ABSTRACT

Title of Thesis: ELECTROPHORETIC REMOVAL OF FINE PARTICULATES FROM AQUCULTURE EFFLUENT

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As larger waste particles breakdown into smaller pieces under the mechanical stress of a recirculating system, it becomes increasingly more difficult to remove these particles through standard methods. This current work explores the possibility of using an impressed electric field as a means of water clarification. In this study aquaculture effluent is passed through an imposed electric field, where the fluid column is divided into two fluid streams: one closest to the positive electrode, and the other closest to the negative electrode. The water quality of each fluid stream is analyzed to determine if any difference results due to its exposure to the electric field. While this study did show that there was a statistically significant difference in certain water quality parameters between the two fluid streams, it was clear that the process was not efficient enough to be considered a viable and effective means of water clarification.

ELECTROPHORETIC REMOVAL OF FINE PARTICULATES FROM AQUCULTURAL EFFLUENT

by

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A = Cross-Sectional area of the fluid column [bxh] (m^2)
D_1 = Initial Dissolved Oxygen of Sample (mg/L)
D_2 = Final Dissolved Oxygen of Sample after 5 Days (mg/L)
E = Electric Field Strength (volts/cm)
m = Mass of Particle (grams)
n = Roughness Coefficient of Material Used (0.013 for plastic/Plexiglas)
P = Decimal Volumetric Fraction of Sample Used
q = Design Flow Rate (m^3/s)
Q = Charge of Particles (coulombs)
$\Re$ = Reynolds Number (dimensionless)
R = Hydraulic Radius [(b x h)/(b + 2h)] (m)
r = Radius of Particle (cm)
S = Channel Slope (m/m)
t = Time (seconds)
v = Fluid Velocity (m/s)
V = Voltage Applied (volts)
x = Variable Distance Between Electrodes (cm) and $0 \leq x \leq Z$
Z = Distance Between Electrodes (cm)
ε = Relative Permittivity
ς = Zeta Potential (mV)
ρ = Density of the Fluid (998.2 kg/m^3 for water at 20^oC)
μ = Viscosity ($1.005x10^{-3}$ Ns/m^2 for water at 20^oC)
η = Viscosity of Suspension (poise)

INTRODUCTION

Large-scale aquaculture production depends on the control and regulation of all environmental parameters pertinent to the optimal growth and development of the fish culture. Without such control, any unregulated element will inevitably fall out of balance and prove harmful to the vitality and health of the fish culture. One such environmental condition is water quality and the removal of waste, specifically fine suspended particles. For the purposes of this paper water quality is defined as the description of the water's suitability and capacity to support the aquatic organism grown and harvested, and to maintain the sanitary conditions necessary for such a production (Wheaton, 1977). Similarly, pollution or waste is defined here as any substance present in the water detrimental to the growth and health of the organism intended for production (Wheaton, 1977).

In recirculating aquaculture systems maintaining acceptable water quality is in itself a significant challenge, but is particularly difficult when addressing the buildup of fine colloid waste less than 50 µm in diameter, because the particles are not easily removed by standard conventional filtration techniques. Libey (1993) showed that the smallest particle sizes (5-10 µm), which cause the greatest problems to the fish culture (Chen et al., 1993), are also the most difficult to remove from the system. For this reason a cumulative effect occurs where the concentration of fine particulates builds up over time (Ebeling et al., 1997).

Research has shown that excessive suspended solids accumulations irritate fish, particularly their gills, causing poor fish health and lower yields (Chen et al.,

1993). If not dealt with, excessive particle buildup leads to gill swelling, which reduces oxygen uptake and slows growth rates. In more extreme cases such irritation can lead to gill damage, increased susceptibility to disease and eventual death (Chen et al., 1993). Chen et al. (1993) have also shown that fine particle accumulation has detrimental physical effects to the system itself, including clogging of the biological filters, the generation of ammonia and increased biochemical oxygen demand. Ammonia production and the increased oxygen demand are a result of the metabolic processes of bacteria cultures that breakdown the suspended organic and inorganic waste.

Even at lower concentrations, it is clear that the presence of accumulated fine particles contributes to the total oxygen demand by sustaining the microorganisms present in the fish culture that decompose organic material. These microorganisms consume oxygen in the process, thereby reducing the overall amount of oxygen available to the fish. It follows that if this major oxygen sink can be reduced or eliminated the capacity of recirculating aquaculture systems to support more fish will correspondingly increase.

Other studies have shown that suspended solids accumulation can lead to an increase in the malodorous 'off-flavor' of the fish product resulting in a noticeably reduced product quality (Schrader and Rimando, 2003). Off-flavors in aquaculture are primarily due to the production and subsequent absorption of one or more metabolic by-products of cyanobacteria (blue green algae) by the fish. The fish may absorb these compounds through their gills and/or skin, or by ingestion during feeding (Schrader and Rimando, 2003). Cyanobacteria cultures thrive under the

heavy organic nutrient loading caused by increased suspended solids (Schrader and Rimando, 2003).

It is evident from the above research that if an effective means of water clarification, beyond normal filtration techniques, can be developed to address the issue of particle accumulation, it will greatly aid in increasing the capacity of the recirculating aquaculture system to support fish; reducing the overall production cost by increasing growth rates; and improve the quality of the fish product. The solution proposed here is the use of an impressed electric field as a means of concentrating the generally negatively charged organic particles to a specific column of water that can then be diverted and removed from the system as waste.

Hiler and Lyle (1970) demonstrated that the process of electrophoresis can be effectively employed as a means of water clarification. In their experiment, a water stream was passed between two electrodes, which induced the suspended negatively charged high density clay particles to bind with, and be deposited on, the positively charged anode, thereby removing the particles from the fluid stream. This present research goes one step further, by first removing the electrodes from the suspension, such that only the electric field acts on the charged particles and no deposition on the anode occurs; and secondly by testing the processes applicability to aquaculture systems where the waste particles are predominately organic in nature, have a wide range of sizes, and have a generally low specific density (Chen and Malone, 1991).

Aquaculture systems are habitats for biological organisms, and as such the sensitivity of the culture and animals must be taken into consideration. It is critical that no electrical charge is conducted through the fluid stream, and therefore the

electrodes cannot come in direct contact with the water. By removing the electrodes from the suspension and inserting an impermeable dielectric wall between the electrode and the water, the unwanted effects of electrolysis and electrochemical reactions, which were encountered in the Hiler and Lyle (1970) experiments, are avoided. In addition, the electrodes will not have to be periodically cleaned of accumulated deposits, nor will the electrodes deteriorate over time.

The objective of this project is to develop a cell that will allow an impressed electric field to act upon the charged colloids in the fluid media. The cell developed consists of an acrylic lined channel separating two electrode plates. It is believed that the negatively charged particles in solution will tend to accumulate near the positively charged plate as the fluid flows through the channel, but will continue to flow with the fluid stream and not bind to the electrode. The portion of the water nearest the positively charged electrode, containing the concentrated suspended particles, can then be diverted from the fluid stream as waste water or undergo further treatment, leaving the remainder of the fluid stream clear of the majority of the contaminants, and suitable for reentry into the aquaculture system.

It should be stressed that the purpose of this study is to test the effectiveness and efficiency of small particle removal by electric fields for incorporation into existing production aquaculture systems, and is not intended to replace existing means of mechanical filtration currently employed. Current filtration techniques are very effective in removing larger suspended particles, but fail to adequately address the problem of fine particles less than 50 to 100 µm in diameter. Economic removal

of these small particles is currently an unsolved weakness in aquaculture production systems.

LITERATURE REVIEW

Before proposing a possible solution to the problem of fine particulate accumulation a basic understanding of the background issues must first be reviewed, beginning with the nature of the particles we wish to target. All filtration and extraction techniques depend upon differences between one or more of the characteristics of the entity you wish to separate, and the surrounding medium that contains it. It follows that once a basic understanding of the nature of the waste particles is obtained, including their physical characteristics, chemical composition, and origin, one can then begin to review current filtration techniques and weigh their relative effectiveness on the range of particulates that need to be addressed. Once the overall system is understood we can then begin to explore the strengths and weaknesses of each technique, and see how this current research fits into the overall scheme of recirculating aquaculture production. Addressing the current weaknesses in the system will provide a firm basis for this proposal. In aquaculture the particle attribute most commonly acted upon, as a means of separation and removal, is particle density and size (Libey, 1993); this paper wishes to explore the possibility of using the electrical or magnetic properties of the aquaculture particles.

In the paragraphs that follow the analysis of the nature of the suspended solids in recirculating aquaculture systems, and the filtration methods and practices currently employed will be examined. Through this review certain characteristics of the particles involved will suggest that they are well suited for separation by an

impressed electric field; while the nature of current recirculating aquaculture practices and the problem of fine particle accumulation will provide justification for the need of additional water clarification methods, and will illustrate how this research aims to serve that purpose. Following this discussion, a review of past research on the use of electrophoresis in water clarification will be presented, including a discussion of how the research done here differs from previous work, and its specific application to aquaculture systems.

Suspended Solids in Aquaculture Systems: General Introduction

Particles that do not settle out of solution contribute to the total suspended solids (TSS) in the recirculating aquaculture system. The TSS is defined as the mass of all particles larger than 2 microns in diameter contained in a known volume of water [ie. mg/L] (APHA, 1995; Chen, 2000). The accumulated TSS originates from three main sources: 1) metabolic waste products of the fish, 2) uneaten fish feed, and 3) the bacterial/algae biomass present in the system (Goddard, 1996).

The majority of the TSS is the result of fish feces production, which can be expressed as a function of the feeding rate. Typically, the conversion rate of fish feed to fish mass is given by the food conversion rate (FCR), which is the ratio of dry weight of feed to fish weight gain (Hardy and Barrows, 2002). The reciprocal of this value is referred to as the feed conversion efficiency (FCE), and is useful in comparing the ability of feed formulas to support weight gain (Hardy and Barrows, 2002). Generally FCR and FCE values vary with the type and digestibility of the feed used, the fish species, and the period in the growth cycle of the fish (Goddard, 1996).

Typical FCR values range from 1.2 to 1.6, however these values can be somewhat misleading as they are a comparison of dry fish feed mass to wet fish flesh mass (the majority of the fish mass being water). A typical 'true conversion' is in the range of 3-4:1 (Goddard, 1996). Higher conversion ratios can be expected for younger more rapidly growing fish.

Goddard (1996) states that up to one third of the feed content may be indigestible, and will eventually be excreted as waste, thereby contributing to the TSS. A mass balance study by Beveridge and Phillips (1993) showed that tilapia retains 23% of the total nitrogen in feed, while the remaining 77% contributed to the total waste in the form of fecal matter, urinary nitrogen, and uneaten food.

The second largest source of TSS is uneaten food, which varies with the type of feed and the hydraulic conditions in the aquaculture system. Warren-Hanson (1982) report that uneaten food varies from 1-5% for dry feed, 5-10% for moist pellets and as much as 10-30% for wet feeds. Again the relative moisture content between dry, moist, and wet feeds has to be considered when comparing the actual mass of feed remaining in the system. Nevertheless the general trend is clear; feed with a higher moisture content usually dissolves more readily in solution, becoming unavailable for fish consumption and resulting in increased suspended particle concentrations.

The quantity of uneaten feed combined with fish waste constitutes the bulk of the organic material in solution. The third highest contributor of suspended solids is the total biomass of the microbial organisms present in the system (Goddard, 1996), which for the purposes here in determining the quantity and sources of the TSS in the

system, can be ignored as they derive their mass, for the most part, from the uneaten feed and fish waste products already in the system. Nevertheless, maintenance procedures should be in place to keep bacteria/algae growth within acceptable levels. This is best done by reducing the concentration of the nutrients and organic material in solution. Suspended solids mass not removed by filtration techniques will eventually be ingested by growing concentrations of microbial agents. However, this is done at great expense to the existing system oxygen supply, and should be avoided. It can be assumed that with increased removal efficiency of TSS, the concentration of microbial agents that depend on waste substrate for growth will correspondingly diminish.

As a generalization, the overall total solid waste mass can be directly related to the feeding rate as determined by this simple mass balance equation: (Total Dissolved and Solid Waste) = (Feed Consumed by the Fish) - (Feed Retained by the Fish) + (Uneaten Food), or alternatively (Total Waste) = (Fish Feed) - (Feed Retained by the Fish). Although a number of factors affect the exact amount of feed consumed and retained by the fish, it is clear that the amount of solid waste produced can be appreciable. This is especially true as the fish culture grows in size and concentration, because the feeding requirements increase correspondingly.

Solid waste production can be illustrated by considering the following example. Stocking densities for mature trout (475-500 g/fish) can reach up to 110 kg/m^3 when optimal growth conditions are achieved (Timmons et al., 2000). Other species may have even higher stocking densities; tilapia for example can reach 147 kg/m^3 for mature fish (950 g/fish) (Timmons et al., 2000). The optimal feeding rate is

calculated as a percentage of the biomass of the fish present, and varies with the fish species, fish size and water temperature (De Silva and Anderson, 1995). The optimal feeding rate is usually the ration size that yields the highest FCR possible, deviating from this optimal ration size will usually result in poorer growth and increase the total waste, resulting in poorer water quality. For mature trout the optimum-feeding rate ranges from 0.9 to 1.6 percent of the fish biomass per day, depending on the water temperature (De Silva and Anderson, 1995). Feeding rates are considerably higher for younger fish. If we assume an average feeding rate of 1.2 percent of the fish biomass per day (corresponding to a water temperature of 12^{o} C) and a stocking density of 110 kg/m^3, then 1.32 kg of feed per cubic meter of water is added to the aquaculture system each day. In total over 75 percent of this feed will eventually end up as waste (1-5 percent of dry fed will not be eaten by the fish, and 70-75 percent of the feed eaten will be excreted by the fish as waste). This is nearly 1.0 kg of waste that will have to be removed from the system per day per cubic meter of water. When you consider that 10 to 15 meter diameter tanks are currently being put into production (Timmons et al., 2000), containing up to 350 m^3, it is clear that waste production is enormous, making proper removal techniques imperative.

Characteristics of Suspended Solids in Solution

Solids removal methods for aquacultural systems depend primarily on differences in particle density and particle size distribution. Particle specific gravity is defined as the ratio of the density of the particle to that of pure water (Timmons, 1994). Generally this value ranges from 1.004 to 1.19 in aquacultural systems (Chen,

1993). This value plays an important role in determining the effectiveness of traditional settling basins, as well as the migratory velocity of the particle in an electric field as will be seen later in this discussion.

Specific information on size distribution is more difficult to generalize, because it depends on the fish size and species, the type of feed used, the water temperature, and the turbulence in the aquaculture system, all of which can vary from system to system (Chen, 2000). However, general trends and research findings are presented here as background information.

Initially, fish fecal matter is relatively large, but is quickly broken apart as a result of the hydraulic conditions present in the aquacultural system. Table 1 below shows the particle size distribution for catfish feces 24 hours after excretion, and is included here as a general representation of particle size that can be expected. The table is reproduced from Chen (2000), who tested four different types of catfish feed (F1, F2, F3 and F4). The results from each test vary slightly, however the general trend is still clear.

Table 1. Particle Size Distribution (%) of Fish Excretion in Response to Four Different Feed Types (Chen, 2000)

Particle Size (µm)	**Feed Types**			
	F1	**F2**	**F3**	**F4**
1-30	18.8	18.5	18.6	18.3
30-105	76.3	77.8	76.5	76.5
105-1000	4.8	3.7	5	5.2

Here the bulk of the total mass of the fish feces results in particles less than 100 µm in diameter after only 24 hours, almost 95% of the total mass for all feed types. Assuming that 70% of the total mass of the feed consumed is execrated from

the fish in the form of feces, then almost 66.5% (95% total waste of 70% excreted waste) of the total mass of feed is converted to fine particles (less than 105 µm in diameter) through this process (Chen, 2000).

Size distribution for dissolved feed pellets varies according the type of feed. Studies show that the majority of uneaten food does not dissolve, but remains as large particles (larger than 1000 µm in diameter), which quickly settle out of suspension (Chen et al., 1994). As such, the majority of uneaten food is easily removed through sedimentation and mechanical filtration. Table 2 shows an analysis of research carried out by Chen et al. (1994) listing the breakdown of catfish feed pellets after being left in solution and stirred for four hours. The percentage calculations are based on particle mass.

Table 2. Particle size distribution (%) of dissolved feed pellets after four hours, based on particle mass.

Particle Size (µm)	**Percentage:**
>1000	50.9
500-1000	21.9
105-500	19.7
60-105	5.3
30-60	1.6
5-30	0.6

As can be seen from the results above only a relatively small percentage of the total feed dissolved into particles smaller than 105 µm in diameter (<7.5%), while the remainder of the pellets held together as larger particles, which would quickly settle out of solution under normal conditions. To illustrate this point, if we assume that no more than 5% of the dry feed introduced into the system is uneaten by the fish (Warren-Hanson, 1982), then considering the information above, less than 0.38% of

the total suspended particles less than 105 µm in diameter after four hours of turbulent conditions, is the result of uneaten feed. Although smaller particles will inevitably build up over longer periods of time, four hours can be considered a representative retention time before the water is passed through the filtration system. From this analysis, it is clear that the bulk of the fine particles in solution results from the metabolic processes of the fish (over 99%), and very little results from uneaten feed. As bacterial biomass is a function of metabolic waste, uneaten food and operating conditions its mass will be neglected in this analysis.

Understanding the process of fine particle accumulation requires that the effects of filtration on particle buildup over time be taken into consideration. While the amount of waste can be determined as a function of feeding rate, feed type and fish species, the actual size distribution cannot be definitively ascertained. This is because larger particles are continually removed from the system through sedimentation and mechanical filtration, while smaller particles, those too small to be removed by typical screens used in production systems (>60 µm), remain in solution, breaking down into finer and finer particle as they are further subjugated to turbulence and acted upon by microbial agents. Likewise, if larger particles are not rapidly removed from the system they may contribute to the formation of finer particles as they continue to breakdown over time. Libey (1993) has shown that high removal efficiencies can be expected for particles greater than 70 µm in diameter, but removal efficiencies gradually decrease for smaller particles. Furthermore, all particles passing through the filtration environment are exposed to shearing forces that increase the relative proportion of smaller particles (Libey, 1993). McMillian et

al. (1996) found that running water through pipes, pumps and nozzles can cause excessive turbulence and contribute to the breakup of larger particles into finer pieces (referred to by McMillian, 1997).

The cumulative effect is that fine particle concentrations tend to build up over the life of the fish culture as larger waste particles are removed and new pollutants, both large and small, are continually introduced into the system. As a result it can be inferred that unless an appropriate and effective means of fine particulate removal is found, capable of keeping pace with the removal of larger particles, the finer constituents of the solution will inevitably reach toxic levels and impair the productivity of the system.

Research has shown that fine suspended particles, less than 50 µm, predominate in intensive aquaculture systems due to the difficulty in removing them through traditional means (Chen, 1994). Chen (1994) refers to a study conducted by Harman (1978) that concluded that the majority of particles were in the range of 6 to 20 µm, while standard settlement techniques were only effective for particle sizes above 100 µm. Studies by Chen et al. (1993), who ran similar test on three different systems, also confirmed this result. The exact particle distribution varies with system conditions, but the general trend remains the same. The summary of their results, presented in Table 3, indicates that on average nearly 70% of the suspended particles are less than 30 µm in diameter.

Table 3. Average Particle Size Distribution for Three Recirculating Trout Aquaculture Systems (Chen et al., 1993)

Particle Size (μm)	**Percentage of Suspended Particles (%):**
>105	22.2
105-70	5.7
70-30	5.2
30-1.5	66.9

The data presented here is offered as a representational particle size distribution for standard intensive recirculating aquaculture systems, and clearly demonstrates the necessity of finding an effective method of fine particle waste removal. This is particularly true in light of the findings of Chapman et al. (1987) who demonstrated that fine suspended particles are more detrimental to fish health than larger particles, which are effectively controlled.

Some efforts have been made to improve waste particle removal efficiency from aquaculture systems by introducing binding agents into the fish feed, intended to increase particle size and thereby facilitate waste filtration (Wheaton et al., 1997). In a study conducted by Wheaton et al. (1997) several binding agents at both high and low concentrations were tested. However, in general the additives did not significantly improve waste removal. Higher binder concentrations were found to be unpalatable for the fish species used, while lower binder concentrations had little to no significant effect. Therefore, it was concluded that the addition of binding agents was not practical for improving waste removal.

Filtration Mechanisms

Before discussing the various filtration techniques currently in use, it would be useful to review the principles involved with each filtration method, in order to better understand the strengths, weaknesses and modes of action for each system. These principles include sedimentation, straining, interception, diffusion and flocculation.

Sedimentation is the process by which particles fall out of solution due to gravitational forces exerted upon them, and is typically responsible for the removal of the greater majority of the waste mass. The rate of sedimentation depends upon the settling velocity of the particles, determined by the particles mass and size, and the viscosity of the solution. As stated previously the specific gravity of the waste material present in aquaculture effluent is only slightly higher than that of pure water. As a result the particles will tend to sink, however their settling velocity will remain low and the particles will be easily subjected to convective currents within the aquaculture tanks. As such, sedimentation is most effective on waste particles greater than 100 µm in diameter (Chen, 2000). Sedimentation techniques consist of influent entering into a settling basis where the larger particles are collected in the lower sludge zone of the basin while the effluent is removed from the top of the basin, and reintroduced into the aquaculture tank.

Straining is the physical process of screening out particles that are larger than the pore size opening of a filter screen or medium (Chen, 2000). This process requires frequent back flushing to ensure proper operation of the filter and prevent blockage, resulting in higher head losses, and higher operating cost. Its range of operation

depends on the pore size, however with smaller pore sizes comes increased maintenance cost. As a result screen filtration is normally used to remove particles no less than 60 µm in diameter (Chen et al., 1994).

Interception is the process by which particles with no significant settling velocity flowing in suspended form along the streamlines of the media collide with and are intercepted by the filter medium (Chen, 2000). The collision of the particle with the medium may result in attachment (Chen et al., 1994). This process differs from that of straining as particles simply collide with and attach themselves to the filter surface, independent of particle size and screen pore diameter. However, finer screen sizes provide more surface area, making interception a more efficient process.

Diffusion is most significant for particles smaller than several microns in diameter (Chen, 2000). Under diffusion, Brownian motion transports particles in the direction of the concentration gradient to areas of lower particle concentration. When effluent particle concentration is high particles will attach themselves to the filter medium, so long as the concentration of particles in solution is greater than the concentration of particles on the filter media. The efficiency of this method is determined by the particle concentration gradient and the particle attachment process, which is controlled by particle size, Reynolds number, and particle surface properties (Chen, 2000).

A final note should be included concerning flocculation, which is an aggregation process that involves the use of chemical additives that alter the interfacial properties of suspended particles allowing coagulation of smaller particles to occur (Hahn, 1995). The resulting larger particles are more readily removed from

the system. The process is commonly used for industrial and large-scale water treatment facilities, however it is generally unacceptable for aquaculture facilities due to the potential adverse affects of the chemical additives. Nevertheless, as it is a commonly used means of water treatment it has been included in the above list of filtration mechanisms.

Standard Filtration Techniques Employed

EIFAC (1980) recommendations suggest that the TSS concentration should be maintained below 15 mg/L for all recirculating aquaculture systems. However, as indicated above, Chapman et al. (1987) demonstrated that finer suspended solids have a more toxic effect than do larger suspended solids in the water column at the same concentration (mg/L), indicating that it is not sufficient to look at TSS concentration alone, without considering particle size distribution. The paragraphs that follow look at some of the standard filtration techniques in use today and their respective ranges of operation, focusing specifically on those procedures employed to remove fine particles.

For the most part particles above 60 µm are generally removed through standard sedimentation and micro-screen filtration. As these are proven techniques for larger particles, and are significantly less effective for finer particles due to their insignificant settling velocity and small size when compared to standard screen hole diameter sizes, only the briefest introduction will be made to them.

Generally the use of a settling basin comprises the first phase in water clarification procedures for recirculating aquaculture systems, where the largest

particles fall out of solution into catchments where they can be removed and treated as waste. This technique is generally only effective for particle sizes above 100 µm (Chen et al., 1994). In certain instances the use of hydro-clones may replace sedimentation basins. This technique employs sedimentation as the primary means of particle separation, however a centrifugal force acts upon the suspended particles, as opposed to gravity alone, pulling the particles out of solution towards the outer edges of the cyclone. The efficiency of this system depends on the density difference between the particles and the water and the centrifugal force in the cyclone, and is generally only effective for particles above 77 µm (Chen et al., 1994). Screen filtration can be used in place of settling basins to remove larger particles in the initial phase of water clarification, but involves the processes of straining and interception as the primary means of filtration, as opposed to sedimentation.

The next class of filters is referred to as granular media filters and include a range of sand and bead filters. Here the influent flows through a caked sand/bead medium where the particles in solution are trapped in or deposited on the granular surface. Granular media filters employ the principle of sedimentation, straining, interception, and diffusion to remove a wide range of particle sizes (Jackson, 1980), and are generally effective in removing fine particles above 20 µm in diameter (Task Committee on Design of Wastewater Filtration Facilities, 1986). The disadvantage to this system is the high head loss and its susceptibility to biofilm development on the sand media at higher levels of organic loading. The formation of a biofilm encourages sand particles to stick together rendering them less effecting in intercepting and straining suspended particles (Chen, 1994). However, proper design

and the use of air injection (Cooley, 1979), water jetting (Wimberly, 1990), and mechanical agitation (Chitta, 1993) can help control biofilm development.

For finer particles a porous media filter can be used as a tertiary filtration stage in conjunction with a granular media filter or some other form of mechanical filtration. Porous media filters consist of a cartridge or vessel containing a medium or film with extremely fine pores through which the influent passes. Because of the fine pore size these filters can strain particles down to less than 1 µm (Chen et al., 1994). The disadvantage of this system is its susceptibility to clogging even at very low TSS concentrations and the prohibitive cost of recharging or replacing the filter cartridges. Due to the high volume of particulate matter in commercial aquaculture systems this process is impractical and/or uneconomical for use in aquaculture systems.

Foam fractionation is another method for removing particles smaller than 30 µm in diameter, whereby fine particles come in contact with and attach themselves to air bubbles rising through a column of water, forming a foam at the water surface that can be skimmed off. Studies show that this technique can remove up to 25 percent of the fine particles in solution per pass (Lawson, 1978). This technique primarily employs the processes of diffusion and interception whereby the particles come in contact with and become adsorbed onto the bubble surface, however, because the particles' attachment to the bubbles surface is dependent on the chemical properties of the particles, removal of particles is also dependent on these properties (Chen et al., 1994).

A relatively new procedure currently under investigation is a process referred to as ozonation, which uses a strong oxidizing agent (ozone) to polymerize organic

particles into larger enmeshed solids that can more readily be removed through one of the mechanical filtration techniques mentioned above. Ozone treatments serve as a disinfectant that kills potential pathogens and provides additional oxygen as a final byproduct (Summerfelt, 2003), however its efficiency depends on concentration and exposure time. Ozonation must be carried out within a separate contact vessel for 1-30 minutes depending on the target microorganism (Summerfelt, 2003). However, care must be taken to ensure that residual ozone levels do not remain, as they are harmful to the fish. Recent studies have shown that residual ozone can cause biochemical changes including lipid peroxidation and reduced glutathione levels in gills for fish (Ritola et al., 2002), and immobility and destruction of gill lamellar epithelium in shrimp (Meunpol et al., 2003). Initial ozonation tests show promise as a means of fine particle removal. However, effectiveness must be balance against the potential toxicity of ozonation, and further research is needed to clearly define the ozone levels that may safely remain in recirculating aquaculture systems (Chen, 1994).

Of the various filtration techniques in use today only porous media filters, fractionation, and ozonation effectively target particles smaller than 30 µm, which can comprise 70% of the TSS in a recirculating aquaculture system (See Table 3 above). However, as mentioned due to the high cost associated with porous media filters, the relatively low effectiveness of foam fractionation per pass (25%), and the experimental use of a strong toxic oxidizing agent associated with ozonation, there is ample justification and need to explore new means of fine particle separation.

Electrophoresis Introduction

With this general background information the method investigated in this study to remove suspended electronegative particles from water can now be discussed more thoroughly. The idea bears its origin with a process known as electrophoresis, which is defined as the movement of charged suspended particles in the presence of a direct current electric field (Andrews, 1986).

Electrophoresis has been used in chemical and biological applications as a separation technique to distinguish between various compounds in solution. This technique capitalizes on the varying characteristic charges or partially charges of the constituents in a solution as a means of distinction and thus separation (Andrews, 1986). The degree to which the element will be pulled to one pole or the other in an electric field varies with the magnitude of the constituent's electric potential. As such, those elements with a stronger negative or positive charge will be pulled more quickly to the opposite positive or negative pole and repulsed from its like charge, causing a stratification in dissolved charged molecules within the fluid column.

Outside of its use in chemical/biochemical applications, the use of electrophoresis as a means of water clarification was not seriously considered until the early 1960's when initial investigations were carried out (Cooper et al., 1965; and Hiler et al., 1965). Among these initial studies Hiler et al. (1965) sought to remove negatively charged kaolin and bentonite ions from solution by depositing them on the anode of an experimental electrokinetic apparatus. He then went further to develop the theoretical equations governing the movement of colloid particles in solution with

an impressed electric field, for both a parallel electrode plate and a rod and cylinder configuration (Hiler et al., 1965).

These equations took a single particle approach in predicting charged colloid movement over time. Their results demonstrated that electrophoresis could successfully be used to remove colloid particles from solution; however their theoretical calculations varied from experimental results. This variation was attributed to the impossibility of accurately measuring the particle electrical potential based on particle size and charge density, which was for the most part assumed (Hiler et al., 1965). Their results also showed that efficiency decreased with increased particle deposition on the anode, at higher voltage potentials due to increased electrolysis of the water causing agitation at the electrode surface, and the introduction of heat convection currents originating from heating at the anode. These problems are avoided in this study by separating the electrodes from the water.

A follow up study was made that took a more stochastic approach (Hiler et al., 1967) where theoretical equations were developed to reflect the build up of charged particle concentration over time. Based on this study a computer simulation program was developed by Hamdy et al. (1968) through which a wide range of variables, including flow, concentration and diffusion, could be altered and analyzed. This study of Hiler et al. (1967) tested both turbulent and psudo-laminar flow conditions for flow through systems. It was found that under turbulent conditions, where turbulent diffusion and currents contributed to the movement of charged colloids towards the anode, effluent concentrations dropped more rapidly before leveling off at a nominal concentration. Under psudo-laminar conditions, where Brownian

diffusion predominated, charged colloid concentrations gradually fell, and eventually resulted in a 'zero concentration,' which was not reached under turbulent flow conditions. This demonstrated that the most ideal circumstances were initial turbulent flow followed by psudo-laminar flow conditions (Hiler et al., 1967), where turbulent conditions result in a more rapid overall decrease in concentration, and complete elimination was achieved when laminar conditions presided.

Further studies involved the theoretical analysis of electrokinetic movement (Hiler et al., 1971), assuming psudo-laminar flow and taking into consideration Brownian diffusion and convective dispersion effects under such conditions. The theoretical equations developed were then verified experimentally by Hiler et al. (1972) and found to adequately describe the electorkinetic movement of charged colloid particles in a flowing water stream. These equations set the basis for predicting the in situ particle concentration over time, by analyzing the migratory velocity of the colloid particles in solution.

Preliminary studies found that the migratory velocity of suspended particles is inversely proportional to the coefficient of viscosity of the suspension and directly proportional to the dielectric constant of the suspension, the electric field strength the particles are exposed to, and the zeta potential of the particle (Helmholtz, 1879, translation 1951 referred to by Hiler, et al., 1965). These relationships have been shown theoretically for inorganic clay particles. However, experimental studies have shown that proteins denature at high field strengths, and as a result the migratory velocity of a protein particle may not increase linearly with increasing voltage potential. As a general rule in biochemical applications proteins are not separated

with field strengths higher than 10-20 volts/cm (Smith, 1979). Further studies concluded that the migratory velocity of the colloid also varied with particle size, increasing with decreasing particle diameter (Abramson, 1931; Hauser and Lebeau, 1941) due to decreased resistance. This was not taken into account with the equations developed by Helmholtz (1897).

The zeta potential of a particle, referred to above, is defined as the electrical potential across the interface of the charged colloid particle and the diffuse ion layer surrounding it (Overbeek and Bijsterbosch, 1979). In general most organic waste is negatively charged, attracting positively charged hydronium ions (or other positively charged ions) to it, forming a diffuse surrounding layer that aids in particle stability. The aggregation of both the negative and positive charges from the colloid and surrounding ions is referred to as the electric double layer, while the potential across the particle surface and the diffuse ion layer is the zeta potential, the strength of which influences the force that will pull the particle to one or the other pole in an electric field (Overbeek and Bijsterbosch, 1979). Figure 1 below illustrates the diffuse ion layer in an aqueous solution associated with charges on a solid particle.

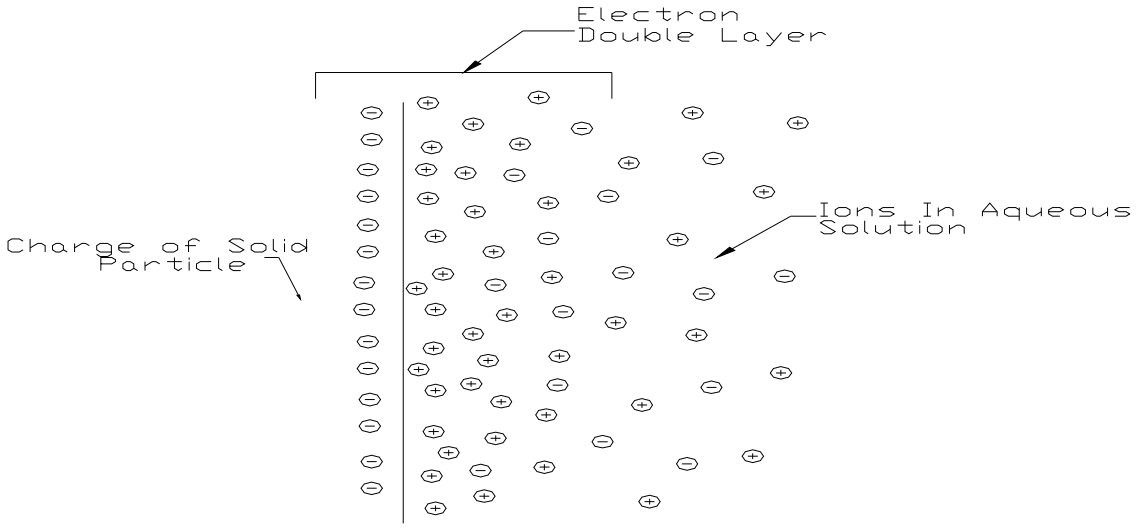


Figure 1. Diffuse ion layer associated with the electron double layer

The resulting electric potential across the double electron layer becomes a critical factor in determining the velocity at which a charged particle moves under the influence of an electric field. The diffuse ion layer that surrounds a charged particle presses into the particle forming a shell around the particle. Under the influence of an electric field this diffuse ion layer becomes distorted as the particle is pulled in one direction and the ions that comprise the difuse ion layer are pulled in the opposite direction. These counter ions pressing against the particle, moving in the opposite direction of the particle, contributes to a retarding effect on the particle movement, often slowing its migratory velocity by one or more orders of magnitude (Overbeek and Bijsterbosch, 1979). This hydrodynamic effect is referred to as the electrophoretic retardation (Overbeek and Bijsterbosch, 1979). An illustration of this effect is shown in Figure 2 below.

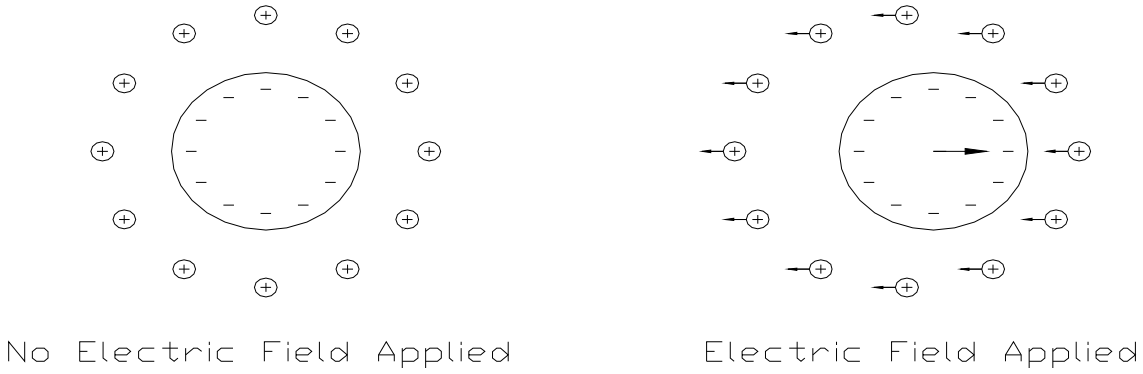


Figure 2. Hydrodynamic effect showing the opposing movement of a charged particle and surrounding diffuse ion layer

This interaction is further complicated when we consider that the distortion of the diffuse ion layer creates its own electric field opposite in direction to the imposed direct current field, reducing the effective strength of the impressed electric field. This is referred to as the relaxation effect (Overbeek and Bijsterbosch, 1979). Generally this effect is not as strong as the electrophoretic retardation, but can reduce the mobility of the particle by 10-50% (Overbeek and Bijsterbosch, 1979).

The cumulative force exerted on the particle under the influence of an electric field is then the force resulting from the effect of the electric field on the charged particle, times a friction factor, minus the retardation effects caused by the double layer. The difficulty in determining the mobility of charged ions under the influence of an electric field, is in determining the strength of the electrical forces and the influence of these two retardation effects. Hiler et al. (1965, 1967) has developed the theoretical equations for modeling the movement of charged colloids in solution and tested these results experimentally. These equations will be used in this study to

predict the movement of particles needed for the design and construction of the electrophoretic cell.

Hiler et al. (1967) make reference to a study conducted by Boyd (1963) who investigated water purification methods using aluminum electrodes exposed to the water source. This initiated an electrochemical reaction producing aluminum hydroxide, which in turn formed flocculation of the colloids in solution, causing them to precipitat out. Bier (1965), Cooper et al. (1967), and Moulik et al. (1967) built upon the research by investigating the use of electrophoresis in the development of water purification systems.

Hiler and Lyle (1970) have conducted the most theoretical research into the use of electrophoretic and electrochemical processes as a means of water purification. Lyle and Hiler (1971) examined the suitability of electrophoresis for individual filtration systems and found that the parallel plate model, which utilized electrophoresis exclusively, was successful for waters of low electrical conductivities. It was found that the operating cost for the procedure was dependent on the electrical conductivity of the water medium, making the procedure economically viable for waters with low to moderate electrical conductivity.

Building upon past research, the current study aims to explore the effectiveness of employing an electrical field imposed upon a flowing media stream, without the electrodes coming into direct contact with the water. In this way the electric field produced is used only as a means of diverting particles in the solution and is not a means of removal in itself, as no deposition occurs. This is done to avoid any electrical current from coming in direct contact with the water, and to eliminate

any unwanted electrochemical products that may find their way into the recirculating water supply of the aquaculture system, and prove harmful to the health and vitality of the fish culture. This also avoids the difficulty of particle deposition on the anode plate itself that would otherwise have to be mechanically removed, as was the case with the experimental models of Hiler et al. (1965, 1967).

Chemical Analysis of Aquaculture Pollutants

When a electrokinetic system is employed, the force utilized to pull colloids out of solution is dependent on the electrical charge, or more specifically the zeta potential, of the particle. Therefore it follows, that an analysis should be made of the chemical compounds that comprise the TSS found in aquaculture systems, as well as the chemical processes involved in the decomposition of these organic compounds.

As demonstrated above fish feces is the primary source of fine particles, the chemical composition of which is dependent on the organic matter in the fish feed and the metabolic reactions at work. Goddard (1996) indicates that fish feces may contain up to 10% of the nitrogen consumed, and 30% of the dietary carbon consumed. The bulk of the dietary carbon is expelled as carbon dioxide, the end product of respiration. Carbon dioxide, given time, will diffuse out of solution and is then of no consequence to the fish culture. At high fish densities carbon dioxide may have to be removed from water to prevent toxicity. The paragraphs that follow are a discussion of the chemical compounds involved, their toxicity to the fish and the degree to which they lend themselves to electrokinetic removal.

The bedrock of all organic compounds is carbon, and as such is the major constituent of organic waste. It forms the backbone of all organic structures upon which functional groups operate. Carbon has four electrons in it outer electron shell or highest energy level and forms strong covalent bonds. Organic compounds consisting solely of carbon and hydrogen bonds are non-polar in nature due to similar electronegative values (Carey, 1992), and are therefore non-soluble in water, forming lipids and oils which separate from the water phase. Organic compounds that contain functional groups such as nitrogen based groups, phosphates and hydroxyl groups, which attach themselves to the carbon backbone and interact with the polar water molecules, can hold a negative or positive polarity. Charges on the compound vary as the release and attachment of protons from the compound is linked to pH levels (Carey, 1992). It is because of these charged regions that the overall organic molecule becomes susceptible to electrokinetic removal, and not because of the nature of the carbon-hydrogen bonds themselves.

Albeit oxidized carbon can form negatively charged carbonate (CO_3^-) and bicarbonate (HCO_3^-) ions, which are the principle ions that contribute to the alkalinity of the water system. Both of these carbon derivatives lend themselves well to electrokinetic removal due to their negative charge. Although carbon forms the bulk of the organic material, the focus here will be on the functional groups that form an integral part of the organic macromolecules, as these sites are responsible for the overall charge of the particle.

Lawson (1995) explains that nearly all of the nitrogen compounds found in aquaculture systems originate from fish feed and are natural products of the metabolic

processes of fish. Broken into its simplest compounds nitrogen can be found in a number of forms including NH_3, NH_4^+, N_2, N_2O, NO, N_2O_3, NO_2^-, NO_3^- and N_2O_5 (Lawson 1995). The majority of the oxidized forms of nitrogen (N_2O, NO, N_2O_3, and N_2O_5) have little significance to aquaculture systems, and for the purposes here, can be ignored. Likewise nitrogen gas (N_2) diffuses in and out of solution from the air, and at normal concentrations is harmless to the fish, and is therefore of no consequence.

The ionized and un-ionized forms of ammonia (NH_4^+, NH_3) are a product of decaying organic nitrogen compounds. It is estimated that 40 to 90% of the nitrogenous waste resulting from fish metabolism is excreted in the form of ammonia (Goddard, 1996). Both forms of ammonia (NH_4^+ and NH_3) exist in equilibrium with one another, the concentration of each is primarily determined by the pH and temperature of the water. The un-ionized form of ammonia (NH_3) is toxic to fish and predominates in higher concentrations with higher pH levels, increasing 10 fold with every unit increase in pH within the pH range important in fish culture (Mead, 1989). Controlling the pH of the system can, therefore, regulate the ratio between the ionized and unionized forms of ammonia and keep ammonia concentrations in check (Goddard, 1996).

In aquaculture systems ammonia is introduced into the system as a by-product of protein metabolism in a process known as deamination (Meade, 1989). Ammonia can be eliminated from the system by nitrifying bacteria *Nitrosomanas* and *Nitrobacter* associated with biofilters, which convert ammonia into nitrite (NO_2^-) and nitrite into nitrate (NO_3^-), respectively (Stickney, 2000). Of the two forms, nitrite is

highly toxic, while nitrate is relatively innocuous at reasonable concentrations (Goddard, 1996). In recirculating aquaculture systems the nitrification processes of *Nitrobacter* control nitrite concentrations, while dissolved nitrate concentrations are usually controlled by continuous water exchange, up to 5% per day (Midlan and Redding, 1998) or by anaerobic filtration. Both of these compounds (NO_2^- and NO_3^-) lend themselves well to removal through electrophoretic means due to their negative charge.

It is important to note here that nitrogen compounds are the building blocks of proteins and amino acids, and are therefore prevalent in larger organic macromolecules and bacterial cells that comprise the TSS. It can be assumed that because of the net negative charge of the molecules the entire particle or bacterial cell can be acted upon by an imposed electric field and pulled out of the stream flow, so long as the cell or macro molecule remains in tact, and does not rip apart on account of an excessively strong imposed electric field. Nevertheless, the net charge of the particles is dependent on pH, which will vary from system to system.

Other Harmful Effects of Fine Particle Accumulation

All suspended waste particles that remain soluble but not yet dissolved contributes to the TSS found in solution, which is, as shown, directly proportional to the feeding rate (Goddard, 1996), but varies with different feed types. This variability in the amount of food that passes through the fish is partially dependent on the digestibility of the feed being used (Goddard, 1996), however higher standards have lead to more consumable feed stocks. Concentrations less than 25 ppm of TSS are

considered safe, while most fish species have poor protection against concentrations exceeding 80 ppm, which may block or cause physical damage to gill surfaces resulting in reduced respiratory function (Goddard, 1996). For intermediary TSS concentrations, 25 ppm to 80 ppm, the fish may or may not be protected, depending on the tolerance of the fish species. Note the European Inland Fisheries Advisory Commission (EIFAC) stipulates a slightly more conservative recommendation of less than 15 mg/L for the concentration of total suspended solids (EIFAC, 1980), indicating that there is some variability or disagreement as to recommended standards.

Other harmful effects of increased fine particle concentration may include: mechanical clogging of the biofilters, increased oxygen demand due to the decomposition of these organic materials, and the introduction of additional ammonia due to mineralization. Mineralization is the process of breaking down organic nitrogen to ammonia by microbial agents (Midlan and Redding, 1998).

Ammonia concentrations in aquacultural system are usually directly proportional to organic pollutant concentration because ammonia is the first inorganic compound resulting from organic material mineralization. This is a natural part of the nitrogen cycle, and one where ammonia concentrations can be minimized through effective removal of the larger organic molecules and particles.

The primary purpose of a biofilter is to remove ammonia and nitrite from the system through the use of nitrifying bacteria, *Nitrosomanas* and *Nitrobacter* (Stickney, 2000). The design, operation and the types of biofilters used in aquaculture systems varies, however each essentially consist of a medium upon which

the nitrifying bacteria cultures are established, and through which the fluid passes. This provides an environment where ammonia present in the water can be converted to nitrite and then to nitrate in a two-step process. With heavy organic loading, the waste present in the system can clog or impair the operation of the biofilter restricting ammonia coming in contact with the bacterial cultures. With proper water clarification methods, an electrophoretic filter may work well in conjunction with a biofilter by eliminating excess particles that could otherwise lead to clogging and ultimately impair the effectiveness of the biofilter.

Focusing the activity of the microbial agents on the ammonia present (nitrification) and the limited quantity of organic material not removed by mechanical or electrophoretic filtration will reduce the total oxygen demand required by the aerobic respiratory processes of the microorganisms needed to break down the remaining waste. In nature, the break down of organic structures by microbial agents is a natural part of the ecological balance, and one that purifies the waters from such pollutants. However, in artificial recirculating aquaculture systems, where fish culture concentrations are maximized for efficiency, these microorganisms compete with the fish for oxygen needed for all aerobic processes, putting undue strain on the fish culture. It can be theorized that reducing the oxygen demand required for microbial activity will increase the capacity of the system to support fish.

OBJECTIVES

The overall goal of this preliminary study is to explore the effectiveness of using an impressed electric field to improve the water quality of closed recirculating aquaculture systems. In order to achieve this the following objectives will be completed.

1. Design and construct an electrophoretic cell using low flow volumes and a range of electric field strengths from 50 millivolts/cm to 30 volts/cm to determine if electric fields can remove fine solids from aquaculture effluent.
2. Use water quality parameters including total suspended solids, particle size distribution, zeta potential and ammonia, nitrite, nitrate and phosphate concentrations to quantify waste removal using electric fields.
3. Based on experimental results, recommend whether this electric field application shows sufficient promise to justify development of a prototype system for fine particle removal from aquacultural systems.

If the procedure proves to be effective, the long-term objective of this project is to develop a working filtration unit for an aquaculture production system that will remove fine particulates, increase the capacity of the recirculating system, and lower overall production cost of the aquaculture operation. These are the practical long-term aspiration for the mostly exploratory analytic work done here.

EQUIPMENT

With the exception of the eletrophoretic cell itself, all equipment needed for the experimental setup and water quality analysis was available at the University of Maryland. The eletrophoretic cell was constructed in the Biological Resources Engineering Project Development Center based upon design specifications and assembly instructions contained within the '*Procedures'* section of this report.

Two direct current (DC) power supplies were used in this research: 1.) a *Hewlett Packard* (Model E3630A) triple outlet 0-20 volt DC power supply and 2.) a *Lambda* (Model LT-804) DC power supply capable of producing a 0-60 volt output with a max rating of 21.5 amps. Also used in the design setup was a *Teel* (Model P809A) 115V, 60 Hz, 4.5 Amp low flow pump capable of pumping a minimum of 18.9 L/hr (5 gal/hr) with a head of 1.5 meters as required in this experimental setup. Additional collection bottles, tubing, needle valves and a suspended bucket with a drain to feed the electrophoretic cell at a constant pressure head were needed for the experimental setup as explained and shown the in '*Procedures*' section.

The above items were needed only for the experimental phase of this study, the subsequent water analysis phase required more sophisticated analytical equipment, the majority of which was found in the Biological Resources Engineering department and included the following:

Coulter Counter LS100:

The Coulter counter (Model LS100) is a particle analyzer that measures particle size and distribution in any given sample. The Coulter counter LS100 is used in conjunction with the 'Micro Volume Module' specifically designed to measure particle distributions of small fluid samples. The Micro-Volume Module serves as a diffraction sample cell contained within the Coulter LS100 instrument. Sample particles are held in suspension by use of a spinning magnetic pin that continually stirs the sample, keeping the fluid in motion. The LS100 passes a thin laser beam with a wavelength of 750 nm through the sample cell, which is then scattered as it passes through sample particles. A Fourier lens system collects the diffracted light and focuses it on a set of detectors at the back of the LS100. Particles of various sizes pass through the laser beam causing specific diffraction patterns, which are projected onto the detector plane and registered by a central computer, a Hewlett Packard 486 (Model: 433DX/Si) running on a *Windows 3.1* platform with Coulter Counter software version 1.53. The deflection pattern is then correlated to a specific particle size, and the frequency of each particle size bracket is tallied over a sample run time of 120 seconds (Coulter Instruments, 1992). The software package then generates a frequency distribution plot and the corresponding statistical data summarized in the results section of this report.

Hach 2000 or 2010:

The majority of the dissolved ion water quality tests were preformed on the Hach system; these tests include ammonia, nitrite, nitrate and phosphorous and are

carried out following Hach method procedures 8038, 8507, 8039 and 8048 (Hach, 2000). This system is a versatile spectrophotometer with wide spread applications. Its principle of operation involves the use of chemical reagents that react with the targeted agent (ammonia, nitrite, nitrate, phosphate etcetera) to produce a visible discoloration in the sample cell. Placed within the spectrophotometer a light beam is passed through the sample cell and the intensity of the light is measured at a specific wavelength (depending on the species concentration being measured), and compared to a blank sample cell. The absorption of the light by the sample is then correlated to a ion concentration. The photometric accuracy of the Hach 2010 varies with each machine but is within ±0.056 Abs, and within in a wavelength accuracy of ±1.00 nm (Hach Company, 2000).

Hach 2100P:

The Hach turbidity meter model number 2100P was used for all sample turbidity measurements. The instrument operates as a nephelometer by directing light from a tungsten-filament lamp through the sample and comparing the light intensity that penetrates the sample with the light deflected from the sample at a 90^{o} angle. The light intensity transmitted through the sample is inversely proportional to the concentration of solid material in the sample, while the light deflected at a 90^{o} angle is directly proportional to the concentration of material in the sample (Hach Company, 1998). The combination of the light intensity deflected at a 90^{o} angle and the light transmitted through the sample is calibrated against Hach turbidity standers ranging in turbidity from <0.1 to 800 NTU (Hach Company, 1998). This information

is stored in the Hach instrument and is correlated against individual sample measurements to determine sample turbidity. The calibration of the Hach 2100P followed the procedures outlined in the operating manual (Hach Company, 1998) using manufacture supplied standards.

ZetaSizer 3000:

Zeta potential measurements were taken using the Malvern ZetaSizer 3000, which measures electrophoretic mobility of a charged colloid in the presence of an electric field of known strength and direction. The ZetaSizer measures particle velocity by a process known as 'Laser Doppler Electrophoresis' (Malvern Instruments, 1996). With this procedure a sample is injected into a chamber between two electrodes to which an electric field is applied. Particle velocity is measured against a stationary plane where two laser beams cross, causing interference fringes that scatter light across the particle and oscillates with time. The light scattered is detected by a photomultiplier connected to a computer and the light oscillation, which helps identify the movement of the particle over time, is correlated to the particle velocity. This value is then used to derive an estimation of the particles' zeta potential.

Electrophoretic mobility is the particle velocity divided by the electric field strength. This value can be related to the effective charge (Q) on the particle using a modification to Stokes law, which equates the electric field strength (the driving force of particle velocity) to the viscosity and drag forces associated with particle movement, shown in equation 1 below (Malvern Instruments, 1996).

$$v / E = \mu_E = \frac{Q}{6\pi r \eta} \quad (1)$$

Where:

v	*= measured particle velocity (m/s)*
E	*= electric field strength (V/m)*
μ_E	*= electrophoretic mobility (m^2/V·s)*
Q	*= the effective charge on the particle (C)*
r	*= the particle radius (m)*
η	*= the viscosity of the fluid (Pa·s)*

As mentioned previously the zeta potential is the voltage potential between the surface of the particle and the diffuse double layer of surrounding ions, the thickness and strength of which is correlated to the effective charge of the particle. By measuring the electrophoretic mobility of the particle in the presence of an electric field yields an estimation of the effective charge of the particle. The electrophoretic mobility of the particle can then be related to the zeta potential by applying the Smoluchowski approximation (Malvern Instruments, 1996), shown in equation 2 below.

$$\mu_E = \frac{\varepsilon \varsigma}{\eta} \quad (2)$$

Where:

μ_E	*= electrophoretic mobility (m^2/V·s)*
ς	*= the zeta potential (V)*
ε	*= the relative permittivity (C/V/m)*
η	*= the viscosity of the fluid (Pa·s)*

Jenco 6071:

The pH measurements were made with the Jenco (model 6071) micro computer based bench pH meter. The meter works by measuring the electrical

potential between an internal reference electrode and a corresponding indicator electrode that is responsive to the presence of hydronium ions. Before operation the meter is calibrated against two solutions of known pH at 7.0 and 4.01, respectively. The instrument uses these two points to plot a linear calibration curve, used to determine pH of an unknown solution. The instruments electrical potential measurements of unknown solutions are compared to this reference calibration curve in determining sample pH.

Electrophoretic Cell Design and Justification

The project began with the design and construction of the electrophoretic cell used throughout the experimentation phase of this project. This piece of equipment had to be custom made to meet the experimental design requirements followed in this study. Below is a list of design and construction objectives and requirements associated with the electrophoretic cell, each of which will be illustrated more thoroughly in the paragraphs that follow:

- Cell design had to ensure that any electric potential applied would be evenly maintained along the length of the cell channel.
- Channel length must be sufficient to ensure that if the electric field applied is to have an effect, on particle movement and stratification along the width of the channel, there will be sufficient exposure time to allow this process to occur.

- Channel width and depth had to allow for uniform distribution of the flow down the length of the channel; while still lending itself to separation into two distinct flow streams.
- System setup had to ensure that consistent flow rates and fluid velocities would be maintained over the length of each run and for consecutive trial runs.
- Cell operation must be easy and safe, as potentially dangerous voltage potentials are to be applied.

Experimental tests are conducted at a flow rate of 7.5 L/hr (2 gal/hr); a value used for design purposes. This nominal flow rate has been selected in order to facilitate the study of particle migration during the experimental phase. In a production system the design flow would be considerably higher, varying with the capacity of the system. However, the purpose of this study is to produce a model from which the suitability of this procedure can be reviewed. The results will have to be projected for a higher capacity system, should such a system be constructed.

The electrophoretic cell used consists of a channel with two electrodes separated from the fluid by thin acrylic sheets. The system is sized such that the cross-sectional area of the channel can accommodate the design flow under all intended operating conditions. Secondly, the length of the channel is sufficiently long such that the greater majority of the suspended charged particles migrate across the width of the channel in less than the time taken for the fluid medium to travel the length of the channel before reaching the outlet.

In sizing the channel the first step is to determine the flow velocity for all intended operating conditions. Hiler et al. (1967) determined that the ideal conditions for electrophoretic separation of colloids from solution is initial turbulent conditions, followed by laminar conditions. However, due to the low flow of this system, laminar flow is maintained at all times and under all testing conditions. This simplifies the design and study by ensuring that the movement of the particle across the channel is controlled entirely by electrokinetic forces. A governing equation predicting lateral movement of charged particles (equation 4) was determined by Hiler et al. (1965), and used later in the design process.

To determine the in-line fluid velocity two equations are applicable, the continuity equation and the Reynolds Number (Streeter et al., 1998). The continuity equation is expressed as:

$$V = \frac{Q}{A} \tag{3}$$

Where:

- V = *fluid velocity (m/s)*
- Q = *design flow rate (m^3/s)*
- A = *cross-sectional area of the fluid column [b x h] (m^2) for a rectangular channel*

The equation for Reynolds Number for open channel flow is expressed as:

$$\Re = \frac{VR\rho}{\mu} \tag{4}$$

Where:

- $\Re$ = *Reynolds Number (dimensionless)*
- V = *fluid Velocity (m/s)*
- R = *hydraulic radius [(b x h)/(b+2h)] (m)*
- ρ = *density of the fluid (998.2 kg/m^3 for water at 20^oC) (Streeter et al., 1998)*
- μ = *viscosity ($1.005x10^{-3}$ Ns/m^2 for water at 20^oC) (Streeter et al., 1998)*

In both of these equations the cross sectional area of the fluid column needs to be determined.

The hydraulic radius in the above Reynolds number equation is defined as the ratio of the cross-sectional area of the fluid over the wetted perimeter (Streeter et al., 1998). This leads us to the difficulty of having three unknown variables (the fluid velocity '*V*', the base width of the channel '*b*', and the height of the fluid column '*h*') and only two simultaneous equations to work with. Inevitably, there are an infinite number of solutions given the number of base height combinations that will accommodate the same flow at a specified velocity. Therefore, the most logical approach is to fix channel base width and solve for fluid depth and velocity.

Given the low design flow rate and previous research experience found in available literature, a fixed channel width of 1.27 cm (½") is selected for cell construction. This width will give sufficient space to allow for particle stratification and movement across the channel, and will give room for a fixed diverting fin to be inserted between the two electrodes that will separate the clean from the dirty water with relative consistency. At the same time the channel will be narrow enough as to not require an unreasonably high voltage.

From physics the force exerted on a charged particle in an electric field is a function of both the electric field strength and the particle's charge. As a result, it is more appropriate to have a fixed and narrow channel with a variable fluid depth, in order that the electric field remains constant for any given voltage, considering that the electric field strength a particle is exposed to decreases with increasing distance

between the electrodes. This will also ensure that all suspended particles are within a predetermined distance from the electrode plates. Theoretically, increasing the electrical potential between the two electrodes will allow for a wider channel, however for practical and safety reasons it would be more advantageous to conduct the study with lower voltage potentials and a narrow channel. In this current study the majority of the experimental runs are conducted using a voltage potential less then 20 volts with the exception of one test conducted at nearly 60 volts in order to verify that similar results are obtained at higher electric field strengths.

Assuming a 1.27 cm (½") channel width, the next step is to determine the range of values possible for fluid flow velocity and channel depth. From a practical standpoint a fluid depth of approximately 5 mm is desirable for two reasons. First, a shallower fluid depth is more likely to interfere with the movement of the particles as a result of surface tension and the friction associated with the channel floor. On the other hand a significantly deeper fluid depth would require a much higher flow rate, which from an experimental perspective would be difficult to maintain.

For calculation purposes a fluid depth of 1.0 mm is assumed, in order to derive a higher than actual fluid velocity, resulting in a more conservative design that will easily accommodate the lower fluid velocities that will be used in practice. In reality a flow depth this shallow will be difficult to maintain as surface tension effects will be high, but for calculation purposes it is acceptable. Using a fluid depth of 1.0 mm and solving for velocity gives us a maximum velocity of 0.166 m/s and a Reynolds number of 142, assuming a flow of 7.57 l/hr (2.0 gal/hr).

Using this maximum fluid velocity in the Manning's equation (Equation 5) below (Streeter et al., 1998), the maximum channel slope can be determined, given the parameters above. This will be the design range for the electrophoretic cell. The Manning's Equation calculates the fluid velocity through a channel as a function of the slope of the fluid stream, the hydraulic radius, and the roughness coefficient of the channel lining. The roughness coefficient (*n*) for the Plexiglas ® lining is assumed to be roughly equivalent to the standard value for plastic (0.013) (Streeter et al., 1998).

$$V = \frac{1}{n} R^{2/3} S^{1/2} \tag{5}$$

Where:

- V = *fluid velocity (m/s)*
- n = *roughness coefficient of material used (0.013 for plastic/Plexiglas ®)*
- R = *hydraulic radius (m)*
- S = *channel slope (m/m)*

Solving for the slope gives a slope of 0.0563 m/m, indicating that for every 10 cm length the tilt must be 0.563 cm high (a 3.3^{o} slope). This gives an indication of range of slopes applicable, lower fluid velocities will require smaller slopes.

Table 4 below lists values for the fluid velocity, the Reynolds number, the hydraulic radius and the required slope for a range of fluid depths from 2.0 to 5.0 mm. The table assumes a fluid flow rate of 7.57 l/hr (2.0 gal/hr) and a channel width of 1.27 cm (½") for all values. Also included in the table is the Reynolds number for each set of parameters, which clearly indicates that the flow is laminar under all conditions (Reynolds Number >> 2000).

Table 4. Fluid Velocity and Channel Depths for a Range of Fluid Depths

Fluid Depth (mm):	Fluid Velocity (m/s):	Slope of Channel (m/m):	Reynolds Number:
2.0	0.083	0.0066	125.1
2.5	0.066	0.0034	118.0
3.0	0.055	0.0020	111.7
3.5	0.047	0.0013	106.0
4.0	0.041	0.0009	100.9
4.5	0.037	0.0006	96.3
5.0	0.033	0.0005	92.0

With the fluid flow velocities calculated above the required channel length can be estimated by noting that there are two velocity vectors associated with the movement of the particles. The first is in the direction of the fluid flow, while the second is perpendicular to the direction of stream flow, and results from the force exerted on it by the electric field. By estimating the perpendicular movement of the particles using the equations developed by Hiler et al. (1965) and the time required to travel the channel width, the corresponding travel length down the channel can be estimated, given the estimated design fluid velocities above.

Hiler et al. (1965) provides us with Equation 6 below, governing the migration of a charged particle between two parallel electric plates. This equation equates the acceleration force exerted on the particle to the force exerted on it by the electric field minus the resistive force due to the viscosity of the fluid.

$$10^7 QE - 6\pi\eta r\frac{dx}{dt} = m\frac{d^2x}{d^2t} \qquad (6)$$

Where:

E	*= electric field strength (volts/cm)*
V	*= voltage applied (volts)*
Z	*= distance between electrodes (cm)*
Q	*= charge of particles (coulombs)*
η	*= viscosity of suspension (poise)*
r	*= radius of particle (cm)*
x	*= variable distance between electrodes (cm) and* $0 \le x \le Z$
m	*= mass of particle (grams)*
t	*= time (seconds)*

The electric field strength above is given by equation 7 below:

$$E = \frac{V}{Z} \qquad (7)$$

Where:

E	*= electric field strength (volts/cm)*
V	*= voltage applied (volts)*
Z	*= distance between electrodes (cm)*

To solve this differential equation a number of variables will have to be determined or estimated (E, Q, η, r and m). The electric field strength is given by the voltage supplied divided by the distance between the electrode plates, which is 1.9 cm (3/4") for the electrophoretic cell model 1. This includes the 1.27 cm (1/2") channel width and two 0.32 cm (1/8") Plexiglas ® sheets inserted between the electrodes and the water. The electric field strength applied varies from 50 mvolts/cm to 30 volts/cm, therefore, for calculation purposes a nominal electric field strength of 50 mvolts/cm is assumed in order to obtain a conservative result. Theoretically, higher electric potentials will result in a higher migratory velocity, and therefore require a shorter channel length unless the organic particles decompose.

The effective charge associated with the particles *(Q)*, is the most difficult value to determine as it varies with the number of charged functional groups associated with the conglomerate particle or bacterial cell. This not only varies with the size of the particle but also with the charge density of the particle determined by the number of charged chemical constituents associated with it per unit of mass. From chemistry, (Barrow, 1996) each single ion charge is 1.6×10^{-19} coulombs (4.8×10^{-10} stat coulombs). However, as noted previously the chemical bonds associated with organic matter are covalent bonds, meaning they share one or more common electron(s). One element may have a stronger tendency to pull electrons to it or borrow another electron from another species, making the electron cloud around it more intense resulting in a more negative region. Nevertheless, this may not be a distinct charge. With this in mind, the objective of this current research is to determine if an imposed electric field has sufficient pull on these electronegative regions associated with the overall macromolecule to be effective. For calculation purposes here it is assumed that the average particle has the equivalent charge of one electron associated with it, which is a very conservative value.

The symbol η represents the viscosity of the fluid in poise and is assumed to be identical with that of distilled water at 20^{o} C, which is 0.01005 cm^2/g (Streeter et al., 1998). For calculation purposes, a particle diameter of 50 μm is assumed, which is equal to the diameter of the majority of fine particles in aquaculture systems are less than 50 μm in diameter. Finally, the mass of the particle is determined by multiplying its specific density by the volume of the particle. As stated previously, the density of colloids in aquaculture systems vary from 1.004 to 1.19 (Chen et al.,

1993), therefore an average value of 1.1 is used for the calculations here, yielding an approximate density of 1100 kg/m^3. By assuming the particle is spherical with a diameter of 50 μm, the approximate volume of a colloid is 6.54 x 10^{-14} m^3, resulting in an estimated mass of 7.18 x 10^{-11} grams. It should be noted that particle shape is not spherical but rather oblong and asymmetrical in nature, further complicating calculations of particle weight and dynamics; however, the assumption that the particles are spherical is used as a rough estimate for the calculations above.

Using the values above in Equation 4 to solve for the terminal migratory velocity (*dx/dt*) yields a velocity of 5.06 cm/s. This value may be significantly higher than the actual velocity due to surface tension effects and relaxation effects not taken into account in the calculations above, but will be used as an overly conservative estimate for calculation purposes here. The indication is that particles suspended within the fluid will travel approximately 5 cm/s in the presence of an electric field of 50 millivolts/cm, thereby traveling the width of the channel (1.27 cm or 1/2") well within half a second. Referring back to Table 4 the velocity of the fluid down the channel with a fluid depth of 1.0 mm and a slope of 3.3 degrees is 16.6 cm/s. Therefore, by the time the particle crosses the width of the channel the fluid will travel approximately 8.3 cm down the channel. This indicates that any channel length above 8.3 cm is sufficient. For practical purposes a considerably longer channel length of 20.3 cm (8”) is selected for the construction of electrophoretic cell.

The calculations above define the justification and guidelines needed to determine the required dimensions of the cell. However due to the difficulty in determining the exact charge associated with a typical particle, and the difficulty of

determining its mass, these estimates can only be used as rough guidelines. Only experimental investigation will validate or invalidate the dimensions used here. After the initial tests, new experimental data can then be used to refine, resize, and modify the design of future cells constructed.

Electrophoretic Cell Construction:

Given the analysis and justification for the dimensions of the fluid channel, the design and construction of the electrophoretic cell itself was initiated. Appendix 1 contains four drawings showing the final design of the electrophoretic cell constructed. Drawing CM1-1 shows the general layout of the cell illustrating the assembled unit once constructed. The cell consists of 19 pieces, 13 of which are unique. Drawings CM1-2, CM1-3, CM1-4 and CM1-5 give the dimensions of each of these 13 pieces and label the pieces Part A - Part M. Table 5 below is a list of all cell parts, their function and the number of pieces required. Parts A-K are constructed from Plexiglas ® sheets and are cut to the specifications shown in the drawings. Part L is cut from a 1.6 mm (1/16”) copper plate and is used as the electrodes on both sides of the channel. Part M is a commercially available plastic barb connecter that is used to connect to inlet and outlet hoses.

Table 5: Cell Model 1 Construction Parts

Part:	Function:	Material:	Quantity Required:
A	Base Plate -channel and cell floor	Plexiglas Sheet 0.95 cm (3/8”)	1
B	Front Plate: Inflow Sidewall -wall at the entrance side of the cell	Plexiglas Sheet 1.27 cm (1/2”)	1
C	Front Plate: Outflow Sidewall -wall at exit end of the cell	Plexiglas Sheet 1.27 cm (1/2”)	1
D	Sidewall Cells Left Hand Side -holds electrode in place	Plexiglas Sheet 0.64 cm (1/4”)	2
E	Sidewall Cells Right Hand Side -holds electrode in place	Plexiglas Sheet 0.64 cm (1/4”)	2
F	Channel Wall Lining -seals electrode from water	Plexiglas Sheet 0.32 cm (1/8”)	2
G	Deflector Pin -stabilizes deflector fin	Plexiglas Rod 1.27 cm (1/2”)	1
H	Deflector Fin -directs flow of stream	Plexiglas Sheet 0.64 cm (1/4”)	1
I	In Flow Sidewall -side wall near entrance	Plexiglas Sheet 0.95 cm (3/8”)	1
J	Out Flow Sidewall -side wall near exit	Plexiglas Sheet 0.95 cm (3/8”)	1
K	Cell Roof -roof of channel gives stability to the structure	Plexiglas Sheet 0.64 cm (1/4”)	1
L	Electrodes	Copper Sheet 0.16 cm (1/16”)	2
M	0.95 cm by 1.91 cm (3/8” by 3/4”) Barb Connector -used to connect the inlet and exit hoses for fluid	Plastic - Commercial	3

Cell construction and assembly is straightforward and needs little explanation. Once all parts have been cut to the specifications shown in the drawings all Plexiglas ® parts were glued together using Weld·On 16 ®, an acrylic epoxy produced by IPS Corporation ®, as shown in the general layout of the cell (Drawing CM1-1). All glued joints were then tested to ensure a watertight seal.

The initial design called for the deflector fin (Part H) position to be adjustable. However, while conducting the experiment it was clear that the deflector fin was not

able to keep water from flowing underneath it as intended. Instead, a significant portion of the water was able to slip under the fin between the two fluid streams. This potential mixing is especially intolerable considering the low flows used. To prevent this from occurring silicone was used to seal the contact between the deflector fin and the floor of the channel. This adjustment ensured that a clear separation between the water flowing along the negative electrode and the water flowing nearest the positive electrode is maintained, and ensures that the two samples do not mix once the fluid stream passes the electrode plates. Figure 3 below is a picture of the final constructed electrophoretic cell.

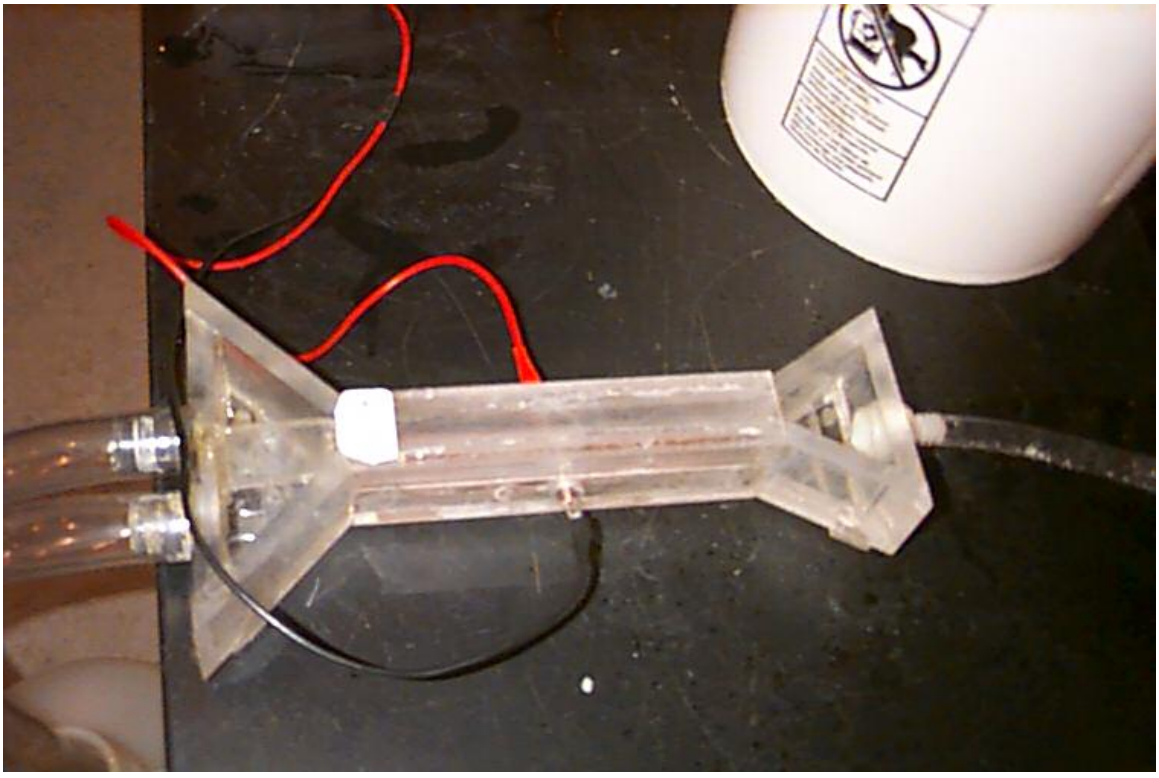


Figure 3. Completed assembly of the electrophoretic cell used in all tests.

PROCEDURE

Experimental Procedure

The effectiveness of the electrophoretic cell is tested using an initial sample obtained from one of the four existing recirculating aquaculture effluent sources (College Park, Frederick, Church Creek and Preston). The testing procedure followed for each sample block is consistent with regards to system setup and operational procedure. In each case sample effluent is pumped from a reservoir, a Nalgene ® container, to a 5 gallon bucket suspended above the electrophoretic cell, which supplies a constant flow rate. The suspended bucket is modified such that it includes a drain hole from which excess water returns to the reservoir when the water level exceeds a predetermined level. At the base of the bucket is a drain connection, and two needle valves used to control flow to the electrophoretic cell. A picture of the experimental setup including reservoir, pump and suspended bucket is seen in Figure 4 below.

Sample effluent is pumped from the reservoir into the suspended bucket at a rate exceeding the flow rate to the electrophoretic cell. The excess water is allowed to drain through an overflow hole at a marked elevation (1 meter) above the electrophoretic cell. The overflow was then siphoned back to the reservoir where it is recirculated through the system again. A constant flow rate to the electrophoretic cell is maintained by a series of two needle valve attached to the exit of the suspended container. The first needle valve is adjusted such that it allows a flow of

approximately 11.3 L/hr (3 gal/hr), and is fixed permanently in this position for all successive tests. The needle valve's initial position had previously been determined by trial and error. While the system was in operation the time taken to fill a 3.8 L (one-gallon) container was noted, and the needle valve position was adjusted until a flow rate of 11.3 L/hr (3 gal/hr) was achieved (ie. 20 minutes to fill a 1-gallon container). Once this position had been determined the needle valve was taped and left in this position for all consecutive runs. A constant head was maintained by inclusion of an overflow drain at a fixed level in suspended bucket.

Figure 4. Setup of system showing pumping system and reservoir containing the initial sample effluent and the suspended container from which a constant flow rate was maintained before entering to the electrophoretic cell beneath it

The position of the second needle valve is left either fully open, during a run, or completely closed, between each run; thereby ensuring that flow rate is determined by the position of the first needle valve. The needle valves predetermined position and the constant pressure head, determined by the overflow drain position in the suspended bucket, ensures that a consistent flow rate is supplied for each consecutive sample run. During testing the flow rate is periodically checked by measuring total volume collected at the end of each run divided by the total run time (20 minutes). This is done to ensure that the needle valve did not fall out of adjustment or that another factor was affecting flow rate.

Once the setup had been completed the same operational procedure was followed for each run within a treatment block. For each run aquaculture effluent is continuously passed through the electrophoretic cell for a period of 20 minutes at a constant flow rate.

During operation the fluid stream passing through the electrophoretic cell is divided into two portions at the end of the fluid channel by the Plexiglas ® divider fin. The divider fin in the stream channel of the electrophoretic cell was set to the ¼ mark and fixed in this position, such that ¼ of the fluid stream was diverted as the waste stream while the remaining ¾ of the fluid stream was diverted as the recirculating stream. The two streams are siphoned into two containers; the stream closest to the positive electrode is marked as the 'waste stream' (S_w), while the fluid stream closest to the negative electrode is marked as the 'recirculating stream' (S_r). The initial hypothesis stated that the fluid stream closest to the positive electrode would contain the majority of the waste particles, as the majority of the suspended

particles have an overall negative charge associated with them, and was for this reason labeled as the waste sample; while the fluid sample closest to the negative electrode was labeled the recirculating sample. This sample operational procedure is followed for each sample run. The end result is a waste and recirculating sample for each treatment level tested for each independent block. The waste and recirculating water samples were collected and stored in the refrigerator until the water analysis tests were conducted.

Experimental Design

The experiment was structured with three independent blocks utilizing water sources from three independently managed recirculating aquaculture systems to which an array of electric field strengths is applied, with a fourth block that looked at the variability in water quality results over time when exposed to a constant electric field. The data from this fourth block are presented as the calculated differences between the waste and recirculating stream samples, instead of measured values, in the '*Results and Discussion*' section of this report as it is intended to look at the variability associated with a single treatment level, as opposed the effectiveness of the procedure over a range of electric field strengths, which is the intended purpose of the initial three trial blocks.

Each trial block corresponds to an independent water source run through the electrophoretic cell, designed and constructed for the purposes of this experiment, over four independent trial periods. In each trial a series of electrical fields strengths varying in intensity from 0.050 V/cm to 30 V/cm is applied to each of the effluent

sources channeled through the cell. Each electric field strength is referred to as a treatment level, while the sample series of nine to ten treatment levels applied to each effluent source is referred to as a block. After passing through the main channel of the electrophoretic cell the fluid stream is divided into two portions, for each treatment level, corresponding to the fluid column closest to the positive electrode, referred to as the waste stream (S_w); and the second fluid stream, corresponding to the fluid column closest to the negative electrode, referred to as the recirculating stream (S_r), intended for recirculation back into the aquaculture system. The resulting samples collected are then analyzed for all or some of the water quality parameters listed in the '*Water Quality Analysis*' section of this report (Table 7). This defines the basic structure of the experimental design.

The effluent sources used for each block include a recirculating aquaculture system located at the University of Maryland at College Park maintained and operated by the Department of Animal Science where striped bass were raised; the second effluent source was obtained from an aquaculture system located near Frederick, Maryland, where stripped bass were also raised; the third effluent source was taken from a farm located near Church Creek, Maryland where tilapia were grown; and the fourth and final effluent source was obtained from a farm located near Preston, Maryland which also raised tilapia. For the remainder of this text the data collected from each of these sources will be referred to as the College Park block, the Frederick block, the Church Creek block and the Preston block, respectively. Each of these experimental treatment blocks were treated under identical experimental conditions, applying varying electric field strengths but following the same

operational procedures in all cases. Table 6 below lists the treatment levels applied to each block.

Table 6. Electric Field Strength Applied to Each Treatment Block

Electric Field Strength Applied (volts/cm):	College Park	Frederick	Church Creek	Preston
0.05	X	X	X	
0.15	X	X	X	
0.25	X	X	X	
0.5	X	X	X	X
1.0	X	X	X	
2.0		X	X	X
3.0	X	X	X	
4.0	X			
5.0	X	X	X	
10.0	X	X	X	
30.0			X	

For the Church Creek block a treatment level of 30 volts/cm was added to verify that a significant change in the results did not occur at a significantly higher voltage potential. Only two treatment levels were applied to the Preston block: 0.50 and 2.0 volts/cm. As noted above the Preston block was used to study the variability in water quality values for samples taken over time, when a constant electric field strength is applied. In this case water samples for the recirculating and waste water streams were collected at 15 minute intervals over a series of 75 minutes, resulting in 5 samples for the recirculating and waste streams at both the 0.5 V/cm and the 2.0 V/cm treatment levels. This allows us to look at the variability of water quality

values for separate samples taken under identical conditions. In the previous three cases, due to the number of treatment levels being tested and the limited volume of aquaculture effluent that could be transported to the lab, each treatment level was run for 20 minutes and the effluent for the recirculating and waste streams was collected over the course of the test, resulting in essentially a single sample for the recirculating and waste streams at each treatment level. The two treatment levels used in the Preston block were chosen after careful consideration of the data obtained in the three previous treatment blocks, which showed that no abnormal behavior occurred at these two levels compared to other treatment levels.

Water Quality Analysis

In order to determine the effectiveness of the electrophoretic cell an array of water quality tests were preformed on each sample. After each run two samples were obtained: one intended for reentry into the aquaculture system referred to as S_r, and the other, which is hypothesized to carry the majority of the waste products and suspended solids, is referred to as S_w. A substantial portion of the experiment revolves around the analysis of water quality parameters for the samples obtained from each run.

However, it should be noted that not all tests listed in the '*Water Quality Analysis*' section of this report were preformed on each block. For example, only a few water quality tests were preformed on the College Park block as it was determined early in the analysis phase that the sample effluent was unsuitable for comparison due to the noticeable lack of pollutants and the relative high water quality

standards maintained, which was required for the pathogen studies being conducted by the Animal Science department at the time. As both the waste and recirculating samples obtained were relatively free of impurities any distinction between the two could not be easily ascertained. However, as the data obtained from this block was used to adjust the research process and revaluate the water quality parameters to be considered, the information is included here for reference purposes. Table 7 below lists all water quality tests preformed on all samples within each treatment block. Also included in Table 7 is the analytical instrument and/or method number used in measuring the given water quality parameter.

Table 7. Water Quality Test Preformed on Each Treatment Block

Water Quality Test:	College Park	Frederick	Church Creek	Preston	Instrument or Method
Alkalinity	X		X	X	SM: 2320*
Electro Conductivity		X	X	X	ZetaSizer 3000
Ammonia-Nitrogen			X	X	HM: 8038**
Nitrate-Nitrogen			X	X	HM: 8039**
Nitrite-Nitrogen			X	X	HM: 8507**
Mobility		X	X	X	ZetaSizer 3000
Particle Size Distribution		X	X	X	Coulter Counter LS100
pH	X		X	X	Jenco 6071
Total Phosphate			X	X	HM: 8048**
Total Solids	X	X	X	X	SM: 2540*
Turbidity	X	X	X	X	Hach 2100P
Zeta Potential		X	X	X	ZetaSizer 3000

* SM: Refers to method number listed in *Standard Methods for the Examination of Water and Wastewater* (APHA, 1995).

** HM: Refers to the Hach method number listed in the *DR/2010 Spectrophotometer Handbook* (Hach, 2000)

In each case the intention is to examine the quality of water being returned to the system (S_r), and verify that the waste materials are being concentrated into the waste water stream (S_w) and removed from the recirculating stream (S_r). For each run it is assumed that the composite sum of the two fluid streams, samples S_r and S_w, is roughly equivalent to the total of S_i entering the cell. However, due to the natural variability in pollutant concentrations and imperfect mixing, S_r and S_w measurements exceed and fall short of S_i levels as samples are taken at various points in the fluid stream. For this reason all test were preformed on all samples.

Table 7 lists the procedures used for each of the water quality tests preformed. In each case the procedure was obtained either from the 'Standards Methods for the Examination of Water and Wastewater' (APHA, 1995) text or from the documentation provided with the analytical instrument used, such as the Hach spectrophotometer standard methods, the Coulter particle counter, or the Malvern ZetaSizer.

Water Quality Test Procedures

Alkalinity (Carbonate/Bi-Carbonate Concentration)

Alkalinity is defined as the sum all titratable bases. It is a measure of the acid-neutralizing capacity of the solution and is primarily a function of the carbonate, bicarbonate and hydroxide concentrations. Insignificant amounts of other bases may be present although alkalinity is generally taken as an indication of these three main constituents (APHA, 1995). Alkalinity is a significant parameter to monitor in aquaculture systems, as it is responsible for buffering pH levels that may otherwise

fall out of balance. However, alkalinity levels must stay within acceptable parameters for fish life.

Alkalinity measurements preformed here follow the titration method outlined in Method 2320 (APHA, 1995). Its principle of operation is simple, acid is added to solution reacting with hydroxyl ions present in solution or that dissociate from alkalinity constituents as pH levels drop. Once all titratable bases present are unable to buffer additional acid a final pH endpoint is reached as indicated by a marked change in pH indicator color. Alkalinity concentrations are calculated from the required volume of standard sulfuric acid (H_2SO_4) of known molarity (0.020 N) added to the pre-measured volume of sample to reach the final pH endpoint.

Conductivity

Conductivity of an aqueous solution is defined as the ability of the solution to carry an electric current, and is a direct reflection of the overall ion concentration. Generally solutions of mostly inorganic compounds such as salts have high conductance, as these compounds dissociate completely into charged constituents. Solutions of organic substances are generally poor conductors, as they do not dissociate as readily. Conductivity is expressed in micro-siemens per centimeter and can be measured directly with a conductivity meter. The conductivity instrument employs a Wheatstone bridge to measure the voltage between two electrodes, and hence the resistance or inversely the conductance of the sample (APHA, 1995).

From conductivity measurements the suitability of the effluent sample, or rather the effectiveness of the electrophoretic cell, which is designed to act upon the

charged particles in the solution, can be indirectly inferred, as conductivity levels are a reflection of charged elements in solution. For this reason conductivity is a valuable parameter to include in this study. Conductivity is not an expression of the total dissolved solids in the solution as it is only relative to the concentration of ions in the sample, whereas not all dissolved solids form ions. However, it will indicate the relative effectiveness in eliminating charged particle from the sample by comparing conductivity levels for the S_r and S_w sample streams after passing through the electrophoretic cell.

Nitrogen

The dissolved concentration of ammonia, nitrite and nitrate is measured using the Hach spectrophotometer model 2000 or 2010, following the methods laid out in Hach standards (Hach, 2000) for methods 8038, 8507 and 8039 for ammonia, nitrite and nitrate, respectively.

The mode of operation for each of these methods is to add a prepackaged and measured reagent to a 25 mL sample, which reacts with the specific nitrogen form of interest, resulting in a discernable color hue, the specific intensity of which correlates to a known ion concentration. The color intensity is measured through the Hach spectrophotometer and compared with a sample blank. Ion concentration is then calculated by the Hach spectrophotometer and displayed on the panel readout.

In the majority of the cases the samples had to be diluted as the specific ion concentration present in the sample was out of range for the Hach method being used. The values were then corrected according to the dilution ratio used.

Phosphate

Nearly all of the dissolved forms of phosphorous exist in solution as phosphates (APHA, 1995), which can easily be determined with a Hach spectrophotometer, using the same principle of operation employed to measure nitrogen concentrations. Reactive phosphorous values were measured using the Hach method 8048 (Hach, 2000), readout values were measured in mg/L of phosphate (PO_4^{-3}). As with the nitrogen sample measurements samples had to be diluted as phosphorous levels were out of range for the Hach method employed.

Particle Size Distribution

Particle size distribution is an important measurement of the effectiveness of the electrokinetic filtration unit used in this research. Particle size distributions were determined using a Coulter counter LS100, which is a light scattering instrument that passes laser light through a sample and measures the scattering effects caused by particles in the sample. The Coulter counter is used in conjunction with the Coulter '*Micro-Volume Module,*' which is a sample cell designed specifically for measuring particle distribution in small fluid samples. Samples were prepared and measured according to the instructions found in the *Coulter® LS Series Operator's Guide* (1992) and the *Addendum to Coulter® LS Series Manuals* (1993).

pH

The acidity of the solution is an important parameter to monitor in all biological systems as it affects the health and integrity of the system, and must be maintained within acceptable levels to ensure the health of the fish culture. Acidity controls the balance between the ionized and unionized forms of ammonia and influences the activity of microbial life. For this reason pH measurements were included for all samples. For all samples pH readings were obtained directly using a Jenco ® (Model 6071) pH meter.

Total Solids

Total solids concentration is a measure of all dissolved and suspended solids in a given sample not including any volatile solids that may be present (APHA, 1995). The total solids concentration was determine using the method prescribed in the *Standard Methods* Section 2540 B (APHA, 1995), which is summarized below.

The mass of the total solids is determined by heating a known volume of sample in the oven at 103-105^{o} C until all water has been removed, leaving a dried solid residual. The difference in weight of the empty evaporation dish and the evaporation dish with the dried remaining solids is taken as the weight of the total solids in the sample. This measured solid mass is then divided by the initial sample volume to determine the concentration of the total solids.

Turbidity

Turbidity, measured in Nephelometric Turbidity Units (NTU), is an expression of the optical clarity of a water sample, and is a measure of the amount of light absorbed or scattered as a result of impurities in the solution that cause negative interference with a known intensity of light passing through the sample (APHA, 1995). While turbidity measurements do not offer any insight into the analytical properties or chemical constituents of the sample, they are a direct measure of sample clarity and an indicator of the water quality, thereby giving a quick confirmation of the effectiveness of the electrophoretic cell. For this reason turbidity measurements are greatly valued in this project.

The turbidity measurements included in this report were measured using a Hach Portable Turbidity Meter (Model 2100P), which utilizes a 'Ratio Optical System' that detects and compares the ratio of light transmitted through the sample with the light deflected at a 90^{o} angle. The instrument was first calibrated following the procedure outlined in the Hach *Instrument and Procedure Manual* (1998), using distilled water and factory prepared formazin solutions of 20, 100 and 800 NTU. After calibration the samples were read per the Hach instrumentation procedures.

Statistical Analysis

Statistical analysis is conducted at two levels: first to determine if an overall treatment effect exist, referred to as a main effect, and second to determine if this treatment effect varies in intensity or effectiveness at different treatment levels, referred to as simple effects. Each treatment block consists of a total of nine to ten

pairs, depending on the experimental block, or 18-20 samples, for the total recirculating and waste streams. On each of these samples a set of water quality parameters is measured, with a minimum of three measurements for each sample. The resulting data is then statistically analyzed using the *SAS* statistical analysis package version 8.0 (SAS, 1999). The complete program code for the data analysis is included as Appendix B. At the end of the program code is a table of all variables used in the program and the parameter that they represent. The discussion below describes how this statistical analysis is conducted and used in determining the effectiveness of the electrophoretic cell. The program statements and procedures referred to below and their function are taken directly from *SAS* (version 8.0) documentation (1999).

The SAS program is broken up into five sections. The first section combines the data from all four treatment blocks into one data set, the remaining four sections looks more closely at patterns found within each of the four treatment blocks. The *SAS* program imports the raw data from Microsoft ® Excel files and assigns variable names as listed at the end of the program code in Appendix B. This is done for each section of the program code depending on if the combined data or data from individual treatment blocks is being analyzed.

The combined data consist of multiple sources of both fixed and random variances including the variance between water sources, variance at various electric field strengths, variance between the two groups (recirculating and waste streams), variance due to experimental error and measurement, and the variance due to the potential interaction of these sources of error. To account for these sources of

variance an analysis of variance was preformed using the *SAS* 'PROC MIXED' procedure that derives an appropriate *f*-value and associated probability for each source of variance, and determines if a significant difference exist or not. For the purposes of this experiment a probability of less than $p \leq 0.05$ will be assumed to be significantly different. This tells us that given the value and number of samples, there is less than a 5% chance that the true population mean for each category is in fact equal. In statistical terms this is the probability of committing a 'Type I' error, of rejecting the null hypothesis that the population means are equal when in fact the null hypothesis is true.

In the 'PROC MIXED' procedure the 'CLASS' statement defines the three main sources of error as variance due to the group (recirculating or waste stream), the treatment level (electric field strength), and the block (source of water). Main effects are overall effects that can be observed by applying the treatment at any level. To determine if a main effect exists we must refer to the *f*-value and probability associated with the variance due to the group, which evaluates the difference between the mean values of the recirculating and waste streams.

Simple effects refer to effects that can be observed at various treatment levels, the electric field strengths being applied. Simple effects are reflected in the *f*-value and probability associated with the variance due to the treatment level. In order to determine what the specific relationship is a regression analysis must be conducted, which will be discussed later in this section.

The analysis of variance test (ANOVA) separates these various sources of variation, between the treatment blocks, the sample groups and treatment levels, and

determines an *f*-value and probability associated with each source of variance. The probability associated with each source of variance tells us if the values associated with each group is statistically different, within a 95% confidence interval. In the case of the variance associated with the treatment blocks, it is known that the sources of water come from different systems under very different loadings and management practices; therefore these values are expected to be statistically difference. The ANOVA test separates this known source of variance and allows for a more meaningful comparison of data between treatment blocks.

It should be noted that the three measurements of each water quality parameter for each sample is averaged into one value when determining main and simple effects above. The three measurements essential represents one sample, and therefore one value needs to be included before analysis occurs. This will avoid overstating the available degrees of freedom. This combining of measurements into one value is also done during the analysis of the main effects found in each treatment block discussed below.

Combining the data in this way increases the number of replications included in the analysis, allowing for increased sensitivity in determining which water quality parameters are significantly affected overall. However, specific information pertinent to each treatment block is lost. Therefore, the remaining four sections of the *SAS* program code corresponds to the four data sets for the College Park, Frederick, Church Creek and Preston blocks, where a closer look at mean values is explored. For presentation purposes the data is graphical presented for each treatment block in the 'Results and Discussion' chapter of this report. As the mean values of each water

quality parameter for each block are drastically different combining the information into one graph would not be as meaningful, therefore the data is presented separately for each treatment block.

In the analysis the main effects of the individual treatment blocks are determined by grouping as either recirculating and waste stream samples without regard for the treatment level. The *SAS* 'PROC TTEST' procedure is then applied, which compares the two sample means, and calculates the associated *t*-value and probability for the comparison. Note that in this case we are only concerned with the variance between the two groups and not with multiple sources of variance due to treatment levels, groups and blocks as with the combined data set. This is done in order to determine the probability of differences in the main effects within each block.

Statistical analysis attempts to describe a population parameter based on a limited number of sample measurements. The *t*-test evaluates the null hypothesis that the two means are equal and assigns a probability to this statement. As with the analysis of variance procedure, the probability value indicates the likelihood that the populations that the sample represent are in fact equal, a low probability value indicates that the true value of the two populations are unlikely to be equal. Again for the purposes of this project a probability of $p \leq 0.05$ is considered to be significantly different, meaning there is a 5% chance of committing a type I error, by rejecting the null hypothesis when in fact it is true. The equation used in calculating the *t*-value for a two sample comparison is shown as equation 8 below. This equation determines the *t*-value by dividing the difference of the two sample means by the standard error.

The sample error depends on the variance of the samples and is calculated somewhat differently if the sample variances are homogeneous or not, referred to as equal or unequal variances.

$$t = \frac{\left(\overline{Y}_1 - \overline{Y}_2\right)}{S_{\bar{y}}} \qquad (8)$$

Where:

t = *t-value*

$\overline{Y}_1$ = *Sample mean (for recirculating samples in this case)*

$\overline{Y}_2$ = *Sample mean (waste stream samples in this case)*

$S_{\bar{y}}$ = *Standard error of the mean*

In determining main effects the 'CLASS' statement groups the data according to the treatment independent variable, which is in this case the group variable (recirculating or waste). The two group means are then compared to determine if any statistical difference exist, for each water quality parameters listed in the 'VAR' statement, given the variance and spread of the samples.

SAS calculates the *t*-value and associated probability using two methods: the first assumes that the sample variances are equal and is the more sensitive and preferred test, the second method is used when sample variances are unequal (Ostle et al., 1996). *SAS* presents the *t*-value and associated probability calculated using both of these methods, and it is up to the user to select the appropriate value based on the results of the analysis of the variance and the specified acceptable error for this analysis, in this case 0.05. The 'PROC TTEST' procedure automatically conducts a test on the homogeneity of the variances using the Folded F method to calculate an *f*-value and associated probability. As with the *t*-test if the probability of the

determined *f*-value is greater than 0.05 it can be assumed that the variances are equal, if the probability is less than 0.05 it can be assumed that the variances are unequal. An unequal variance indicates that the average variance between the sample value and mean of one group is not equal to the variance of the other group being compared in the analysis, therefore the variances of both groups can not be pooled together and must be treated separately.

For the sake of simplicity, in the results section of this report the variances are reported as either equal or unequal, without inclusion of the specific *f*-value or associated probability. If the variances are equal the *t*-value and probability, calculated using the more sensitive pooled method, is reported, in those cases where the variances are found to be unequal, the *t*-value and probability calculated using the Satterthwaite method for unequal variances is reported.

If the homogeneity of variance assumption is met the standard error used in equation 8 above is calculated using the pooled variance of both the recirculating and waste stream samples as shown in equation 9 below. If the homogeneity of variance assumption is not met, the pooled variance can not be used and the calculated variance values for the recirculating and waste streams must be used separately in calculating the standard error of the mean, which is done using equation 10 below.

$$S_{\bar{y}} = \sqrt{S_p^2 \times \left[\frac{1}{n_1} + \frac{1}{n_2}\right]} \qquad (9)$$

Where:

$S_{\bar{y}}$ = *standard error of the mean (for equal variance)*
n_1 = *sample size (for recirculating stream samples)*
n_2 = *sample size (for waste stream samples)*
S_p^2 = *pooled estimate of variance given by equation 11*

$$S_{\bar{y}} = \sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}} \qquad (10)$$

Where:

$S_{\bar{y}}$ = *Standard error of the mean (for unequal variance)*

S_1 = *Sample variance (recirculating stream samples)*

S_1 = *Sample variance (waste stream samples)*

The pooled variance used in equation 9 above is given by the following equation.

$$S_p^2 = \frac{\left[(n_1 - 1) \times S_1^2\right] + \left[(n_2 - 1) \times S_2^2\right]}{(n_1 - 1) + (n_2 - 1)} \qquad (11)$$

Where:

$S_{\bar{y}}$ = *Standard error of the mean (for unequal variance)*

S_1 = *Sample variance (recirculating stream samples)*

S_1 = *Sample variance (waste stream samples)*

SAS presents the *t*-value and associated probability calculated using both of these methods, and it is up to the user to select the appropriate value base on the specific acceptable probability error, in this case 0.05.

Simple effects are determined in a similar manner by comparing only the three replications associated with the recirculating and waste streams at each treatment level for each treatment block. These individual pairs at each treatment level are compared to determine if any simple effects are present at certain treatment levels and not others. The SAS program in Appendix B does this by performing the 'PROC MEANS' procedure on the difference between the recirculating and waste stream samples (S_w - S_r). By specifying specific options at the end of the 'PROC MEANS' statement, SAS calculates a number of useful statistics including the mean, standard

error, *t*-value and associated probability. These specific options are *mean*, *stderr*, *t* and *prt* commands respectively.

If a statistical difference between the recirculating and waste streams is found to exist, for any of the water quality parameters tested, the secondary objective is to determine if there is a linear relationship between the treatment effects observed and the electric field strength applied. This is done using the 'PROC REG' procedure of *SAS*. In this analysis the electric field strength is the independent variable and the difference between the recirculating and waste streams for any given water quality parameter is the dependent variable. *SAS* utilizes the least squares linear regression method in determining a regression. This method determines the regression line where the sum of the squared deviations from the regression line is at a minimum. In this way *SAS* derives a best-fit regression line and the corresponding equation, along with the adjusted root mean square error (R^2), which is an indication of the goodness of fit of the data to the regression line.

In determining a regression line *SAS* generates a value for the *y*-intercept and the slope of the line, along with the associated *t*-value and probabilities that determine the likelihood that these values are equal to zero. For the purposes of this report a regression line is determined to be significant if the associated probability for the slope of the regression line is less than 0.05. As with the *t*-test above a probability of less then 0.05 indicates that the slope of the line is significantly different then zero, and it will be assumed that a true regression does exist. This indicates that the difference between the values obtained for the recirculating and waste streams either

increases or decreases with increasing electric field strength as indicated by the determined slope of the line.

In determining regression there are a number of assumptions that must be met before the derived regression line can be validated. First the sample values taken must be randomly drawn and independent of one another, and the *x* variable must be fixed. Each of these assumptions are ensured during the experimental design and testing phase. However, it must also be shown that the sample values obtained are normally distributed and that the variances are homogenous. A plot of the residual variance values verses predicted values can be generated to visually inspect the variance data, which is also reflected in the derived *f*-value. As with the *t*-test if the *f*-value is greater than 0.05 then it can be assumed that the homogeneity of variances assumption is met. Normality can be validated by running the PROC UNIVARIATE procedure on the data and observing the generated Shapiro-Wilk value, if this value is greater than 0.05 it can be assumed that the data is normally distributed. These steps are conducted for any parameter shown to have a regression associated with it.

RESULTS AND DISCUSSION

The analysis of variance (ANOVA) conducted included three fixed sources of variance: the variance between the recirculating and waste streams, referred to as the group; the variance due to varying intensity in the electric field strength applied, referred to as the treatment level; and the variance between the treatment blocks. The ANOVA information presented in Table 8 below separates each of these sources of variance. It is expected that a significant difference should exist between water quality parameters between the various treatment blocks as the water sources for each block were obtained from independent aquaculture systems at differing loading capacities and operational conditions. The *SAS* (SAS, 1999) analysis confirmed this clear difference for all water quality parameters measured; therefore, this information is of little significance and will not be discussed further. However, of considerable concern is the variance that is observed between the two groups, which will be referred to as the main effect or group effect; secondly, if a main effect is observed then the variance between the treatment levels, referred to as a simple effect or treatment effect, must be evaluated in order to determine if a measurable relationship exists between electric field strength and the variance between the two groups.

Table 8 presents the ANOVA results for the combined data showing the calculated *f*-value for each fixed source of variance: group, treatment level, and block. The total variance is then the sum of these three sources of variance plus the

random experimental error obtained with each sample measurement. This is represented by equation 12 below.

$$S_p^2 = \frac{\left[(n_1 - 1) \times S_1^2\right] + \left[(n_2 - 1) \times S_2^2\right]}{(n_1 - 1) + (n_2 - 1)} \quad (12)$$

Where:

$S_{\bar{y}}$ = *Variance due to each treatment block (i)*

S_1 = *Variance due to the group (j)*

= *Variance due to each treatment level (k)*

= *Variance as a result of the experimental error*

Also presented in the Table 8 are the probability values for the group and treatment level sources of variance associated with the calculated *f*-values and the degrees of freedom associated with each calculation. The denominator degrees of freedom equals the total number of samples measured minus one. The numerator degrees of freedom refer to the degrees of freedom associated with each fixed source of variance being analyzed, for example there are two catagories associated with the group class, recirculating and waste, therefore there is one degree of freedom; there are four categories associated with the treatment block, therefore there are at most 3 degrees of freedom assuming that the specific water quality parameter was measured for the samples of each of the 4 blocks. Both of these values are used by *SAS* in determining the *f*-value and associated probability.

Table 8. ANOVA Table showing Fixed sources of variation for all water quality parameters tested

Source of Variance	**Numerator Degrees of Freedom**	**Denominator Degrees of Freedom**	***f*-Value**	**Probability (*f*)**
ANOVA Table for Alkalinity:				
Group	1	43	7.29	0.0099
Treatment Level	10	43	1.19	0.3247
Block	2	43	22539.0	<0.0001
ANOVA Table for Ammonia:				

Group	1	27	1.00	0.3273
Treatment Level	9	27	0.11	0.9992
Block	1	27	22.33	<0.0001
ANOVA Table for Conductivity:				
Group	1	44	4.02	0.0511
Treatment Level	9	44	0.27	0.9801
Block	2	44	35142.6	<0.0001
ANOVA Table for Mobility:				
Group	1	44	0.61	0.4374
Treatment Level	9	44	1.65	0.1321
Block	2	44	306.99	<0.0001
ANOVA Table for Nitrate:				
Group	1	27	0.35	0.5608
Treatment Level	9	27	1.06	0.4220
Block	1	27	144.65	<0.0001
ANOVA Table for Nitrite:				
Group	1	27	0.05	0.8177
Treatment Level	9	27	0.40	0.9344
Block	1	27	463.81	<0.0001
ANOVA Table for Particle Size:				
Group	1	58	4.57	0.0367
Treatment Level	10	58	1.88	0.0664
Block	3	58	103.38	<0.0001
ANOVA Table for Particle Skewness Factor:				
Group	1	41	0.74	0.3936
Treatment Level	10	41	0.82	0.6073
Block	2	41	1.59	0.2155
ANOVA Table for Particle Standard Deviation:				
Group	1	41	0.00	0.9863
Treatment Level	10	41	1.89	0.0755
Block	2	41	3.98	0.0263
ANOVA Table for pH:				
Group	1	43	5.69	0.0215
Treatment Level	10	43	3.54	0.0018
Block	2	43	4.72	0.0140
ANOVA Table for Phosphate:				
Group	1	27	3.99	0.0560
Treatment Level	9	27	0.90	0.5362
Block	1	27	30311.2	<0.0001
ANOVA Table for Total Solids:				
Group	1	60	1.03	0.3131
Treatment Level	10	60	4.52	<0.0001
Block	3	60	1543.06	<0.0001
ANOVA Table for Turbidity:				
Group	1	44	1.73	0.1954

Treatment Level	10	44	2.81	0.0088
Block	3	44	657.54	<0.0001
ANOVA Table for Zeta Potential:				
Group	1	44	0.46	0.4999
Treatment Level	9	44	1.64	0.1348
Block	2	44	245.95	<0.0001

The analysis of variance on the combined data showed that overall a significant difference is seen between the recirculating and waste streams of the alkalinity, mean particle size, and pH measurements. However it should be noted that the difference in the conductivity and phosphate concentrations were found to be nearly significant with probability values of 0.0511 and 0.0560, respectively. These main effects shown to be statistically different within a 95% confidence interval are listed in Table 9 below. Also presented in Table 9 are the simple effects shown to be statistically different within a 95% confidence interval. This list includes those water quality parameters where the measured values vary significantly between treatment levels. This combined information presented in Table 9 lists significant main and simple effects gleaned from Table 8 above.

Table 9. Main and simple effects found to be significantly different with the combined data

Water Quality Test:	**Numerator Degrees of Freedom**	**Denominator Degrees of Freedom**	***f*-Value:**	**Pr (*f*):**
Main Effects Found to be Significantly Different:				
Alkalinity	1	43	7.29	0.0099
Particle Size	1	60	4.53	0.0363
pH	1	43	5.69	0.0215
Simple Effects Found to be Significantly Different:				
pH	10	43	3.54	0.0018
Total Solids	10	60	4.52	0.0001
Turbidity	10	44	2.81	0.0001

It should be noted that an observed simple effect does not give any information on which treatment levels vary, and how they vary, rather it simply states that the measured difference associated with at least one treatment level differs significantly from the values associated with the other treatment levels. A closer examination of the individual treatment values for each treatment block will have to be conducted in order to better understand any relationship that may exist between the measured values and the electric field strength intensity, as will be explored later in this section.

The information above shows that only the pH values were seen to vary significantly between the recirculating and waste streams as well as between the individual treatment levels. There are several contributing factors to this observation. First, the variance associated with the water quality parameters of different treatment levels have both fixed and random sources of variation, which may contribute to the observation that there is an apparent difference in effect at varying electric field strengths. The water used in each block was obtained from the same tank at the same time, nevertheless due to the large volume of water required and extended duration of the tests, there were naturally occurring random variation in the water quality parameter being measured as the test proceeded, despite efforts made to ensure proper mixing at the start of each test. Therefore, there may be a significant difference in the water quality parameter mean value at one treatment level when compared to the next treatment level. This sort of natural fluxuation will inevitably affect the measured difference values between the reciruculating and waste streams, especially when compounded over several treatment blocks. This natural variation over the duration

of the testing phase may explain why a significant difference was observed with the turbidity and total solids values measured at different treatment levels even though a main effect between the recirculating and waste streams was not observed.

Conversely, it can be anticipated that a main effect may be observed between the recirculating and waste streams even when there is no apparent change in this observed effect in intensity or degree between treatment level, indicating that the application of an electric field at any intensity may have a nearly equivalent observed affect on the water quality parameter being observed. In this case a consistent difference between the recirculating and waste streams may be observed, and yet the natural variations swings in the measured water quality parameter between individual samples may overshadow any difference in degree as a result of changing electric field strength. As a result, the natural variation is too great to discover any significant difference between treatment levels.

As a result only the pH values appear have a noticeable difference between the recirculting and waste streams as well as a noticeable change in the intensity of the effect between treatment levels. Therefore, a regression analysis will be conducted on this water quality parameter in order to better define this relationship, following a more detailed examination of the water quality data obtained.

The data above looks solely at the differences in the relative concentrations for all samples taken, however, it is constructive to take a closer look at the data obtained for each treatment block. A graphical presentation of the data for each treatment block is discussed below. The data presented in the remainder of this chapter is grouped according to the water quality parameter being tested. Each graph

presents the results of a single water quality parameter for one of the independent water sources, referred to here as treatment blocks, and includes the average values for each treatment level for the recirculating and waste samples (S_r and S_w) for the College Park, Frederick and Church Creek treatment blocks. Graphs presenting the Preston block data show the difference between the recirculating and waste samples at each 15-minute time interval for which measurements were taken, for both the 0.5 V/cm and 2.0 V/cm treatment levels. A separate graph is presented for each treatment block in which the water quality test was preformed. Note that the data for each water quality parameter from separate blocks can not be lumped together on a single graph since each water source is associated with a separate fish tank operated under different management practices for fish cultures at varying stocking densities and stages in their development, resulting in distinct water quality levels.

Each point on a graph represents the mean value of at least three measurements. The standard error bars presented in the graphs were generated by Microsoft ® Excel (2000) and are used here as an indication of the variance associated with each sample, however the statistical probability values and variances discussed here were calculated using the statistical analysis software package *SAS*, version 8 (SAS, 1999). Following the graphical presentation of the data, a more quantitative analytical presentation of the statistical data associated with each measurement is included at the end of this chapter.

Zeta Potential Data:

Figures 5, 6 and 7 below present the mean zeta potential measurements obtained for the Frederick, Church Creek and Preston blocks. As discussed previously the zeta potential of a particle is the electric potential created between the particle surface and the ion diffuse layer surrounding the particle; as such it plays a significant role in determining the particles' susceptibility to electrokinetic movement in the presence of an imposed electric field.

Note that figures 5 and 6 present actual zeta potential measurements for both the recirculating and waste streams at each voltage potential, where as figure 7 presents a difference between the recirculating and waste streams ($S_w - S_r$) for each time interval a sample was taken. As mentioned previously only two voltage potentials were applied to the Preston block (0.5 and 2.0 volts/cm), however five recirculating and waste stream samples were taken over a period of an hour and 15 minutes in order to look at variability in sample values over time. As a result of this change in the data structure a change in the graphical presentation of all water quality measurements taken for the Preston block was necessary and is repeated throughout the remainder of this chapter.

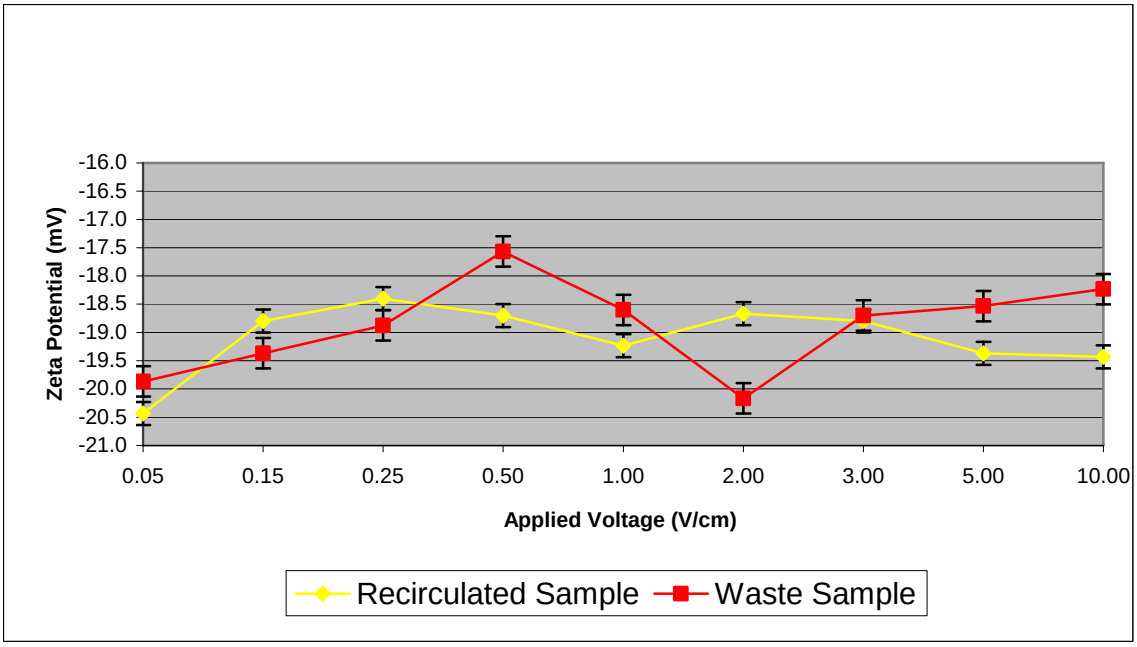


Figure 5. Zeta potential measurements for the Frederick block

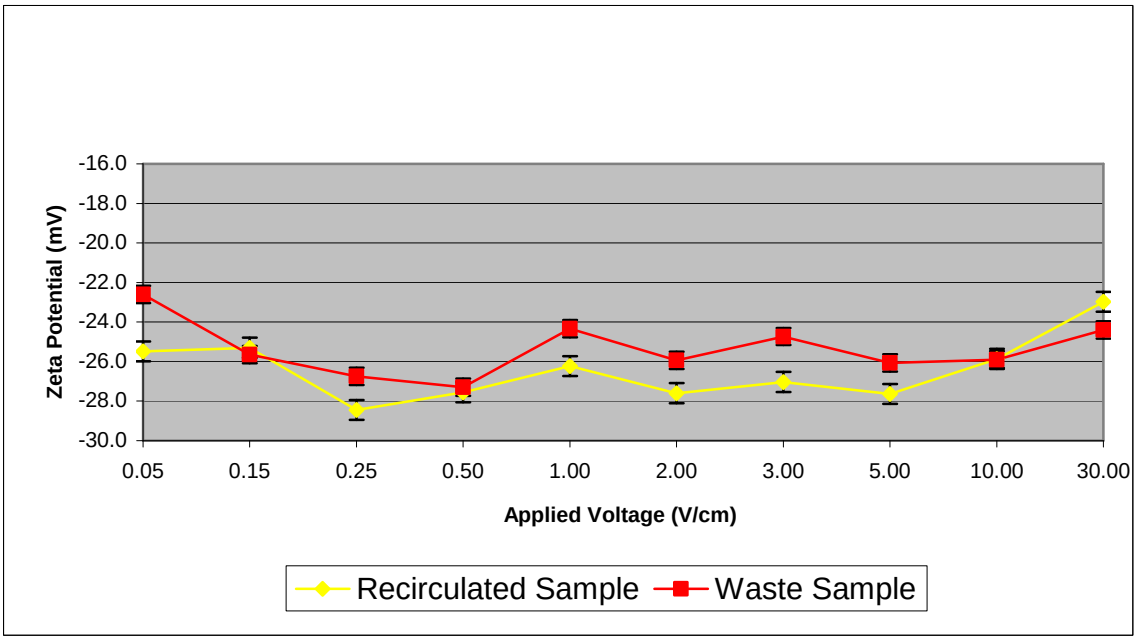


Figure 6. Zeta potential measurements for the Church Creek block

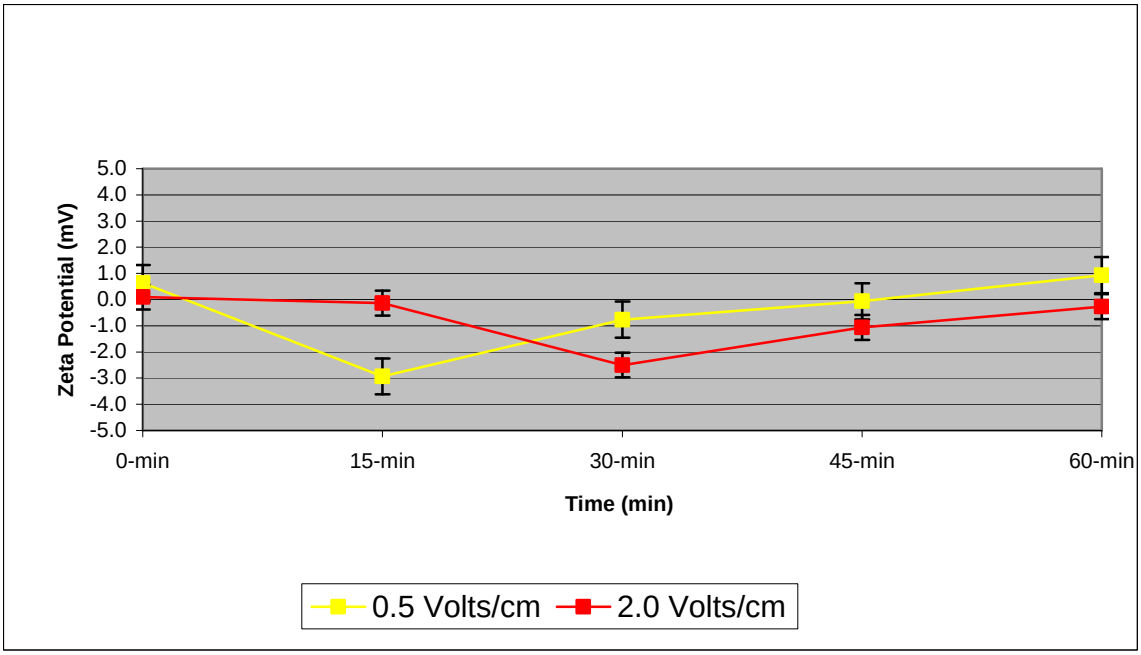


Figure 7. Zeta potential measurement differences ($S_w - S_r$) for the Preston block

Although a cursory glance at the mean zeta potential measurements between the recirculating and waste samples at individual treatment levels appear to be different, an overall treatment effect was not observed. Surprisingly, the mean difference between the zeta potential values of the two groups, the recirculating samples of the same treatment block and the waste samples of the same treatment block, were not found to be statistically different, within a 95% confidence interval. The probability that the two sample groups were the same, for the Frederick, Church Creek and Preston blocks, was found to be 0.5360, 0.1412 and 0.1749 respectively, each were within a 95% probability indicating that none were significantly different.

It is clear that the overall zeta potential of the aquaculture effluent particles in all samples were negatively charged, although the specific distribution and range of zeta potential measurements differs between the three treatment blocks. This

difference undoubtedly reflects a variation in feed and management practices carried out at the three respective sites. The negative zeta potential values indicate that the overall surface charge of the particles is negative, and therefore the particle will have a tendency to move toward a positively charged electrode in the presence of an electric field. As the zeta potential is calculated from the measured mobility the observation that particles will tend to move toward the positive electrode is again confirmed in that the average mobility for all samples is also clearly negative, as discussed in the subsequent section.

The fact that a significant difference is not observed as a result of the treatment effect may be explained by noting that the fish were fed a uniform diet of specifically formulated fish feed. There being no other significant input of solid materials, it can be theorized that the solid wastes, consisting of uneaten food and feces, are fairly homogenous and subjected to the same environmental conditions, resulting in a relatively uniform charge density associated with all suspended particles. If this is the case, then all aquaculture effluent particles, found in either the recirculating or waste stream, should have roughly equivalent zeta potential measurements. Note that the zeta potential measurements indicate only the average observed zeta potential of the particles within a sample, and do not necessarily imply a difference in particle concentration. Therefore it can be expected that the particles in both the recirculating and waste streams should have roughly the same zeta potential associated with them, even if the concentration of particles is different between the two groups.

Combining all the samples for both the recirculating and waste streams yields a mean and standard deviation of –19.0 ±1.1 mV, –25.9 ±2.5 and –17.6 ±1.9 mV for the Frederick, Church Creek and Preston blocks, respectively. The low variance in each case supports the idea of particle zeta potential homogeneity, and possibly explains why a significant difference does not exist between streams. Had a large spread in zeta potential values been observed, it would be expected that the particles with the highest negative zeta potential would accumulate in the waste stream, while those particles with the lower negative zeta potential would appear in higher concentration in the recirculating stream.

It was hypothesized that the particles with the higher negative effective charge will be held within the fluid stream nearest the positive electrode, and will therefore have a higher concentration in the waste water stream. However, considering the overall homogeneity of the particles, the zeta potential difference in the two streams (S_w and S_r) was not statistically different.

Mobility Data:

Closely related to the zeta potential is the average mobility of the particles, which is used by the ZetaSizer in the calculation of the zeta potential values. Electrophoretic mobility is a measure of the particles velocity divided by the electric filed strength applied, and gives an indication of the particles susceptibility to electrophoretic movement. Figures 8, 9 and 10 below present the mean mobility values measured for each sample. Noting again the homogeneity of the sample values, no significant difference was found for the overall treatment effect associated

with the individual treatment blocks; however, the probability associated with the Church Creek and Preston blocks were found to be almost significant with an associated probability of 0.0815 and 0.0773, respectively. A probability of 0.5091 was determined for the paired comparison of the recirculating and waste streams for the Frederick block, indicating that a significantly difference does not exist.

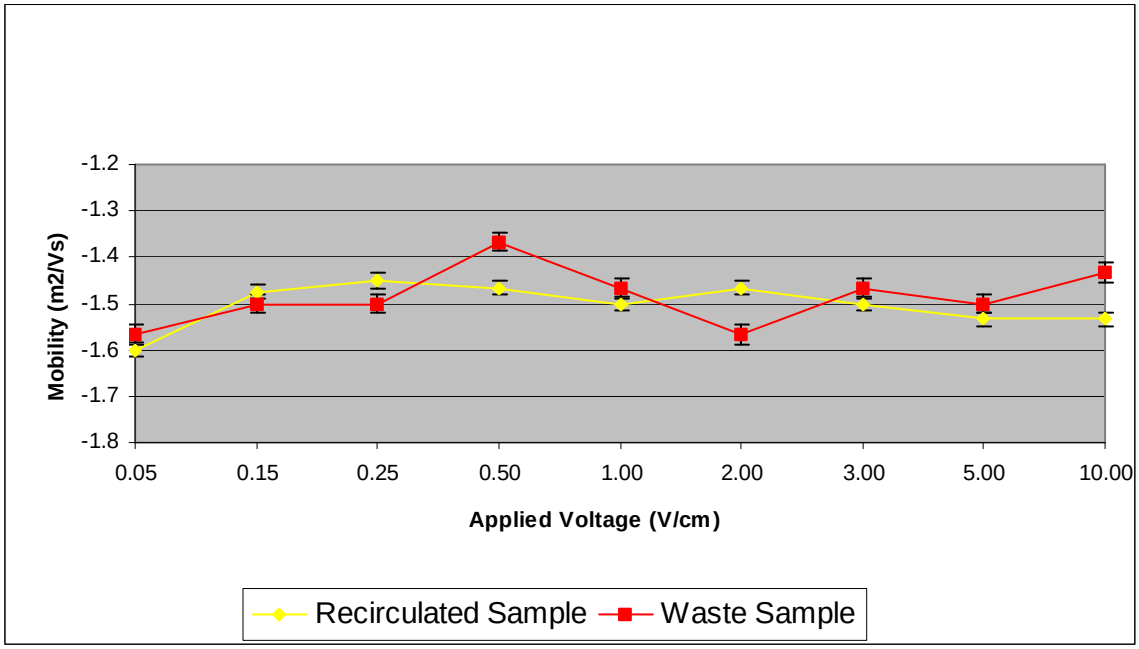


Figure 8. Mobility measurements for the Frederick block

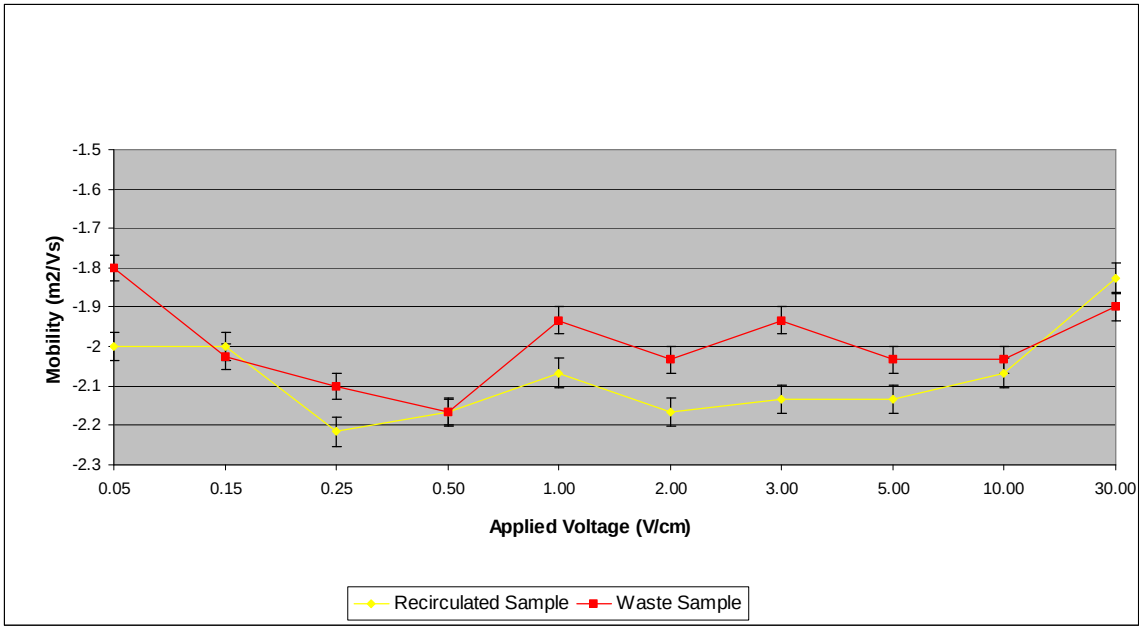


Figure 9. Mobility measurements for the Church Creek block

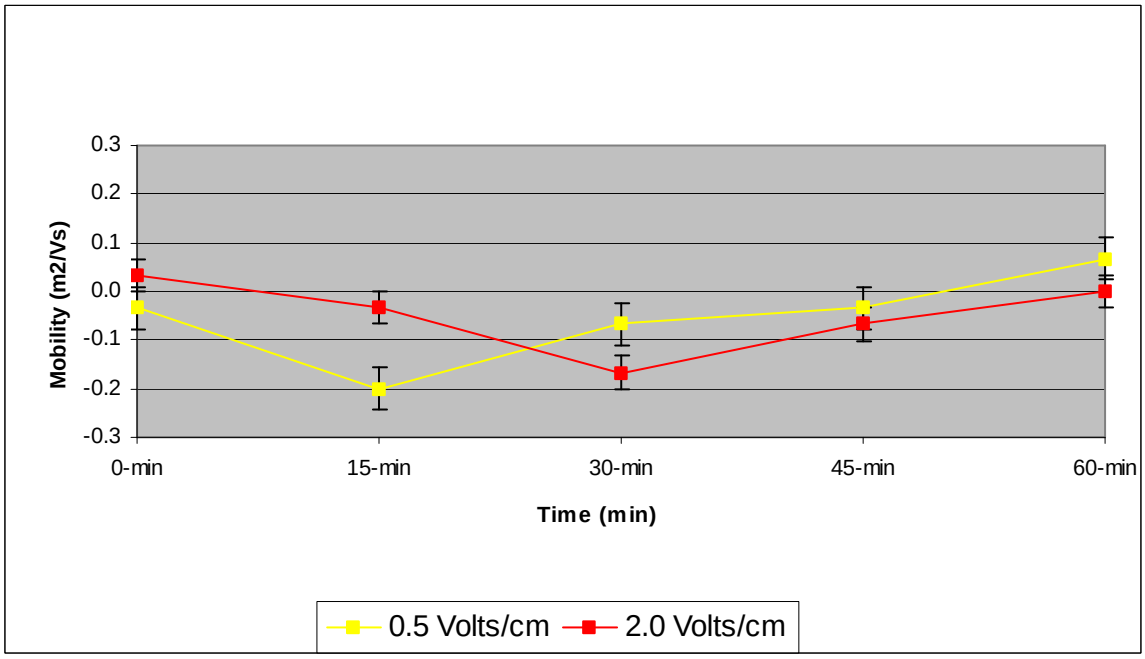


Figure 10. Mobility difference (S_w-S_r) measurement for the Preston block

Turbidity Data:

The zeta potential and mobility data above tells us that there is little if any qualitative difference with respect to the charge density and electrical susceptibility of the individual suspended particles themselves; however, to examine the quantitative aspects of the particle distribution and water quality we must look towards other tests. The simplest of these tests is the turbidity test that provides information on any qualitative difference in the transparency of the fluid, a measure that is correlated to the concentration of the opaque suspended solids in each sample. Figures 11, 12, 13 and 14 below present the turbidity test results for the College Park, Frederick, Church Creek and Preston blocks, respectively.

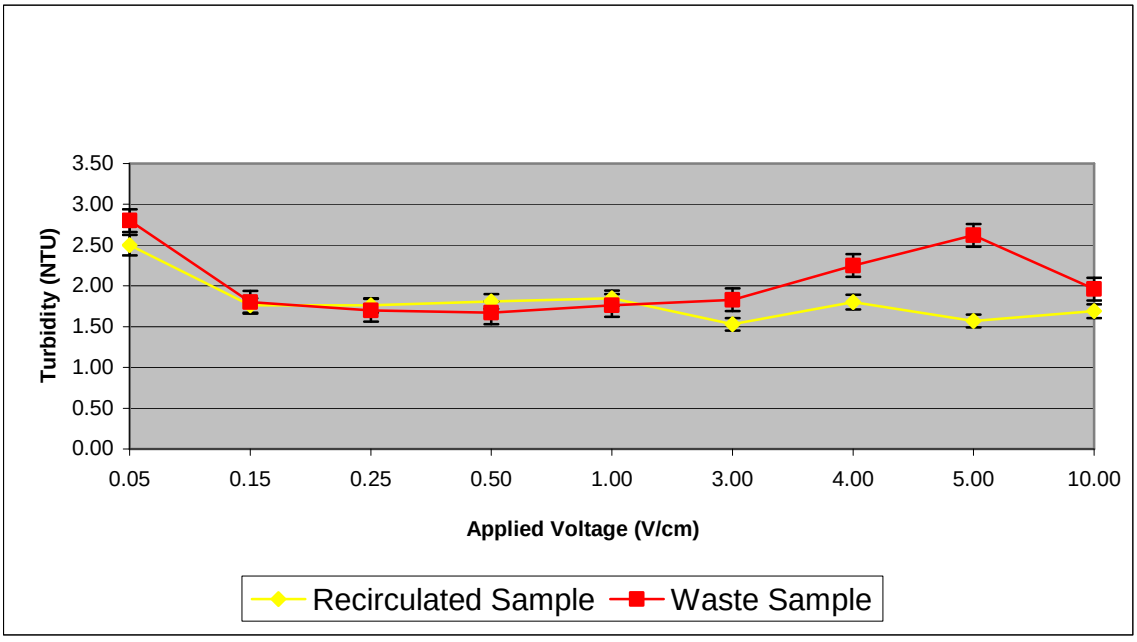


Figure 11. Turbidity measurements for the College Park block

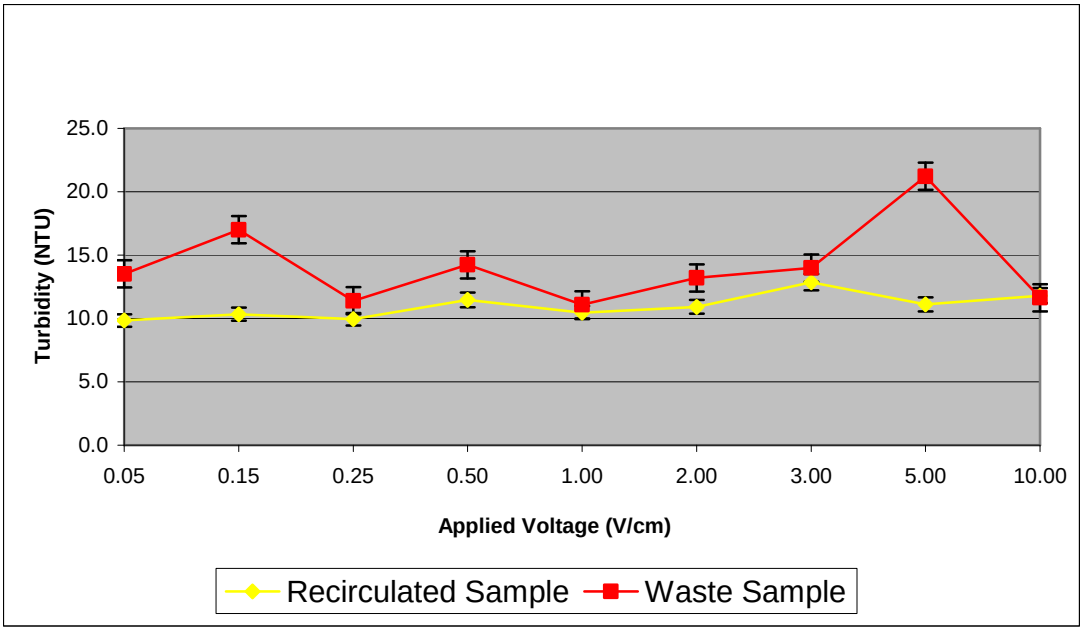


Figure 12. Turbidity measurements for the Frederick block

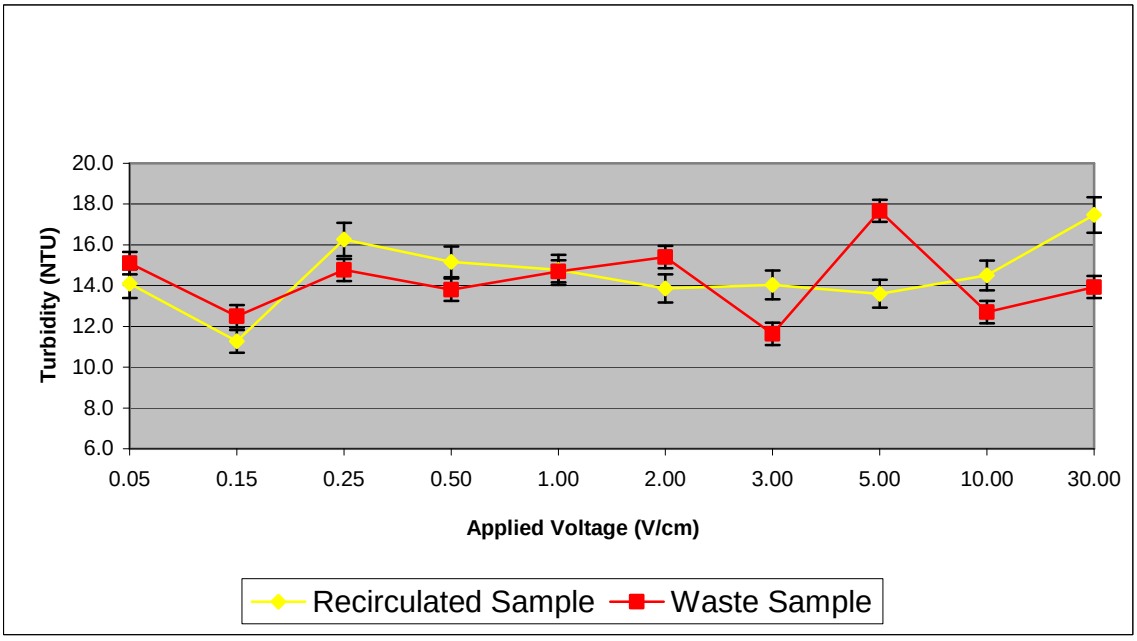


Figure 13. Turbidity measurements for the Church Creek block

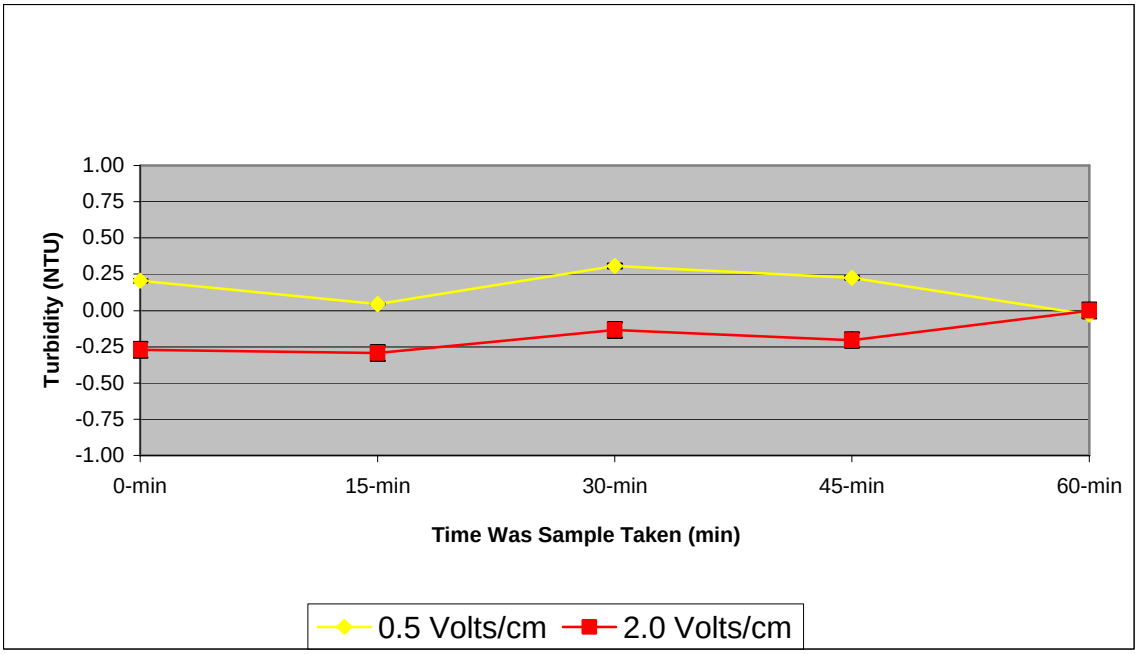


Figure 14. Turbidity difference (S_w-S_r) measurement for the Preston block

Of the three treatment blocks a significant overall treatment effect was found only with the Frederick block, as indicated by the *t*-value probabilities of 0.1798, 0.0269, 0.7118 and 0.9557 for the College Park, Frederick, Church Creek and Preston blocks respectively. In this case only, the Fredrick block was shown to be significantly different. However, given the poor significance of the other three blocks, and the fact that a significant difference was not found with the combined data may indicate that the low probability of the Frederick occurred by chance.

However, a noteworthy observation is the relatively low and narrow range of turbidity values associated with the College Park and Preston blocks when compared to the remaining two treatment blocks, ranging in turbidity from 1.5-3.0 NTU for the College Park sample and between 3.0-6.0 NTU for the Preston block, as compared to the much higher values of the two remaining treatment blocks, ranging from 7-25

NTU. The very low and narrow range of the College Park and Preston blocks is undoubtedly one of the contributing factors that make a significant difference more difficult to determine if indeed a true difference does exist. Also noteworthy is the relatively narrow range of the turbidity values of the Church Creek block compared to that of the Frederick block. The low turbidity values of the College Park and Preston blocks and the narrow range of the Church Creek block shed some light on why a significant difference was not detected.

Total Solids:

This outcome is again repeated with the 'total solids' test, where none of the four overall comparisons were found to be significantly different. Figures 15, 16, 17 and 18 below present the total solids data for the College Park, Frederick, Church Creek and Preston blocks, respectively. Lack of a significant difference may be due in part to the relatively large experimental error associated with the total solids test. In order to determine the mass of the total solids within a fluid volume, the mass of the evaporating dish must be subtracted from the total mass of the dish and solids residue after complete evaporation has occurred. As a result the error associated with both the initial and final measurements is compounded making a significant difference more difficult to detect, this is especially true if the difference is minute, and the weight of the residue solids is significantly less than that of the evaporating dish, the usual case with solids in this project. However, a significant difference was found with the Frederick block as indicated by a probability of 0.0418. The probabilities of the remaining College Park, Church Creek and Preston blocks were

found to be 0.9803, 0.6409 and 0.0704, respectively, indicated that a significant difference was not associated with any of the remaining treatment blocks.

Special notice should be given to figure 18 where the difference in the total solids concentration for the Preston block appears to increase for both electric field strengths tested as the test proceeds; indicating that more time may be needed in order for the effects of the electric field to appear. However, this is the only instance where this trend appears, and therefore it is considered to an anomaly rather than the rule. Other tests for the Preston block show fluctuating results as the test proceeds, indicating that time is not likely to increase or decrease the effectiveness of the procedure, rather there will continue to be some natural variation.

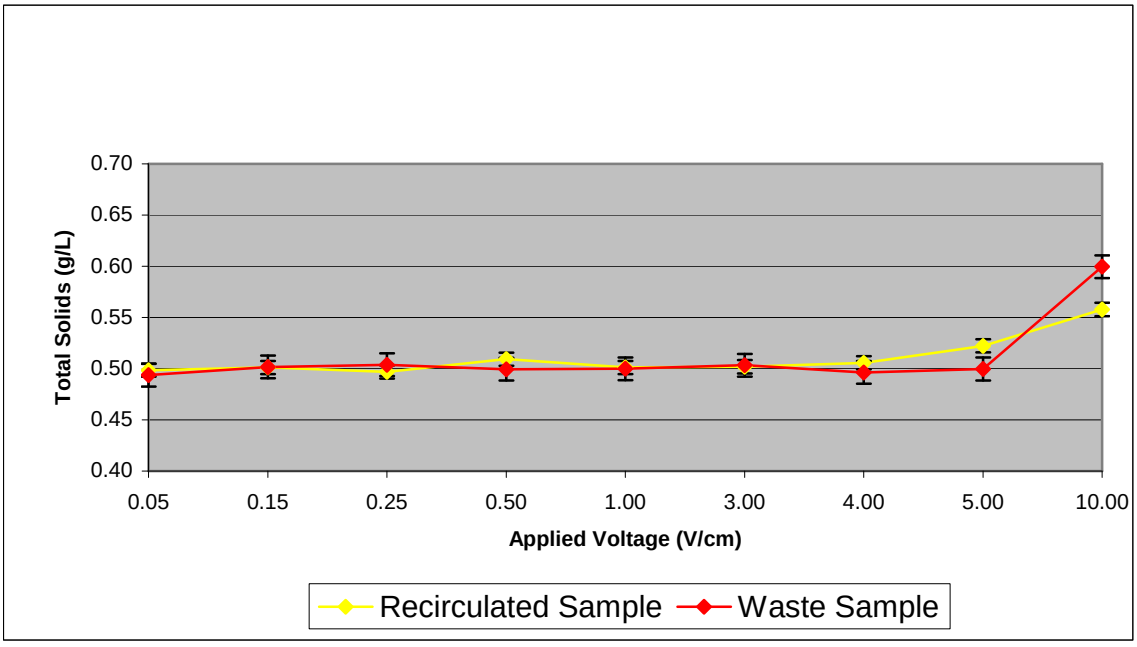


Figure 15. Total solids (g/L) for the College Park block

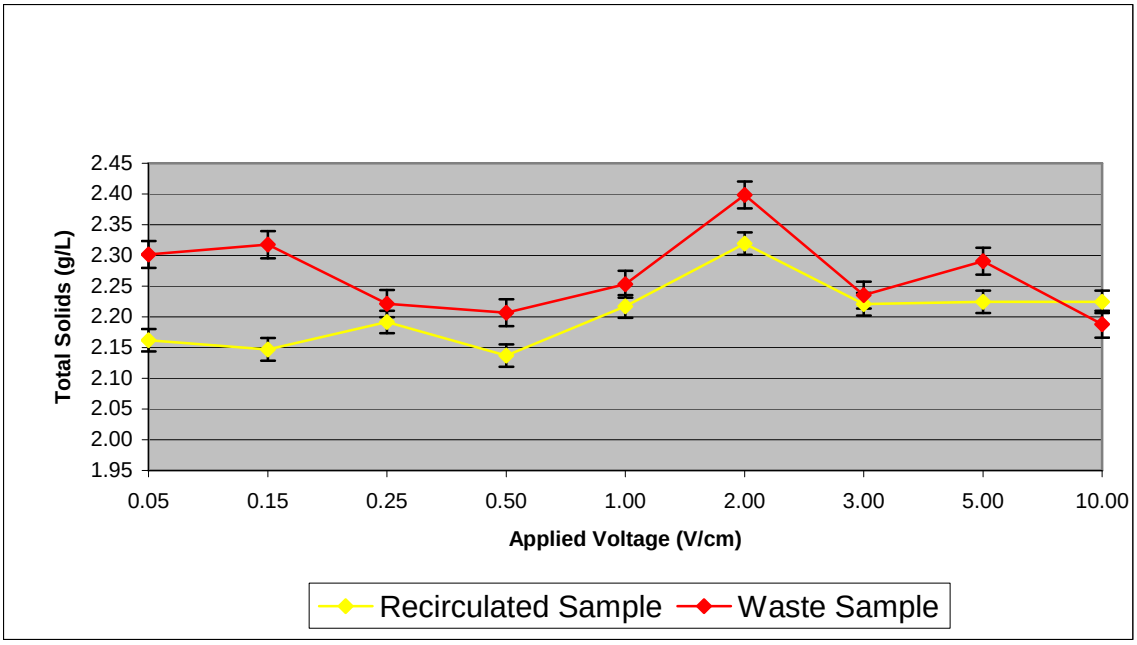


Figure 16. Total solids (g/L) for the Frederick block

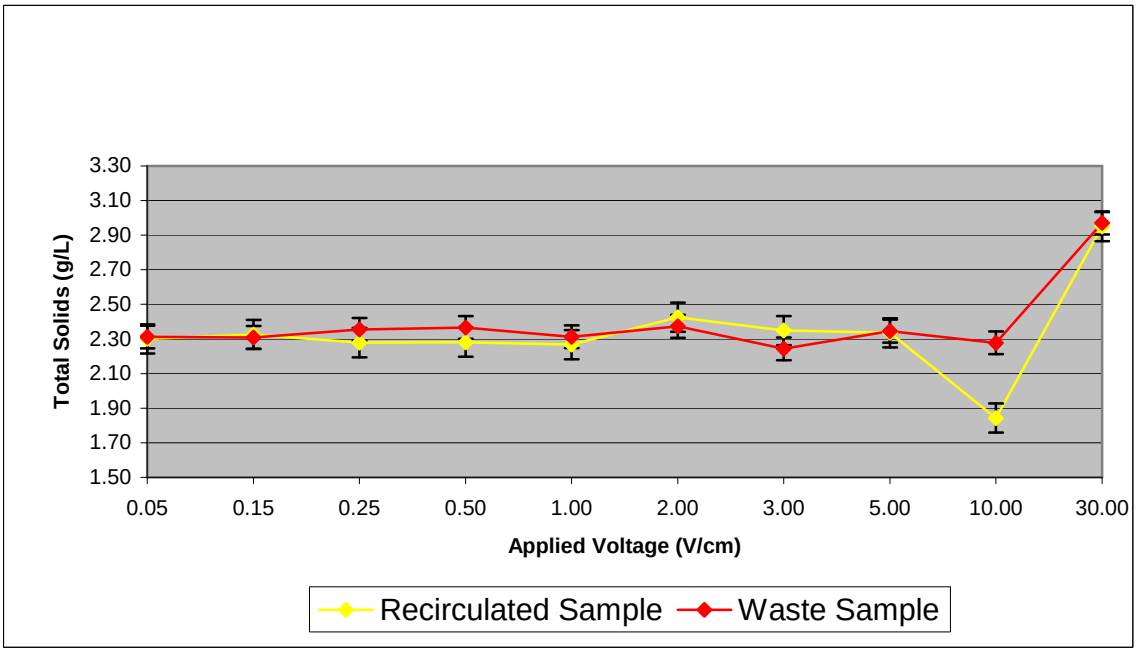


Figure 17. Total solids (g/L) for the Church Creek block

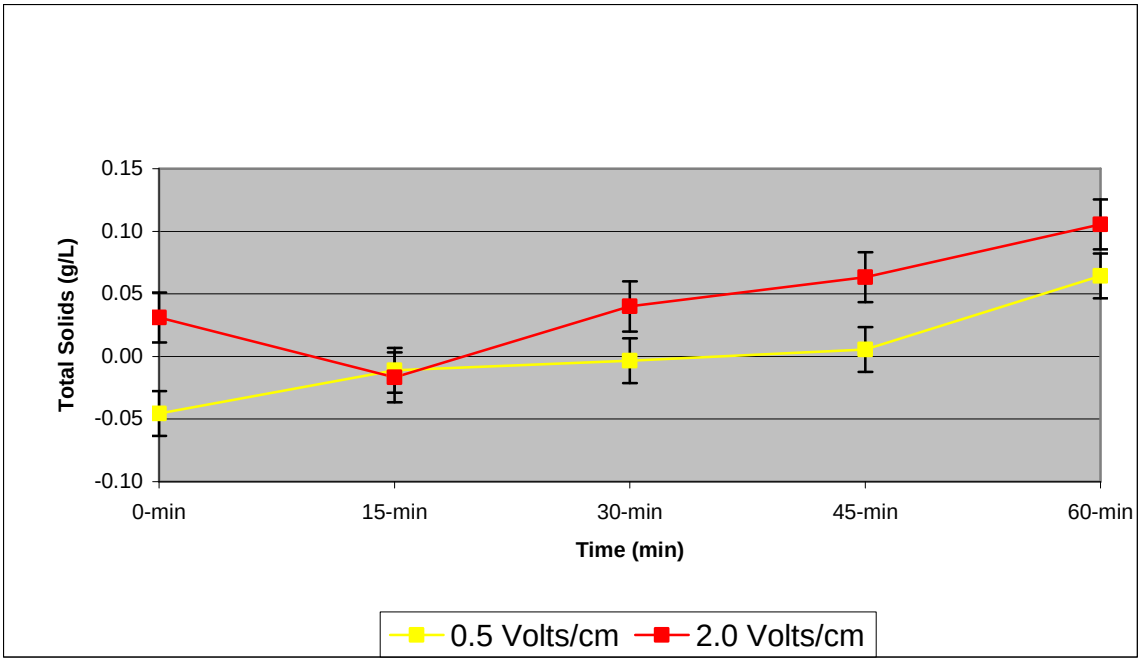


Figure 18. Total solids difference (S_w-S_r) in (g/L) for the Preston block

In light of the natural tendency of solute concentration to invade areas of lower concentration, it can be speculated that, as the negatively charged particles are acted upon by the electric field and begin to accumulate near the positive electrode, the increasing charge density within this portion of the fluid stream begins to repulse new particles from approaching; this tendency toward disorder thereby counteracts any tendency for additional negatively charged particles to move toward the positive electrode. This may be especially true if the particles do not bind to the charged electrode, as is the case here where the electrodes are separated from the fluid stream. If this is true, the freely moving particles in the fluid column that are not effectively bound to the electrode may move against this electrical potential, down its concentration gradient, thereby maintaining a spatial equilibrium that prevents any significant accumulation in a given portion of the fluid column, from occurring. This

may be one explanation as to why significant separation was not observed; an alternative explanation is simply that an electric field is not effective in moving organic waste particles from found in aquaculture effluent.

Particle Size and Distribution Data:

Another distinctive significant physical characteristic is that of particle size and distribution. As explained previously particle mobility is a function of charge density as well as particle mass and form. Particle size can be a factor in mobility as the drag force associated with the suspended particles is a function of the particles projected area, with larger particles having greater resistance.

Figures 19, 20, 21, and 22 below present the mean particle size for the College Park, Frederick, Church Creek and Preston blocks, respectively. The ANOVA analysis shows that the overall particle size difference between the recirculating and waste streams was not statistically different for any of the treatment blocks individually. The probabilities associated with the College Park, Frederick, Church Creek and Preston blocks are found to be 0.1713, 0.1379, 0.5299 and 0.9074 respectively, indicating that no significant difference was apparent with any of the treatment blocks individually. However, the combined data, with its increased sensitivity, did show that a statistical difference does exist, as indicated by the combined probability of 0.0367 associated with the combined data.

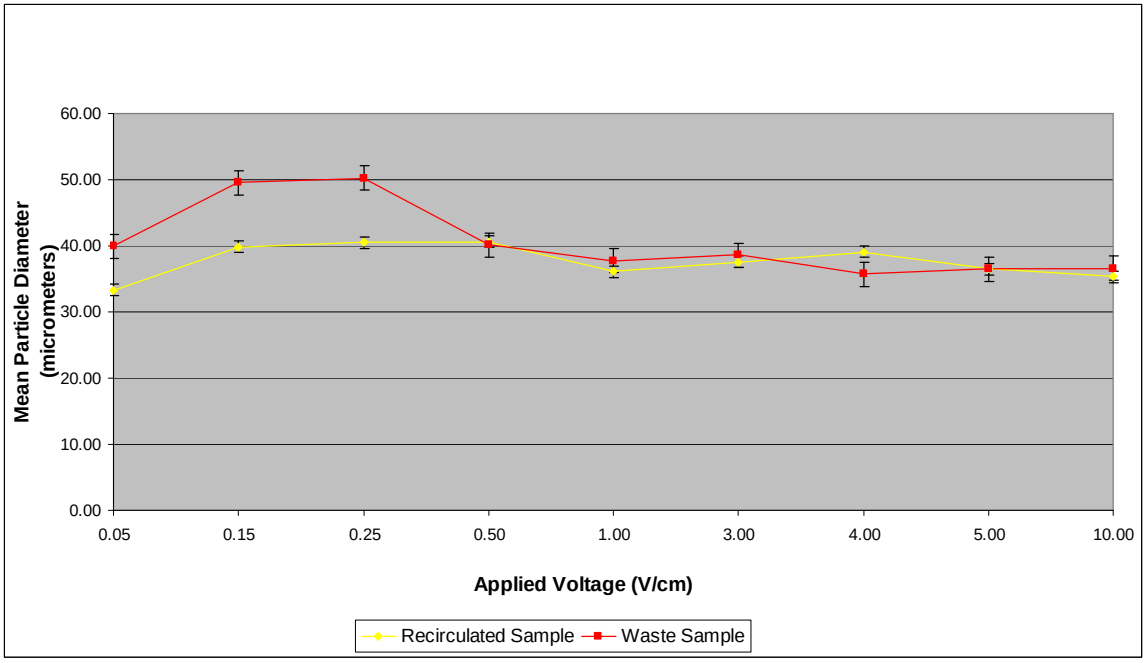


Figure 19. Mean particle size for the College Park block

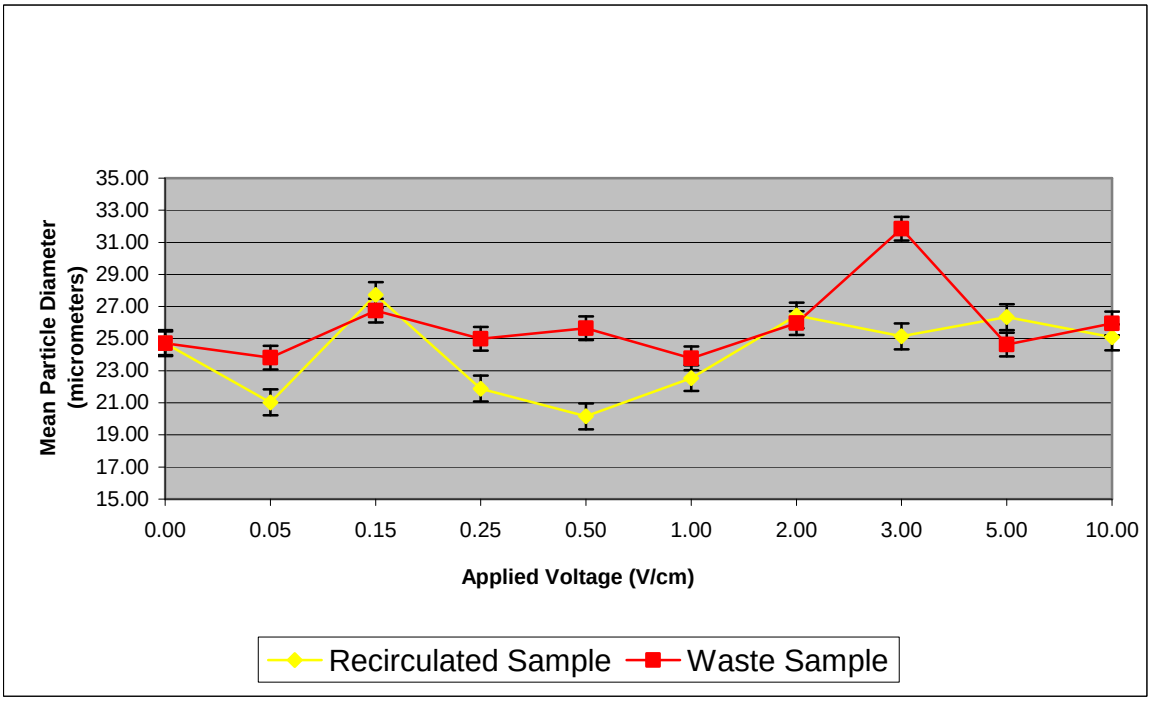


Figure 20. Mean particle size for the Frederick block

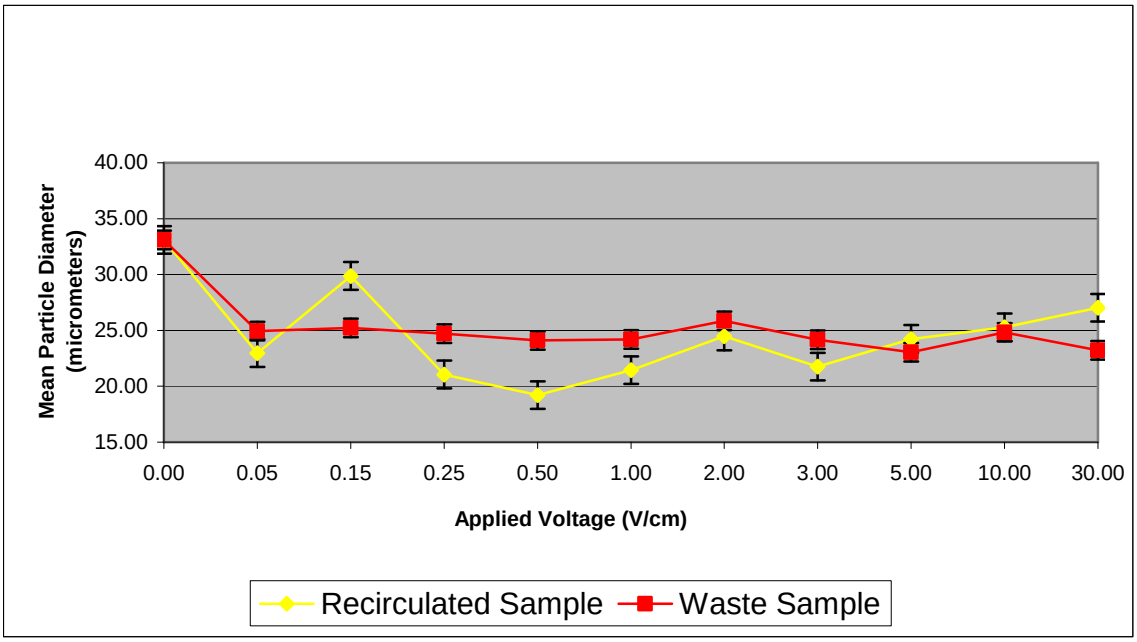


Figure 21. Mean particle size for the Church Creek block

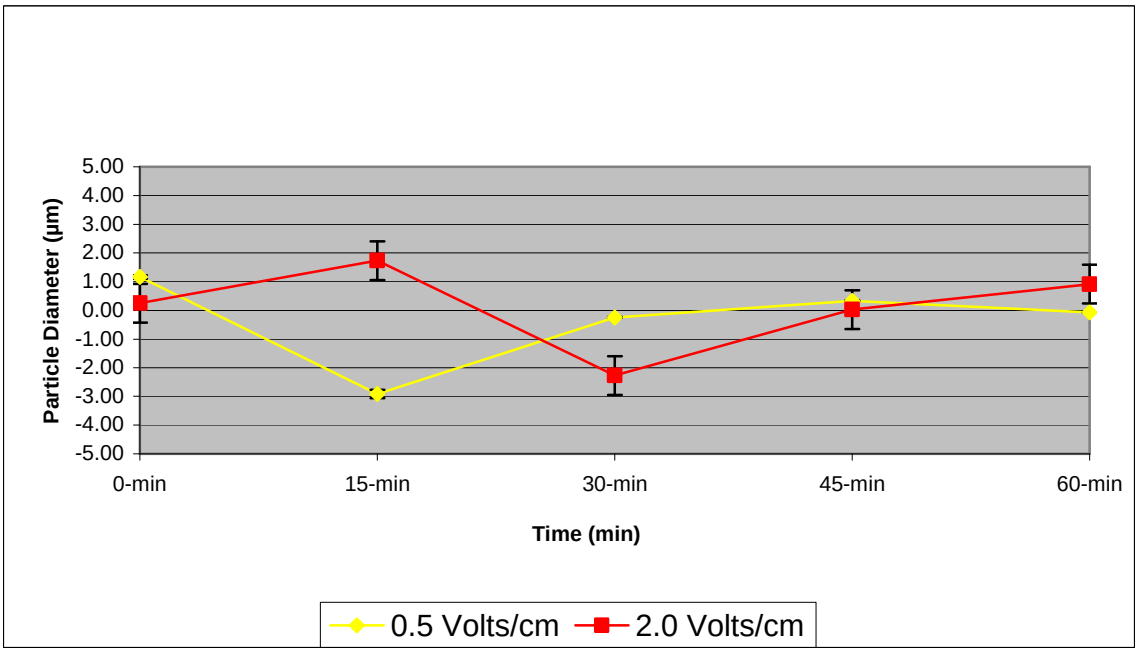


Figure 22. Mean particle size difference (S_w-S_r) for the Preston block

The four graphs above show only the mean particle size and do not give any indication as to particle size distribution. In order to determine if there is a difference in particle size distribution between the recirculating and waste streams we must look to other factors that characterize distribution. Figure 18 below presents a typical graph of the particle size distribution for the aquaculture effluent from the Church Creek site before being passed through the electrophoretic cell. This general right skewed pattern is seen in virtually all samples taken, and was generated using the Coulter Counter software.

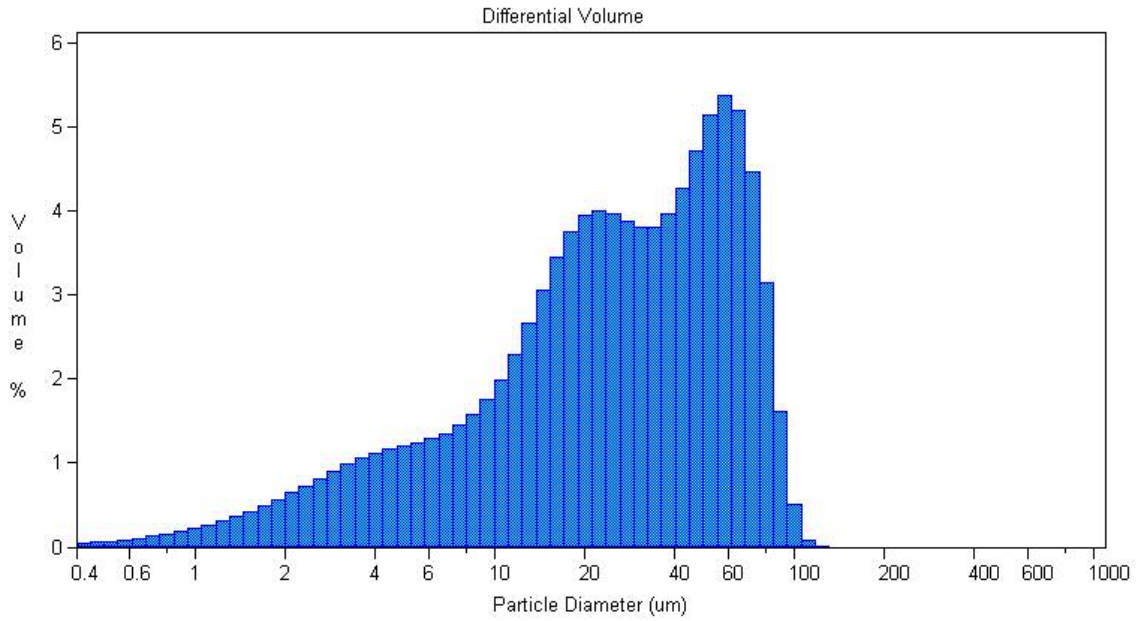


Figure 23. Typical particle distribution graph for aquaculture effluent showing particle distribution for the Church Creek sample before being passed through the electrophoretic cell.

A minimum of three particle size distribution plots were made for each sample taken, resulting in literally hundreds of such plots, making a full presentation of this data impractical and the analysis of statistical differences more difficult. However, the Coulter Counter does provide a number of bits of information that make the

analysis possible. First, virtually all plots obtained in this study are right skewed to a varying degree, characterized by a skewness factor calculated by the Coulter Counter software. Secondly, the Coulter Counter calculates a standard deviation for each plot, thereby characterizing the spread of the particle size distribution. In determining if a difference in particle distribution exists between the recirculated and waste streams, the skewness factor and standard deviation values obtained from the Coulter Counter data for each particle distribution plot were separately averaged and compared using *SAS* to determine if any statistical difference occurs. The skewness factor and standard deviation values were treated in the same manner as other measured water quality parameters and underwent the same statistical analysis.

Graphical presentation of skewness factors and standard deviation values is less meaningful, therefore Tables 10 and 11 below are included to show both the mean calculated skewness factor and the mean particle distribution plot standard deviation for the grouped recirculating and waste streams for each treatment block. Also included in the table is the standard error (SE) associated with the measured mean values and probabilities that the paired means are statistically different. In the tables below a high positive skew value indicates a heavy pull to the larger particle size diameter values in the plot distribution, while a high standard deviation indicates a wider spread in particle diameter size.

Table 10. Mean particle distribution plot skewness factors and paired *t*-test probability values for each treatment block.

Treatment Block:	Grouped Sample:	Skew Factor Mean Value ±SE:	Variance:	*t*-Value Probability:
College Park	Recirculating	0.4004±0.105	Equal	0.0862
College Park	Waste	0.2518±0.127		
Frederick	Recirculating	1.3890±0.131	Equal	0.0003
Frederick	Waste	0.9913±0.114		
Church Creek	Recirculating	1.1145±0.178	Unequal	0.3438
Church Creek	Waste	4.4860±0.962		

Table 11. Mean particle distribution plot standard deviation values and paired *t*-test probability values for each treatment block.

Treatment Block:	Grouped Sample:	Mean Value (μm) ±SE:	Variance:	*t*-Value Probability:
College Park	Recirculating	19.49±1.471	Equal	0.7305
College Park	Waste	19.87±1.683		
Frederick	Recirculating	22.24±1.387	Equal	0.5141
Frederick	Waste	21.37±2.258		
Church Creek	Recirculating	22.30±3.313	Unequal	0.6930
Church Creek	Waste	20.8±0.614		

The paired probability data presented above show a significant difference was observed for the skew value of the Frederick block, as indicated by the obtained *t*-value probability of 0.0003, however no significant difference was seen for the College Park or Church Creek blocks. Likewise, none of the treatment blocks showed a significant difference for the plot standard deviation values.

Dissolved Ions:

The results presented above deal exclusively with the effects of the electric field on the suspended particles themselves. However, equal consideration should be

extended to the effects of the electric field on the dissolved or dissociated ions within the fluid stream. These ions are also influenced by the presence of an electric field but lack the mass associated with larger suspended solids. For the most part this aspect of the study was not explored until the Church Creek sample was taken, and therefore for most of the tests there exist only the Church Creek and Preston treatment blocks to evaluate.

It is hypothesized that ions with a negative charge will tend to accumulate near the positive electrode thereby showing up in higher concentration in the waste stream; while ions with a positive charge will appear in higher concentration in the recirculating stream. Figures 24, 25 and 26 show pH levels for the College Park, Church Creek and Preston treatment blocks. A significant treatment effect was observed for the College Park and Preston blocks, as indicated by the calculated probabilities of 0.0023 and 0.0001, respectively; but was not found to be significant for the Church Creek block, which had a probability of 0.2125. However, as would be expected, a significantly difference was shown for the overall combined data. This is consistent with the hypothesis in that the waste stream is clearly more basic, higher (OH^-) concentration; while the recirculating stream was more acidic, higher (H_3O^+) concentration.

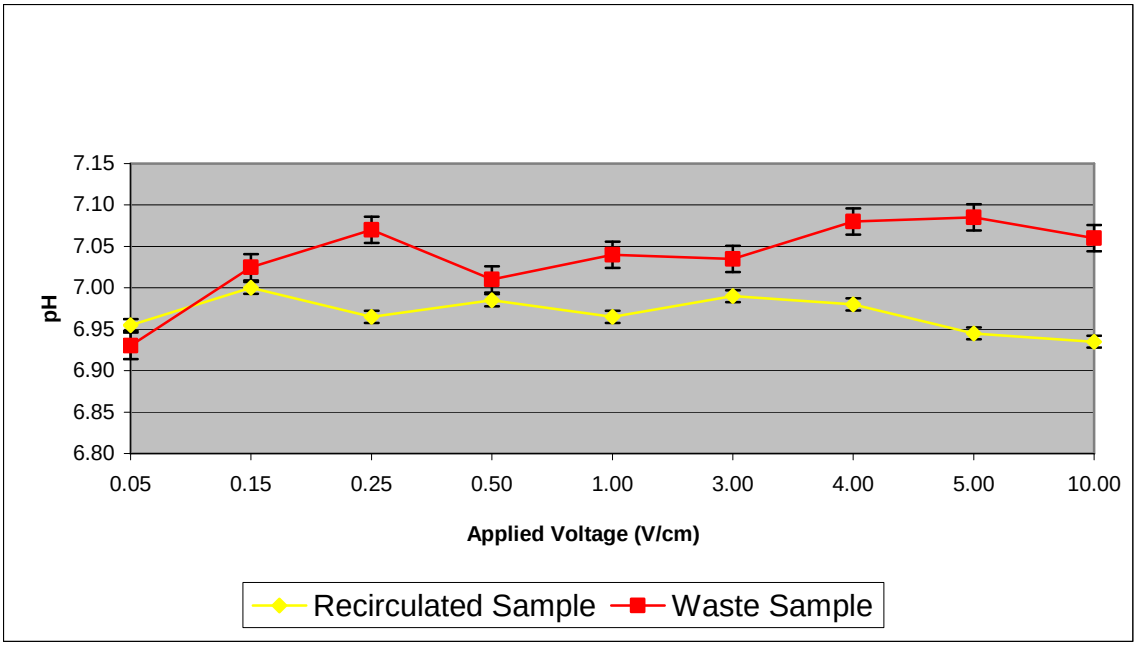


Figure 24. Mean pH for the College Park block

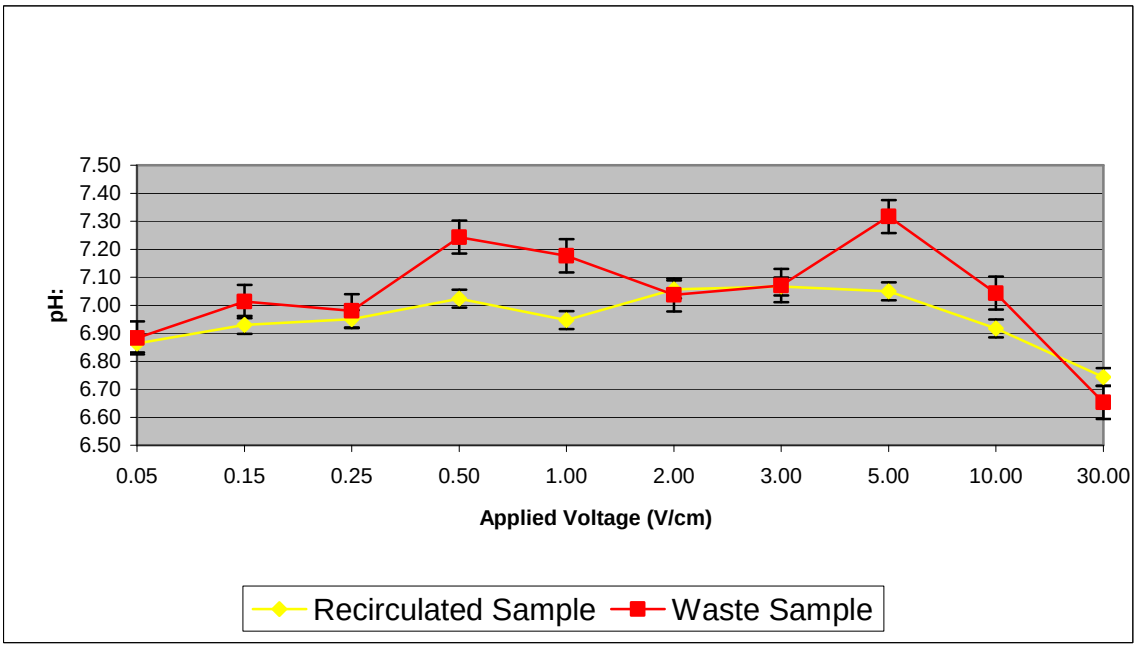


Figure 25. Mean pH for the Church Creek block

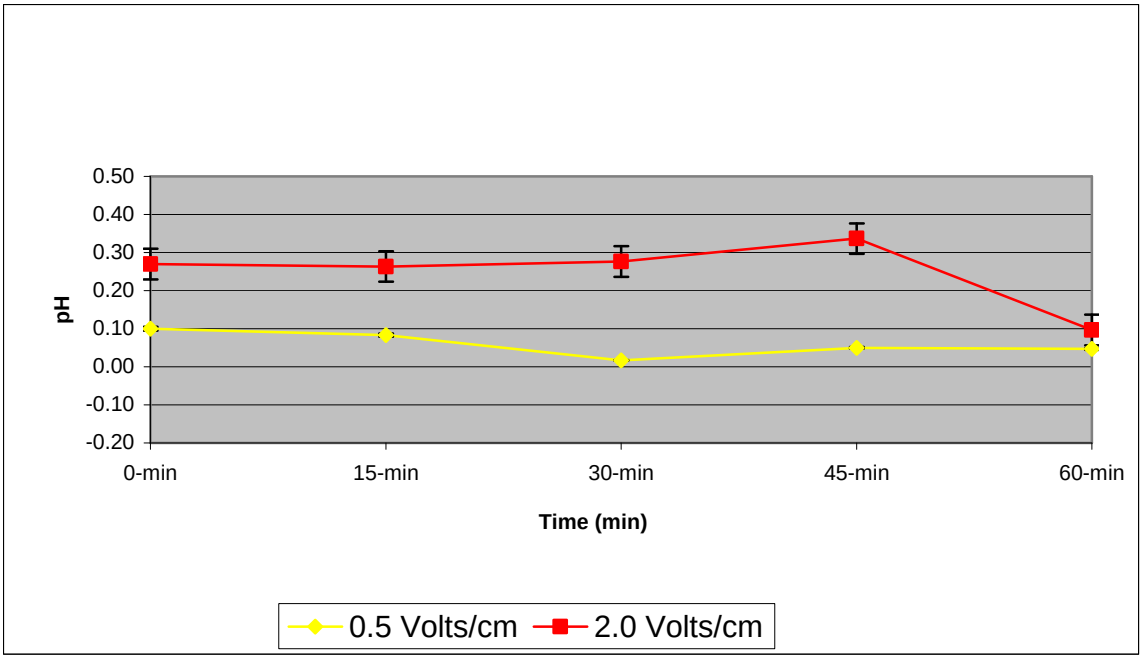


Figure 26. Mean pH difference (S_w-S_r) for the Preston block

Alkalinity values appeared to be significantly different for the Church Creek block (0.0017), but did not appear significantly different for the College Park and Preston blocks, 0.4884 and 0.4653. However, the combined data did show an overall significant probability of 0.0099. Figures 27 and 28 show alkalinity concentrations results for the Church Creek and Preston blocks, respectively.

Phosphate concentrations were measured for the Church Creek and Preston blocks, and the probability associated with the difference between the recirculating and waste streams was found to be 0.0987 and 0.1325, and was not found to be significant overall. However it is worth noting that both of these values are relatively low albeit not significantly different. Figures 29 and 30 compare phosphate concentrations for the Church Creek and Preston treatment blocks, respectively

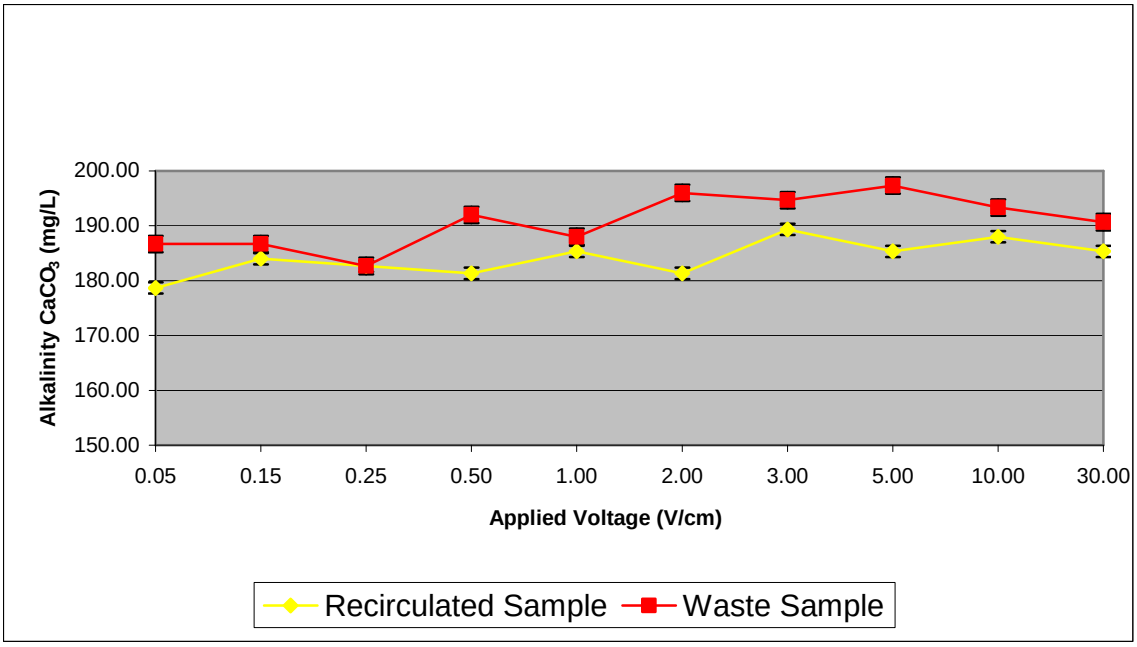


Figure 27. Alkalinity concentration for the Church Creek block

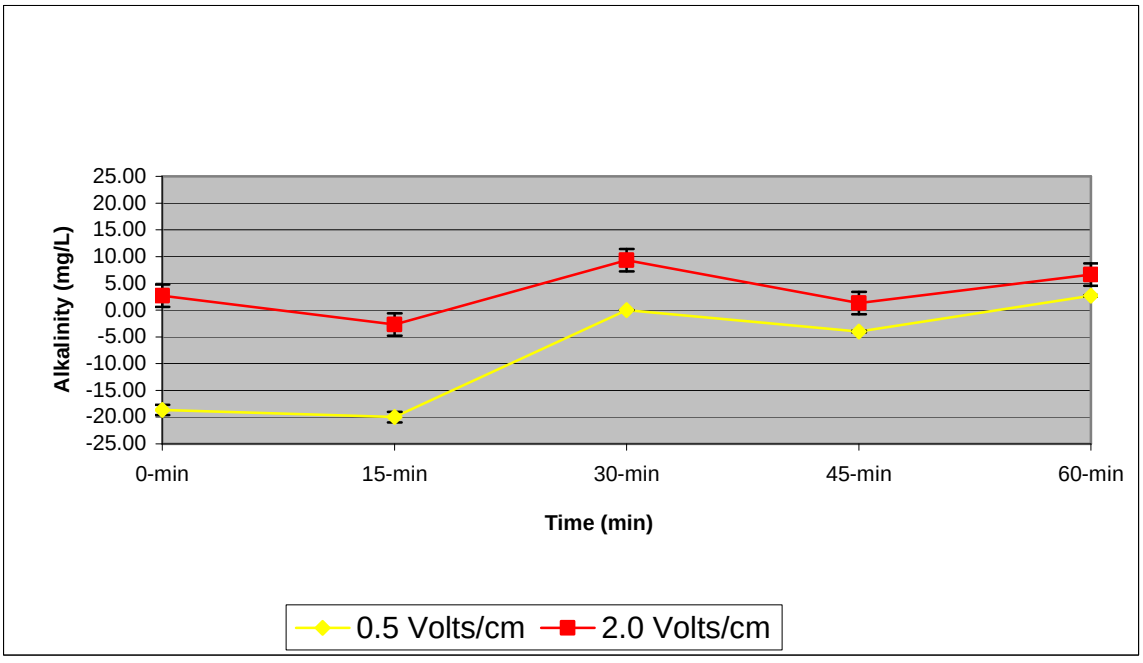


Figure 28. Alkalinity concentration difference (S_w-S_r) for the Preston block

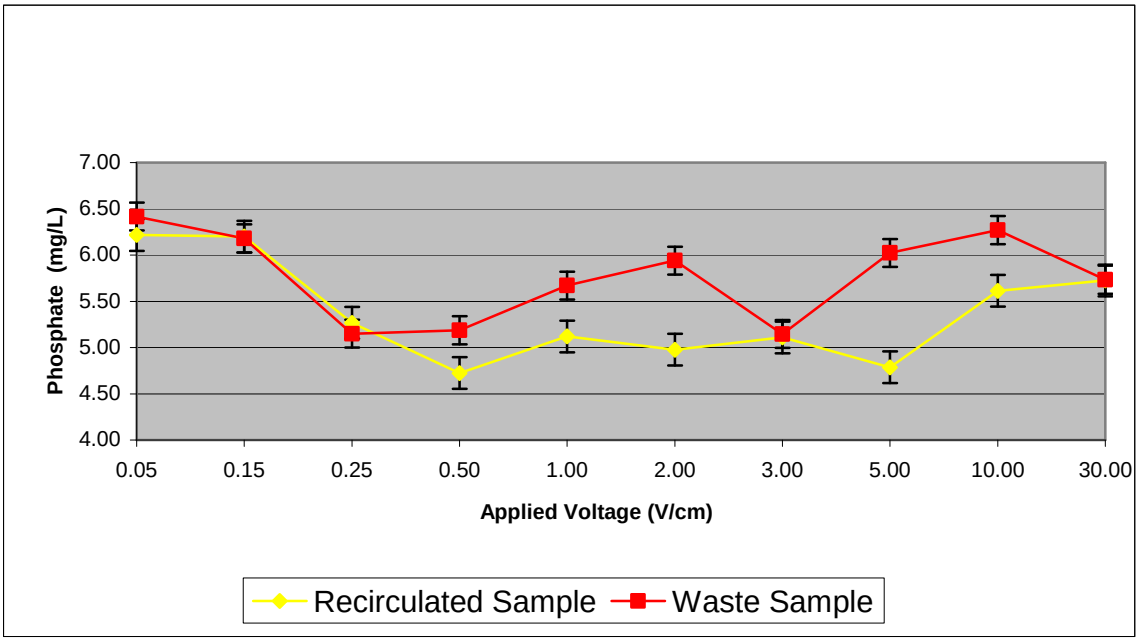


Figure 29. Phosphate concentration for the Church Creek block

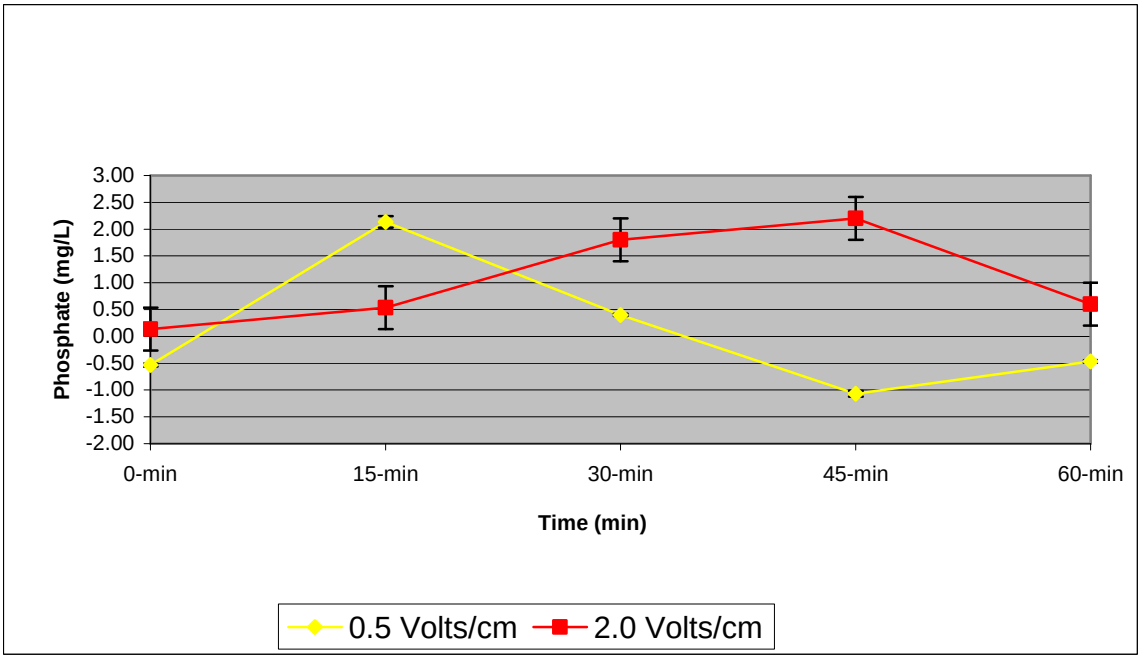


Figure 30. Phosphate concentration difference (S_w-S_r) for the Preston block

The overall the dissolved nitrogen concentrations for ammonia, nitrate and nitrite for the Church Creek and Preston blocks were not significantly different between recirculating and waste stream samples. The *t*-test results comparing the average S_w with S_r had probability values of 0.5625 and 0.3057 for the ammonia tests, 0.0259 and 0.7710 for the nitrate test, and 0.3640 and 0.7710 for the nitrite tests for the Church Creek and Preston blocks, respectively. Only the concentration difference for the nitrate values for the Church Creek block was found to be significantly different, but none of these values was found to be significant overall with the combined data. One possible explanation for this is that both nitrite and ammonia appear in relatively low concentrations, compared to the nitrate and alkalinity levels in particular, making a concentration distinction more difficult. Furthermore, ammonia exists in both the ionized (NH_3) and un-ionized (NH_4^+) forms, and only the ionized form is subject to migration in the presence of an electric field. Figures 31 and 32 below present ammonia concentrations for the Church Creek and Preston blocks respectively; figures 33 and 34 show nitrate concentrations; while figures 35 and 36 present nitrite concentrations for each treatment block, respectively.

It should be noted that the results presented for the ammonia concentrations show that, in general, the ammonia concentration appears to be higher in the recirculating stream when compared to the waste stream. In figure 32 this is seen by the mostly negative differential concentrations ($S_w - S_r$). This is expected as the ionized form of ammonia is positively charged and therefore is likely to be pulled toward the negatively charged electrode, and therefore into the recirculating stream. The actual Hach method used (Method # 8038: Nessler Method) measures the

concentration of the un-ionized form of ammonia (NH_3), nevertheless the ionized and un-ionized forms of ammonia exist in equilibrium, and therefore it can be expected that the concentration of the ionized from of ammonia is proportional to that of the un-ionized form given a constant pH.

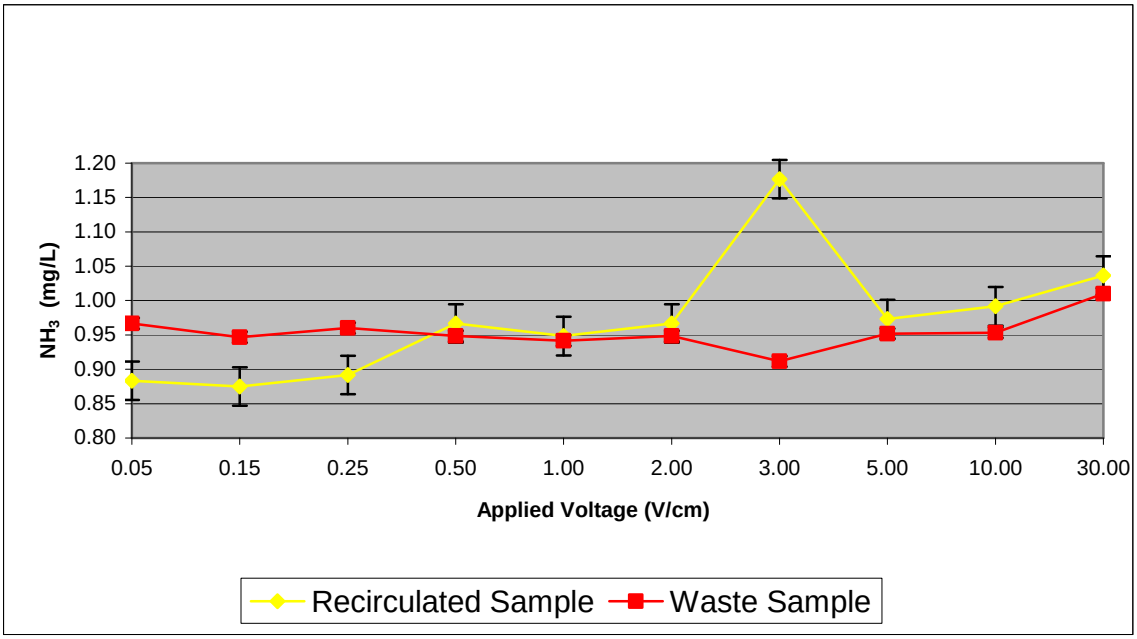


Figure 31. Ammonia concentration for the Church Creek block

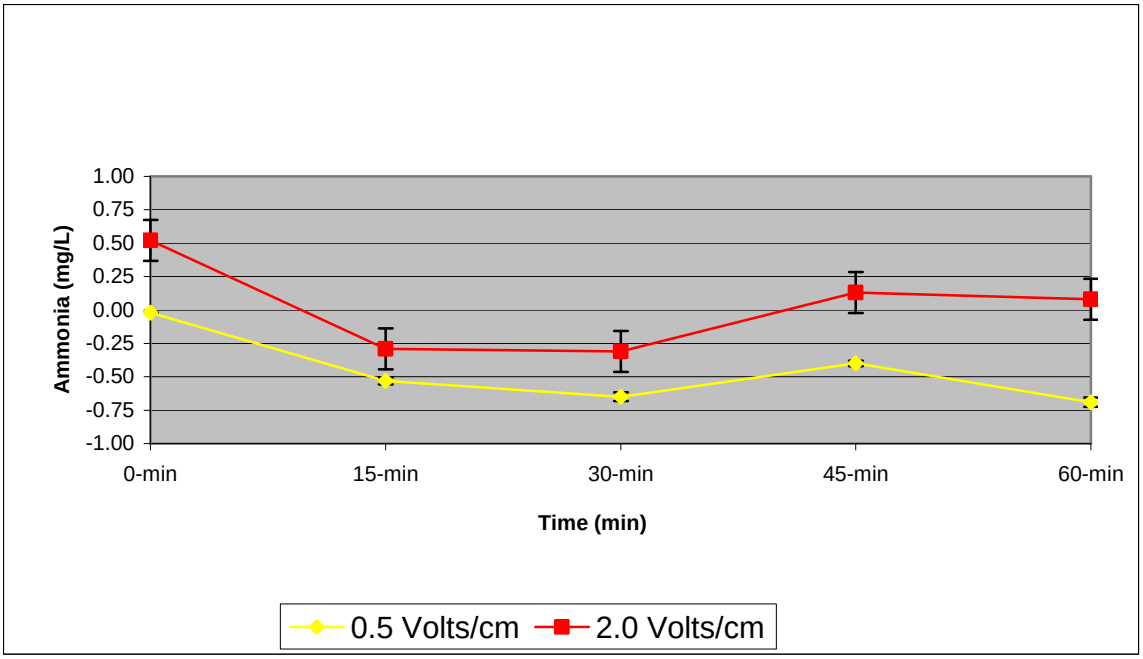


Figure 32. Ammonia concentration difference (S_w-S_r) for the Preston block

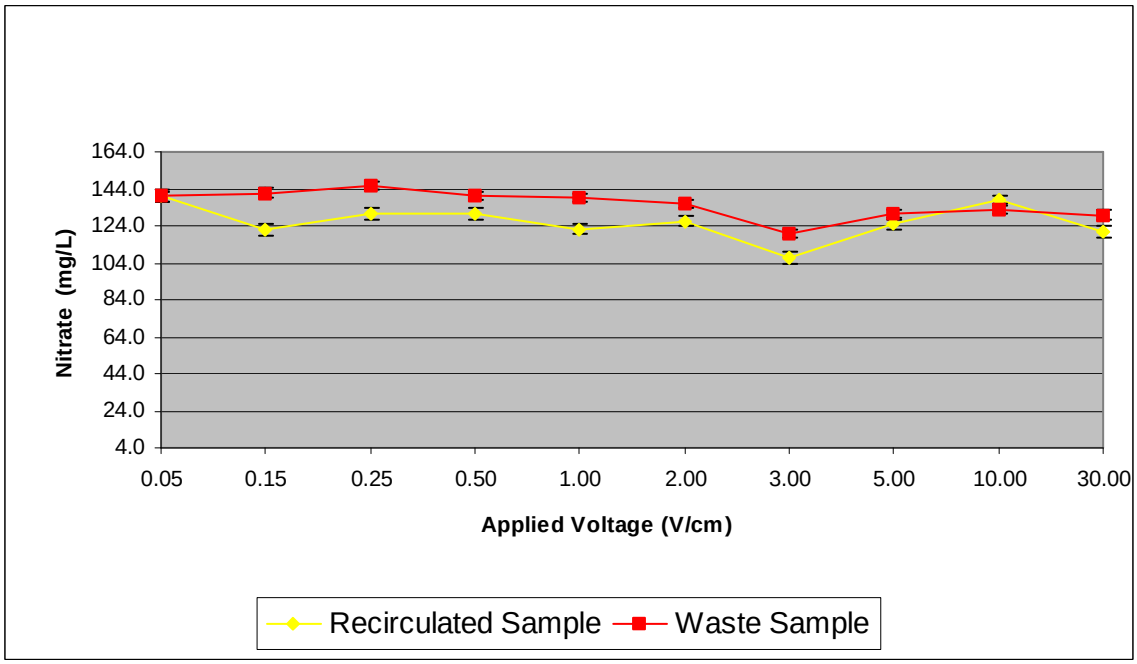


Figure 33. Nitrate concentration for the Church Creek block

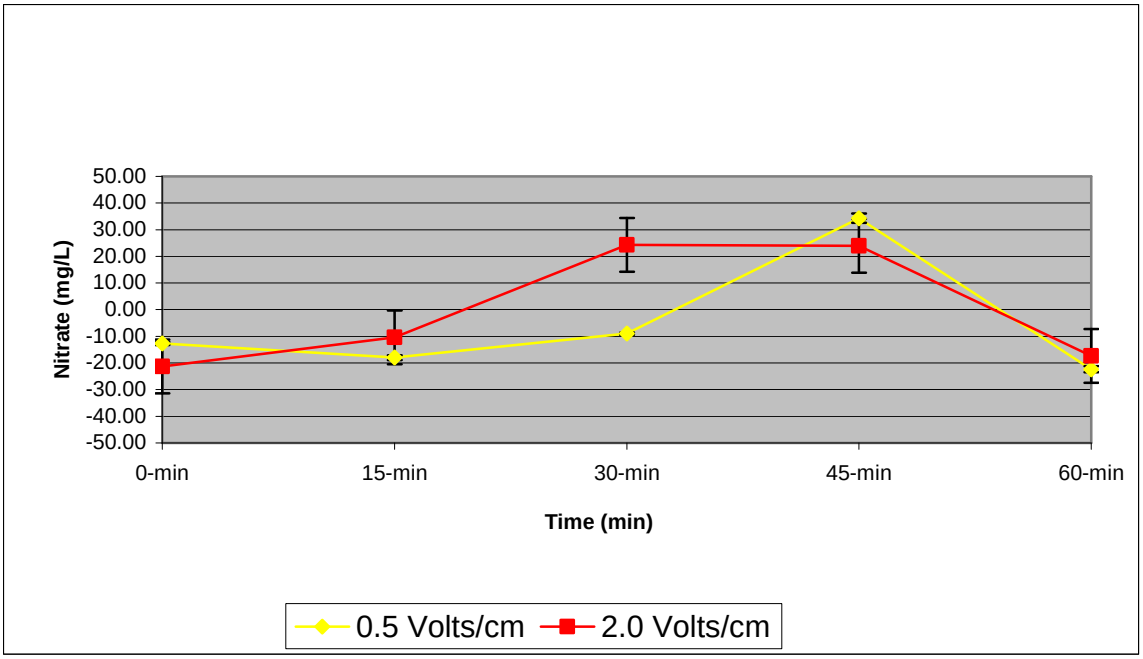


Figure 34. Nitrate concentration difference (S_w-S_r) for the Preston block

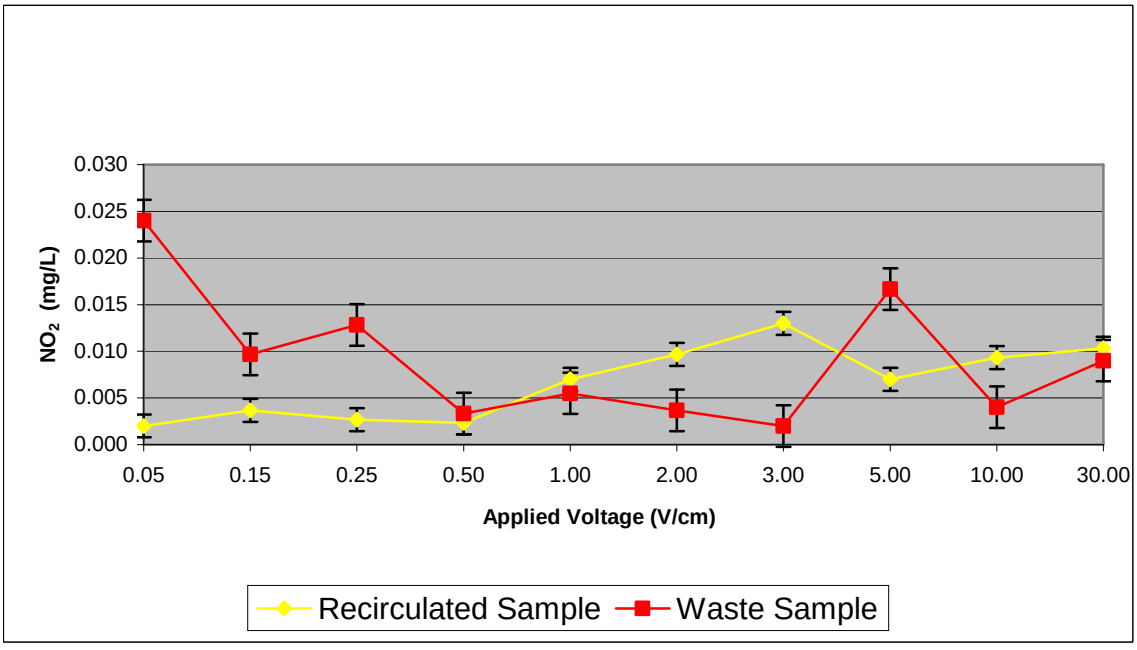


Figure 35. Nitrite concentration for the Church Creek block

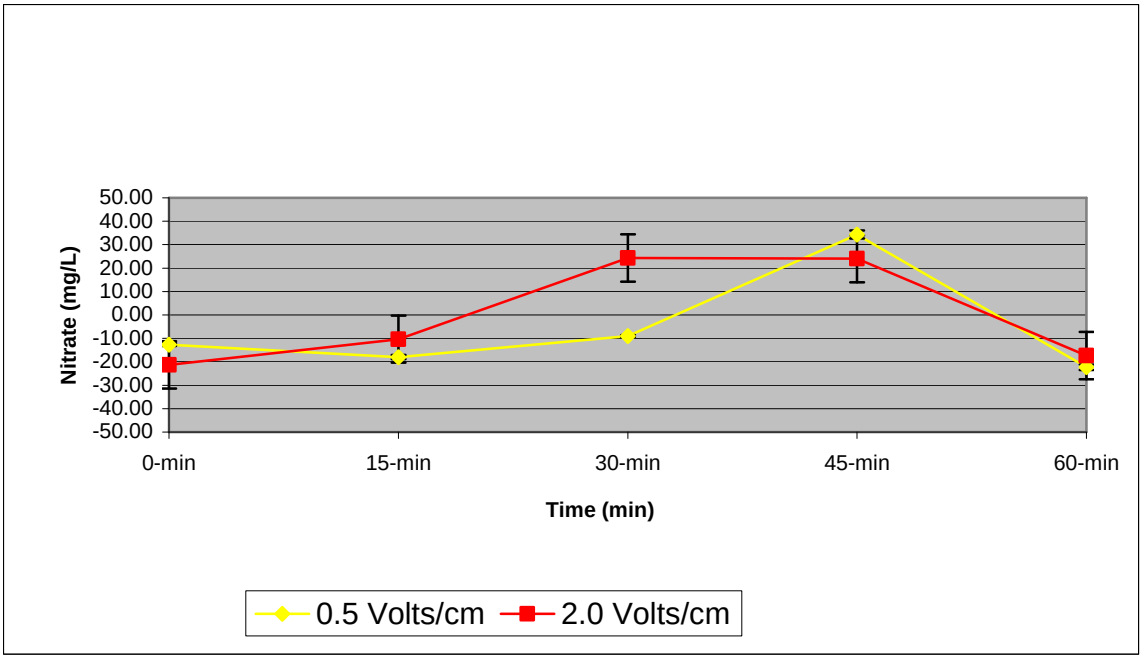


Figure 36. Nitrite concentration difference (S_w-S_r) for the Preston block

Conductivity Data:

Another important yet non-specific measure of dissolved ion content is the associated conductivity values of the water samples. Conductivity is a measure of the fluid's ability to conduct an electrical current and as such it is a good indication of the overall dissolved ion concentration of the sample. Conductivity measurements were shown to have significantly different values for the Frederick and Church Creek blocks but not for the Preston block as indicated by the probability values of 0.0002, 0.0177 and 0.2192, respectively. The probability associated with the combined data was just barely outside of being significant as indicated by the 0.0511 probability value. Figures 37, 38 and 39 below show the spread of conductivity values for the Frederick, Church Creek and Preston blocks, respectively.

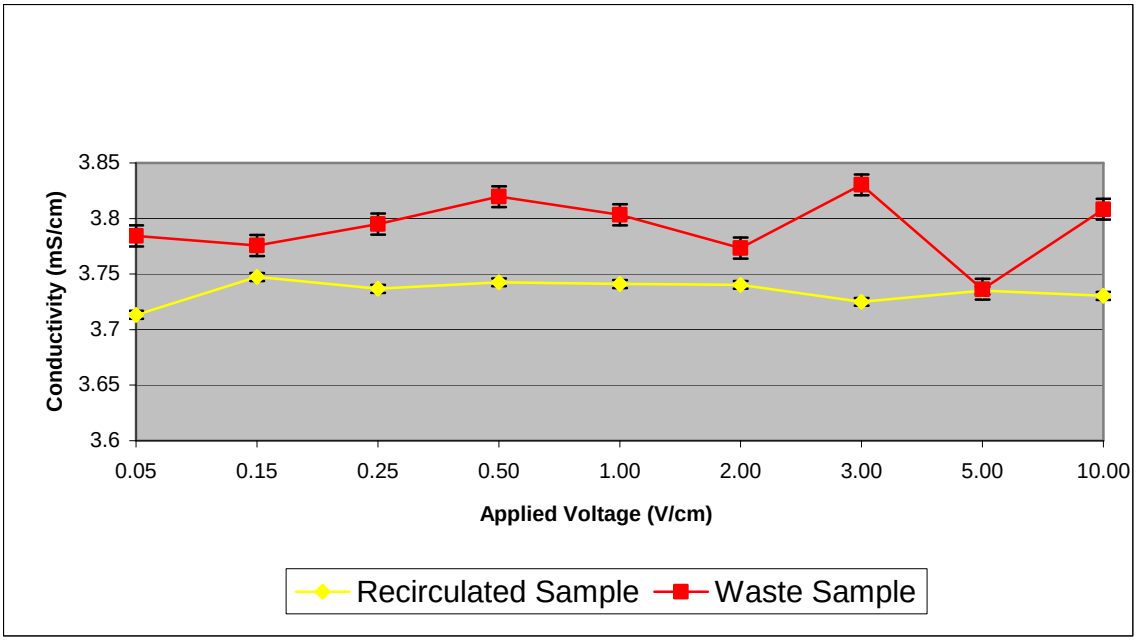


Figure 37. Conductivity values for the Frederick block

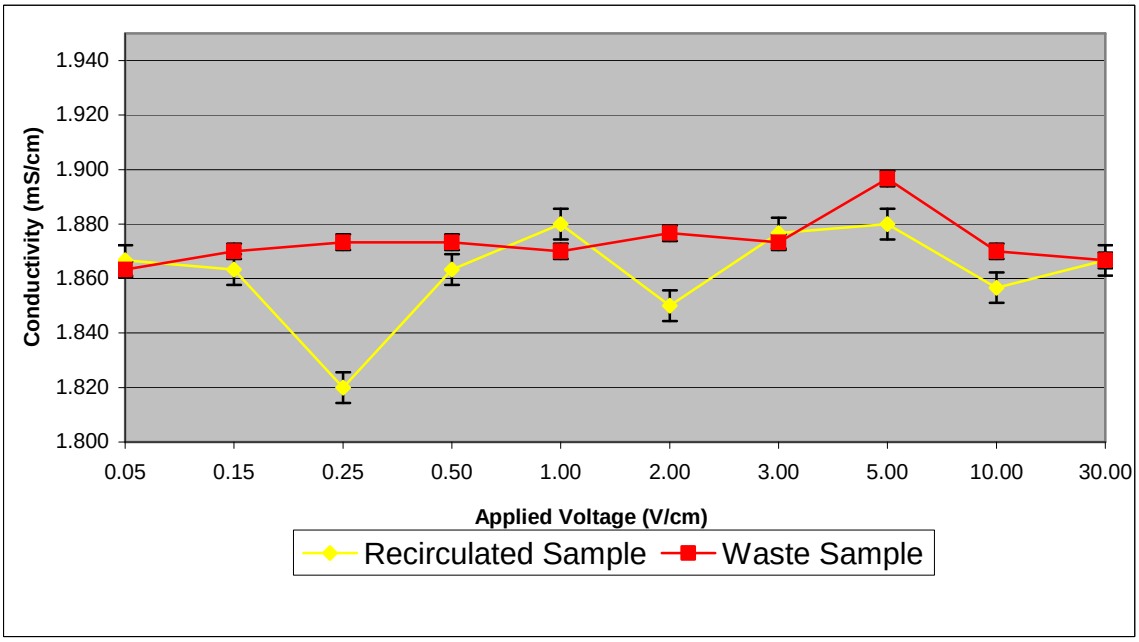


Figure 38. Conductivity values for the Church Creek block

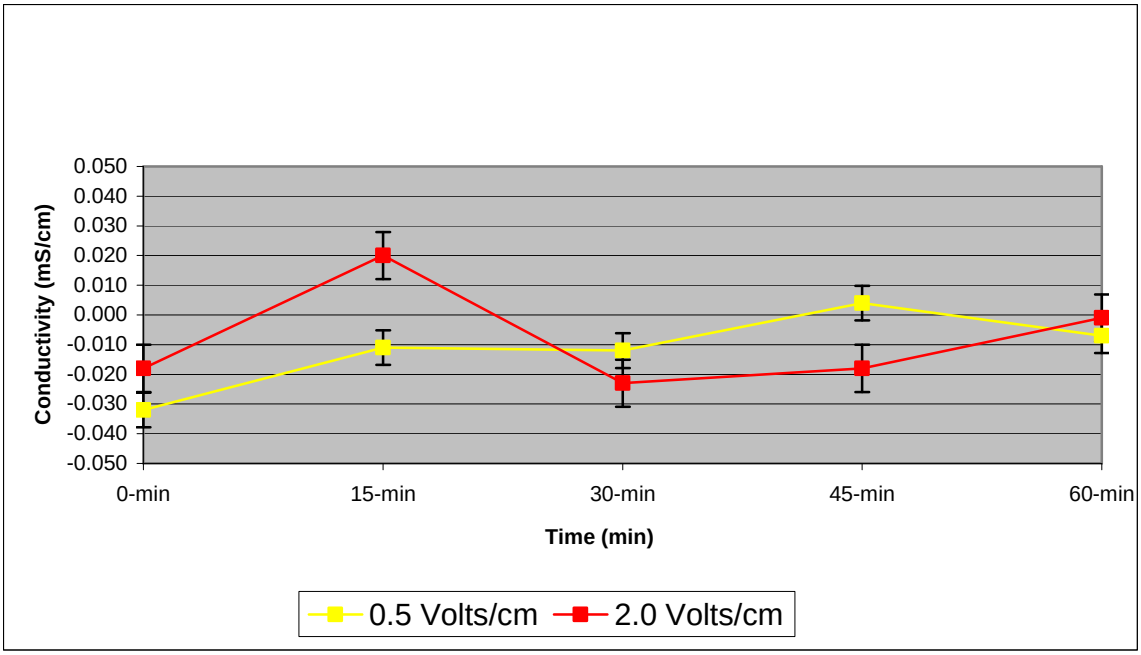


Figure 39. Conductivity difference (S_w-S_r) values for the Preston block

Summary of Statistical Data:

The above graphs show visually the spread and division of sample means between the recirculating and the waste streams. A more analytic assessment of filtration effectiveness must involve a summary of statistical information. At the start of this section the ANOVA results for the combined data defined those water quality parameters that where shown to be statistically significant overall. However, it is constructive to look closer at each individual treatment block in order to determine statistically differences within each block, thereby eliminating any biases that may have appeared due to a single treatment block.

To this end a paired *t*-test was performed between the recirculating and waste streams as a whole for each treatment block and on each individual treatment level in order to determine if any main effect or simple effect exists. The validity of this

statistical evaluation requires that the data both be normally distributed and have equal variances. If variances are found to be equal within a 95% probability the *t*-value calculated using pooled variances is reported here, if variances are not found to be equal within a 95% confidence interval a more conservative *t*-value is reported here calculated by *SAS* using the Satterthwaite method (SAS, 1999). Table 12 below presents those water quality parameters that were found to be significantly different between the grouped recirculating and waste stream data within a 95% probability for each treatment block. The probability values presented are taken for equal or unequal variances; variance values are said to be equal if the probability of the '*F*' value determined by *SAS* using the 'Folded F' method is within a 95% probability (SAS, 1999). Those water quality tests that were found not to be significantly different are presented in Table 13.

Table 12. Water quality parameters found to be significantly different for each treatment block ($p \leq 0.05$).

Block:	**Water Quality Test:**	**Variance:**	**Degrees of Freedom:**	**Pr (*t*):**
College Park	pH	Unequal	17	0.0023
Frederick	Conductivity	Unequal	28	0.0002
Frederick	Total Solids	Equal	26	0.0418
Frederick	Turbidity	Unequal	44	0.0269
Church Creek	Alkalinity	Equal	29	0.0017
Church Creek	Conductivity	Unequal	29	0.0177
Church Creek	Nitrate	Equal	29	0.0259
Preston	pH	Equal	29	0.0001

Table 13. Water quality parameters found not to be significantly different for each treatment block.

Block:	Water Quality Test:	Variance:	Degrees of Freedom	Pr (*t*):
College Park	Alkalinity	Unequal	8	0.4884
College Park	Particle Distribution	Unequal	44	0.1713
College Park	Total Solids	Equal	26	0.9803
College Park	Turbidity	Equal	26	0.1798
Frederick	Mobility	Equal	27	0.5091
Frederick	Particle Distribution	Equal	29	0.1379
Frederick	Zeta Potential	Equal	27	0.5360
Church Creek	Ammonia	Unequal	29	0.5625
Church Creek	Mobility	Equal	43	0.0815
Church Creek	Nitrite	Equal	29	0.3640
Church Creek	Particle Distribution	Equal	29	0.5299
Church Creek	pH	Equal	29	0.2125
Church Creek	Phosphate	Equal	29	0.0987
Church Creek	Total Solids	Equal	29	0.6409
Church Creek	Turbidity	Equal	29	0.7118
Church Creek	Zeta Potential	Equal	44	0.1412
Preston	Ammonia	Equal	29	0.3057
Preston	Alkalinity	Equal	29	0.4653
Preston	Conductivity	Equal	29	0.2192
Preston	Mobility	Equal	29	0.0773
Preston	Particle Distribution	Equal	29	0.9074
Preston	Phosphate	Equal	29	0.1325
Preston	Nitrate	Equal	29	0.7710
Preston	Nitrite	Unequal	29	0.8299
Preston	Total Solids	Unequal	29	0.0704
Preston	Turbidity	Equal	29	0.9557
Preston	Zeta Potential	Equal	29	0.1749

Tables 14, 15, 16 and 17 below present the probability values for those individual comparisons that were found to be significantly different for the College Park, Frederick, Church Creek and Preston treatment blocks, respectively. Probability values for treatment levels not found to be significantly different were not included in the tables due to the large number of entries.

Table 14. List of individual comparisons for each electric field potential found to be significantly different for the College Park treatment block ($p \leq 0.05$).

Block:	**Electric Field (V/cm):**	**Water Quality Test:**	**Sample Size (n):**	**Pr (*t*):**
College Park	4.0	pH	3	0.0635
College Park	5.0	pH	3	0.0454
College Park	0.05	Particle Distribution	5	0.0031
College Park	0.15	Particle Distribution	5	0.0116
College Park	0.25	Particle Distribution	5	0.0001

Table 15. List of individual comparisons for each electric field potential found to be significantly different for the Frederick treatment block ($p \leq 0.05$).

Block:	Electric Field (V/cm):	Water Quality Test:	Sample Size (n):	Pr (*t*):
Frederick	0.05	Conductivity	3	0.0308
Frederick	0.25	Conductivity	3	0.0068
Frederick	0.50	Conductivity	3	0.0024
Frederick	1.0	Conductivity	3	0.0112
Frederick	2.0	Conductivity	3	0.0187
Frederick	3.0	Conductivity	3	0.0057
Frederick	10.0	Conductivity	3	0.0001
Frederick	0.05	Particle Distribution	3	0.0124
Frederick	0.25	Particle Distribution	3	0.0022
Frederick	0.50	Particle Distribution	3	0.0017
Frederick	1.0	Particle Distribution	3	0.0051
Frederick	3.0	Particle Distribution	3	0.0021
Frederick	5.0	Particle Distribution	3	0.0018
Frederick	0.15	Total Solids	3	0.0112
Frederick	0.05	Turbidity	5	0.0001
Frederick	0.15	Turbidity	5	0.0001
Frederick	0.25	Turbidity	5	0.0004
Frederick	0.50	Turbidity	5	0.0009
Frederick	1.0	Turbidity	5	0.0032
Frederick	2.0	Turbidity	5	0.0001
Frederick	3.0	Turbidity	5	0.0027
Frederick	5.0	Turbidity	5	0.0001
Frederick	10.0	Turbidity	5	0.0001
Frederick	0.50	Zeta Potential	3	0.0489

Table 16. List of individual comparisons for each electric field potential found to be significantly different for the Church Creek treatment block ($p \leq 0.05$).

Block:	Electric Field (V/cm):	Water Quality Test:	Sample Size (n):	Pr (*t*):
Church Creek	0.05	Ammonia	3	0.0122
Church Creek	0.25	Ammonia	3	0.0376
Church Creek	3.0	Ammonia	3	0.0201
Church Creek	30.0	Ammonia	3	0.0001
Church Creek	1.0	Conductivity	3	0.0076
Church Creek	3.0	Conductivity	3	0.0062
Church Creek	0.05	Particle Distribution	3	0.0050
Church Creek	0.25	Particle Distribution	3	0.0072
Church Creek	0.50	Particle Distribution	3	0.0257
Church Creek	1.0	Particle Distribution	3	0.0083
Church Creek	3.0	Particle Distribution	3	0.0100
Church Creek	5.0	Particle Distribution	3	0.0091
Church Creek	0.15	pH	3	0.0202
Church Creek	0.50	pH	3	0.0108
Church Creek	1.0	pH	3	0.0118
Church Creek	5.0	pH	3	0.0020
Church Creek	10.0	pH	3	0.0285
Church Creek	30.0	pH	3	0.0001
Church Creek	0.500	Phosphate	3	0.0182
Church Creek	3.0	Phosphate	3	0.0396
Church Creek	0.25	Nitrate	3	0.0329
Church Creek	1.0	Nitrate	3	0.0138
Church Creek	2.0	Nitrate	3	0.0249
Church Creek	3.0	Nitrate	3	0.0239
Church Creek	0.15	Nitrite	3	0.0453
Church Creek	2.0	Nitrite	3	0.0288
Church Creek	5.0	Nitrite	3	0.0193
Church Creek	10.0	Nitrite	3	0.0290
Church Creek	3.0	Total Solids	3	0.0135
Church Creek	0.05	Turbidity	3	0.0225

Church Creek	0.50	Turbidity	3	0.0111
Church Creek	2.0	Turbidity	3	0.0170
Church Creek	3.0	Turbidity	3	0.0040
Church Creek	5.0	Turbidity	3	0.0001
Church Creek	10.0	Turbidity	3	0.0121
Church Creek	30.0	Turbidity	3	0.0010

Table 17. List of individual comparisons for each electric field potential found to be significantly different for the Preston treatment block ($p \leq 0.05$).

Block:	Electric Field (V/cm) –Time (min):	Water Quality Test:	Sample Size (n):	Pr (*t*):
Preston	0.05-30 min	Ammonia	3	0.0073
Preston	0.05-45 min	Ammonia	3	0.0006
Preston	0.05-60 min	Ammonia	3	0.0364
Preston	2.0-15 min	Ammonia	3	0.0349
Preston	2.0-45 min	Ammonia	3	0.0279
Preston	2.0-60	Alkalinity	3	0.0377
Preston	2.0-30 min	Conductivity	3	0.0029
Preston	0.5-15 min	Particle Distribution	3	0.0182
Preston	0.05-0 min	pH	3	0.0225
Preston	0.05-15 min	pH	3	0.0202
Preston	0.05-45 min	pH	3	0.0131
Preston	2.0-0 min	pH	3	0.0059
Preston	2.0-15 min	pH	3	0.0006
Preston	2.0-30 min	pH	3	0.0040
Preston	2.0-45 min	pH	3	0.0016
Preston	2.0-60 min	pH	3	0.0047
Preston	0.5-15 min	Phosphate	3	0.0343
Preston	2.0-30 min	Phosphate	3	0.0276
Preston	2.0-45 min	Phosphate	3	0.0082
Preston	2.0-60 min	Phosphate	3	0.0351
Preston	0.05-45 min	Nitrite	3	0.0055
Preston	2.0-15 min	Nitrite	3	0.0002
Preston	2.0-45 min	Nitrite	3	0.0187
Preston	2.0-45 min	Total Solids	3	0.0488
Preston	0.05-0 min	Turbidity	3	0.0206
Preston	0.05-30 min	Turbidity	3	0.0043
Preston	2.0-0 min	Turbidity	3	0.0014
Preston	2.0-15 min	Turbidity	3	0.0047
Preston	2.0-30 min	Turbidity	3	0.0117

The tables above look at both the main and simple effects for each treatment block. This analysis eliminates the block effects associated with the combined data and allows us to take a closer look at individual differences. Table 12 lists those water quality parameters that were shown to be significantly different for each treatment block. In these instances the average of all waste stream values and the average of all recirculating stream values for each water quality parameter is used to determine if a main effect exist. However, in these cases a simple effect is not necessarily observed at all treatment levels as seen in tables 14-17.

Also of significance is the appearance of significantly different comparisons at a number of treatment levels where a significant difference was found for one or more treatment levels for the same water quality parameter, and yet an overall treatment effect was not observed. This is especially true for the Preston block were an overall significant effect was found for only the pH and phosphate tests and yet a large number of individual comparisons are significantly different for a number of distinct water quality parameters being tested.

When we look at the data more closely, specifically with the Preston block, we see that at certain time comparisons for the same electric field strength the recirculating concentration may be higher than that of the waste stream even though in the majority of the cases the opposite is true. Referring back to the graphs presenting the Preston data this is easily seen; in these graphs a positive value indicates that the concentration or measured value was higher in the waste stream than in the recirculating stream, while a negative value indicates that the opposite is

true. In all cases there is at least one occurrence of this switch, associated with either the 0.5 V/cm or 2.0 V/cm treatment levels, with the exception of the pH values where higher pH values are always associated with the waste stream.

We would expect that the difference between the recirculating and waste streams at each 15-minute time step for the same treatment level would be approximately equal. However, the fact that the recirculating measured values are at times higher and at times lower than that of the waste stream at the same electric field strength, combined with the fact that a statistical difference could not be determined for the majority of the water quality parameters, is a clear indication that the natural variation in sample values rivals in magnitude any apparent treatment effect. This means that if a true treatment effect does exist, the number of replications and the sensitivity of the measurements are insufficient to detect a difference in all cases. However, more importantly, the difficulty in detecting a significant difference is a clear indication that the filtration process proposed is not adequate to affect a useful change in the water quality values examined here.

Regression Analysis:

A secondary objective of this study is to examine if a functional relationship exists between the measured water quality parameter and electric field strength, if indeed a measurable effect is observed. To do this a regression analysis was conducted in SAS, which showed that a regression was found only with the particle size difference values between the waste and recirculating streams.

The linear relationship determined using the least squares linear regression calculated in *SAS* was found to be: $y = -0.176x + 1.56$ with an adjusted R-square value of 0.22, where x represents the applied electric field strength (V/cm) and y represents the mean difference ($S_w - S_r$) in particle size (µm). The regression analysis tests the hypothesis that the slope of the best fit line is equal to zero. In the case of the particle size distribution the probability that this is true is determined to be 0.005, indicating that well within a 95% confidence interval a linear regression does exist. No other significant regression was found. Figure 40 below plots the average difference in particle size between the waste and recirculating stream for the combined data. The graph and best-fit line is generated using Microsoft ® Excel, while the equation for the regression line above is generated using *SAS* (version 8.0).

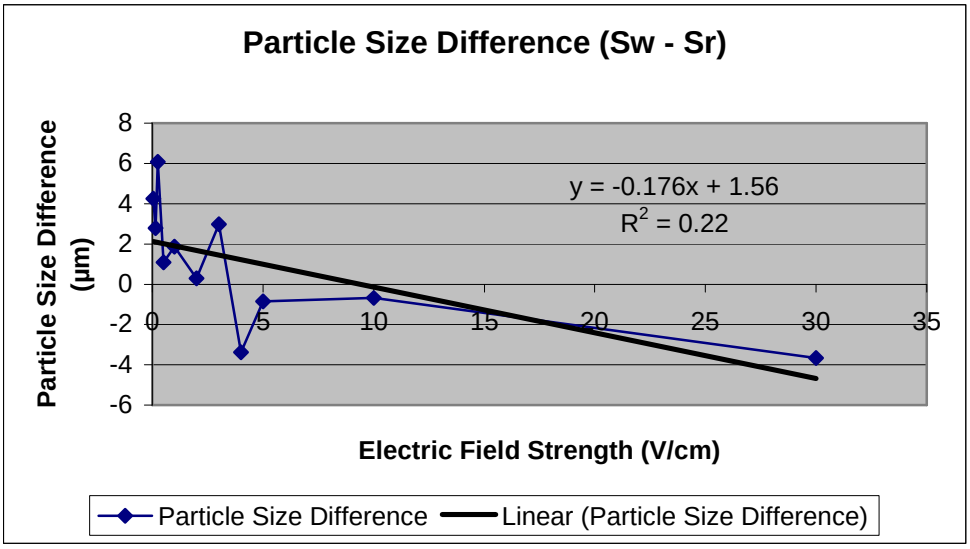


Figure 40. Linear regression of particle size difference (S_w-S_r) values for the combined data.

Interestingly, the relationship was found to have a negative slope indicating that the difference in particle sizes decreases as the electric field strength increases. This relationship, although unexpected, is very reasonable if one considers that under a weak electric field, the applied force on the particle will effectively move only very small particles, while larger heavier particles will remain relatively inert. As a result the relative difference in particle size between the waste and recirculating streams may appear greater, noting that the particle size is not an indication of concentration, although no significant change in concentration was observed. As the electric field strength increases, the applied force on charged particles can be expected to have an effect on both large and small particles alike, making the relative difference in particle size less significant. If this is correct then a corresponding increase in total solids may have also been observed had the sensitivity of the analysis been greater.

It is important to note that the electric field strengths used in this study were not evenly distributed over the given range 0-30 v/cm. Instead more readings are found within the range of 0-5 v/cm than in the remaining 25 v/cm span. As a result the measurements associated with the higher treatment levels, particularly the 30 v/cm treatment level, have a disproportionately higher weight in determining the intercept and the slope of the line associated with the regression curve. Therefore, the regression analysis was run a second time omitting the measurement values taken at an electric field strength of 30 v/cm in order to determine if a regression curve emerges without the predominating influence of the measurements obtained at 30 v/cm.

In this second regression analysis two additional regression curves emerge that were not seen when all of the data was included. The first significant regression is associated with the total solids as indicated by the *f*-value and probability of 9.79 and 0.0044, respectively. The equation of the regression line determined in *SAS* is $y = 0.034x - 0.03$, with an associated adjusted R-square value is 0.2527. A second significant regression curve is seen with the nitrate measurements as indicated by the *f*-value and associated probability of 7.88 and 0.0095, respectively. The equation for this regression line is $y = -1.63x + 13.33$, with an associated adjusted R-square value of 0.2093.

In the ANOVA test neither the total solids nor the nitrate concentrations measurements showed a clear significant difference between the recirculating and waste streams or between the various treatment levels, therefore the fact that a regression curve emerges here may inconsequential. Nevertheless, it is interesting to note that as would be expected there is a positive slope to the regression line associated with total solids indicating that as the electric field strength increases so does the difference in the total solids between the recirculating and waste streams with the higher concentration being associated with the waste stream. The fact that this regression line did not emerge when the data associated with the 30 v/cm treatment level was included may indicate that there is a leveling off of the effectiveness of this trend.

This pattern is not seen with the nitrate concentrations where the regression line equation indicates that increasing the electric field strength will decrease the difference between the recirculating and waste streams. As discussed previously this

may be due in part to the assumption that the migration of dissolved ions such as nitrate does not require the higher electric force, and therefore may be equally effective at both the higher and lower electric field strengths.

The fact that no other significant regression occurred with other water quality parameters even though some significant differences were found overall, may indicate that the application of an electric field has only a limited effect. The fact that greater accumulation did not occur at higher electric field strengths may also be an indication that electrophoretic forces are not effective in overcoming the diffusion and mixing that naturally occurs.

It should also be noted that of the three water quality parameters found to be statistically different for the combined data (alkalinity, particle size and pH) only the particle size data measures a physical characteristic of the suspended particles. Alkalinity and pH both measure ion concentrations within the fluid, and lack the mass associated with the suspended particles. For this reason, increasing the electric field strength may not play an important role in significantly changing the magnitude of the observed effect; rather both high and low electric field strengths may have a comparable observed effect. As a result, any linear regression may be more difficult to define for ion concentrations, if indeed a linear relationship exists.

CONCLUSIONS

The following are conclusions and observations gleamed from the results of the data obtained during this study:

1. An applied electric field is not effective in removing suspended particles from aquaculture effluent. The primary objective of this study was to determine if an applied electric field could be used as a means of water clarification, and although a significant difference was statistically shown for some water quality parameters used in the analysis, a dramatic water clarification was not observed as would be required for any reasonable filtration mechanism proposed. It is clear that the nature of the organic aquaculture particles do not lend themselves to electrophoretic removal.
2. A functional relationship between water quality improvement and the electric field strength applied was not determined. Although a linear regression was observed with the relative difference in suspended particle size, a quantitative regression between the concentration of any water quality parameter and electric field strength was not observed. This indicates that an applied electric field has only limited influence and the effect is not increased or decreased with a change in electric field strength.
3. An applied electric field has a more obvious effect on dissolved ion concentrations, as observed by differences in hydronium ions (pH), and alkalinity, than it does on actual suspended particles as would be indicated by a change in the total solids concentration and turbidity.

4. Further development of an electrophoretic filtration system for industrial aquaculture use is not be advisable. As indicated in the objectives of this paper this was intended as a pilot study to determine if further development would be warranted. The discouraging and mostly inconsequential results of this study do not warrant further investigation.

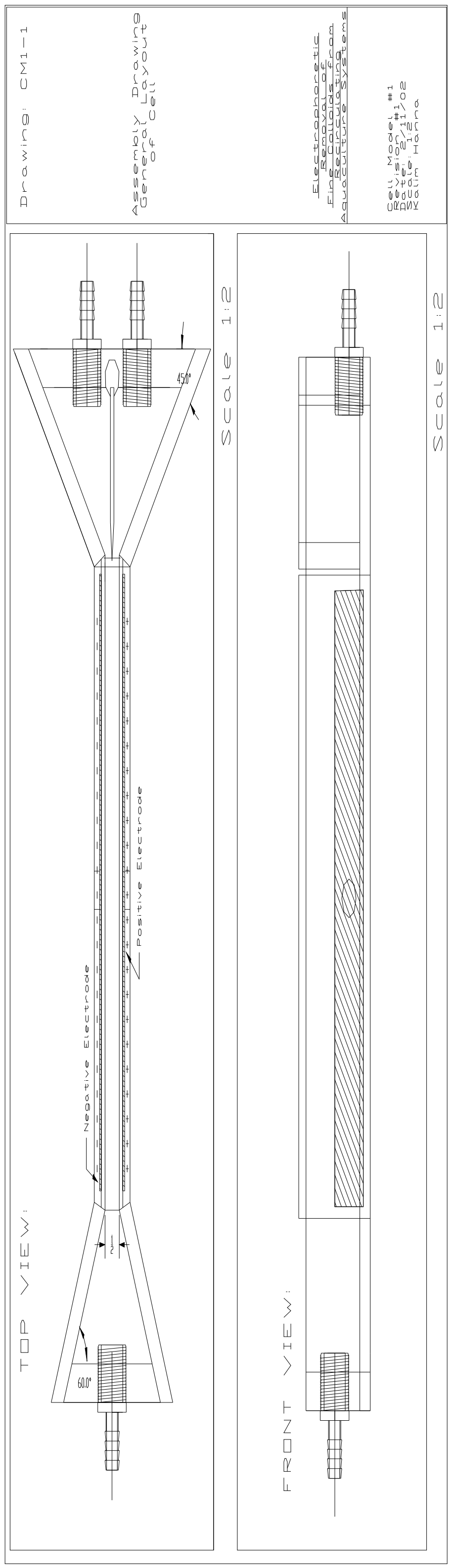
SUGGESTIONS FOR FURTHER STUDY

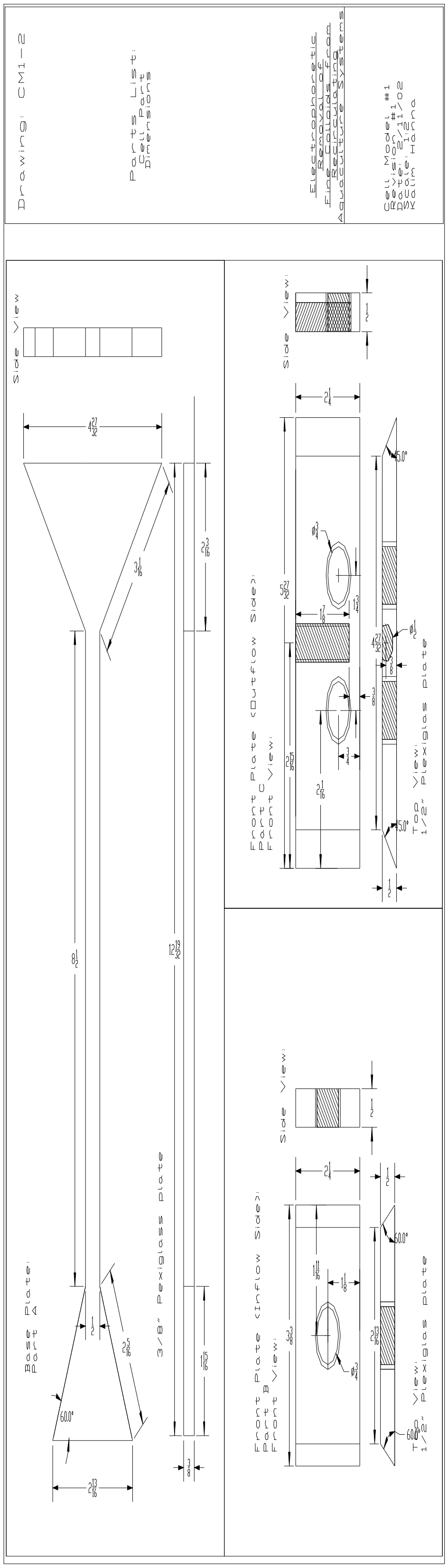
Possible areas for further development and investigation may include the following:

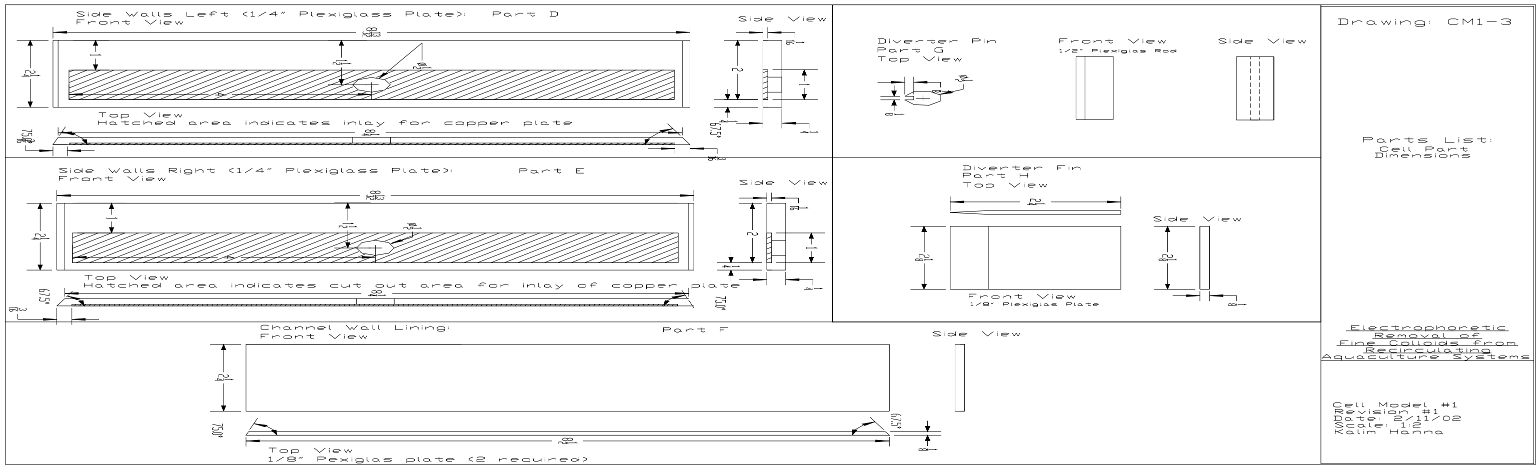
1. Although the focus of this research centered on the removal of suspended solids a more thorough analysis of a similar process may be explored to remove dissolved ions, such as ammonia, nitrite and nitrate that may accumulate over time.
2. The experiment here involved shielding the electrodes behind a thin Plexiglas sheet, in order to prevent the possibility of metal ion dissociation from the electrodes into the fluid stream, and exposure to an electrical current. However, it would be constructive to compare the effectiveness of the process as well as the potential hazards, to determine if such a process is suited for aquaculture systems. It may be especially useful to explore the effects of aluminum electrodes, as aluminum is a known flocculent that may aid in fine particle removal.
3. For a fuller exploration of the potential of electrophoretic removal, particle mobility of aquaculture effluent suspended solids should be compared over a range of pH levels. It is known that the pH level of the surrounding fluid affects the charge density on the surface of suspended solids, which determines particle mobility. Therefore, it will be informative to compare the relative effectiveness of the electrophoretic process for effluent sources of varying pH levels. Such a study may have broader implications for possible treatment regimes for wastewater treatment facilities.

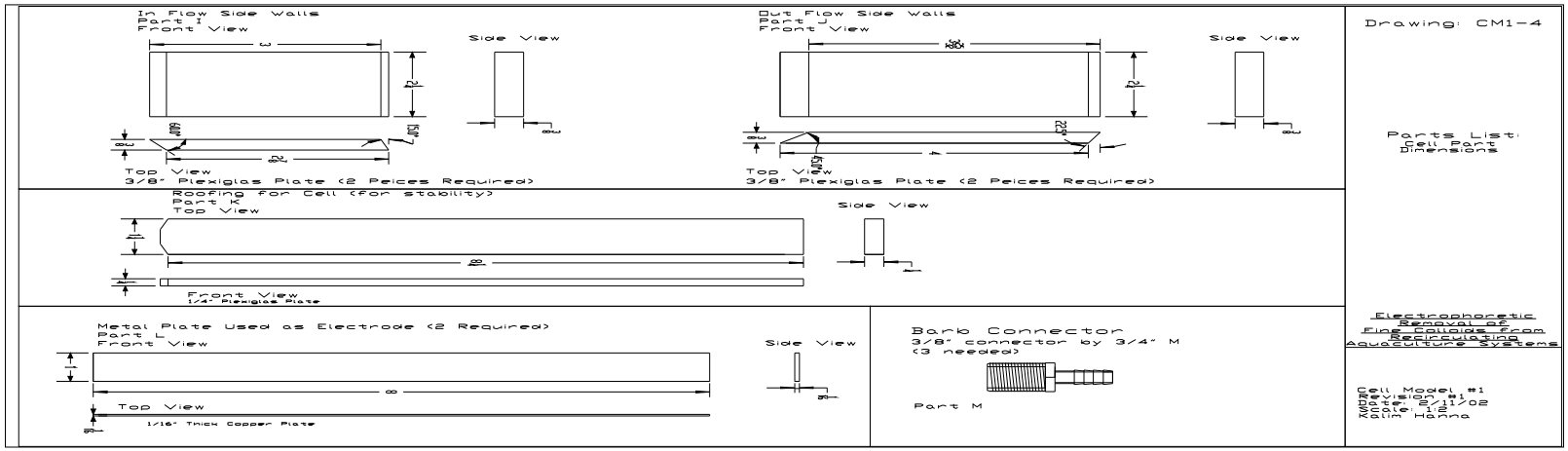
APPENDIX

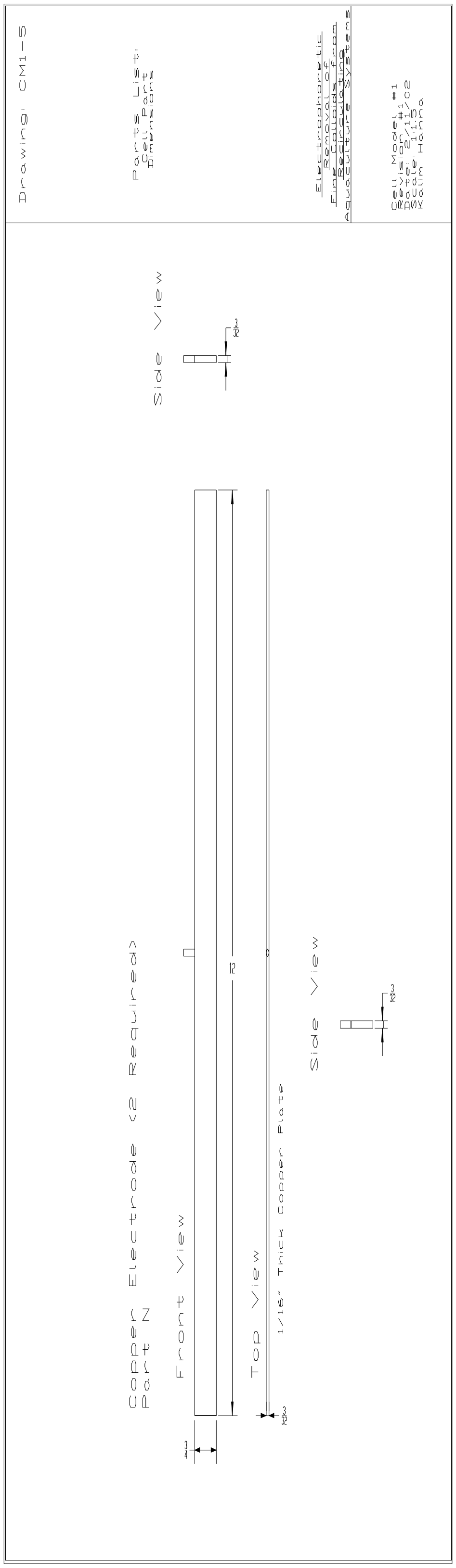
Appendix A: Construction Drawings


Drawing: CM1-1
Assembly Drawing
General Layout
of Cell
Electrophoretic
Removal of
Fine Colloids from
Recirculating
Aquaculture Systems
Cell Model #1
Revision #1
Date: 2/11/02
Scale: 1:2
Kalim Hanna
TOP VIEW:
Scale 1:2
45.0°
60.0°
Negative Electrode
Positive Electrode
FRONT VIEW:
Scale 1:2


Drawing: CM1-2
Parts List:
Cell Part
Dimensions
Electrophoretic
Removal of
Fine Colloids from
Recirculating
Aquaculture Systems
Cell Model #1
Revision #1
Date: 2/11/02
Scale: 1:2
Kalim Hanna
Side View:
Base Plate:
Part A
3/8" Pexiglass plate
Front Plate (Outflow Side):
Part C
Front View:
Side View:
Top View:
1/2" Plexiglas plate
Front Plate (Inflow Side):
Part B
Front View:
Side View:
Top View:
1/2" Plexiglas plate


Side Walls Left (1/4" Plexiglass Plate): Part D
Front View
Side View
Top View
Hatched area indicates inlay for copper plate
Side Walls Right (1/4" Plexiglass Plate): Part E
Front View
Side View
Top View
Hatched area indicates cut out area for inlay of copper plate
Channel Wall Lining:
Front View
Part F
Side View
Top View
1/8" Pexiglas plate (2 required)
Diverter Pin
Part G
Top View
Front View
1/2" Plexiglas Rod
Side View
Diverter Fin
Part H
Top View
Front View
1/8" Plexiglas Plate
Side View
Drawing: CM1-3
Parts List:
Cell Part
Dimensions
Electrophoretic
Removal of
Fine Colloids from
Recirculating
Aquaculture Systems
Cell Model #1
Revision #1
Date: 2/11/02
Scale: 1:2
Kalim Hanna


In Flow Side Walls
Part I
Front View
Side View
Top View
3/8" Plexiglas Plate (2 Peices Required)
Out Flow Side Walls
Part J
Front View
Side View
Top View
3/8" Plexiglas Plate (2 Peices Required)
Drawing: CM1-4
Parts List:
Cell Part Dimensions
Roofing for Cell (For stability)
Part K
Top View
Side View
Front View
1/4" Plexiglas Plate
Metal Plate Used as Electrode (2 Required)
Part L
Front View
Side View
Top View
1/16" Thick Copper Plate
Barb Connector
3/8" connector by 3/4" M
(3 needed)
Part M
Electrophoretic
Removal of
Fine Particle Scrap
Recirculating
Aquaculture Systems
Cell Model #1
Revision #1
Date: 8/11/02
Scale: 1:8
Drawn: Hanna


Drawing: CM1-5
Parts List: Cell Part Dimensions
Electrophoretic Removal of Fine Colloids from Recirculating Aquaculture Systems
Cell Model #1
Revision #1
Date: 2/11/02
Scale: 1:1.5
By: Jim Hanna
Side View
Copper Electrode (2 Required)
Part N
Front View
12
Top View
1/16" Thick Copper Plate
Side View

Appendix B:* SAS *Program Code

```
DM 'Log; clear; out; clear;';
options ls=100 ps=1000 pageno=1;

Title1 Combined Data;
Data combined;
PROC IMPORT OUT= WORK.combinedgrp
        DATAFILE= "d:\thesis\thesis material\thesis data\SAS-combined.xls"
        DBMS=EXCEL2000 REPLACE;
    GETNAMES=YES;
RUN;
Title4 ANOVA test for TOTAL SOLIDS (ts)variable for the Combined Data PROC
MIXED;
Proc MIXED;
class grp ef block;
model ts = grp ef block /DDFM=KR outp=resids;
Quit;

Title4 ANOVA test for TURBIDITY (tur) variable for the Combined Data PROC
MIXED;
Proc MIXED;
class grp ef block;
model tur = grp ef block /DDFM=KR outp=resids;
Quit;
Title4 ANOVA test for MEAN PARTICLE SIZE (par) variable for the Combined
Data PROC MIXED;
Proc MIXED;
class grp ef block;
model par = grp ef block /DDFM=KR outp=resids;
Quit;
Title4 ANOVA test for PARTICLE SKEWNESS FACTOR (pskew) variable for the
Combined Data PROC MIXED;
Proc MIXED;
class grp ef block;
model pskew = grp ef block /DDFM=KR outp=resids;
Quit;
Title4 ANOVA test for PARTICLE STANDARD DEVIATION (pstdev) variable for
the Combined Data PROC MIXED;
Proc MIXED;
class grp ef block;
model pstdev = grp ef block /DDFM=KR outp=resids;
Quit;
```

```
Title4 ANOVA test for ZETA POTENTIAL (zeta) variable for the Combined Data
PROC MIXED;
Proc MIXED;
class grp ef block;
model zeta = grp ef block /DDFM=KR outp=resids;
Quit;
Title4 ANOVA test for CONDUCTIVITY (con) variable for the Combined Data
PROC MIXED;
Proc MIXED;
class grp ef block;
model con = grp ef block /DDFM=KR outp=resids;
Quit;
Title4 ANOVA test for MOBILITY (mob) variable for the Combined Data PROC
MIXED;
Proc MIXED;
class grp ef block;
model mob = grp ef block /DDFM=KR outp=resids;
Quit;
Title4 ANOVA test for ALKALINITY (alk) variable for the Combined Data PROC
MIXED;
Proc MIXED;
class grp ef block;
model alk = grp ef block /DDFM=KR outp=resids;
Quit;
Title4 ANOVA test for pH (ph) variable for the Combined Data PROC MIXED;
Proc MIXED;
class grp ef block;
model ph = grp ef block /DDFM=KR outp=resids;
Quit;
Title4 ANOVA test for PHOSPHOROUS (phos) variable for the Combined Data
PROC MIXED;
Proc MIXED;
class grp ef block;
model phos = grp ef block /DDFM=KR outp=resids;
Quit;
Title4 ANOVA test for NITRATE (nat) variable for the Combined Data PROC
MIXED;
Proc MIXED;
class grp ef block;
model nat = grp ef block /DDFM=KR outp=resids;
Quit;
Title4 ANOVA test for NITRITE (nit) variable for the Combined Data PROC
MIXED;
Proc MIXED;
class grp ef block;
model nit = grp ef block /DDFM=KR outp=resids;
```

```
Quit;
Title4 ANOVA test for AMMONIA (amm) variable for the Combined Data PROC
MIXED;
Proc MIXED;
class grp ef block;
model amm = grp ef block /DDFM=KR outp=resids;
Quit;


Title1 Combined Data Regressoin Analysis;
Data combinedef;
PROC IMPORT OUT= WORK.combined
          DATAFILE= "d:\thesis\thesis material\thesis data\SAS-combined.xls"
          DBMS=EXCEL2000 REPLACE;
    GETNAMES=YES;
RUN;

Title4 Regression Analysis of Electric Field Strength on Water Quality Parameter;
Proc REG DATA=combined;
MODEL tsd turd pard pskewd pstdevd zetad cond mobd alkd phd phosd natd nitd
ammd = ef;
OUTPUT OUT = assumps
       P=pred R=resid
       U95M=u95m
       L95M=l95m;
Quit;

Title4 Checking Assumptions for Regression Analysis for Combined Data;
PROC UNIVARIATE;
BY ef;
VAR tsd turd pard pskewd pstdevd zetad cond mobd alkd phd phosd natd nitd ammd;
Quit;
PROC PLOT DATA=assumps;
PLOT resid*pred
        resid*ef       / VREF=0;
QUIT;

Title1 College Park Block;
Data animal_sci;
PROC IMPORT OUT= WORK.animal_sci
          DATAFILE= "d:\thesis\thesis material\thesis data\SAS-animal.xls"
                    /*DATAFILE= "a:\SAS-animal.xls"*/
          DBMS=EXCEL2000 REPLACE;
    GETNAMES=YES;
RUN;
```

```
Title4 Paired T-Test for grouped variables for the College Park block;
PROC TTEST;
CLASS grp;
VAR alk do ph par pskew pstdev ts tur;
QUIT;

Title4 Paired T-Test for variables at each treatment level for the College Park block;
PROC MEANS N MEAN STDERR T PRT;
VAR alkd dod phd pard pskewd pstdevd tsd turd;
BY ef;
QUIT;

Title1 Fredrick Block;
Data fredrick;
PROC IMPORT OUT= WORK.fredrick
        DATAFILE= "d:\thesis\thesis material\thesis data\SAS-fredrick.xls"
                /*DATAFILE= "a:\SAS-fredrick.xls"*/
        DBMS=EXCEL2000 REPLACE;
    GETNAMES=YES;
RUN;

Title4 Paired T-Test for grouped variables for the Fredrick block;
Proc ttest;
class grp;
var con mob par pskew pstdev ts tur zeta;
Quit;
Title4 Paired T-Test for variables at each treatment level for the Fredrick block;
PROC MEANS N MEAN STDERR T PRT;
VAR cond mobd pard pskewd pstdevd tsd turd zetad;
BY ef;
QUIT;

Title1 Church Creek Block;
Data church_creek;
PROC IMPORT OUT= WORK.church_creek
        DATAFILE= "d:\thesis\thesis material\thesis data\SAS-church.xls"
                /*DATAFILE= "a:\SAS-church.xls"*/
        DBMS=EXCEL2000 REPLACE;
    GETNAMES=YES;
RUN;

Title4 Paired T-Test for grouped variables for the Church Creek block;
Proc ttest;
class grp;
var alk amm con con2 mob par pskew pstdev ph phos nat nit ts tur zeta;
Quit;
```

```
Title4 Paired T-Test for variables at each treatment level for the Church Creek block;
PROC MEANS N MEAN STDERR T PRT;
VAR alkd ammd cond con2d mobd pard pskewd pstdevd phd phosd natd nitd tsd turd
zetad;
BY ef;
QUIT;

Title1 Preston Block;
Data preston;
PROC IMPORT OUT= WORK.preston
        DATAFILE= "d:\thesis\thesis material\thesis data\SAS-preston.xls"
                /*DATAFILE= "a:\SAS-preston.xls" */
        DBMS=EXCEL2000 REPLACE;
    GETNAMES=YES;
RUN;

Title4 Paired T-Test for grouped variables for the Preston block;
Proc ttest;
class grp;
var amm alk con mob par ph phos nat nit ts tur zeta;
Quit;

Title4 Paired T-Test for variables at each treatment level for the Preston block;
PROC MEANS N MEAN STDERR T PRT;
VAR ammd alkd cond mobd pard phd phosd natd nitd tsd turd zetad;
BY ef time;
QUIT;
```

Table B1. List of variables used in *SAS* program code.

Variable Name:	Variable Definition:
alk	Alkalinity data
alkd	Alkalinity difference data ($S_w - S_r$)
amm	Ammonia concentration data
ammd	Ammonia concentration difference data ($S_w - S_r$)
con	Conductivity data
cond	Conductivity difference data ($S_w - S_r$)
do	Dissolved oxygen data
dod	Dissolved oxygen difference data ($S_w - S_r$)
ef	Electric Field strength
grp	Sample group (recirculating or waste stream)
mob	Mobility data
mobd	Mobility difference data ($S_w - S_r$)
nat	Nitrate concentration data
natd	Nitrate concentration difference data ($S_w - S_r$)
nit	Nitrite concentration data
nitd	Nitrite concentration difference data ($S_w - S_r$)
par	Mean particle size data
pard	Mean particle size difference data ($S_w - S_r$)
ph	PH data
phd	PH difference data ($S_w - S_r$)
phos	Phosphorous concentration data
phosd	Phosphorous concentration difference data ($S_w - S_r$)
pskew	Particle skewness factor data
pskewd	Particle skewness factor difference data ($S_w - S_r$)
pstdev	Particle distribution standard deviation data
pstdevd	Particle distribution standard deviation difference data ($S_w - S_r$)
ts	Total solids data
tsd	Total solids difference data ($S_w - S_r$)
tur	Turbidity data
turd	Turbidity difference data ($S_w - S_r$)
zeta	Zeta potential data
zetad	Zeta potential difference data ($S_w - S_r$)

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