separate wells. After incubation, the number of positive wells is converted to an MPN using a table provided. Quanti-Tray/2000 counts from 2 to 2,419 MPN / 100 ml.

Sample Collection and Storage

Collect samples in autoclave-sterilized bottles.

Seal each sample bottle individually in a plastic bag.

Transport in a cooler with ice to the lab and place immediately in the refrigerator at 4^0C.

Maximum storage time is 24 hours.

Equipment

a. 100 ml autoclave-sterilized Pyrex vials with lids
b. Quanti/Tray 2000® trays
c. Quanti-Tray® Sealer
d. Incubator
e. UV light lamp

Reagents

Colilert® Powder
Sterile water

Method

a. Turn sealer on to warm up for 20 min.
b. Pipette 99 mL of sterile water into the pyrex bottles.
c. Add 11mL of sample from the sampling container to one pyrex bottle (a 10 fold dilution).
d. Shake for 20 seconds.
e. Transfer 11mL from the first bottle to another pyrex bottle (another 10 fold dilution).
f. Repeat steps c-e until a dilution bottle has an expected concentration of 10-1000 MPN/100mL.
g. Pipette off 10mL to achieve 100mL in the dilution bottle.
h. Add Colilert® Powder reagent to sample and shake until fully dissolved.
i. Pour sample/reagent into Quanti-Tray®/2000 (counts from 1-2,419).
j. Seal in Quanti-Tray® Sealer and place in 37°C incubator
k. 24 hours later count positive wells and refer to MPN table.
    - Yellow wells = positive for total coliforms
    - Yellow and UV-fluorescent wells = positive for *E. coli*

Calculations

Count large and small wells that have turned yellow and also fluoresce magenta under the UV light. Consult MPN table provided and record results as MPN/100mL. Use the number of dilutions, n, to calculate the sample concentration by dividing by 10^{-n}.

ex) # of dilutions, $n = 3$

Large wells positive = 40

Small wells positive = 7

Lookup MPN on IDEXX table = 90.8 MPN / 100 mL

$$\text{Sample Conc} = \frac{\text{Conc in dilution}}{10^{-n}} = \frac{90.8\text{MPN/100mL}}{10^{-3}} = 9.08 \times 10^{4}\,\text{MPN/100mL}$$

Quality Control

Run negative controls (sterile water + reagent) and positive controls (sterile water spiked with *E. coli* + reagent) every time.

References

http://www.idexx.com

Standard Operating Procedures
ULTRAVIOLET ABSORBANCE (UV_{254})

Principle

Beers Law states that absorbance is proportional to the concentration of the analyte for a given absorption pathlength at any given wavelength. UV absorbance at 254 nm is a useful surrogate parameter for estimating the raw water concentrations of organic carbon and THM precursors (*Standard Methods* 2006).

Apparatus

a. Hitachi UV2000 spectrophotometer
b. Cuvettes, 1cm path length, 3 ml volume, matched quartz cells (Suprasil ®, Fisher Sci.)

Reagents and materials

Collection of Samples

Collect samples in 40 mL amber TOC vials that have been washed with chromic acid and baked 90 min. in a muffle furnace at 550^0C to mineralize all organic matter.

Store at 4^0C.

Holding time: < 48 hours.

Method

a. Remove samples from refrigerator and allow to warm to room temp.
b. Set spectrophotometer to measure wavelength 254 nm.
c. Zero machine on RO lab water blank.
d. Rinse cuvette with RO water twice; then fill with at least 1.5 ml of sample.
e. Wipe cuvette with kimwipe to be sure it is dry and free of smudges.
f. Measure and record absorbance.
g. Analyze sample aliquots in duplicate (triplicate if discrepancy).

Quality Control

a. Blanks every 8 samples to check for drift.
b. Run duplicate samples from a random source each round of sampling.
c. For this method (not same instrument) the standard deviation of duplicate samples was ± 0.011 cm^{-1}. The standard deviation of duplicate measurements was ± 0.002 cm^{-1}. (Collins et al. 1989)

Hitachi UV2000 Specifications		
Range	Reproducibility	Accuracy
0-0.5 abs.	± 0.001	± 0.002
0.5-1.0 abs.	± 0.002	0.004 ±

Care for cuvettes

a. Periodically clean cells by rinsing with methanol then RO water, or use phosphate free soap.

b. Take care not to drop, scratch or in any way damage the cells.

Instrument Setup

a. Select Photometry in Main Menu using arrow keys; press ENTER.
b. Select Test Setup: set/check set to 254 nm wavelength.
c. Press FORWARD; machine will align to 254 nm. Wait for 30 minutes for the lamp to warm up.
d. Press AUTOZERO to zero on blanks.
e. Press start to measure absorbance of samples.

References

APHA, AWWA, WEF (2006). *Standard Methods for the Examination of Water and Wastewater*. 21st Ed.

Page T. G. 1997. "GAC Sandwich Modification to Slow Sand Filtration for Enhanced Removal of Natural Organic Matter" Masters thesis, University of New Hampshire, Durham, NH.

Standard Operating Procedures
TOTAL ORGANIC CARBON

Principle

Organic carbon is oxidized to carbon dioxide by persulfate in the presence of ultraviolet light. The carbon dioxide produced is measured directly by a non-dispersive infrared analyzer.

Sample Collection and Storage

Collect samples in 40-mL amber TOC vials that have been washed with chromic acid and combusted at 550 degrees Celcius for 90 minutes to remove all organic matter.

Preserve with concentrated H_3PO_4 to pH < 2.

Refrigerate.

Holding time: < 2 weeks with acid preservation.

Equipment

a. Sievers Model 800 TOC Analyzer with Autosampler
b. Aluminum foil
c. Vials, 40 mL amber glass TOC vials

Reagents

a. Potassium persulfate solution, 15%. Shelf life: approximately 90 days.
b. Potassium acid phthalate (KHP), $KHC_8H_4O_8$ for standards

Method

Prepare KHP standards:

1. Prepare 1000 mg/L stock: dissolve 2.1254 g $KHC_8H_4O_8$ (dried to constant weight at 103 degrees Celcius) in RO lab water and dilute to 1000 mL.
2. Make standards according to the table below.

Volumes of standard stock and RO lab water diluent to make TOC standards.

Standard Concentration, mg/L	Volume of 1000 mg/L Stock	Dilute to:
0.5	1 mL	2 L
1.0	1 mL	1 L
2.0	2 mL	1 L
5.0	5 mL	1 L
10.0	5 mL	500 mL

Run TOC Analyzer

a. Start TOC analyzer, autosampler, computer, and printer.
b. Open TOC analyzer software program.
c. Fill TOC vials with standards: 1 for each point on the calibration curve and 1 standard of random concentration for every 8 samples.
d. Cover each vial with a small piece of aluminum foil in place of the cap. Be careful not to leave fingerprints on the foil over the vial opening. Fingerprints will be detected by the analyzer as the probe punctures the foil.
e. Arrange samples and standards. A typical run has the following sequence:

Run order for TOC samples and standards.

Position	Sample or Standard
1-2	RO blank
3-7	Standards: one of each, randomized
8-15	Samples and/or sample duplicates, randomized
16	Randomly selected standard readback
{repeat 8 samples and 1 standard until all samples and duplicate have been analyzed}	
{last 3 spots}	RO blanks

f. Mount the samples and standards in the autosampler and enter their labels into the computer software.
g. Enter the oxidation and acid rates for each sample and standard:

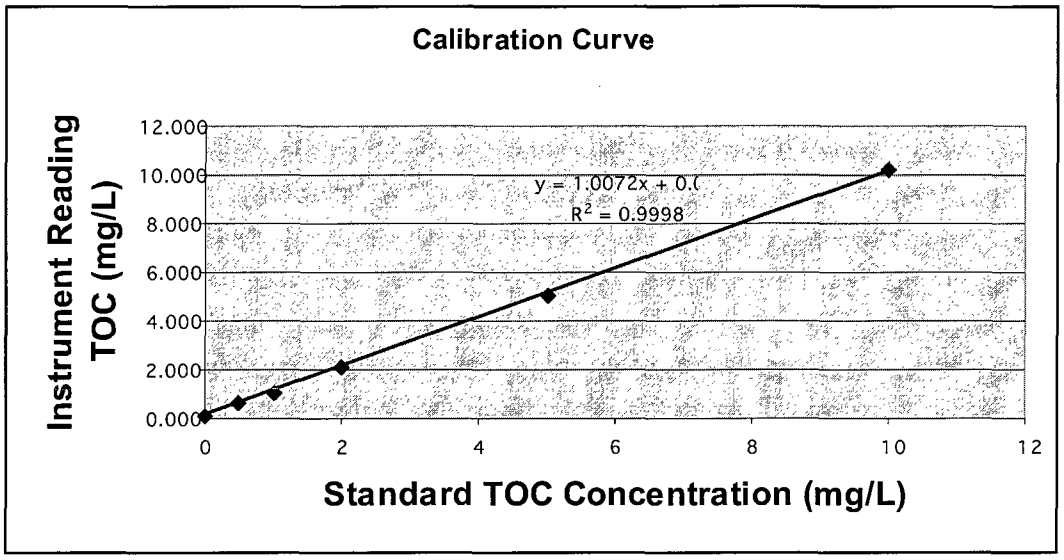
Acid and oxidation rate settings for standard or sample concentrations.

Concentration	Acid Rate	Oxidation Rate
RO blank	0.5	0.5
0.5 mg/L standard	0.5	1.0
All others	1.0	2.0

h. Run the collection program. The analyzer will take three readings from each sample or standard and calculate an average and standard deviation.

Calculations

a. Calibration Curve: Plot the measured concentrations against the expected standard concentrations and fit a calibration curve using linear regression as shown below.
b. Calculate the sample concentration by substituting the instrument reading (average of 3 readings for each sample) into the calibration curve equation.



Sample TOC calibration curve (June 22, 2005).

Quality Control

Readbacks: random standard after every 8 samples.

Duplicates: analyzed at least 2 duplicate every run.

References

Mercier, David J (1998). Characterization and treatability of natural organic matter from the Croton Reservoir – Pilot Study II. M.S. Thesis. Univ. of New Hampshire.

Standard Operating Procedures
ENUMERATION OF PROTISTS BY PRIUMLIN STAINING FROM WATER SAMPLES

Principle

Protists in water samples are fixed with glutaraldehyde buffered with cacodylic acid and stained with primulin stain. Primulin causes eukaryotic cells to fluoresce yellowish-brown under UV light.

Equipment

a. Vortex mixer
b. 10 mL sterile centrifuge tubes
c. Sterile microscope slides and coverslips
d. Microscope equipped with Hg fluorescent lamp
e. Cellulose nitrate backing filters: 0.45 µm, 25 mm diameter
f. Black protist filters: Micronsep, cellulosic, 0.8 µm, 25 mm diameter
g. 12-well Millipore filtration apparatus with vacuum pump

Reagents

a. 10 %Glutaraldehyde and 10% Cacodylic Acid fixative solution
    i. 5 g cacodylic acid
    ii. 20 mL of 25% gludaraldehyde stock
    iii. 30 mL distilled water
    iv. filter through 0.22 micron syringe filter into sterilized bottle
b. Sterile Phosphate Buffer pH = 7 (Sinclair and Ghiorse, 1987)
    b. 2.2 mM KH_2PO_4 x 1 L x 136.1 g/mol = 0.299 g KH_2PO_4 / L
    c. 4.02 mM K_2HPO_4 x 1 L x 174.2 g/mol = 0.700 g KH_2PO_4 / L
    d. Dilute with distilled water to 1 L in a volumetric flask.
    e. Transfer to a chromic acid-washed wide-mouthed amber bottle.
    f. Autoclave 20 min at 121^0C.
c. Primulin Stain
d. Tris-HCl

Collection of Samples

a. Collect samples in sterile containers.
b. Transport and store at 4^0C.
c. Fix immediately after returning to the lab.
    - Use 10 %Glutaraldehyde and 10% Cacodylic Acid fixative solution in a ratio of 1:10, fixative : sample.
    - Fix the smallest volume aliquot of the sample as necessary to minimize waste.
d. Holding time: < 6 hours.

Method

Filtering

a. Mount a nitrate cellulose backing filter with a drop of sterile RO water. Use the desired number of filters, leaving well 12 free.
b. Mount a protist filter dark side up over the backing filter.
c. Set the top section of the 12-well filtration apparatus in place on the blue base, and tighten the screw.
d. Plug each well that does not contain a filter with a blue plug except for well 12.
e. Fill each well with a filter with a few mL of sterile RO water.
f. Turn on the vacuum pump. **Never use a vacuum above 5 psi to avoid rupturing the filter!**
g. Plug well 12 to create a vacuum and filter the RO water.
h. Remove the plug from well 12 whenever adding a new reagent, and plug well 12 to filter.
i. Filter 2 mL Tris-HCl through each filter. Wait 2 minutes.
j. Filter another 2 mL Tris-HCl through each filter. Wait 2 minutes.
k. Filter desired volume of fixed protist extract (typically the entire fixed amount).
l. Add 2 mL primulin stain to each well. Cover the entire apparatus with aluminum foil to prevent light degradation.
m. Let primulin stand for 10 minutes. Check periodically to ensure filters remain wet with primulin. Add extra as needed to keep filters from drying out.
n. After 10 minutes, filter remaining primulin.
o. Place filters stained side up in weigh dishes in a drawer overnight (or until dry).

Mounting on Slides

a. Place a large drop of immersion oil in the center of a slide.
b. Using tweezers, place the filter on the oil drop. Avoid air bubbles.
c. Place another large drop of immersion oil on top of the filter.
d. Mount a coverslip. Use tweezers to press air bubbles out edge of coverslip.
e. Ensure enough oil has been used to saturate filter.

Counting

a. Allow the UV lamp to warm up for 15 minutes.
b. Mount the scanning jig on the microscope stage. Using the 60x Nikon objective lens, the scan length is 11.10 mm.
c. Use Nikon filter cube B-2H.
d. Perform the necessary number of scans to count 300 protists. Select the location of each scan randomly.
e. Criteria to count a protist:
    a. fluoresces yellow-green
    b. has even edges
    c. is roughly ellipsoid
    d. is larger than 3 μm

Calculations

a. Determine the area multiplier, *M*, for scans:
a. The filtration apparatus wells have a diameter of 18.22 mm and, thus, and area of 260.7 mm^2.
b. The scans have a length of 11.10 mm. Their width is 0.0725 mm (the width of the whipple disk set in the microscope eyepiece). Thus, the scan area is 0.80475 mm^2.
c. *M* = filtration area / scan area = 324.0
b. Determine the dilution factor, *D*:
d. *D* = total volume of buffer extract generated / volume of extract filtered = 45 mL / volume of extract filtered
c. Determine number of protists, *N*, on original sand sample:
e. *N* = average count per scan * *D* * *M*
d. Calculate number of protists per gram dry weight sand:
f. Determine dry weight of sand be drying Bag 1 with washed sand at 103^0C for 24 hours and subtracting weight of bag.
g. Number of protists / gram dry weight = *N* / dry weight

References

a. Hines, L. E. (1998). The Response of Subsurface Bacteria and Protists to an Organic Perturbation: Column Studies. Durham, NH, University of New Hampshire Master's Thesis

Standard Operating Procedures
ENUMERATION OF PROTISTS ON SAND AND SCHMUTZDECKEN BY PRIMULIN STAINING

Principle

Protists associated with the biological mat of the schmutzdecke or sand within a biological sand filter are fixed with glutaraldehyde buffered with cacodylic acid and stained with primulin stain. Primulin causes eukaryotic cells to fluoresce yellowish-brown under UV light.

Equipment

a. Vortex mixer
b. 10 mL sterile centrifuge tubes
c. 150 mL Whirl-Pak bags (Nasco Inc., Loves Park, IL)
d. Sterile microscope slides and coverslips
e. Microscope equipped with Hg fluorescent lamp
f. Cellulose nitrate backing filters: 0.45 μm, 25 mm diameter
g. Black protist filters: Micronsep, cellulosic, 0.8 μm, 25 mm diameter
h. 12-well Millipore filtration apparatus with vacuum pump

Reagents

a. 10 %Glutaraldehyde and 10% Cacodylic Acid fixative solution
    i. 5 g cacodylic acid
    ii. 20 mL of 25% gludaraldehyde stock
    iii. 30 mL distilled water
    iv. filter through 0.22 micron syringe filter into sterilized bottle
b. Sterile Phosphate Buffer pH = 7 (Sinclair and Ghiorse, 1987)
    b. 2.2 mM KH_2PO_4 x 1 L x 136.1 g/mol = 0.299 g KH_2PO_4 / L
    c. 4.02 mM K_2HPO_4 x 1 L x 174.2 g/mol = 0.700 g KH_2PO_4 / L
    d. Dilute with distilled water to 1 L in a volumetric flask.
    e. Transfer to a chromic acid-washed wide-mouthed amber bottle.
    f. Autoclave 20 min at 121^{0}C.
c. Primulin Stain
d. Tris-HCl

Collection of Samples

a. Pre-weigh one 150 mL Whirl-Pak bag for each sample.
b. Fill each Whirl-Pak bag with 25 mL sterile phosphate buffer.
c. Drain filter supernatant using either feed port (for pilot filter) or pipette (for lab-scale columns) to slightly above top of schmutzdecke.
d. Cut the tip from a 5mL pipette tip and measure the diameter. Using the suction provided by the pipette, withdraw the top 5mm of the schmutzdecke and underlying sand.
e. Transport field samples in sterile 150mL Whirl-Pak bags submerged in 25 mL sterile phosphate buffer in coolers with ice packs. Immediately after returning to the lab, fix samples according to steps below.

Method

Fixing

a. Start with sand in 25 mL S&G buffer in Whirl-Pak bag as collected during sampling (see above).
b. Shake gently for 30 sec.
c. Decant buffer into fresh Whirl-Pak (Bag 2).
d. Add 10 mL buffer to sand sample.
e. Shake gently again for 30 sec.
f. Decant buffer, adding to first 25 mL.
g. Repeat steps d-f to achieve a total of 45 mL buffer with dislodged protists in Whirl-Pak Bag 2.
h. Set Bag 1 aside for sand dry weight analysis.
i. Pipette 2 mL S&G buffer into each of 2 sterile centrifuge tubes.
j. Shake Bag 2 30 sec, and pipette 1 mL into each of the tubes from step h.
k. Add 0.3 mL of the filter-sterilized 10% glutaraldehyde solution to each tube.
l. Vortex for ~3 sec. and allow to sit for at least 10 min.
m. Fixed protists can be stored up to 5 days.

Filtering

a. Mount a nitrate cellulose backing filter with a drop of sterile RO water. Use the desired number of filters, leaving well 12 free.
b. Mount a protist filter dark side up over the backing filter.
c. Set the top section of the 12-well filtration apparatus in place on the blue base, and tighten the screw.
d. Plug each well that does not contain a filter with a blue plug except for well 12.
e. Fill each well with a filter with a few mL of sterile RO water.
f. Turn on the vacuum pump. **Never use a vacuum above 5 psi to avoid rupturing the filter!**
g. Plug well 12 to create a vacuum and filter the RO water.
h. Remove the plug from well 12 whenever adding a new reagent, and plug well 12 to filter.
i. Filter 2 mL Tris-HCl through each filter. Wait 2 minutes.
j. Filter another 2 mL Tris-HCl through each filter. Wait 2 minutes.
k. Filter desired volume of fixed protist extract (typically the entire fixed amount).
l. Add 2 mL primulin stain to each well. Cover the entire apparatus with aluminum foil to prevent light degradation.
m. Let primulin stand for 10 minutes. Check periodically to ensure filters remain wet with primulin. Add extra as needed to keep filters from drying out.
n. After 10 minutes, filter remaining primulin.
o. Place filters stained side up in weigh dishes in a drawer overnight (or until dry).

Mounting on Slides

f. Place a large drop of immersion oil in the center of a slide.
a. Using tweezers, place the filter on the oil drop. Avoid air bubbles.

b. Place another large drop of immersion oil on top of the filter.
c. Mount a coverslip. Use tweezers to press air bubbles out edge of coverslip.
d. Ensure enough oil has been used to saturate filter.

Counting

a. Allow the UV lamp to warm up for 15 minutes.
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4. *M* = filtration area / scan area = 324.0
5. Determine the dilution factor, *D*:
6. *D* = total volume of buffer extract generated / volume of extract filtered = 45 mL / volume of extract filtered
7. Determine number of protists, *N*, on original sand sample:
8. *N* = average count per scan * *D* * *M*
9. Calculate number of protists per gram dry weight sand:
10. Determine dry weight of sand be drying Bag 1 with washed sand at 103^0C for 24 hours and subtracting weight of bag.
11. Number of protists / gram dry weight = *N* / dry weight

References

a. Hines, L. E. (1998). The Response of Subsurface Bacteria and Protists to an Organic Perturbation: Column Studies. Durham, NH, University of New Hampshire Master's Thesis

Standard Operating Procedure
For
CHROMIC ACID WASH STATIONS

Chemical Name(s): Chromic Acid; Chromerge and Sulfuric acid

Engineering Controls:

Always use in fume hood and keep in secondary containment.

Personal Protective Equipment:

Goggles
Face Shield
Nitrile gloves with Neoprene gloves over them. Tuck arm sleeves into cuffs of gloves. Fold/roll glove cuffs forward to prevent acid from running onto clothing.
Rubber apron

Proper Use:

1. Read MSDS

2. Always add ACID to WATER

3. Rinse dirty glassware at least 3 times with RO water to remove gross contamination and minimize acid use.

4. Working in secondary containment, pour a small amount of concentrated chromic acid into glassware to be washed. Swirl and then pour and continue to swirl as it is poured (pour-n-swirl) into next glassware to be acid washed. Repeat until all glassware is coated with acid. When finished, pour the remaining acid from the glassware back into the concentrated acid container until the glassware is completely empty (i.e., no more dripping coming out). [Note: the concentrated chromic acid is spent when the color turns green.]

5. With a wash bottle, spray rinse around the container mouth letting the rinse water flow over the inside surface of the container. Pour rinse into a properly labeled (yellow label) 4L hazardous waste bottle until completely empty (stops dripping). REPEAT 2X. Minimize water use. The key to efficient contaminant removal and hazardous waste minimization is multiple rinses using small quantities of water with complete drainage between rinses.

6. If gloves or exterior surface of glassware become contaminated with acid or neutralizer, rinse with RO wash bottle spray into a beaker. Pour rinse into hazardous waste rinse bottle until the beaker is completely empty.

7. Finally, rinse glassware at least 6 times with RO water or 3 times with RO and 3 times with better quality water if appropriate. Discharge rinse water to the sink drain.

8. If a spill occurs, cover with neutralizer until reaction stops (excess neutralizer). With spatula, scoop the neutralizer into tray and discard into hazardous waste bucket labeled "spent chromic acid neutralizer". Use a yellow hazardous waste label. Make sure to put respective cover securely back on the waste bucket.

9. Wet paper towels and sponges should be used to clean spent neutralizer from hood surfaces. Used wipers must be disposed in the hazardous waste bucket labeled spent chromic acid neutralizer. Immediately clean up any acid or spent neutralizer spills to the floor using a wet sponge or paper towel and place in hazardous waste bucket labeled spent chromic acid neutralizer.

10. Keep areas clean at all times. Contamination is a health and safety hazard and is considered a hazardous waste release by the USEPA and State of New Hampshire Department of Environmental Services.

First Aid:

1. WATER, WATER, AND MORE WATER

2. For skin contact – immediately flush contaminated areas for 15 minutes to ensure removal

3. For eye contact – immediately eye wash 15 minutes

4. For inhalation – fresh air

5. For ingestion – get medical attention and provide MSDS sheet of chemical swallowed

6. GET MEDICAL ATTENTION

7. Refer to MSDS located in laboratory for further information

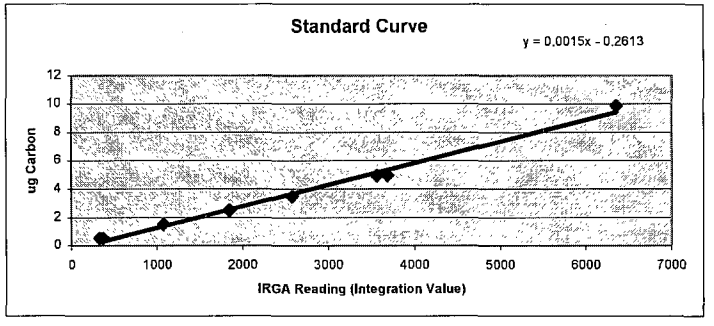
APPENDIX B – DATA TABLES

Ripening Challenge 1: CO2 Respiration Data

Vial Measurements					Respiration Measurements					
	Vial Empty (g)	Dried Sar	Dried Sand (g)			Readings	ug C (from curve)	CO2 respi	Averages	Error Calcs
6 wk Top 1	18.242	18.902	0.66		6wk1	5368.4	7.7913	89.1671		3.3168 <= St. Dev.
6 wk Top 2	18.311	18.83	0.519		6wk2	4473.6	6.4491	93.8578	91.5125	2.3454 <= St. Error
3 wk Top 1	18.309	19.595	1.286		3wk1	5217.7	7.56525	44.4346		10.4638 <= St. Dev.
3 wk Top 2	18.323	19.071	0.748		3wk2	4084.7	5.86575	59.2326	51.8336	7.3990 <= St. Error
2 wk Top 1	18.288	19.311	1.023		2wk1	3252.4	4.6173	34.0919		0.0799 <= St. Dev.
2 wk Top 2	18.282	19.2	0.918	Top	2wk2	2927.3	4.12965	33.9789	34.0354	0.0565 <= St. Error
1 wk Top 1	18.295	19.286	0.991		1wk1	2502.5	3.49245	26.6192		4.7711 <= St. Dev.
1 wk Top 2	18.322	19.3745	1.0525		1wk2	2020.2	2.769	19.8719	23.2456	3.3737 <= St. Error
3 day Top 1	18.297	19.351	1.054		3day1	1886.3	2.56815	18.4043		5.4678 <= St. Dev.
3 day Top 2	18.302	19.117	0.815		3day2	2054.3	2.82015	26.1369	22.2706	3.8663 <= St. Error
6 wk Bottom 1	18.346	19.312	0.966		6wk1	1233.1	1.58835	12.4196		1.4472 <= St. Dev.
6 wk Bottom 2	18.424	19.236	0.812		6wk2	917.61	1.115115	10.3729	11.3963	1.0233 <= St. Error
3 wk Bottom 1	18.354	19.459	1.105		3wk1	869.92	1.04358	7.1335		0.2743 <= St. Dev.
3 wk Bottom 2	18.3	19.221	0.921		3wk2	785.61	0.917115	7.5215	7.3275	0.1940 <= St. Error
2 wk Bottom 1	18.438	19.408	0.97		2wk1	773.53	0.898995	7.0004		1.1909 <= St. Dev.
2 wk Bottom 2	18.369	19.64	1.271		2wk2	770.58	0.89457	5.3163	6.1583	0.8421 <= St. Error
1 wk Bottom 1	18.336	19.291	0.955	Bottom	1wk1	659.33	0.727695	5.7555		0.2325 <= St. Dev.
1 wk Bottom 2	18.389	19.247	0.858		1wk2	585.15	0.616425	5.4266	5.5911	0.1644 <= St. Error
3 day Bottom 1	18.347	19.319	0.972		3day1	572.74	0.59781	4.6455		0.3010 <= St. Dev.
3 day Bottom 2	18.376	19.43	1.054		3day2	566.76	0.58884	4.2198	4.4327	0.2128
					Water1	560.57	0.579555			
					Water2	568	0.5907			

Standards

mL CO2 gas	Reading	uMol CO2	ug C
1	370.36	0.0410	0.4930
1	330.31	0.0410	0.4930
3	1073.1	0.1231	1.4789
5	1845.8	0.2052	2.4649
5	1847.9	0.2052	2.4649
7	2579.1	0.2873	3.4508
10	3566.3	0.4104	4.9298
10	3691.2	0.4104	4.9298
20	6360.6	0.8208	9.8595



Ripening Challenge 1: Phospholipid Data

Replicate	20mL Empty Vials	20mL Dried Sand + Vial	Dry Sand Weight	Absorbance (610nm)	PO4 From Curve (uL)	PO4 per gram dry weight (moles)	Averages	Error Calcs	
6 wk Top 1	13.639	14.249	0.61	2.159	18.518	151.783		19.6646	<= St. Dev.
6 wk Top 2	13.549	14.167	0.618	2.137	18.310	148.142	161.267	11.3534	<= St. Error
6 wk Top 3	13.638	14.109	0.471	2.032	17.321	183.876			
3 wk Top 1	13.523	14.372	0.849	1.236	9.822	57.847		12.2785	<= St. Dev.
3 wk Top 2	13.525	14.41	0.885	1.174	9.238	52.194	61.919	7.0890	<= St. Error
3 wk Top 3	13.563	14.374	0.811	1.497	12.281	75.716			
2 wk Top 1	13.488	14.498	1.01	0.974	7.354	36.407		7.5774	<= St. Dev.
2 wk Top 2	13.591	14.584	0.993	1.154	9.050	45.568	44.472	4.3748	<= St. Error
2 wk Top 3	13.594	14.426	0.832	1.102	8.560	51.442			
1 wk Top 1	13.468	14.235	0.767	0.589	3.727	24.297		1.3944	<= St. Dev.
1 wk Top 2	13.562	14.451	0.889	0.617	3.991	22.447	23.974	0.8051	<= St. Error
1 wk Top 3	13.593	14.567	0.974	0.714	4.905	25.179			
3 day Top 1	13.596	14.458	0.862	0.575	3.595	20.855		3.9846	<= St. Dev.
3 day Top 2	13.427	14.224	0.797	0.667	4.462	27.993	25.447	2.3005	<= St. Error
3 day Top 3	13.637	14.577	0.94	0.742	5.169	27.492			
6 wk Bottom 1	13.593	14.435	0.842	0.413	2.069	12.287		1.6696	<= St. Dev.
6 wk Bottom 2	13.473	14.552	1.079	0.413	2.069	9.589	11.506	0.9639	<= St. Error
6 wk Bottom 3	13.479	14.331	0.852	0.422	2.154	12.641			
3 wk Bottom 1	13.397	14.353	0.956	0.414	2.079	10.871		1.4544	<= St. Dev.
3 wk Bottom 2	13.597	14.348	0.751	0.408	2.022	13.463	12.549	0.8397	<= St. Error
3 wk Bottom 3	13.596	14.444	0.848	0.433	2.258	13.311			
2 wk Bottom 1	13.583	14.519	0.936	0.383	1.787	9.544		0.3695	<= St. Dev.
2 wk Bottom 2	13.665	14.658	0.993	0.391	1.862	9.375	9.667	0.2133	<= St. Error
2 wk Bottom 3	13.624	14.538	0.914	0.389	1.843	10.083			
1 wk Bottom 1	13.348	14.379	1.031	0.375	1.711	8.299		2.1756	<= St. Dev.
1 wk Bottom 2	13.643	14.352	0.709	0.271	0.731	5.159	7.598	1.2561	<= St. Error
1 wk Bottom 3	13.662	14.538	0.876	0.367	1.636	9.337			
3 day Bottom 1	13.563	14.556	0.993	0.407	2.013	10.134		3.6701	<= St. Dev.
3 day Bottom 2	13.873	14.629	0.756	0.274	0.760	5.025	6.058	2.1189	<= St. Error
3 day Bottom 3	13.666	14.582	0.916	0.252	0.552	3.016			

Ripening Challenge 1: Total Organic Carbon Data

Sample	TOC ppm
Blank	78.2
Blank	41.1
1	1.04
1	1.19
2	2.03
5	5.11
5	4.96
10	9.86
Raw 1	6.84
Raw 2	6.78

"Top"=Influent Water	TOC ppm	
3 Day Top Rep 1	6.9	0.02 <= St. Dev.
3 Day Top Rep 2	6.87	0.02 <= St. Error
1 Wk Top Rep 1	6.9	0.05 <= St. Dev.
1 wk top rep 2	6.97	0.03 <= St. Error
2wk Top Rep 1	6.72	0.15 <= St. Dev.
2 Wk Top Rep 2	6.93	0.11 <= St. Error
3 wk Top Rep 1	6.96	0.06 <= St. Dev.
3wk Top Rep 2	6.88	0.04 <= St. Error
6 Wk Top Rep 1	12.4	0.00 <= St. Dev Outlier
6 Wk Top Rep 2	6.99	0.00 <= St. Error
"Bottom" = Effluent Water	TOC ppm	
3 Day Bot Rep 1	6.8	0.04 <= St. Dev.
3 day bot rep 2	6.74	0.03 <= St. Error
1 Wk Bot Rep 1	6.69	0.14 <= St. Dev.
1 Wk Bot Rep 2	6.89	0.10 <= St. Error
2 wk bot rep 1	6.69	0.01 <= St. Dev.
2 Wk Bot Rep 2	6.7	0.00 <= St. Error
3 Wk Bot Rep 1	6.53	0.05 <= St. Dev.
3 wk bot rep 2	6.6	0.03 <= St. Error
6 wk bot rep 1	6.25	0.06 <= St. Dev.
6 Wk Bot rep2	6.33	0.04 <= St. Error

Average influent: 6.902222

Average Influent Error
0.04875

Averages	Time
6.90	Influent
6.77	3 day
6.79	7 day
6.70	14 day
6.57	21 day
6.29	42 day

Ripening Challenge 1: Microsphere Data

Filtered 10mL each time.

	1	2	3	4	5	6	7	8	Counts	Ave Counts	Ratio Remaining	Log Removals
3day1	0	0	0	0	0	0	0	0	0			
3day2	0	1	0	2	0	1	1	0	20.25	10.13	0.0006	3.2
1wk1	0	0	0	0	0	0	0	0	0			
1wk2	0	0	0	0	1	1	0	0	8.10	4.05	0.0002	3.6
2wk1	0	0	0	0	0	0	0	0	0			
2wk2	0	0	0	0	1	0	1	0	8.10	4.05	0.0002	3.6
3wk1	0	0	0	0	0	0	0	0	0			
3wk2	0	0	1	0	0	0	0	0	4.05	2.03	0.0001	3.9
6wk1	0	0	0	0	0	0	0	0	0			
6wk2	0	0	0	0	0	0	0	0	0	0	0	All removed
Inf1	540								17496.00			
Inf2	549								17787.60	17641.80		
Raw1	0	0	0	0					0			
Raw2	0	0	0	0					0			

Ripening Challenge 1: UV Data

Rep	254nm Reading	Error Calcs	
Influent 1	0.318	0.0055	<= St. Dev.
Influent 2	0.328	0.0032	<= St. Error
Influent 3	0.327		
6 wk 1	0.279	0.0092	<= St. Dev.
6 wk 2	0.267	0.0053	<= St. Error
6 wk 3	0.261		
3 wk 1	0.27	0.0150	<= St. Dev.
3 wk 2	0.253	0.0087	<= St. Error
3 wk 3	0.24		
2 wk 1	0.263	0.0071	<= St. Dev.
2 wk 2	0.254	0.0041	<= St. Error
2 wk 3	0.249		
1 wk 1	0.283	0.0047	<= St. Dev.
1 wk 2	0.281	0.0027	<= St. Error
1 wk 3	0.274		
3 day 1	0.291	0.0040	<= St. Dev.
3 day 2	0.287	0.0023	<= St. Error
3 day 3	0.295		

Averages	
Influent	0.3243
3day effluent	0.2910
1wk effluent	0.2793
2wk effluent	0.2553
3wk effluent	0.2543
6wk effluent	0.2690

Ripening Challenge 1 - E.coli Data

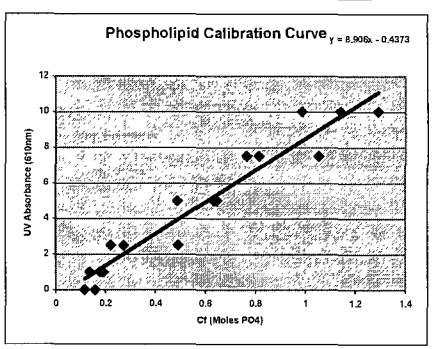
	Large Yellow	Small Yellow	Large Blue	Small Blue	Dilution	Total Coliforms	E.coli	Other than e.coli	Final Counts: Total	Final Counts: E.coli	Final Counts: Other	Error Calcs	
In 10-1	48	48	48	48	0.1	1011.1	1011.1	0	10111	10111	0		
3 day 10-1	48	48	48	48	0.1	1011.1	1011.1	0	10111	10111	0		
3 day 10-1	48	48	48	48	0.1	1011.1	1011.1	0	10111	10111	0		
3 day 10-2	48	24	48	24	0.01	328.2	328.2	0	32820	32820	0	3550	<= St. Dev.
3 day 10-2	48	27	48	27	0.01	378.4	378.4	0	37840	37840	0	2510	<= St. Error
1wk 10-1	48	44	48	44	0.1	829.1	829.1	0	8291	8291	0	292	<= St. Dev.
1wk10-1	48	45	48	45	0.1	870.4	870.4	0	8704	8704	0	207	<= St. Error
2wk10-1	47	17	47	17	0.1	83.5	83.5	0	835	835	0	45	<= St. Dev.
2wk10-1	48	14	46	14	0.1	77.1	77.1	0	771	771	0	32	<= St. Error
3wk10-1	25	0	25	0	0.1	33.5	33.5	0	335	335	0	71	<= St. Dev.
3wk10-1	24	1	22	1	0.1	33.1	23.5	9.6	331	235	96	50	<= St. Error
6wk10-1	45	11	45	11	0.1	139.6	139.6	0	1396	1396	0	84	<= St. Dev.
6wk10-1	46	11	46	11	0.1	151.5	151.5	0	1515	1515	0	59	<= St. Error
6wk10-1 Redo	46	12	46	12	0.1	156.5	156.5	0	1565	1565	0		
6wk10-1 Redo	45	12	44	12	0.1	143.9	133.4	10.5	1439	1334	105		
6wk10-2	4	2	4	2	0.01	6.2	6.2	0	620	620	0		
6wk10-2	16	1	16	1	0.01	20.1	20.1	0	2010	2010	0		
In 10-3	33	9	33	9	0.001	67.6	67.6	0	67600	67600	0	11102	<= St. Dev.
In 10-3	40	4	40	4	0.001	83.3	83.3	0	83300	83300	0	7850	<= St. Error
In 10-5	0	1	0	1	0.00001	1	1	0	100000	100000	0		
In 10-5	2	0	2	0	0.00001	2	2	0	200000	200000	0		
Raw1 100%	48	39	26	4	1	658.6	41.3	617.3	658.6	41.3	617.3		
Raw2 100%	48	40	27	8	1	689.3	49.6	639.7	689.3	49.6	639.7		

	Averages	Log Removals	Averages		
Influent	112725				
3 day	32820	0.5359	0.5050	0.0437	<= St. Dev.
	37840	0.4741		0.0309	<= St. Error
1 week	8291	1.1334	1.1229	0.0149	<= St. Dev.
	8704	1.1123		0.0106	<= St. Error
2 week	835	2.1303	2.1476	0.0245	<= St. Dev.
	771	2.1650		0.0173	<= St. Error
3 week	335	2.5270	2.6040	0.1089	<= St. Dev.
	235	2.6810		0.0770	<= St. Error
6 week	1396	1.9071	1.8894	0.0251	<= St. Dev.
	1515	1.8716		0.0178	<= St. Error

Springfield Challenge 1 - Phospholipid Analysis Data

Filter	Sample	Top or Bottom of Core	Replicate	Empty 20mL Bottles (g)	Bottle + Dry Sand (g)	Dry Sand Weight (g)	UV Spec Readings (610nm)	Moles PO4 (from curve)	Moles PO4/gdw
A	1	T	1	13.536	14.388	0.852	0.392	3.054	17.922
A	1	T	2	13.589	14.425	0.836	0.445	3.526	21.088
A	1	B	1	13.612	14.478	0.866	0.142	0.827	4.777
A	1	B	2	13.604	14.393	0.789	0.296	2.199	13.935
A	2	T	1	13.559	14.459	0.9	0.219	1.513	8.406
A	2	T	2	13.548	14.314	0.766	0.249	1.780	11.621
A	2	B	1	13.521	14.805	1.284	0.216	1.486	5.788
A	2	B	2	13.297	14.293	0.996	0.143	0.836	4.198
A	3	T	1	13.528	14.377	0.849	0.18	1.166	6.866
A	3	T	2	13.515	14.445	0.93	0.165	1.032	5.549
A	3	B	1	13.584	14.411	0.827	0.129	0.712	4.302
A	3	B	2	13.647	14.446	0.799	0.382	2.965	18.553
B	1	T	1	13.421	14.325	0.904	0.7	5.797	32.063
B	1	T	2	13.486	14.261	0.775	0.542	4.390	28.321
B	1	B	1	13.343	14.393	1.05	0.348	2.662	12.676
B	1	B	2	13.612	14.497	0.885	0.388	3.018	17.052
B	2	T	1	13.588	14.435	0.847	0.547	4.434	26.176
B	2	T	2	13.572	14.348	0.776	0.427	3.366	21.685
B	2	B	1	13.592	14.794	1.202	0.57	4.639	19.298
B	2	B	2	13.466	14.574	1.108	0.435	3.437	15.509
B	3	T	1	13.666	14.859	1.193	0.596	4.871	20.414
B	3	T	2	13.634	14.648	1.014	0.393	3.063	15.102
B	3	B	1	13.589	14.864	1.275	0.233	1.638	6.423
B	3	B	2	13.645	14.567	0.922	0.396	3.089	16.754

Standards (uL PO4)	UV Absorbance (610nm)	Cf (PO4 before spec)
0	0.117	0
0	0.159	0
10	0.136	1
10	0.178	1
10	0.191	1
25	0.222	2.5
25	0.492	2.5
25	0.273	2.5
50	0.636	5
50	0.648	5
50	0.489	5
75	1.052	7.5
75	0.765	7.5
75	0.814	7.5
100	0.987	10
100	1.142	10
100	1.292	10



Springfield 1 Challenge - Select Coliform Data

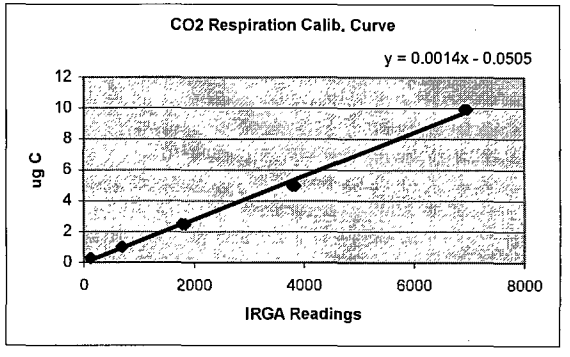
Date	Raw	SS #9	SSF #12
1/2/07	360	2	0
1/3/07	70	0	0
1/4/07	140	1	0
1/5/07	80	1	0
1/8/12	110	0	0
1/9/12	400	1	0
1/10/12	200	0	1
1/11/12	110	0	0
1/12/12	210	0	0
1/16/07	320	0	no sample
1/17/07	210	0	0
1/18/07	100	1	0
1/19/07	160	0	0
1/22/07	70	0	0
1/23/07	200	1	0
1/24/07	100	0	0
1/25/07	80	0	2
1/26/07	120	0	1
1/29/07	56	1	0
1/30/07	625	1	0
1/31/07	12	0	1
7/1/07	300	no sample	21
7/2/07	160	no sample	7
7/3/07	220	no sample	4
7/5/07	180	0	2
7/6/07	65	no sample	3
7/11/07	280	0	0
7/12/07	160	1	1
7/18/07	120	0	no sample
7/19/07	100	1	1
7/25/07	160	0	0
7/26/07	180	0	0
8/1/07	80	0	0
8/2/07	100	0	0
8/9/07	160	0	1

Courtesy Springfield Water & Sewer

Springfield Challenge 1 - CO2 Respiration Data

Filter	Sample	Top or Bottom of Core	Replicate	Empty 20mL Bottles (g)	Bottle + Dry Sand (g)	Dry Sand Weight (g)	IRGA Readings	ugC (from curve)	ugC/gdw*day
A	1	T	1	18.297	19.034	0.737	1639.8	2.245	23.011
A	1	T	2	18.306	19.07	0.764	1598	2.187	21.619
A	1	B	1	18.28	19.164	0.884	1089.9	1.475	12.606
A	1	B	2	18.281	18.9	0.619	750.6	1.000	12.207
A	2	T	1	18.308	19.039	0.731	1073	1.452	15.000
A	2	T	2	18.336	19.142	0.806	1037.1	1.401	13.133
A	2	B	1	18.348	19.121	0.773	859.45	1.153	11.264
A	2	B	2	18.385	19.136	0.751	813.54	1.088	10.947
A	3	T	1	18.338	19.259	0.921	1885.8	2.590	21.238
A	3	T	2	18.294	18.996	0.702	1439.9	1.965	21.147
A	3	B	1	18.329	19.116	0.787	869.02	1.166	11.192
A	3	B	2	18.309	19.449	1.14	1052.8	1.423	9.431
B	1	T	1	18.552	19.055	0.503	2179.6	3.001	45.064
B	1	T	2	18.293	19.025	0.732	1582.3	2.165	22.337
B	1	B	1	18.297	19.055	0.758	1066.7	1.443	14.378
B	1	B	2	18.309	19.742	1.433	1250.3	1.700	8.960
B	2	T	1	18.322	19.508	1.186	2064	2.839	18.082
B	2	T	2	18.286	19.104	0.818	1875.9	2.576	23.784
B	2	B	1	18.35	19.863	1.513	1425.8	1.946	9.713
B	2	B	2	18.367	19.24	0.873	954.07	1.285	11.120
B	3	T	1	18.299	19.361	1.062	1514.3	2.070	14.719
B	3	T	2	18.241	19.051	0.81	1580	2.162	20.156
B	3	B	1	18.39	19.238	0.848	1021.1	1.379	12.283
B	3	B	2	18.31	19.503	1.193	1248	1.697	10.742

Standards (mL CO2)	umol CO2	ugC	IRGA Readings
0.5	0.021	0.248	124.54
0.5	0.021	0.248	130.09
2	0.083	0.991	682.72
2	0.083	0.991	703.74
5	0.206	2.479	1840.6
5	0.206	2.479	1777.6
10	0.413	4.957	3791.2
10	0.413	4.957	3854.4
20	0.825	9.915	6975.6
20	0.825	9.915	6908.2



Springfield Challenge 2 - Phospholipid Analysis Data

Not Ripe = Filter 15 (A) Ripe = Filter 16 (B)

Filter	Core	Replicate	Empty 20mL Bottles (g)	Bottle + Dry Sand (g)	Dry Sand Weight (g)	UV Spec Readings (610nm)	Moles PO4 (from curve)	Moles PO4/gdw
A	1	1	13.422	14.263	0.841	0.625	2.536	15.074
A	1	2	13.523	14.409	0.886	0.629	2.578	14.547
A	2	1	13.486	14.685	1.199	0.718	3.516	14.663
A	2	2	13.616	14.592	0.976	0.828	4.676	23.956
A	3	1	13.542	15.064	1.522	0.863	5.045	16.574
A	3	2	13.645	14.29	0.645	0.612	2.398	18.593
B	1	1	13.368	14.143	0.775	1.985	16.877	108.882
B	1	2	13.594	14.107	0.513	1.62	13.028	126.977
B	2	1	13.297	13.896	0.599	1.466	11.404	95.191
B	2	2	13.342	13.673	0.331	0.885	5.277	79.716
B	3	1	13.528	14.45	0.922	1.916	16.149	87.577
B	3	2	13.588	14.233	0.645	1.681	13.671	105.977

AVERAGE

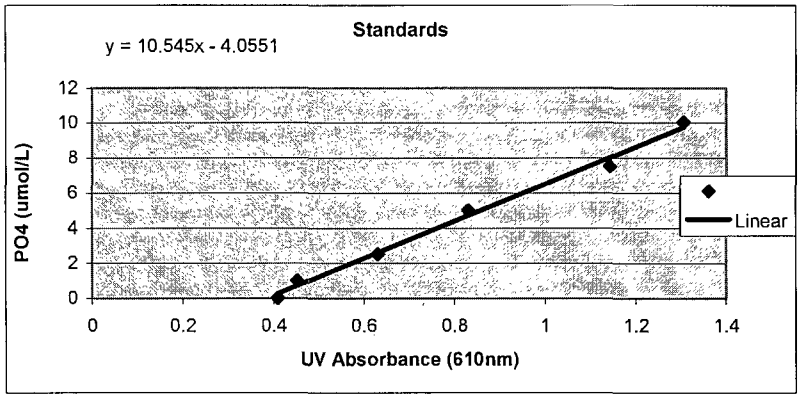
Not Ripe	SD	Std. Error
17.23	3.63198	1.48275

AVERAGE

Fully Ripe	SD	Std. Error
104.92	14.9897	6.703579

Standards

Known PO3 Conc. (ug/L)	UV Spec Reading (610nm)	Known PO3 Conc. (ug/L)	UV Spec Reading (610nm)
0	0.421	50	0.859
0	0.398	50	0.817
0	0.541	50	0.818
10	0.431	75	1.227
10	0.507	75	0.858
10	0.397	75	1.058
25	0.63	100	1.067
25	0.639	100	1.463
25	0.624	100	1.15



Springfield Challenge 2 - CO2 Respiration Data

Filter	Core	Empty 20mL Bottles (g)	Bottle + Dry Sand (g)	Dry Sand Weight (g)	IRGA Readings	ugC (from curve)	ugC/gdw*day
A	1	18.314	19.845	1.531	1782.85	2.824	13.933
A	2	18.304	19.402	1.098	1456	2.301	15.830
A	3	18.292	19.388	1.096	1012	1.591	10.963
B	1	18.346	19.16	0.814	4173	6.648	61.692
B	2	18.3	18.735	0.435	2124.5	3.371	58.531
B	3	18.186	18.807	0.621	2573	4.088	49.728

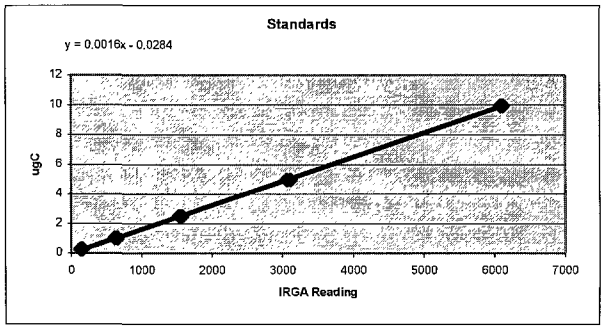
Averages

Not Ripe	SD	Std. Error
13.576	2.453125	1.416312234

Ripe	SD	Std. Error
56.650	6.199868	3.579495478

Standards

ml CO2	umol CO2	ugC	IRGA Reading
0.5	0.021	0.248	157
0.5	0.021	0.248	131
2	0.083	0.991	658
2	0.083	0.991	619
5	0.206	2.479	1571
5	0.206	2.479	1531
10	0.413	4.957	3060
10	0.413	4.957	3112
20	0.825	9.915	6118
20	0.825	9.915	6091



Springfield Challenge 2 - Coliform readings for February

SSF 15			SSF 16			Raw water	
						2/1/08	80
						2/2/08	140
						2/3/08	260
						2/4/08	280
						2/5/08	130
			2/6/08	0		2/6/08	200
			2/7/08	4		2/7/08	130
						2/8/08	480
						2/9/08	170
						2/10/08	240
						2/11/08	320
start-up						2/12/08	50
2/15/08	6						
2/16/08	4					2/16/08	70
2/17/08	3					2/17/08	20
2/18/08	13					2/18/08	170
2/19/08	18					2/19/08	190
2/20/08	8		2/20/08	3		2/20/08	40
2/21/08	10					2/21/08	30
2/22/08	13		shutdown			2/22/08	90
2/23/08	3					2/23/08	30
2/24/08	2					2/24/08	20
2/25/08	4					2/25/08	20
2/26/08	5					2/26/08	70
2/27/08	0					2/27/08	0

All units: cfu/100 mL Courtesy Springfield Water & Sewer

Springfield Challenge 2 - Filter Characteristics

All information on this page courtesy Springfield Water & Sewer
Both filters are 0.82 acres

SSF 15

Days on line = 19 (2/9/08 - 2/27/08)
Turbidity = 0.14 ntu at start-up, 0.10 ntu at time of shutdown for coring
Flow rate = about 0.75 MG/Day

SSF 16

Days on line = 356 3/14/07 - 2/22/08
Turbidity = 0.15 at start-up, after 1 month below 0.1 ntu, 90% of daily readings 0.05 - 0.06, end reading = 0.05 ntu
Flow rate = 1.5 - 2.0 MG/Day

Filters	Year completed	Acres per filter	Sq. feet per filter
9-10	1925	0.82	35720
11-14	1952	0.5	21780
15-18	1966	0.82	35720

1 acre = 43,560 sq.ft.

Filters	Year completed	Acres per filter	Sq. feet per filter
9-10	1925	0.82	35720
11-14	1952	0.5	21780
15-18	1966	0.82	35720

1 acre = 43,560 sq.ft.

Slow Sand Filter Flow Rate (MG/day)

Filter area	0.5	1	1.5	2	3	4
0.5 acres	23	46	69	92	138	184
0.82 acres	14	28	42	56	84	112

Flow rate in gals./sq.ft./day

"Under normal operating conditions the slow sand filters maintain a constant output of between 1 to 2 million gallons per day. They can support a filter rate of 4 to 4.5 million gallons per day for short periods (see Slow Sand Filters Status sheet). The filters can consistently produce water ≤ 0.1 NTU, even at the higer rates of filtration (see Slow Sand Filters Turbidities sheet).
Each filter is washed once per year, usually during the winter months."

Select SSF Combined Effluent Temperatures
July 2007...10.8 C
Aug 2007...11.2 C
Sept 2007...11.0 C

Dec 2007...3.9 C
Jan 2008...2.5 C
Feb 2008...2.2 C

Springfield Challenges 1 and 2

Influent turbidity (raw water) ntu

	August-07	February-08
1	0.58	0.54
2	0.48	0.51
3	0.53	0.56
4	0.53	0.48
5	0.50	0.51
6	0.65	0.49
7	0.49	0.81
8	0.54	1.10
9	0.58	0.50
10	0.52	0.49
11	0.49	0.49
12	0.46	0.53
13	0.62	
14	0.44	0.70
15	0.48	
16	0.44	0.60
17	0.47	0.84
18	0.50	0.59
19	0.45	0.80
20	0.53	0.55
21	0.45	0.55
22	0.5	0.63
23	0.54	0.62
24	0.57	0.56
25	0.48	0.64
26	0.50	0.61
27	0.47	0.59
28	0.54	0.60
29	0.50	0.61
30	0.48	
31	0.47	
Average	**0.51**	**0.61**

Seeding Challenge and Ripening Challenge 2 - E.coli Data

Filter	IDEXX Reading	Dilution	Concentration (MPN/100mL)	Average Influent Conc.	% Removal	Ratio Remaining	Log Removal		Average Log Removal	StDev	StError
6wk	178.9	0.01	17890		97.87	0.021	1.672				
6wk	248.9	0.01	24890		97.04	0.030	1.529	44 (no seed)	1.601	0.101	0.072
4wk	272.3	0.01	27230		96.76	0.032	1.490				
4wk	231	0.01	23100		97.25	0.027	1.561	30 (no seed)	1.526	0.051	0.036
2wkA	791.5	0.01	79150		90.59	0.094	1.027				
2wkA	755.5	0.01	75550		91.02	0.090	1.047	16 (no seed)	1.037	0.014	0.010
2wkB	755.5	0.01	75550		91.02	0.090	1.047				
2wkB	755.5	0.01	75550		91.02	0.090	1.047	16 (seed)	1.047	0.000	0.000
1wkA	416	0.001	298700		64.50	0.355	0.450				
1wkA	416	0.001	360900		57.11	0.429	0.368	5 (no seed)	0.409	0.058	0.041
1wkB	313	0.001	272300		67.64	0.324	0.490				
1wkB	416	0.001	298700		64.50	0.355	0.450	5 (seed)	0.470	0.028	0.020
3dA	298.7	0.001	416000		50.56	0.494	0.306				
3dA	360.9	0.001	416000		50.56	0.494	0.306	2 (no seed)	0.306	0.000	0.000
3dB	272.3	0.001	313000		62.80	0.372	0.430				
3dB	298.7	0.001	416000		50.56	0.494	0.306	2 (seed)	0.368	0.087	0.062
Challenge	77.1	0.0001	771000								
Challenge	88.4	0.0001	884000								
Tube	69.7	0.0001	697000								
Tube	101.4	0.0001	1014000	841500 (8.42*10^5)							

A=not seeded
B=seeded

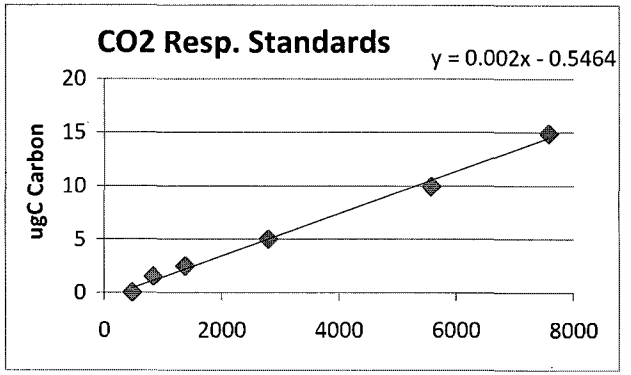
Seeding Challenge and Ripening Challenge 2 - Phospholipid Data

	Empty Vial Weights	Dry Sand Vial Weights	Sand Weight	Spec Reading 610nm	PO4 (uL) from chart	PO4 per gdw		Average	StDev	StError
6wk1	13.345	14.263	0.918	2.092	x					
6wk2	13.549	14.616	1.067	0.589	4.346	20.364				
6wk3	14.02	15.358	1.338	0.751	6.401	23.921	44.000	22.142	2.515	1.778
4wk1	13.464	14.614	1.15	0.917	8.507	36.989				
4wk2	13.344	14.846	1.502	0.773	6.680	22.238				
4wk3	13.405	14.976	1.571	0.746	6.338	20.171	30.000	26.466	9.171	5.295
2wkA1	14.029	15.366	1.337	0.829	7.391	27.640				
2wkA2	13.377	15.319	1.942	0.927	8.634	22.230				
2wkA3	13.552	x		0.738	6.236		16 (no seed)	24.935	3.825	2.705
2wkB1	13.379	14.961	1.582	0.909	8.406	26.567				
2wkB2	13.434	15.386	1.952	1.453	15.308	39.211				
2wkB3	13.659	15.009	1.35	x	x		16 (seed)	32.889	8.941	6.322
1wkA1	14.074	16.28	2.206	0.711	5.894	13.358				
1wkA2	13.528	14.277	0.749	0.491	3.102	20.710				
1wkA3	13.675	15.124	1.449	x	x		5 (no seed)	17.034	5.198	3.676
1wkB1	13.564	14.821	1.257	1.525	x					
1wkB2	13.681	15.954	2.273	0.591	4.371	9.615				
1wkB3	13.542	15.116	1.574	0.423	2.240	7.114	5 (seed)	8.365	1.769	1.251
3dayA1	13.499	14.856	1.357	1.089	x					
3dayA2	13.681	15.184	1.503	0.453	2.620	8.716				
3dayA3	13.471	14.897	1.426	0.526	3.546	12.435	2 (no seed)	10.576	2.629	1.859
3dayB1	13.61	14.574	0.964	0.57	4.105	21.290				
3dayB2	13.976	14.938	0.962	0.509	3.331	17.311				
3dayB3	13.646	15.186	1.54	0.675	5.437	17.652	2 (seed)	18.751	2.205	1.273

Seeding Challenge and Ripening Challenge 2 - CO2 Respiration Data

	Vial Weights	Vial + Sand	Sand Weight	IRGA Reading	ugC from chart	C02 respired/gd w/day	Averages	StDEv	Error
6wk1	18.311	19.235	0.924	4472.6	8.4	82.7			
6wk2	18.293	19.072	0.779	4203.9	7.9	91.8	87.3	6.4	4.6
4wk1	18.359	19.486	1.127	3931.5	7.3	59.1			
4wk2	18.339	19.251	0.912	3927.3	7.3	72.9	66.0	9.8	6.9
2wkA1	18.371	19.532	1.161	2520.6	4.5	35.2			
2wkA2	18.395	19.991	1.596	2425.5	4.3	24.5	29.9	7.6	5.3
2wkB1	18.375	19.126	0.751	2345.9	4.1	50.2			
2wkB2	18.279	19.729	1.45	3100.2	5.7	35.5	42.9	10.4	7.4
1wkA1	18.336	19.681	1.345	2302.4	4.1	27.5			
1wkA2	18.348	19.647	1.299	1883.9	3.2	22.6	25.0	3.5	2.4
1wkB1	18.301	19.079	0.778	1497.4	2.4	28.6			
1wkB2	18.382	20.3	1.918	2483.6	4.4	21.0	24.8	5.4	3.8
3dayA1	18.389	19.379	0.99	1749.5	3.0	27.1			
3dayA2	18.382	19.675	1.293	1631.1	2.7	19.1	23.1	5.7	4.0
3dayB1	18.312	19.673	1.361	2792.2	5.0	33.7			
3dayB2	18.381	19.569	1.188	2939.9	5.3	40.9	37.3	5.1	3.6

Standards	RGA Readir	Averages	umol CO2	ugC
0	476	476	0	0
3	843			
3	839	841	0.12	1.49
5	1389			
5	1375	1382	0.21	2.48
10	2767			
10	2835	2801	0.41	4.96
20	5579			
20	5584	5581.5	0.83	9.91
30	7490			
30	7675	7582.5	1.24	14.87



Potential Total Protistan Uptake

Assume: 1.1 bacteria/protist/hour (Kinner, 1998)
And $2x10^3$ protist/mm^2

$A = pi*r^2$
4.8cm diameter filter column
$A = 1808\ mm^2$

$1808mm^2 * (2x10^3\ protist/mm^2) = 3.6x10^6$ protist/filter

$3.6x10^6$ protist * (1.1 bacteria/protist/hour) = $3.9x10^6$ bacteria consumed/hour

Or, $5.9x10^6$ bacteria consumed over 90 minute challenge application period.

At 10 mL/min = 900mL/90min

900 mL @ 104MPN/100mL

$9x10^4$ bacteria added over 90 minute period.

$5.9x10^6 > 9x10^4$ bacteria (meaning much greater capacity for uptake than added by challenge)