Treatment of Wet Fish Sludge with Vermicomposting

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ABSTRACT

TREATMENT OF AQUACULTURE WASTEWATER WITH VERMICOMPOSTING

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Aquaculture, the cultured production of fish, is growing at a rapid pace worldwide. The industry is generating approximately 140,000 cubic meter wastewater per year. For this industry to flourish, viable methods for treating the resulting waste stream must be identified. The various methods were tried by many researchers like sand filtration method, recirculating aquaculture system, intermittent filtration methods. The most of the industries use sand filtration methods for treating aquaculture wastewater and the problems associated: the reduction in hydraulic conductivity, accumulation of solid due to which anaerobic conditions developed. This study investigated possible treatment technologies for wastewater and sludge produced from Blue Ridge Aquaculture (BRA), an indoor, recirculating aquaculture facility where tilapias (Oreochromis) are raised. Research focused on the use of vermicomposting in conjunction with sand bed filtration to filter aquaculture waste and treat the resulting solids. Two experiments were conducted: a feedstock acceptability test and a filter bed test.

The feedstock acceptability test evaluated the suitability of the fish sludge (mixed with cardboard) as a feedstock for the worms involved in the vermicomposting process. The results

showed that as the percentage of fish sludge in the feed increased from 0 to 50%, there was a corresponding increase in the growth rate of *E.fetida* biomass.

The filter bed test appraised the feasibility and effectiveness of incorporating vermicomposting in sand filter beds to directly treat aquaculture wastewater. Popular in early wastewater treatment systems, sand filtration has seen a resurgence in recent years. To test the potential for even more effective filtration, sixteen sand filter beds were established—twelve that included worms and four that did not. Wastewater (1.5 % total solids) from BRA was applied to the sand beds at loading rates of 400 to 1000 grams of volatile solids/m^2/week. Filter beds containing worms exhibited no ponding over the 70-day experimental period. However, all units without worms failed (exhibited ponding) by the 24^{th} day of operation.

Removal efficiencies obtained from the filter bed study for total solids (TS), volatile solids (VS), total suspended solids (TSS), chemical oxygen demand (COD), total phosphorus (TP), sulfate, chlorides, and ammonia-N were greater in filter beds with worms than beds without worms. The worms were crucial to maintaining porosity in the filter beds, hence keeping the filters functioning over time. Worm filter beds removed approximately 100% of the TS, VS, TSS and Ammonia-N, 90% of the TP, 50% of the chlorides, 80% of the sulfate and 70% of the COD. Maximum hydraulic conductivity of 35 cm/day was achieved at the maximum application rate. All the worm filter beds therefore had greater hydraulic conductivity than filter beds without worms. The potential impact is to treat the wastewater effectively, to increase the flow of water, and may be to maintain the aerobic conditions on the worm filter-beds.

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Acronyms and Nomenclature

AFW = Artificial Fish Wastewater

BOD_5 = Biological Oxygen Demand, mg/L

BRA = Blue Ridge Aquaculture

BW = Backwash Water

C:N = Carbon:Nitrogen Ratio

COD = Chemical Oxygen Demand, mg/L

DO = Dissolved Oxygen, mg/L

K = Hydraulic Conductivity, cm/day

LSVS = Liquid Sludge Vermistabilization Studies

PVC = Poly Vinyl Chloride

SAS = Statistical Analysis Software

SBW = Sieve Backwash Water

SP = Soluble Phosphorus, mg/L

TKN = Total Kjeldhal Nitrogen, mg/L

TN = Total Nitrogen

TP = Total Phosphorus, mg/L

TS = Total Solid, mg/L

TSS = Total Suspended Solids, mg/L

VS = Volatile Solids, mg/L

w.b. = wet basis

ρ = Density of the Fluid, gm/cm^3

η = Viscosity of the Fluid, poises

g = Acceleration of Gravity, cm/sec^2

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1. Introduction

The aquaculture industry is growing at a rapid pace worldwide. Although the industry provides vital protein for many cultures throughout the world, the waste generated by these facilities has become an environmental hazard. The average aquaculture facility produces approximately 140,000 cubic meter of waste per year, overloading waterways with ammonia, nitrogen, and potential disease-causing bacteria. The effects of this have been seen in places like Alaska, where salmon aquaculture facilities have caused massive die-offs in aquatic vegetation and have buried the seafloor in a thick coat of nutrient-rich sludge. For the industry to flourish, therefore, the problem of waste stream management must be addressed.

In Virginia, BRA produces 1600 m^3 of wastewater daily (Brazil, 2001). Due to the high costs associated with pollution, BRA is interested in installing a wastewater treatment facility and wishes to investigate possible treatment technologies for both the wastewater and the resulting sludge. BRA's goal is to devise a waste management scheme that will be environmentally sound and ideally generate an additional source of income for the business in the form of a value-added product produced from the waste stream. Edwards (1988) reported that, "Increasingly strict water pollution regulations throughout the world have resulted in the generation of increasing amounts of sludge for ultimate disposal. Many alternative management systems for stabilizing sludge have been developed, such as composting, land application, and the use of earthworms to help stabilization".

Composting is a process of conversion of organic materials from unstable products to increasingly more stable ones. A range of microorganisms and invertebrates, called decomposers, perform this process. Generally, the term composting refers to a system where heat-loving microorganisms predominate. Vermicomposting is another composting process which utilizes worms as the primary decomposers.

Worms, when present in aerobic sludge, increase the sludge volatile solids destruction rate, relative to microorganisms alone. "The worms maintain aerobic conditions in the mixture,

ingest solids, convert a portion of the organic waste into worm biomass and respiration products, expel the remaining partially stabilized matter as discrete materials (castings) and increase the respiration of the microorganisms in the mixture". Many studies have been conducted on the vermicomposting of animal excreta, sewage sludge, and agro-industrial waste (Butt, 1993; Mitchell, 1997; Edwards, 1998), but none have focused on aquacultural sludge. Such a technology may be the most cost-effective method for aquacultural waste treatment. As Raymond (1988) noted "Vermicomposting or vermistabilization represents a technology that is environmentally sound, need not be energy, capital or equipment intensive, and should not require extensive management."

The worm species most commonly used for degrading organic wastes is *Eisenia fetida*. Raymond (1998) reported many reasons why this species is preferred:

- "It is ubiquitous, and many organic wastes become naturally colonized by this species;
- It tolerates a wide temperature range;
- Lives in wastes with a wide range of moisture content;
- Is robust and tolerates handling well;
- In mixed cultures it usually becomes dominant, so systems that begin with other species often end up with a large proportion of *E.fetida*; and,
- It exhibits a rapid growth rate and high reproductive potential".

This study consists of two parts: The first part was conducted to evaluate the acceptability of fish sludge as a feedstock for the vermicomposting process. The second study was designed to evaluate the feasibility and effectiveness of sand-bed filter-bed with worms for treating aquaculture wastewater from drum filter (0.5 to 1.5% solid). If successful, aquaculture facilities could reduce costs and mitigate pollution by eliminating the need to directly discharge wastewater to municipal sewage systems. Furthermore, the resulting compost can be sold to increase economic returns to aquacultural producers.

Widrig (1996) reported that "In addition to treating aquacultural sludge, treatment methods are needed to separate the solids from the wastewater. There is considerable debate as to the most appropriate biological filter technology for intensive aquaculture applications. The four major filter types used are: sand bed, trickling filters, rotating biological contractors, and floating bead filters. Sand bed filtration for wastewater treatment is an established technology that has attracted renewed interest due to its potential to satisfy many of the current needs for wastewater treatment." Sand bed filters are typically constructed of beds of sand two to three feet deep. The filter material (called media) is contained in either a plastic liner or concrete tank. Depending on soil conditions, sand filters can be constructed above ground, partially above ground, or entirely below the surface.

In both Europe and the United States, domestic wastewater treatment for the latter half of the nineteenth century used intermittent sand filters. Sand filtration systems were widely used at the turn of the century in the northeastern United States, particularly by communities in Massachusetts. However, as the population density in urban areas increased, wastewater flows also increased and the use of sand filtration declined due to large land requirements and introduction of activated sludge processes. Recently, intermittent sand filters were reintroduced to the field of wastewater treatment techniques. Widrig reported that "The continuing general need for reliable, cost-effective wastewater treatment in many rural regions, as well as increased land development pressures in environmentally sensitive areas such as shorelines and high relief terrains, are among the forces compelling this renewed interest in intermittent sand filters." Thus, intermittent sand filtration offers a promising and proven technology for low-cost, low-maintenance wastewater treatment. The best suited areas are where conventional septic tank-soil absorption systems are not practical due to seasonal high water table, shallow soils over bedrock and low permeable soil as well as where re-use of water is desired.

1.1 Objectives

This study examines the effectiveness of vermicomposting of aquacultural wastes based on the following:

1) Evaluation of the acceptability of fish sludge as a feedstock for the vermicomposting process, and
2) Examination of the feasibility and effectiveness of treating aquacultural wastewater using sand filters that incorporate vermicomposting.

2. Literature Review

2.1 Waste Generated by the Aquaculture Industry

According to department of environmental conservation (1987), the discharge volume of wastewater from an aquaculture facility approximately 140,000 cubic meters per year. Aquaculture wastewater may contain chemicals that are harmful to the development of plants. It may also contain bacteria and other organisms that are detrimental for plants and animals. Wastewater may even contain bacteria and other organisms which, when eaten by animals, may in turn infect the people who eat the contaminated meat. Managing waste generated from aquaculture wastewater represents a tremendous problem; Kristiansen, (1996) stated that "Handling wastewater is a major problem in all animal agriculture systems, but there are substantial differences between aquaculture wastewater and manure from dairy or hog systems. The latter are typically in the range of 5 – 15 % suspended solids, while fish wastewater can be anywhere from 0.2 – 4.0 % suspended solids". Typically, suspended solids concentrations from drum filters used in intensive aquaculture operations are around 0.5 %. Waste production from aquaculture systems will be from 0.2 to 0.5 kg of waste per kg of feed (Drennan et al., 1995; Chen et al., 1997.

2.2Possible Methods of Wastewater Treatment

The two most common methods used to recycle solid wastes from aquaculture facilities are land application and composting. Aquaculture sludges are good for use in both crop and created wetland. Nevertheless, if transportation costs make sludge disposal on crop land uneconomical, disposing of the sludge on-site within created wetlands might be the next best alternative.

Due to an increasing environmental concern regarding the aquaculture wastewater, various methods for treating wastewater have been implemented. Four main stages associated with aquaculture wastewater management are waste collection, fish-fed loss reduction, suspended

particle separation, and sludge treatment. The later phase has rarely been considered because of the large increase in sludge volume originating from such sources (Bergheim et al., 1993a), however, it is becoming an increasingly significant problem.

Loehr et al. (1984a) studied the feasibility of using earthworms to stabilize wastewater treatment sludge and similar wastes. The study concluded that both excessive and inadequate moisture content can adversely impact earthworm growth. Worm growth at high and low total solids contents (7.9, 18.4, 18.6, 20.5, and 25.1% solids) was statistically different from worm growth occurring in the middle range of solids (9.3 to 17.1% solids). The best worm growth occurred over a range of total solids, wet basis (w.b.), of about 9 to 17% solids. Hence, the identified appropriate range of moisture content (91 to 83%) is the total solids content to which the worms were exposed. Loehr (1984) extended the experiment to determine whether the vermistabilization process could be self supporting, e.g., whether the aerobic condition was maintained by worms (*E. fetida*) over a long period of time. Results showed that at the application rate (200 grams/week) after about 12 months, volatile solids reduction of 10- 15% was achieved. No apparent adverse effects were observed as worms and cocoons were distributed throughout the accumulation. No major difference in the performance of the vermistabilization was observed as a function of time. High concentrations of nitrate-N in the effluent in the worm reactors imply the aerobic conditions were maintained.

Edward et al. (1988) based a study on Loehr's which researched the potential of earthworms to manage sewage sludge with a 10% solid content. Results showed that *E. fetida* can increase the Volatile Sludge Destruction (VSD) rate when present in aerobic sludge (Table 2-1). The increase in the VSD rate reduces putrefaction due to anaerobic conditions. The worms aid in the more rapid degradation of organic matter through increased aeration as they move through the sludge.

Table 2- 1 Effect of *E.fetida* on Volatile Solids Destruction Rate Present in Aerobic Condition

Time, Days	Volatile Solids Destruction, %	
	E. fetida	**Without Worms**
1	3.5	1.2
2	5.0	1.2
3	9.8	1.0
4	12.5	3.5
7	16.0	4.5
10	21.0	5.0
17	24.5	9.0
22	25.0	11.0

The use of sand filter beds in wastewater treatment has been studied with regards to municipal wastewater, sewage sludge, and industrial wastewater. Very few studies have focused on aquaculture wastewater. Currently, there is very little information available on treating the higher solid content (11 % solid) of the fish sludge and separating fish sludge from the higher moisture content (98.5 %) of aquaculture wastewater from a drum filter.

Kristiansen and Cripps (1996) conducted a study on the treatment of fish wastewater using sand filtration. This study's objective was to evaluate the feasibility of sand as a renovation, stabilization, and drying system for sludge derived from the first stage treatment of aquacultural wastewater. Feasibility was assessed in terms of hydraulic capacity and treatment efficiency. The pilot study comprised coarse sand-filled infiltration beds loaded with either an artificial fish farm wastewater (AFW), backwash water from a micro sieve (BW), or sediment micro sieve backwash water (SBW) collected daily from a settling chamber. Results obtained from the study showed that the hydraulic conductivity of all the columns was reduced from an initial 2000 cm / day, measured as infiltration rate, to < 100 cm / day after 40 days of usage. A large reduction in hydraulic conductivity was caused by the establishment of a clogging mat on the sand filter surface. Kristiansen stated that "About 60 % of the sludge total organic C was removed by the filters. Nitrogen in the effluent from

the SBW loaded filter was predominately organic, and nitrate concentrations were significant (< 0.03 mg NO_3^-N L^{-1}). Effluent ammonium concentration decreased from 97 % of the effluent total nitrogen (TN) after 1month of loading, to 10 % after 2 to 3 months, with an attendant increase in nitrate to about 65 % of the TN. The P binding capacity of the test sand volume was exceeded after 1 to 2 months of SBW loading. This capacity was not exceeded during the experiment, using the two other effluent types (BW and AFW). Filter effluent P concentrations were about 1.4 mg / l. At a SBW loading of 1 cm / day, to coarse sand, with a hydraulic head of > 10 cm, it was expected that 2 to 3 months loading could occur before maintenance or change of filter surface sand would be required." The use of sand infiltration for treating salmon farm sludge was therefore shown to be feasible.

Wridge et al., 1996 conducted a study on intermittent sand filtration for domestic wastewater treatment, focusing on the effects of filter depth and hydraulic parameters. Wridge stated that "The objective of the study was to operate a series of pilot-scale intermittent sand filters for the treatment of domestic wastewater, and evaluate their performance based on current discharge standards for wastewater quality in the state of Ohio. Specific objectives were to relate sand filter depth, hydraulic conductivity, and infiltration rate to treatment performance. Results show that the 60 cm filter constantly produced an effluent that met the Ohio department of health regulations for BOD_5 of 20 mg/L after nine weeks of operation. Filter depth also influenced total suspended solids removal, but to a lesser degree than for BOD_5 removal. The effect of filter depth was also clearly evident for ammonia-N removal. Estimates were made of saturated hydraulic conductivity and infiltration rate for the laboratory filters. In general, as filter run increased, both the hydraulic conductivity and infiltration rate decreased".

While many studies have examined the effects of vermicomposting process on domestic wastewater, municipal wastewater, sewage sludge, and industrial wastewater, few have examined effects on aquaculture wastewater. Scientific studies have helped establish the technical basis for vermistabilization. Research (Kristiansen et al., 1996; Wridge et al., 1996; Edwards et al., 1988; Loehr et al., 1984) has shown that sand filtration method with worms can be effective for separating solids from aquaculture wastewater. In addition, studies have identified the effect of filter media, depth of filter-bed, hydraulic conductivity, and various

variable concentrations such as ammonia, biological oxygen demand (BOD) and nitrate for separating solids and treating wastewater. Therefore; these studies may be helpful in designing an experiment for treating aquaculture sludge with higher percentage of solid (10 – 20%) and wastewater from drum filter at higher moisture content (98.5 to 99.5).

2.3 Vermicomposting

Composting is a biological decomposition of organic matter under controlled aerobic conditions into humus-like stable products. The processing of organic wastes into organic fertilizers via composting is a technique that has been used to address the issues of environmental pollution, reliance on chemical fertilizers, sustainable natural fertility, and minimizing the development of new landfills. Vermicomposting uses worms to convert animal, agricultural, and industrial wastes to useful fertilizers.

Raymond, 1988 reported that "Earthworms have been used for waste stabilization for many years, especially in the Philippines and other countries in Southeast Asia. The process is also being used in Italy, England, and the Netherlands not only to stabilize wastes, but also to produce castings for horticultural purposes. In the United States, feasibility studies (Camp et al., 1981; Pincince et al., 1980) evaluated the process for sludge management and indicated that the operating costs of a practical system may be competitive with other sludge management options for certain communities".

Worms maintain aerobic conditions in the waste mixture, ingest solids, convert a portion of the organic media into biomass and respiration products, and expel the remaining, partially stabilized matter as discrete material (castings). Worms and microorganisms act symbiotically to accelerate and enhance the decomposition of the organic matter. Degradation is a function of the portion of waste that is biodegradable, maintenance of aerobic conditions, and avoidance to toxic conditions. Earthworms perform physical/mechanical and biochemical actions through substrate aeration, mixing, and grinding as they process waste. Thus, vermicomposting lowers operational costs, making it a very economical method for waste treatment. Hand et al. (1988)

defined vermicomposting as "a low cost technology for the processing or treatment of organic wastes".

Edwards (1988) noted that "At the same time earthworms promote microbial activity since the fecal material or 'casts' that earthworms produce is more fragmented and microbially active than what earthworms consume". During this process, the important plant nutrients in the material, particularly nitrogen (N), potassium (K), phosphorus (P), and calcium (Ca) are released and converted through microbial action into forms that are much more soluble and available to plants than those in the parent compounds.

However, Ndegwa and Thompson, 2000 similarly reported that "The major drawback in the vermicomposting process is that…vermicomposting processes must be maintained at temperatures below 35^{o}C. Exposure of worms to temperatures above this is lethal. During the vermicomposting process therefore, the temperatures are not high enough for acceptable pathogen kill and hence the process does not meet EPA rules for pathogen reduction. In some cases, depending on the feed substrate, some forms of quick composting will be needed for the vermicompost to meet EPAs process to further reduce pathogens (PERPs) guidelines for pathogen destruction for class-A compost (Class-A compost is said to be satisfactory for general distribution)".

2.4 Efficiency of Eisenia fetida in Vermicomposting

Many earthworm species have potential in vermicomposting (Neuhauser et al., 1979b; Kaplan et al., 1980a, b). Edward (1988) studied five earthworm species (*Dendrobaena veneta, E. fetida, E. eugeniae, Perionyx excavatus and Pheretima hawayana*) for their use in vermicomposting of sludge, evaluating growth and reproduction as indicators of their fitness. Of these species, *E. fetida* produced more live worms per cocoon than any of the other species tested. *E. fetida* thus appears to be an appropriate species to use in vermistabilization studies since it produced the greatest number of young worms per initial parent worm. Environmental Resource Systems at the University of Arkansas also reported that "The most beneficial worm to soil is *Eisenia foetida*—also known as the red worm, brandling worm, red wiggler, or

manure worm. Most worms live and die within the same year, but in culture can live up to four and a half years."

The growth of *E. fetida* and other species shows that litter-dwelling earthworms can be easily bred in vermiculture (Loehr et al., 1985). Dominguez and Edwards (1996) concluded that *E. andrei*, a close relative of *E. fetida*, could be cultured in pig manure grew and matured between 65% and 90% moisture content, the optimum being 85%. There is a direct relationship between the moisture content and the growth rate of earthworms, and *E.fetida* can survive in moistures between 50% and 90% (Edwards et al., 1985; Sims and Gerard, 1985), growing more rapidly between 80% and 90% in animal wastes (Edwards et al. 1985). Reinecke and Venter (1985) concluded that the optimum moisture content for *E.fetida* is well above 70% in cow manure, making it perfect for vermicomposting of sludge with high liquid contents.

Many researchers have therefore focused on *E. fetida* as a suitable species for vermicomposting sludge. Graff (1974), Watanabe and Tsukamoto (1976), Hartenstein (1978), Loehr et al. (1984) and Hartenstein and Bisesi (1989) suggested using *E.fetida* for managing labile organic wastes on soil. Neuhauser et al. (1980a) demonstrated that "biological sludge derived from municipal wastewater treatment facilities is a very favorable substrate and growth medium for *E.fetida"*.

It is clear, however, that *E. fetida* survives best within a certain temperature range. Kaplan et al. (1980) reported that "*E.fetida* grew most rapidly on biological sludges when incubated at fixed-temperatures of 20^{o}C, 25^{o}C or 28^{o}C depending on the moisture content of the sludge substrate". Loehr et al. (1984) indicated that the "maximal rate of growth for *E. fetida* was observed when incubated at fixed-temperatures of 25^{o}C". Temperature must be taken into consideration for any waste treatment operation that wishes to make use of vermicomposting as a possible treatment strategy.

2.5 Biology of E. fetida

The Sustainable Agriculture Research and Educational Program at the University of California describes *E. fetida* as having a red, cylindrical body with red color; 35 – 130 x 3 – 5 mm. *E. fetida* can live a maximum of 4 – 5 years, and can reproduce sexually, producing up to about 900 eggs (cocoons) per worm per year. Each cocoon or worm egg can have 2 to 20 worms (Urban Agriculture Notes, Published by City Farmer, Canada's Office of Urban Agriculture). Figure 2-1 shows the physical appearance of *E. fetida*. Each worm weighs between 0.2 to 0.3 gm.

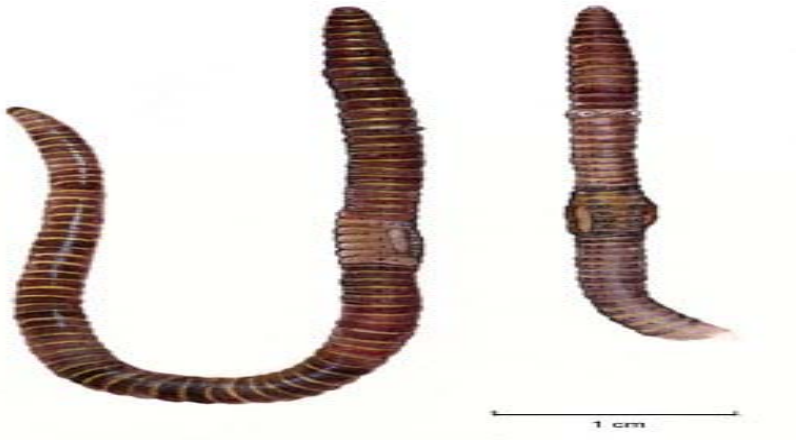


Figure2- 1 *Eisenia fetida*

Edwards (1988) conducted a study on the breakdown of animal, vegetable and industrial organic wastes by earthworms. Five species of earthworms were considered. Three species—*E.fetida, Dendrobaena veneta, Lumbricus rubellus*—were of temperate climate origin and two, *Eudrilus eugeniae* and *Perionyx excavatus,* came from the tropics. The survival, growth, and reproduction of these species were studied in the laboratory in pig, cattle, duck, turkey, poultry, and paper waste and compared with that in activated sewage sludge. Growth of the worms in different animal and vegetable wastes was studied in relation to a range of environmental factors. All species required the wastes in which they lived to be aerobic. As soon as anaerobicity developed, the worms moved out of the waste. All the species differed considerably in terms of their response to and tolerance of different temperatures. The optimum temperature for *E. fetida* was 25^{o}C.

2.6 Feedstocks

Many feedstocks such as cow slurry, pig manure, liquid municipal sludge, poultry litter, cattle manure, etc., are suitable for vermicomposting. Hand (1988) used cow slurry to assess its suitability as a substrate for vermicomposting, to investigate the changes brought about in slurry by *E. fetida*, and to examine the specific relationship between *E. fetida* and microorganisms isolated from the slurry. The results showed that cow slurry is a suitable substrate for vermicomposting, both when mixed with solid materials and when applied to the surface of bedding materials containing earthworms. Mixtures of cow slurry with paper tissue waste produced greater earthworm growth and cocoon production per unit of slurry consumed than mixtures with peat. The growth of *E. fetida* was not affected by the proportion of slurry mixed with tissue waste; the number of cocoons produced was proportional to the amount of slurry present. The author concluded that the earthworm growth rate was maximal in a 2:1 tissue waste/slurry mixture, and the earthworms for used increased amounts of slurry in the substrate for reproduction. Similar results were reported by Neuhauser et al. (1980) when sewage sludge was added to soil containing *E. fetida*; little difference in growth rate was observed from the addition of 10 – 100% sludge.

In a study closely related to aquaculture wastewater treatment, Decker et al. (1999) studied the influence of exchangeable ammonium on the survival of earthworms during the vermicomposting of fish offal mixed with peat. The study's aim was to determine the ideal percentage of peat mixed with fish offal for vermicomposting and to determine the maximum amount of ammonium which would still give a 100% survival rate of *E. fetida* during vermicomposting. Decker stated that "Peat was chosen as the best bulking agent to examine vermicompost of fish offal. Peat has good odor control characteristics and excellent water absorption capacity, which renders it very suitable for fish offal, which has high moisture content. Peat is also best for fish offal in terms of nitrogen conservation". Results showed that the maximum amount of fish offal that can be used during vermicomposting to get a 100% survival rate was 13% fish offal (dry weight). Fish offal of 15% or more (dry weight) was fatally toxic to the earthworms. This was true even when the pre-composting period was extended more than two weeks. Peat, the bulking agent, absorb much of the ammonia (NH_3) produced from the decomposition of fish offal as ammonium (NH_4^+). Results indicated that

when earthworms are initially added to a compost mixture, the level of ammonium ion should not exceed 1.0 mg / kg to allow for an earthworm survival rate of 100 %.

2.7Parameters for Feedstocks

Different parameters have been examined for various feed stock treatments such as stocking density, pH, ammonium content, salt content, carbon-nitrogen ratio (C:N), temperature etc. Ndegwa and Thompson (1999) studied effects of stocking density and feeding rate on vermicomposting of biosolids. The study focused on treatment of fresh biosolids amended with paper mulch and *E. fetida* and investigated two important system-design parameters: stocking density and feeding rate. Four stocking densities (0.8, 1.20, 1.60, and 2.00 kg-worms/m^2) and three feeding rates (0.75, 1.0, and 1.25 kg-feed/kg-worm/day) were investigated. Effects or responses to be investigated were: product stability, worm biomass, pH, nutrients, N, and P. For the bioconversion of biosolids into earthworm biomass, a stocking density of 1.60 kg-worm/m^2 at a feeding rate of 1.25 kg-feed/kg-worm/day was optimal for vermicompost production. The same stocking density at a feeding rate of 0.75 kg-feed/kg-worm/day resulted in the most commonly digested vermicompost. For all the stocking densities and feeding rates investigated in the study, substantial reduction of both total solids (TS) and total bulk weights were obtained, ranging from 22% to 42%.

Feedstocks high in ammonia and inorganic salts are fatal to earthworms. Edwards (1988) studied the sensitivity of ammonia and stated that earthworms will not be able to survive in organic wastes containing much ammonia, e.g., fresh poultry litter. Large amount of inorganic salts in wastes will also not suitable for the survival of earthworms. Both ammonia and organic salts have very sharp cut-off points between being toxic and non-toxic. Wastes that have too much ammonia became acceptable after this was removed by a period of composting or both excessive ammonia and salts can be washed out of the waste. Worms are relatively tolerant with regard to pH, but when given a choice in a pH gradient, they moved to pH = 7.0 with a preference.

The summary table (Table 2-2) mentions the optimum requirements for *E.fetida*.

Table 2- 2 Optimum Conditions for *E. fetida* (Edwards, 1988)

Temperature	15^{o} – 20^{o}C for breeding
Moisture Content	80% to 90%
Oxygen Requirement	Aerobic
Ammonium Content of Wastes	Low < 0.5mg/g
Salt Content of Wastes	Low < 0.5%
pH	Between 5 and 9

Hand (1988) reported that "The presence of worms had a marked effect upon nitrogen transformations in a tissue waste/cow slurry mixture. Nitrogen mineralization was greater in the presence of earthworms, and this mineral nitrogen was retained in the nitrate form. This suggested that *E. fetida* produced conditions which favored nitrification. Earthworms assimilate organic nitrogen and excrete approximately equal amounts of nitrogen as ammonium and muco-proteins (Needham, 1957). This is a possible explanation for the reduction in organic nitrogen in the substrate containing worms".

In conjunction with studying optimal C:N ratios on the vermicomposting of biosolids, Ndegwa (2000) studied the percent change in Volatile Solids (VS) and Total Solids (TS) and reported that the percent VS reduction for the four C:N ratios were significantly different ($\alpha = 0.05$), with the higher reduction occurring in the C:N ratio of 25 while the lowest occurred in the C:N ratio of 10. In all C:N ratios, substantial reductions ranging between 36 % and 41 % were observed in TS; however, no significant difference ($\alpha = 0.05$) was observed among the four levels. VS decreased over the duration of these experiments. The reduction in VS increased with rising C:N ratios while the trend for TS was exactly opposite. Thus, VS can be a better indicator of the stabilization of biosolids. The highest degree of stabilization occurred when the C:N ratio was 25. Increase in the concentration in P was observed at higher C:N ratios. However, statistically, no significant difference ($\alpha = 0.05$) was observed among the four treatments. Vermicomposting decreased N concentration in the solids, except at a C:N ratio of 25. A significant difference ($\alpha = 0.05$) in the N content was observed between the treatments

with a C:N ratio of 25 and the other three treatments. The pH of the products was lower than the pH of the feedstocks in all cases. The final pH of the products was 5.32, 5.17, 5.27, and 6.17 for substrates with initial C:N ratios of 10, 15, 20, and 25, respectively. There was generally a lowering of original pH by as much as 18 – 32 % during the five weeks of vermicomposting. By the end of experiment, the most favorable pH = 6.0 for the growth of worms was in the feed stock whose initial C:N ratio was 25. This can be used as a general guide in the formulation of wide range of substrates for vermicomposting.

3. Feedstock Acceptability Test

Research began in August 2002 and concluded in May 2003. The first and second phase of the feedstock acceptability test was conducted at the Water Quality Laboratory in the Department of Biological Systems Engineering at Virginia Polytechnic Institute and State University. Earthworms (*E. fetida*) were obtained for first experiment from Vermicycle, Inc. in Wilson, North Carolina. The worms for the second experiment were acclimated for the fish sludge feedstocks, raised and collected at the Blue Ridge Aquaculture facility (BRA). At the BRA, 13.6 kg of worms were raised on 1.2 m x 2.4 m bed and fed a mixture of cardboard and fish sludge.

3.1 Materials and Methods

3.1.1 Feed Treatment Preparation

Two experiments were conducted in the first phase. The first consisted of five treatments with six replicates of each treatment, for a total of thirty experimental units. The five treatments (Table 3-1) were 0%, 5%, 10%, 15% and 20% fish sludge with the remaining percentage being cardboard. Percentages were on a dry weight basis. Based on the results of the first experiment, a second experiment of three treatments (15%, 25%, and 50% fish sludge) with six replicates (Table 3-2) was conducted. For each treatment, enough distilled water was added to achieve an 80 % moisture-content (w.b.) feed.

Fish sludge was collected from BRA prior to each experiment and frozen in batches sufficient for a weekly feeding. The cardboard was collected from corrugated cardboard boxes and was ground into small pieces using a hand-held coffee bean grinder for ten minutes. To assure that the desired mixture of fish sludge and cardboard was achieved, the moisture content of the fish sludge was determined prior to freezing based on the average moisture content of five samples. The wet weight of five grab samples were recorded; then, the samples were oven-dried for 24 hours at 104^{o}C in a Fisher Scientific Isotemp Oven Model 630-G; and the resulting dry weights were recorded. For the first experiment, the fish sludge was frozen at 95% moisture w.b. The moisture content of the fish sludge for the second experiment was 89% w.b. The cardboard was oven-dried for 24 hours prior to feedstock preparation to remove moisture. Distilled water

was added to each feed mix to achieve a final moisture content of 80% w.b. Fifty grams of feed were added to each experimental unit each week.

3.1.2 Worm Feeding Boxes

Plastic containers (Rubbermaid Sandwich Boxes, 0.767 L capacity) were modified for use as growing boxes. The center of the container lid was cut out, leaving only the rim. A nylon cloth was draped over the top of the box and held in place by this rim piece (Figure 3-1). This allowed for aeration within the container. For the first experiment, 50 g of well-composted dairy manure, which was saturated with water and allowed to gravity drain for 24 hours, was placed in the bottom of each box to serve as a bedding material for the worms. For the second experiment, 50 g of a mixture of 75% peat moss and 25% worm castings (volume basis), which was saturated with water and gravity drained for 24 hours, was used as the bedding material.

Figure 3 - 1 Experimental Setup for Feedstock Acceptability Test

Ten worms were placed in the bedding material in each box. A piece of fiberglass mesh (standard window screening, available at hardware stores) was placed on top of the bedding material. Fifty g of feed was placed on top of the screening material. After placing the bedding, worms, screening and feed in a box, the total container weight was measured and

recorded. Twice a week the containers were reweighed and misted with distilled water to return the box to its original weight, thus assuring that the feedstock remained moist for worm consumption. The boxes were placed inside a cabinet drawer to keep the worms in the dark. The boxes were randomly placed in the drawers to maintain a complete random design.

3.1.3 Worm Biomass Measurement

Worm biomass in each box was measured at the beginning and end of the experiments and prior to feeding each week. To accomplish this, worms were removed from the bedding by hand, gently rinsed with distilled water to remove the bedding, briefly drained on a paper towel, and weighed using a Mettler Toledo PG 5002-S scale. The ten worms initially placed in each box were weighed as a unit. Once a week for the ten weeks of the experiment, the worms were removed from each box and weighed (using the same procedure described above) to track change in biomass over time. The number of live worms found in each container each week was also recorded. No additional worms were added during the experiment, regardless of the mortality rate. After weighing the worms, old feed was discarded and new feed was placed on the screen. This simulated feeding in a commercial operation.

Table 3 - 1 Composition of feeding treatments for the first experiment (95 % Moisture Content of Fish Sludge)

Compost Mixture	Composition	Weight Basis Mixture
1	0 % fish sludge, 100 % cardboard	0 g sludge, 280 g water, 70 g cardboard
2	5 % fish sludge, 95 % cardboard	78 g sludge, 206 g water, 66 g cardboard
3	10 % fish sludge, 90 % cardboard	155.5 g sludge, 131.5 g water, 63 g cardboard
4	15 % fish sludge, 85 % cardboard	233 g sludge, 57 g water, 60 g cardboard

5	20 % fish sludge, 80 % cardboard	294 g sludge, 56 g cardboard, 0 g water

Table 3 - 2 Composition of feeding treatments for the second experiment (89% Moisture Content of Fish Sludge)

Compost Mixture	Composition	Weight Basis Mixture
1	15 % fish sludge, 85 % cardboard	96 g fish sludge, 59 g cardboard, 195 g water
2	25 % fish sludge, 75 % cardboard	159 g fish sludge, 53 g cardboard, 138 g water
3	50 % fish sludge, 50 % cardboard	315 g fish sludge, 35 g cardboard, 0 g water

3.1.4 Statistical Analyses

All statistical tests were analyzed at the $\alpha = 0.05$ level to determine if statistical differences existed. The SAS (Statistical Analysis Software) program using the GLM was used.

Analysis was conducted on the basis of average worm mass and number of worms alive in each container. A two-step analysis was done. The first step calculated a linear regression of average worm weight per time for each box. The first step analysis resulted in 30 graphs (average worm mass versus time) from five treatments and six replicates of each treatment. The slope and intercepts of all 30 graphs were calculated. Based on the results of the first step, a second step was also similar to the first (ANOVA analysis) but to determine the significant difference among the treatments a Tukey's Studentized Range (HSD) Test for the 30 intercepts and slope achieved to determine significant differences between the various treatments. In these tests, the intercept was the worm weight at the starting of the experiment and slope was the growth rate (g/week).

In the first step, regression analysis was used. Its model was:

$$Y = \beta_0 + \beta_1(x) + \varepsilon$$

A linear equation was customized for each worm box within a treatment so that there was a relationship for each worm box over time. Then, the slope (β_1) from those regressions was used as the dependent variable in a very similar model,

$$Y = \mu + \alpha_i + \varepsilon_{ij}$$

Where i=1to 5, or the number of treatments (fish solids) and
j=1 to 6, or the number of replicates per treatment.

A similar statistical analysis was done for the second experiment. The SAS output for both experiments is discussed in Appendix A.

3.2 Results and Discussion

3.2.1 Experiment 1

As the percentage of fish sludge increased in the feed mixtures, there was a corresponding increase in biomass of *E. fetida*. The different ways of comparing results are average mass per worm and worm mortality rates. Mortality rates are the number of worm deaths over a given time. A mixture of 0% fish sludge has a lower mortality rate whereas 10% and 20% have higher mortality. However, statistically there were no differences on mortality rate among the treatments. During the acclimation period, higher mortality occurred during the first two weeks. But after their acclimation period, the worms increased their biomass and mortality declined. Figure 3-2 shows the percent mortality of the worms.

Figure 3-3 summarizes the average worm mass in each container at different time intervals. Fish solids of 20% have the maximum average worm mass and 0% fish sludge has the minimum average mass per worms. Comparing both the graphs shows that 20% fish sludge has fewer numbers of worms present but achieved the maximum gain in average mass per worm, whereas in 0% fish sludge, the maximum numbers of worms were alive with minimum gains in average mass per worm. This indicates that the worms gain weight as the amount of fish sludge increases. The second experiment was conducted to examine whether the worms can survive on higher percentage of solids.

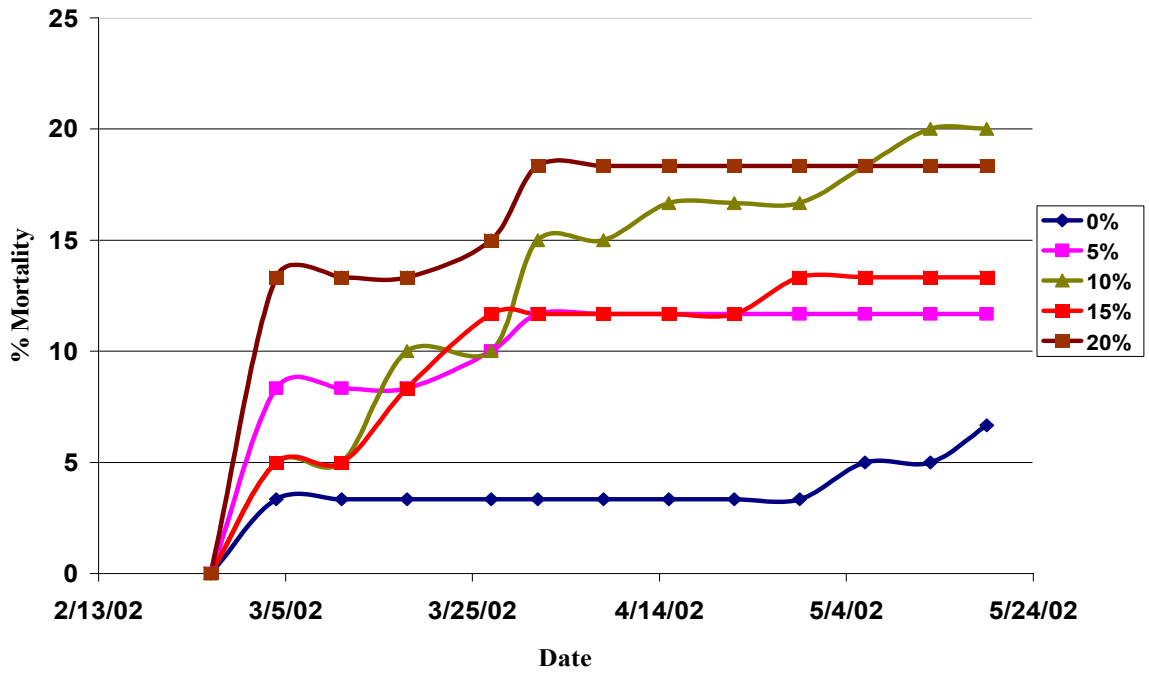


Figure 3 - 2 Percent Mortality of Worms

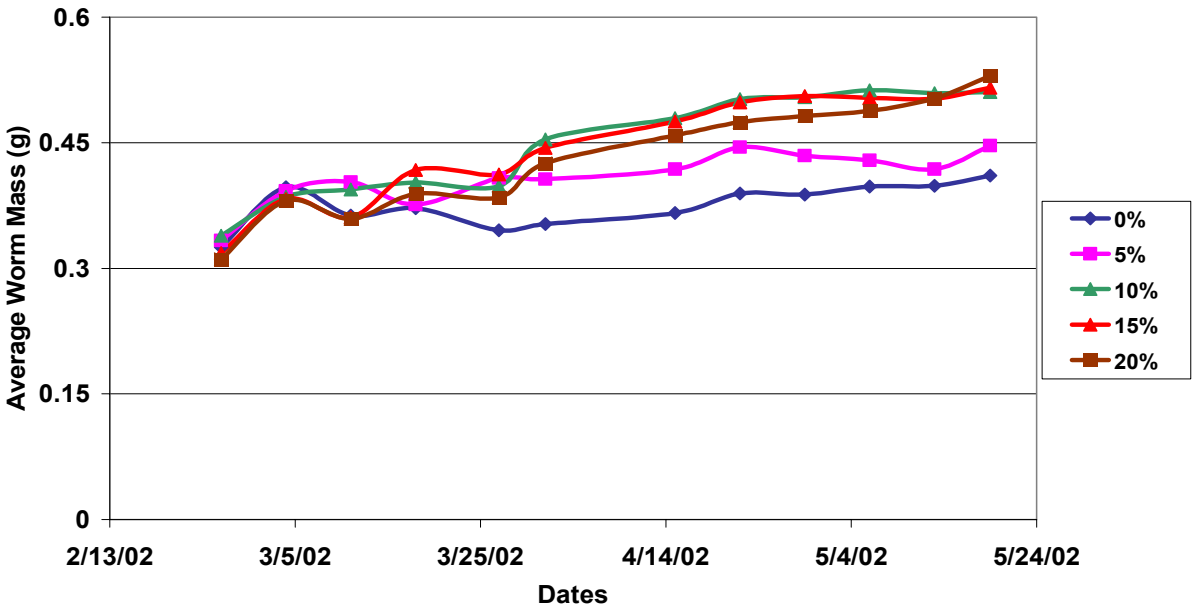


Figure 3 - 3 Average Mass per Worm for Various Treatments on Weekly Basis

3.2.2 Experiment 2

Based on the results of the first experiment, three treatments with higher percentages of fish sludge were examined (i.e. 15%, 25%, and 50% fish sludge). Results obtained from the second experiment are summarized in Figure 3-4. The first two weeks were an acclimation period due to the change in environment and physical holding conditions. However, immediately after acclimation, worm biomass increased and no mortality occurred during the 11 week treatment period. Cocoon production occurred from the fourth week onwards in 50% fish sludge, whereas for 15% and 25%, cocoon production began in the sixth week.

Figure 3-4 summarizes the average worm mass at different time intervals. Average mass per worm for all treatments increases with time. Average worm biomass at the end of 11 weeks was greatest for the 50% fish solids treatment and was smallest for the 15% fish solids treatment. The figure shows the biomass over time for all of the treatments. According to the average worm biomass, the 15%, 25% and 50% fish sludge gave good results, but it was observed that for greater percentages of fish sludge worm weight gain was greater. There was a significant difference ($P \leq 0.05$) between 50% fish solids and 15%, 25% fish solids. The data's were also analyzed by using linear regression and Tukey's Studentized Test as it was mentioned in earlier section.

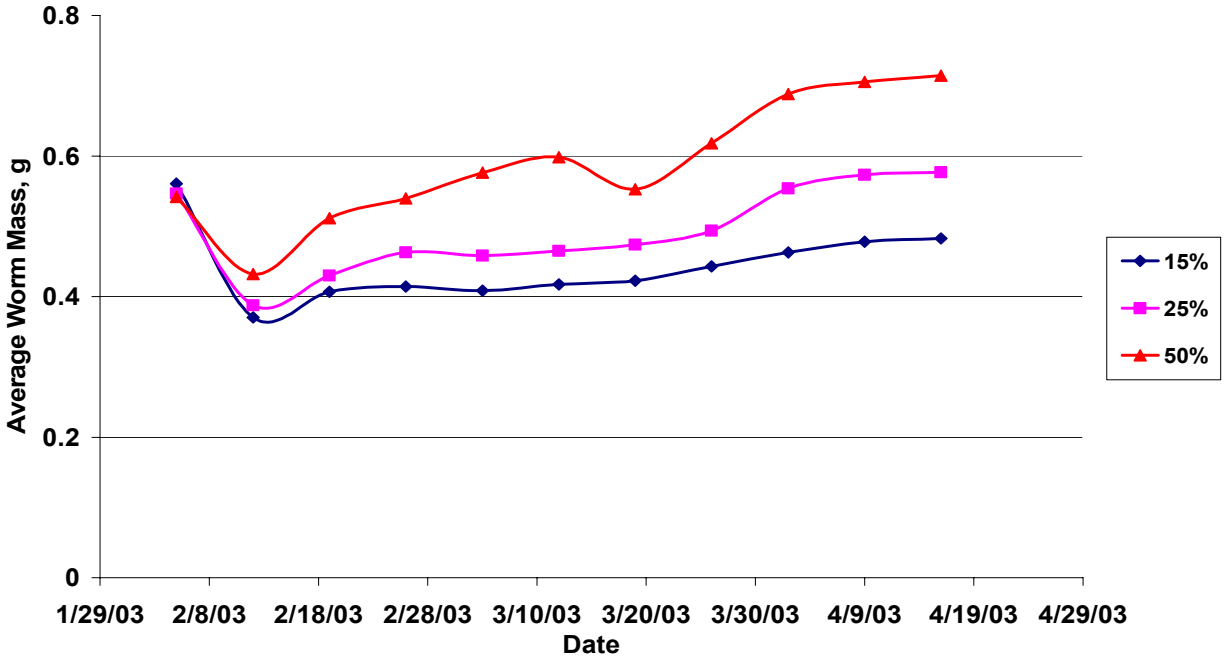


Figure 3 - 2 Average mass per worm for various treatments

Table 3-3 shows the statistical analysis results obtained using Tukey's Studentized Range test for the first experiment. There were no significant differences in growth rate between 10%, 15%, and 20 % fish sludge, but the growth rates for 0% and 5% fish sludge are significantly less than the higher percentage of fish sludge (10%, 15%, and 20 %). Therefore, it can be concluded from the result of the first experiment that the growth rate of worms increases with higher percentages of fish solids. The second experiment also indicated that the worm growth rates increased with higher percentages of fish solids (Table 3-4). The SAS output for both experiments is discussed in Appendix B.

Table 3 - 3 Tukey's Studentized Range (HSD) Test for slope for the first experiment

Tukey's Grouping	Mean (gm / wk)	N	Fish solids
A	0.016	6	20%
A	0.015	6	15%
A	0.014	6	10%
B	0.006	6	5%
B	0.004	6	0%

Table 3 - 4 Tukey's Studentized Range (HSD) Test for slope for the second experiment

Tukey's Grouping	Mean (gm / wk)	N	Fish solids
A	0.017	6	50%
A	0.012	6	25%
B	0.002	6	15%

Means with the same letters are not significantly different. N represents the number of replications.

3.3 Summary and Conclusions

Vermicomposting is an effective method for stabilizing fresh fish sludge. The results showed that with an increase in fish sludge feed mixtures (cardboard and fish sludge), there was a corresponding increase in worm biomass. According to the average worm biomass, the 15%, 25% and 50% fish sludge gave the best results. There were no significant differences on mortality rate among the treatments during the first experiment and for the second experiment there was no mortality.

4. Filter Bed Test

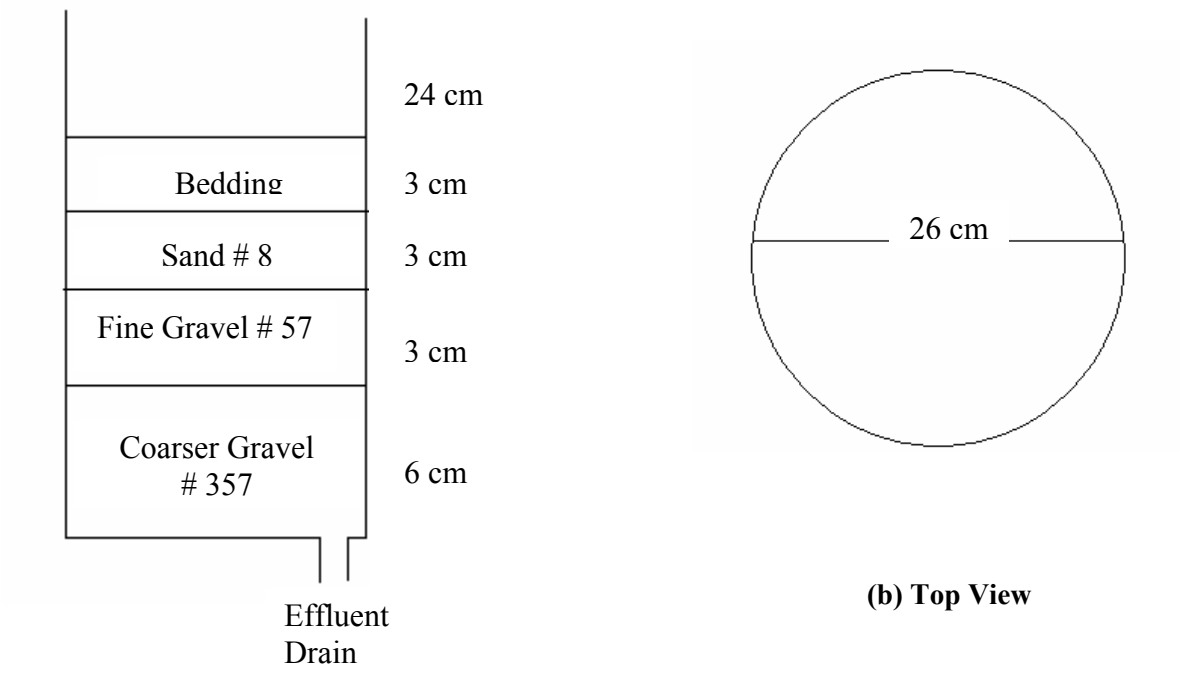
4.1 Materials and Methods

4.1.1 Experimental Design

An experimental setup was constructed in a greenhouse at the Virginia Tech Aquaculture Facility, Blacksburg, VA. Sixteen sand bed filters were constructed—twelve with worms and four without worms. Worm filter-beds had four application rates, each with three replications, and the control filter-beds had two application rates each with two replications. The fish sludge was obtained from Blue Ridge Aquaculture, Martinsville, Virginia. This facility produces tilapia (*Oreochromis spp.*) in an indoor, recirculating system and discharges approximately two million liters of wastewater per day. The facility is interested in installing a wastewater treatment facility and wished to investigate possible treatment technologies for the wastewater that would be produced.

4.1.2 Filter Bed Construction

The sand filter beds were constructed of Poly Vinyl Chloride (PVC) pipe 26 cm in diameter and 39 cm high. The bottom of each sand-bed filter was covered with a lid from a 19 L container. A two cm PVC pipe was connected to the nozzle at the bottom of each filter bed to drain the effluent into an 11.3 L plastic can (Rubbermaid®). Sand and gravel layers were placed in the PVC pipes to act as filter bed to retain solids and allow drainage of effluent water to the plastic can. The filter beds had layers of coarse gravel, fine gravel, and sand, from bottom to top, respectively (Raymond et al., 1988). The worm filter beds also had a three cm layer of bedding material on top of the sand layer. Nylon fiber mesh was placed between the bedding and sand layer to prevent the movement of worms from the bedding to other layers. Approximately 100 kg of worms of mixed ages were added to each worm filter bed (Raymond et. al., 1988). The bedding layer helped the worms to survive and maintain the proper porosity. Figure 4-1 shows side and top views of a filter bed.



(a) Side View

(b) Top View

Figure 4 - 1 Side view and top view of filter bed layers

4.1.3 Bedding Material

A mixture of ¾ well-composted dairy manure and ¼ peat moss by volume with a pH between 6 and 7 was used as the bedding material for the worm filter beds. Distilled water and calcium hydroxide (Ca $(OH)_2$) were added to the mixture to get the appropriate pH of 6.5. To accomplish the pH adjustment, the mixture was mixed manually and allowed to rest a day, at which time the pH level was measured. This procedure continued until the desired pH was achieved. After excess water was drained from the mixture, the bedding material was placed on the worm filter bed. Table 4-1 shows the analysis of the well-composted dairy manure. Well-composted dairy manure was chosen as a percentage of the bedding material because it has good water absorption capacity and will not heat due to microbial decomposition, thus killing off the worms.

Table 4 - 1 Biosolids analysis report of well-composted dairy manure

Variable	Result (%)	Result (mg/g)	Detection Limit (mg/g)
Calcium	3.49	34900	100
Ammonia-N	Low 0.01	100	100
Nitrate-Nitrite-N		96	10
Solids	43.79	437900	100

4.1.4 Frame Construction

A cubical frame made of plywood 2.5 m x 1.25 m x 1.25 m was constructed (Figure 4-2) to maintain the proper temperature for the worms. Four sections of plywood 1.25 m x 2.5 m x 0.01 m thick were used to construct the frame. Three sections of plywood of 2.5 m by 1.25 m each were used: two pieces for the each side of the frame and one to cover the top of the frame. The fourth section of plywood of 2.5 m x 1.25 m was cut into two equal parts of 1.25 m x 1.25 m: one of them used at the back of the frame and other one used at the front of the frame. All the sections of plywood were joined by screws and the front door of the frame was joined by 4 two-door hinges of 88 mm. Inside the frame, two 2.5 m x 0.3 m wooden plates were screwed on the left and right sides 1.25 m above the ground. The filter-beds were placed in a randomized block design on top of the wooden plates. Sixteen plastic cans (Rubbermaid, 11.36 L) were placed below each filter bed and the drain pipe from the filter-bed was inserted into the can. Between the two wooden plates inside the frame, an electric oil-filled radiator (DeLonghi Safe Heat) was placed and a maximum-minimum thermometer (SPER Scientific 736680) was hung on the wall of the frame to maintain the optimum temperature of $20^{o} \pm 5^{o}C$ for *E.fetida*, as shown in Figure 4-3.

Figure 4 - 2 Experimental setup at Virginia Tech Aquaculture Facility

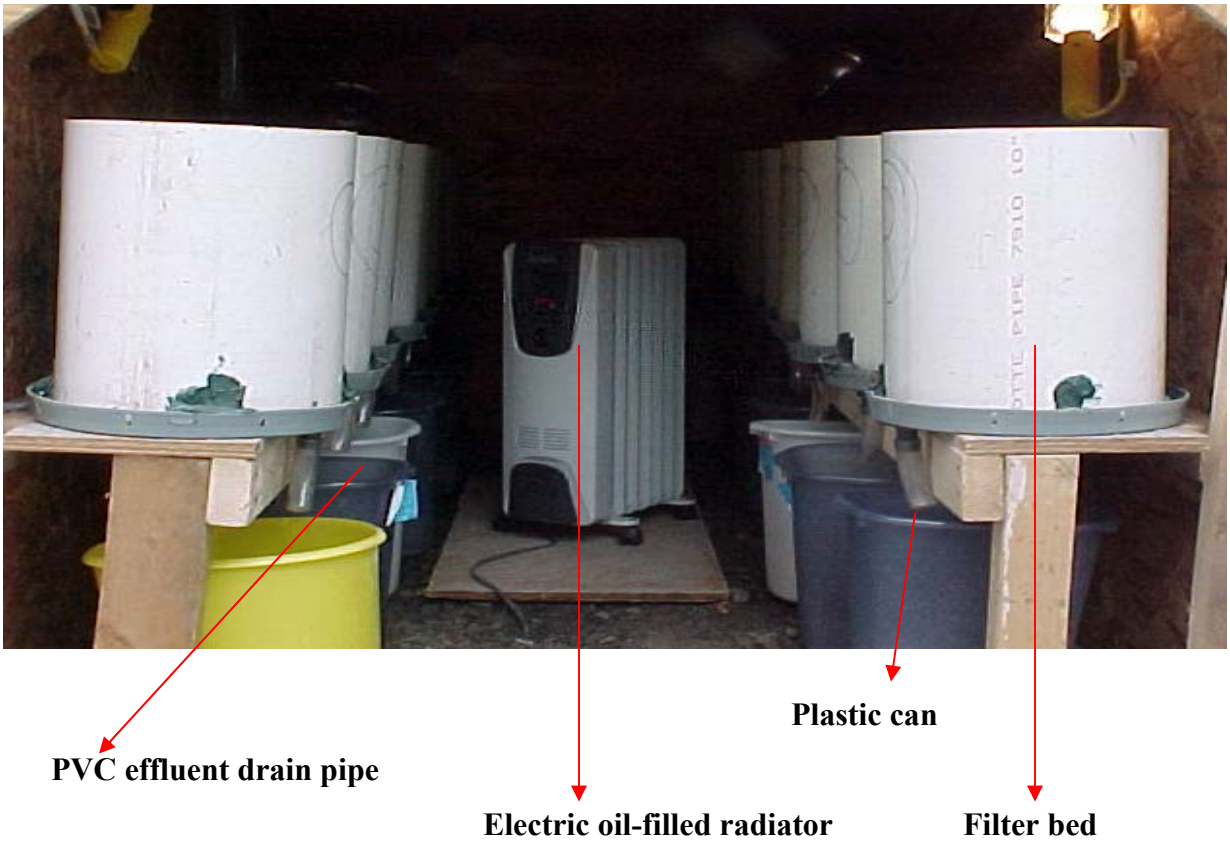


Figure 4 - 3 Labeled diagram of experimental setup at Virginia Tech Aquaculture Facility

4.1.5 Application Method

Application rates of fish waste water were calculated on the basis of results obtained from the study done by Raymond et al. in 1988. The study was done for municipal sludge of 0.6 to 1.3% total solids, wherein the maximum VS loading rate at which the worm filter bed performed satisfactorily was 1000 gm / m^2/week, and for control filter-bed it was 400 gm /m^2 / week.

BRA fish sludge was measured for the TS and VS content and their ratio was calculated. The amount of VS was calculated for 1.5 gm of TS in 100 gm of sludge on the basis of this ratio. This value and the waste application frequency were used to calculate the maximum application rate as determined by Raymond et al. in 1988. The detailed calculation is shown in Appendix D. Three more application rates less than the maximum value were selected. Application rates for the control treatment were also decided according to the maximum value suggested by Raymond et al. in 1988. Table 4-2 shows the application rates as calculated for each treatment and control.

Table 4 - 2 Relationship between number of filter beds and application rates

Treatment Designation	Worms (Yes/No)	Number of Replicate	VS Loading in gm / m^2 / wk	Wastewater Application Rate (l/m^2)*	Wastewater Application Rate (l/application)*
W1	Yes	3	1000	24	1.2
W2	Yes	3	800	20	1.0
W3	Yes	3	600	16	0.8
W4	Yes	3	400	10	0.5
C1	No	2	400	10	0.5
C2	No	2	300	8	0.4

* Note: All treatments received an application of wastewater three times per week (Monday, Wednesday, and Friday).

4.1.6 Preparation of Feed

Fish sludge obtained from BRA was stored in the refrigerator in a Ziploc Sandwich Bag to prevent decay. The moisture content was measured prior to freezing. Prior to each application

of influent to the beds, the feed was prepared by adding water to the sludge to reduce the solid content to 1.5 % solids by weight. This moisture content was selected to simulate wastewater coming off of a drum filter. Total feed needed for each application was 12.3 L, the sum of the application rates mentioned (Table 4-2). The sample calculation for all 16 filter beds of one feeding day is shown in Appendix E.

4.1.7 Sampling and Analysis

Fish sludge was applied to each filter bed three times a week for 10 weeks at the aforementioned application rates. The effluent was collected from each bed once a week in 250 mL sampling bottles for analysis. Effluents from the additional two application periods each week were discarded. Following variables were analyzed in both the influent and the effluent: VS, TS, pH, TSS, soluble P, total P, chloride, sulfate, alkalinity, ammonium-N, nitrate-N, chemical oxygen demand (COD), hydraulic conductivity, and dissolved oxygen.

4.1.8 Procedure for Different Variables

The principles related to the test are discussed below and complete procedures are given in Appendix C. The first five laboratory analyses were done with the help of **HACH** Spectrophotometers Odyssey DR/2500. All the tests except the dissolved oxygen test were performed in the Land and Water Resources Lab, Biological Systems Engineering Department, Virginia Tech. Dissolved oxygen tests were performed in Virginia Tech's Aquaculture Facility.

Total Phosphorus (TP), Soluble Phosphorus (SP), and Phosphate (PO_4^3)

The procedure for both the tests was same. For SP, the filtrate of the sample effluent was used. HACH DR/2500 Method number 10127 Molybdovanadate Method with Acid Persulfate Digestion Method HR 1 to 100 mg/L PO_4^{3-} was used to measure the TP and SP in the influent as well as the effluent wastewater.

Sulfate (SO_4^{2-})

HACH DR/2500 Method number 8051 SulfaVer 4 Method HR 2 to 70 mg/L was used to measure the SO_4^{2-} in the influent as well as the effluent wastewater. Program 680 was selected from the menu of the HACH Spectrophotometers. The readings were over range, so to get the appropriate value the sample was diluted with deionized water until the apparatus read a value. The exact dilution was 2 ml of samples with 8 ml of deionized water, at which point the readings were in range. Results were multiplied by five to get exact readings in mg/L SO_4^{2-}.

Nitrate-N (NO_3–N)

HACH DR/2500 Method number 8039 Cadmium Reduction Method HR 0.3 to 30 mg/L NO_3-N was used to measure the NO_3–N in the influent as well as the effluent wastewater. Program 355 N was selected from the menu of the HACH Spectrophotometers. The readings were over range, so to get the appropriate value the sample was diluted with deionized water until the apparatus read a value in the proper range. The exact dilution was 0.5 ml of samples, with 9.5 ml of deionized water, at which point the readings were in range. Results were be multiplied by 20 to get the exact readings in mg/L NO_3–N.

Ammonium-N (NH_3–N)

HACH DR/2500 Method number 10031 Salicylate Method HR 0.4 to 50 mg/L NH_3-N was used to measure the NH_3–N in the influent as well as the effluent wastewater. Program 343 N, Ammonia HR TNT, was selected from the menu of the HACH Spectrophotometers.

Chemical Oxygen Demand (COD)

HACH DR/2500 Method number 8000 Reactor Digestion Method HR 20.0 to 15000 mg/L COD was used to measure the Nitrate-N in the influent as well as the effluent wastewater. Initially, COD reactor was turned on and heated to 150^{o}C. Program 435 HR was selected from the menu of the HACH Spectrophotometers.

Chlorides (Cl^-)

For chloride test method 8225 from the *HACH WATER ANALYSIS HANDBOOK*, a silver nitrate burette titration method (0 to 25,000 mg/l Cl^-) was used. Volume samples and standard titrants were selected (Table 4-3) that corresponded to the expected chloride (Cl^-) concentration.

Table 4 - 3 Relationship of multiplier and sample volume based on expected chloride range

Range (mg/l as Cl^-)	Sample Volume (ml)	Digital Multiplier
0-125	100	5
100-250	50	10
200-500	25	20
500-1250	100	50
1000-2500	50	100
2500-10,000	25	200
5000-25,000	10	500

Total Suspended Solids

Principle- A well-mixed sample was filtered through a weighed standard glass-fiber filter and the residue retained on the filter was dried to a constant weight at 103 to 105^oC. The increase in weight of the filter represented the total suspended solids. If suspended materials clogged the filter and prevented filtration, the difference between the total solids and total dissolved solids provided an estimate of the total suspended solids.

Volatile Solids

Method 8276 from the *HACH WATER ANALYSIS HANDBOOK* was used to determine the volatile solids. An aluminum dish was weighed and 50 mL from a mixed sample was added to the aluminum dish. The sample was kept in a preheated oven and evaporated at 103 to 105^oC for approximately six hours. After six hours, the dish was removed from the oven and allowed

to cool to room temperature in a desiccator. Again the dish with the sample was weighed, transferred to the aluminum dish into a pre-heated muffle furnace at 550^{o}C and kept there for 30 minutes. The dish was removed and cooled to room temperature in a desiccator. The sample was weighed again for total volatile solids.

Total Solids

Method 8271 from the *HACH WATER ANALYSIS HANDBOOK* was used to determine total solids. An aluminum dish was weighed and 50 mL from a mixed sample was added to the aluminum dish. The sample was kept in a preheated oven and evaporated at 103 to 105^{o}C for approximately six hours. After six hours, the dish was removed from the oven and allowed to cool to room temperature in a desiccator. Again, the sample was weighed. The procedure was repeated until results did not differ by more than 0.4 mg.

Alkalinity

For the alkalinity test, method 8221 from *HACH WATER ANALYSIS HANDBOOK*, silver nitrate burette titration method (0 to 5,000 mg/l $CaCO_3$) was used. The volume sample 25 mL (Table 4-4) was measured with a graduated cylinder or pipette and transferred to a 250 ml Erlenmeyer flask. The final sample was prepared by mixing the contents with one phenolphthalein indicator powder pillow. Next, a 25 ml burette was filled with 0.020 N sulfuric acid standard solutions. The prepared sample was titrated while swirling the flask until the color changed from pink to colorless. Since the solution was colorless before titrating with sulfuric acid, the phenolphthalein alkalinity was zero. One Bromcresol green-methyl red indicator powder pillow was added and the sample was mixed by swirling the flask. The titration was continued until a light pink endpoint was reached.

Table 4 - 4 Relationship of multiplier and sample volume based on expected Alkalinity range

Range (mg/l as $CaCO_3$)	Sample Volume (ml)	Digital Multiplier
0-500	50	20
400-1000	25	40
1000-2500	10	100
2000-5000	5	200

Dissolved Oxygen:

The YSI Model 55 Handheld Dissolved Oxygen System, a micro-processor based, digital meter with an attached YSI dissolved oxygen probe, was used to determine dissolved oxygen in mg/l. The system has a calibration function built into the instrument and was calibrated every time prior to testing collected samples.

pH

A Corning 360 – I measured pH. Before measuring the pH, the meter was calibrated by using solutions of pH 7 and 4.

Hydraulic Conductivity

The infiltration rate was measured for all treatments at the end of the experiment (week 10). Once the media in all the filter beds was saturated, various flow rates were applied to the designated filter beds. Filter beds were saturated by applying wastewater at the specified rates for four consecutive days. The volume of waste collected at the bottom of each filter bed over a specific period of time defined the flow rate. The hydraulic conductivity of each treatment was calculated by dividing the flow rate with the product of area (A) and hydraulic gradient ($I = \Delta H/L$), where ΔH is the change in head and L is the length of sample.

$$K = (V * L) / (A * t * \Delta H)$$

Where; K = Hydraulic conductivity (cm/day), V_{out} = Volume of effluent (cm^3), L = Length of filter bed (cm), A = Cross-Sectional area of the filter bed (cm^2), t = Time to drain water (day), V_{in} = Influent volume, L, ΔH = Change in hydraulic head (cm) = V_{in} / Surface area (A).

4.1.9 Statistical Analysis

All statistical tests were analyzed at the $\alpha = 0.05$ level to determine differences. The SAS (Statistical Analysis Software) program using the MIXED procedure was used to analyze the data of various parameters. A repeated measures statement was used to account for the change in concentration of various parameters over time in the effluent. Another statement with contrast was used to account for the difference between the worms and control filter beds. The

second statement was used only when the value of P for the interaction between sample and time from the first statement was significant ($P \leq 0.05$). The SAS codes for both the statement are shown in Appendix F.

The statistical model used to analyze the data for the first statement is given below:

$$\mathbf{Y = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}}$$

Where

μ = overall mean

α_i = filter bed affect with i^{th} term, (i = 16).

β_j = time affect with j^{th} term, (j = 10).

$(\alpha\beta)_{ij}$ = interaction affect

ε_{ijk} = error term

The statistical model used to analyze the data for the second statement for each week is given below:

$$\mathbf{Y = \mu + \alpha_i + \varepsilon_{ij}}$$

Where,

μ = overall mean

α_i = filter bed affect with i^{th} term, (i = 16)

ε_i = error term

4.2 Results and Discussion

The objective of the filter bed test was to determine if the addition of worms to a sand filter bed could improve the filter's effectiveness.

Wastewater was added on the surface of each filter bed three times a week. Generally within one day, earthworm activity leveled the surface. Throughout the study there were no odors, other than an earthy smell, from any of the filter beds. In addition, there were no indications of stressed conditions for the worms, i.e., no worms attempted to escape from the filter beds and there were no dead worms on the surface of the filter beds. Raymond (1984) observed similar results with municipal sludge at 1.6 % TS.

With time, there was a buildup of residual solids in the filter beds. The solids increase was related to both the length of time a filter bed was in operation and the waste application rate. After ten weeks, about 1.3 cm to 2.54 cm solids accumulated on the surface of the filter beds. However, worm filter beds functioned satisfactorily until the project ceased. The control filter beds failed in a short period of time when the liquid no longer flowed through the accumulated sludge solids for more than ten minutes. Ponding resulted and may have contributed to anaerobic conditions. For example, control filter beds receiving an application rate of 0.5 L per feeding per pipe (C1.1 and C1.2) failed after 24 days and control filter beds at application rate 0.4 L per feeding per pipe (C2.1 and C2.2) failed after 35 days. Although they had failed, applications were continued on the control filter beds to compare water quality in the effluent with the worm filter beds.

The greatest loading rate tested was 1000 grams of VS /m^2 / week. The vermistabilization process functioned satisfactorily at this rate. When the worm filter beds functioned well, most of the water added with the liquid sludge drained quickly (within 60 minutes) and aerobic conditions were maintained. It appears that worms also increased the pore space of the accumulated solids to increase hydraulic conductivity.

The total quantity of drainage and its characteristics were determined weekly in tandem with characteristics of the influent wastewater (Table 4-5). It was observed that the influent concentration of TS was less than the TSS. The influent concentration for all the variables was determined twice during the experimentation period, and the value mentioned (Table 4-5) was the average of the two readings. The difference between the value of TSS and TS (denoted by *) can be attributed to test performance error at the first reading. TSS in the influent during the first week was 8850 mg / L, whereas TS was 7000 mg / L. Theoretically, it is not possible to have a concentration of TSS greater than TS. The test for the influent performed on the fifth week had less TSS concentration (6920 mg / L) than TS. As a result, the fifth week value of TSS is acceptable. The error occurred on the first week of sampling.

Table 4 - 5 Characteristics of sludge added to filter beds

Parameter	Concentration
Total Solids, mg / L	7000*
Volatile Solids, mg / L	6000
Total Suspended Solids, mg / L	7886*
Total Phosphorus, mg / L	210
Soluble Phosphorus, mg / L	19.3
Chemical Oxygen Demand, mg / L	680
Sulfate, mg / L	365
Nitrate-N, mg / L	130
Ammonia-N, mg / L	15
pH	6.67
Alkalinity as $CaCO_3$, mg / L	380
Chloride, mg / L	156
Dissolved Oxygen, mg / L	11

4.2.1 Cocoon Production

Worms reproduce by producing an egg case, called a cocoon, which contains several eggs. Cocoon production was hindered at lower application rates. Cocoon production occurred in filter bed 1, which received the highest application rate (1.2 L per feeding per pipe), after 24 days. Filter beds 2 and 3, which received 1.0 and 0.8 L per feeding per pipe, exhibited cocoon

production after 38 days. Cocoons were produced in filter bed 4, which received the lowest application rate (0.5 L per feeding per pipe) after 65 days.

4.2.2 Drainage and Vermistabilized Sludge

Results of interest in this experiment are: (a) the average characteristics of the liquid that drained from the filter beds, (b) the characteristics of the vermistabilized sludge, and (c) the upper limit of the loading rate at which the filter beds functioned successfully. Average drainage characteristics determine whether the resulting wastewater can be returned to the production facility or if it must be further treated. Characteristics of vermistabilized sludge are of interest because ultimately the sludge must also be managed (used as compost or disposed of). The upper limit of the loading rate will determine the size of filter beds required to treat a specified volume of wastewater. In the following paragraphs, the performance of the liquid vermistabilization process is discussed in terms of the aforementioned points.

The liquid that drained from the filter beds following the liquid sludge application contained particles not captured by the filter beds, as well as any soluble constituents that leach through the beds. Leached constituents may be in the liquid sludge application or may result from the vermicomposting of solids captured from previous applications. The concentrations of the various parameters in the filter bed effluents over time are illustrated in the figures that follow.

The average characteristics of the drainage can be put into perspective by comparing them to the characteristics of the liquid sludge applied to the filter beds. With each portion of liquid sludge, a sizable fraction (approximately 100-150 ml) of the applied material was retained in the filter beds and stabilized by the worms and microorganisms. Table 4-6 lists all of the parameters tested in the effluent and indicates whether or not the observed differences among the worm filter beds and between the worm and control filter beds were statistically significant. Among the worm filter beds, statistical analysis was calculated for the last three weeks of the experiment.

Table 4 - 6 Significant differences in various effluent parameters

Parameter	**Significant Difference Among Worm Filter Bed @ ($P \leq 0.05$)**	**Significant Difference Between Worm and Control Filter Bed (Yes/No) @ ($P \leq 0.05$)**
Total Solid	No ($P \geq 0.1312$)	Yes ($P \leq 0.0001$)
Volatile Solid	No ($P \geq 0.2203$)	Yes ($P \leq 0.0001$)
Total Suspended Solid	No ($P \geq 0.0956$)	Yes ($P \leq 0.0034$)
Ammonia-N	No ($P \geq 0.1945$)	Yes ($P \leq 0.0001$)
Nitrate-N	Yes ($P \leq 0.0021$)	Yes ($P \leq 0.0008$)
Chemical Oxygen Demand	No ($P \geq 0.2421$)	No ($P \geq 0.1752$)
Chloride	No ($P \geq 0.3242$)	No ($P \geq 0.2503$)
Sulfate	No ($P \geq 0.1026$)	No ($P \geq 0.1517$)
Total Phosphorus	No ($P \geq 0.310$)	Yes ($P \leq 0.0061$)
Soluble Phosphorus	Yes ($P \geq 0.1301$)	Yes ($P \leq 0.0015$)
Dissolved Oxygen	Yes ($P \geq 0.1301$)	Yes ($P \leq 0.0005$)
pH	No ($P \geq 0.1301$)	Yes ($P \leq 0.0001$)
Alkalinity	No ($P \geq 0.1301$)	Yes ($P \leq 0.0001$)

4.2.2.1 Total Solids (TS), Volatile Solids (VS), & Total Suspended Solids (TSS)

Figure 4-4 shows the performance of the filter beds in terms of average total solids discharged in the effluent. The TS present in the liquid sludge (influent) applied to the filter beds, throughout the experiment (1.5%). It can be seen from Figure 4-4 that over the course of the experiment, TS in the effluent dropped to nearly zero for all worm treatments. TS in the effluent from the control filter beds increased over time and actually exceeded the TS content of the influent, indicating that accumulated solids were being carried into the effluent. As the experiment proceeded, solids started accumulating on the surface of both the worm and the control filter beds. However, the worms present on the surface of the filter beds appear to have increased the pore size of the medium (keeping the hydraulic conductivity high) and allowing the solids in subsequent liquid sludge applications to be trapped in the filter bed. In the control filter beds, the solids that accumulated on the surface restricted the movement of wastewater.

Ponding occurred, and solids escaped in the effluent. During week ten, there were no differences in the concentration of TS in the effluent between the filter beds with worms; all four loading rates preformed similarly. However, the TS concentrations in the effluent from worm filter beds were significantly lower than the TS concentrations in the control filter effluents.

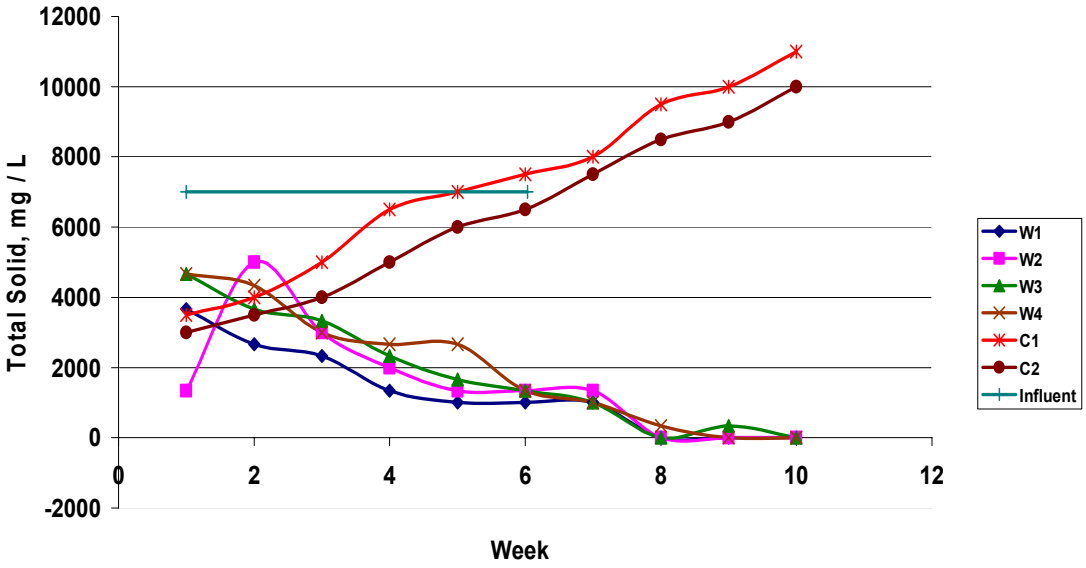


Figure 4 - 4 Average TS concentrations over time in the effluent for various treatments

The VS concentration in the effluent over time (Figure 4-5) was very similar to what was observed with TS. Again, as time proceeded, concentration in the effluent from the worm filter beds decreased, becoming effectively zero by week four for all loading rates. However, in the control filter beds, VS concentrations in the effluent increased after the third week for both loading rates. This corresponded with failure of the filter beds. Again, there was no difference between the various worm filter bed treatments, but the difference in filters with worms and without worms was statistically significant. The more rapid degradation of the organic matter was probably due to increased aeration and other factors brought about by the earthworms (Raymond et al. 1988).

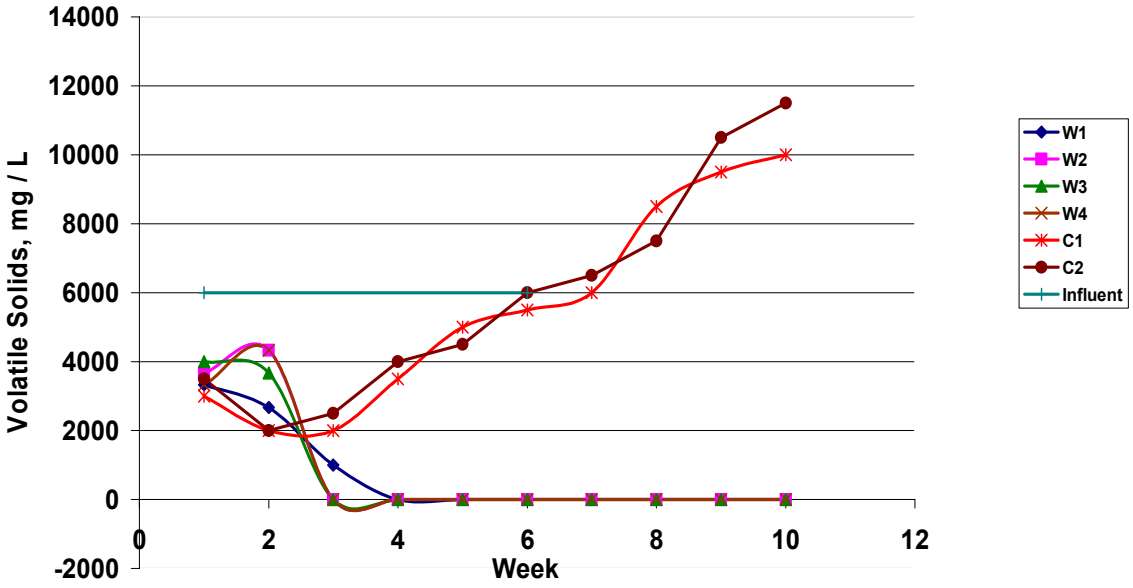


Figure 4 - 5 Average VS concentrations over time in the effluent for various treatments

Figure 4-6 shows the TSS in the effluent over time. The TSS concentration in the effluent was reduced in the worm filter beds after the fourth week. The worm filter beds with the highest application rate (W1) showed the greatest TSS concentration reduction rate. After the eighth week, the TSS concentrations in effluent from filter beds 1, 2, 3 and 4 were negligible, whereas the concentrations of TSS in the effluent from the control filter beds increased after the fourth week due to an accumulation of solids on the surface layer. Once again, there was no difference between the treatments with worms, but there were differences between worm and control treatments.

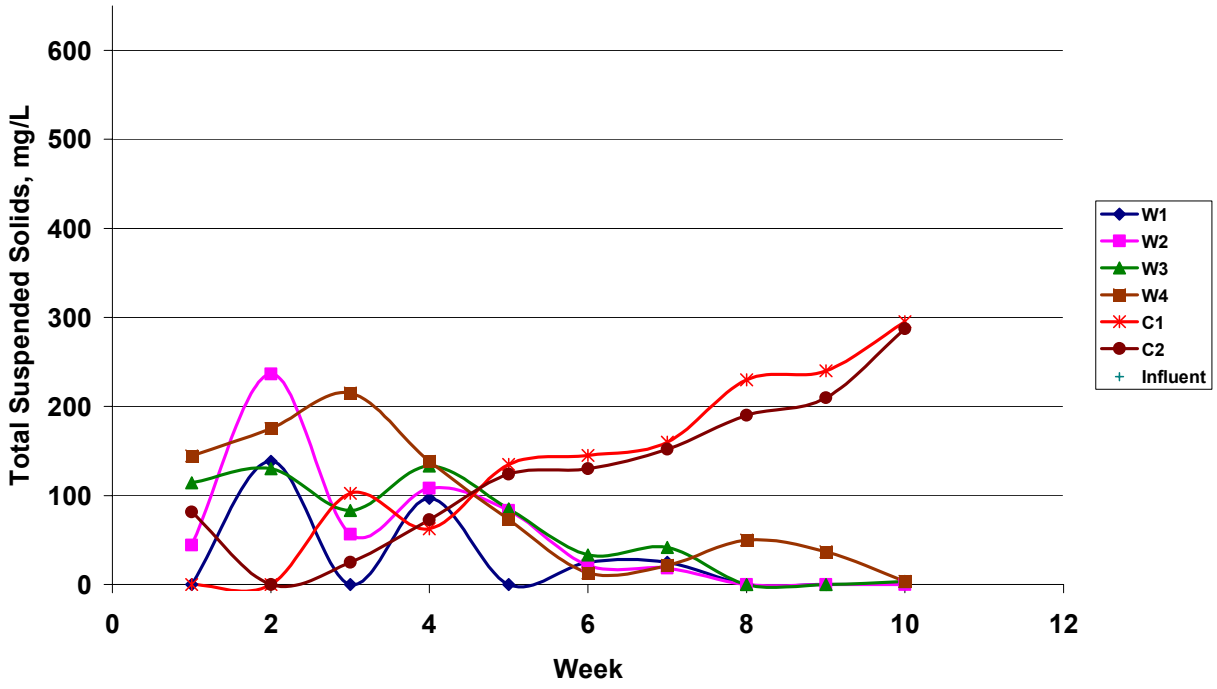


Figure 4 - 6 Average TSS concentrations over time in the effluent for various treatments

It can be concluded that the filter beds with worms were statistically more effective than filter beds without worms in reducing TS, VS, and TSS concentrations in effluent. However, there were no differences in the concentrations of TS, VS, or TSS in the effluent among the worm filter beds, implying that within the loading rates tested, filter efficiency was not a function of loading rate. Apparently the worm filter beds retained the applied solids and the drainage consisted only of soluble constituents. The removal efficiencies for TS, VS, and TSS were approximately 100% for the worm filter beds. Raymond (1984) conducted a study for treating municipal sludge (0.3 to 1.6 % total solids) using earthworms and observed that the vermistabilization process reduces approximately 75% TS and 80% VS at the maximum application rate of 1000 gm VS/m^2/week. The data for TS, VS, and TSS are shown in Appendix G.

4.2.2.2 Ammonia and Nitrate Nitrogen

Nitrogen analyses indicated an interesting pattern. Figure 4-7 summarizes ammonia-N removal from aquaculture wastewater. All the worm filter beds had higher concentrations of ammonia-N in the effluent at the beginning of the experiment, with perhaps some ammonia-N leaching from the dairy manure in the bedding material. The reduction of ammonia-N concentration in the effluent was greater in the control filter beds until week three, but as the experiment continued, the ammonia-N concentration in the effluent dropped to near zero by week nine for all worm treatments, probably due to nitrification of nitrate or immobilization of organic-N. As the experiment proceeded, the solids accumulated on the surface of both the worm and control filter beds. At the fourth week, a cross-over occurred between worm filter beds and control filter beds and concentration on most of the variables start reducing on worm filter-beds. The worms present on the surface of the filter beds appear to have increased pore size of the medium, keeping the hydraulic conductivity high, trapping the ammonia-N, and converting it to nitrate-N in subsequent liquid sludge applications. Raymond (1984), Parle (1963), and Syers (1979) also noted a simultaneous increase in nitrate-N and decrease in ammonia-N as castings age.

A possible explanation for the ammonia concentration increases in the control filter bed effluent is ammonification. In ammonification, microorganisms decompose the organic-N present on the accumulated solids and produce ammonia. Kristiansen (1981b) studied the treatment of fish wastewater using sand filtration and observed that ammonia concentration in the effluent was due mostly to the ammonification process. In contrast, worm filter beds removed approximately 100% of ammonia-N from the wastewater.

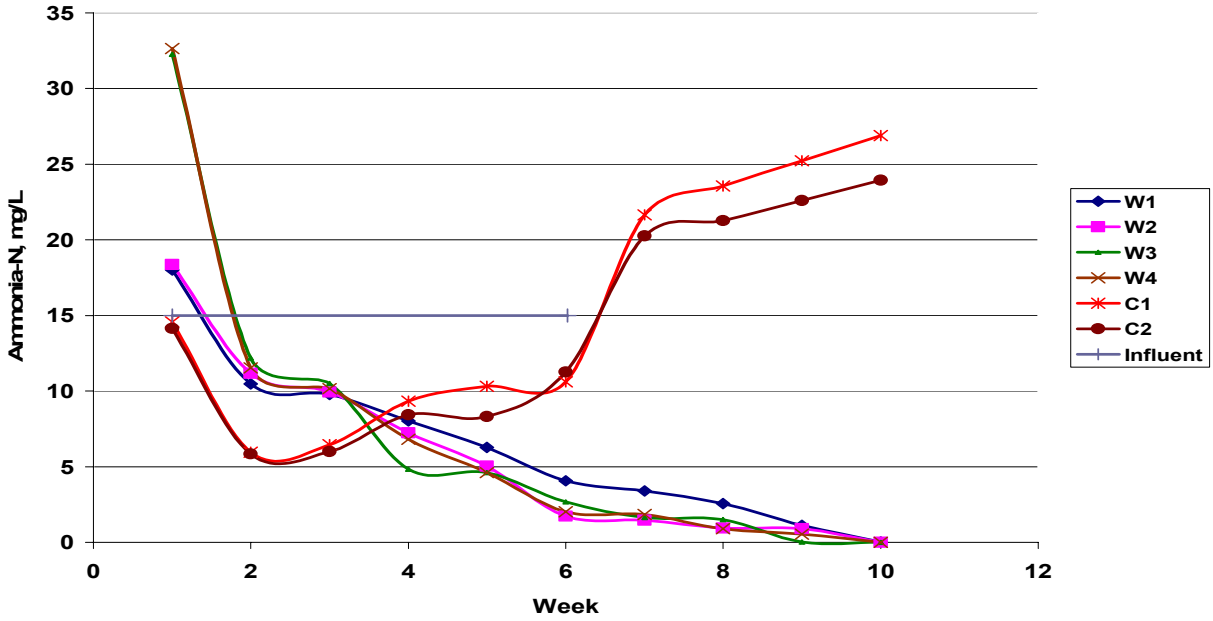


Figure 4 - 7 Average ammonia-N concentrations over time in the effluent for various treatments

The filter beds with worms were statistically more effective than filter beds without worms in reducing ammonia-N concentrations in the effluent. However, there were no differences in the concentrations of ammonia-N in the effluent between the worm filter beds, implying that within the loading rates tested, filter efficiency was not a function of loading rate. The removal efficiency for ammonia-N was approximately 100% for the worm filter beds.

Figure 4-8 shows the reduction of nitrate-N concentration in the effluent. The nitrate-N reductions in the control filter beds were considerably higher than the worm filter beds. It appears that the worms convert the ammonia-nitrogen into nitrate, which caused the concentration of nitrate-N to be higher in the effluent from the worm filter beds. This suggests that *E. fetida* produced conditions that favored nitrification. The nitrate-N concentration in the effluent was nearly stable in worm filter beds after the eighth week, whereas the nitrate-N concentration in the effluent from the control filter beds decreased effectively to zero by week seven for all loading rates tested. It appears that the denitrification process occurred between the application periods. Another possibility for the reduction of nitrate-N concentration on the control filter-beds is that nitrate-N may have been taken up by bacteria present on the

accumulated solids at the surface and converted to organic-N in their cell tissues; and, due to an ammonification process, organic-N may have decomposed and produced ammonia-N.

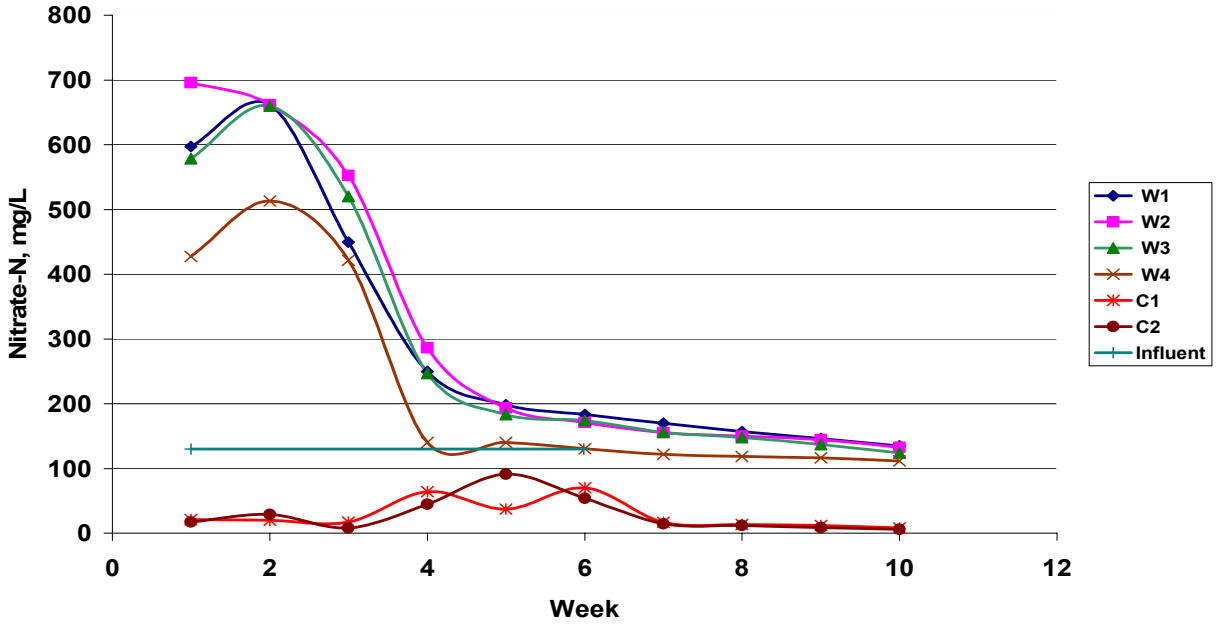


Figure 4 - 8 Average nitrate-N concentrations over time in the effluent for various treatments

Filter beds without worms were statistically more effective in reducing nitrate-N concentrations than worm filter beds. However, there were no differences in the concentrations of nitrate-N in the effluent of the worm filter beds, implying that within the loading rates tested, filter efficiency was not a function of loading rate. The data for ammonia-N and nitrate-N are shown in Appendix G.

4.2.2.3 Chloride and Sulfate

Figure 4-9 shows the performance of the filter beds in terms of average chlorides discharged in the effluent. The chlorides present in the influent to the filter beds were the same throughout the experiment. Worm filter beds had higher concentrations of chloride in the effluent than control filter beds for the first three weeks, probably due to the presence of chlorides in the

bedding material. However, as the experiment continued, solids started accumulating on the surface of the both the worm and control filter beds. The fourth week was the crossover for worm filter beds and control filter beds. The worms present on the surface of filter-beds appear to have increased the pore size of the medium (keeping the hydraulic conductivity high) and trapping chloride concentration in subsequent liquid sludge applications. Bacterial activity in the accumulated solids and bedding material may also have been a factor. In the control filter beds, the solids that accumulated on the surface restricted the movement of wastewater, ponding occurred, and chlorides leached into the effluent. However, there were no statistical differences observed either between the worm filter beds or between the worm filter beds and control filter beds.

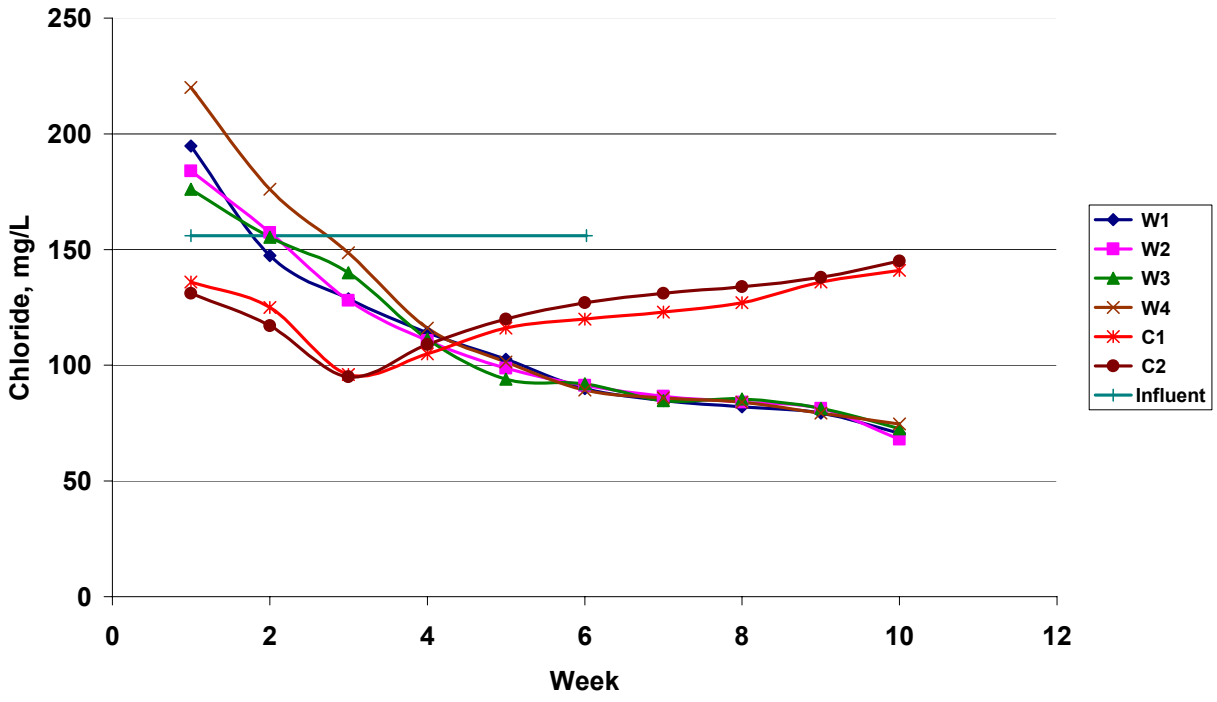


Figure 4 - 9 Average chloride concentrations over time in the effluent for various treatments

Figure 4-10 shows the sulfate concentration in the effluent over time. All filter beds had higher concentrations of sulfate in the effluent at the beginning of the experiment. The reduction of sulfate concentrations in the effluent was greater in the control filter beds until week four. This may have led to the formation of calcium sulfate, because of the presence of calcium in the

sand; but, as the experiment continued, the sulfate concentration in the effluent decreased for all worm treatments. By contrast, the sulfate concentration in the effluent from the control filter beds increased after the seventh week due to an accumulation of solids on the surface layer, ponding occurred, and sulfate leached in the effluent. The sulfate reduction in the worm filter beds was probably due to precipitation with calcium present in the dairy manure along with peat-moss, which formed the mixture for the bedding material. This may have led to the formation of calcium sulfate.

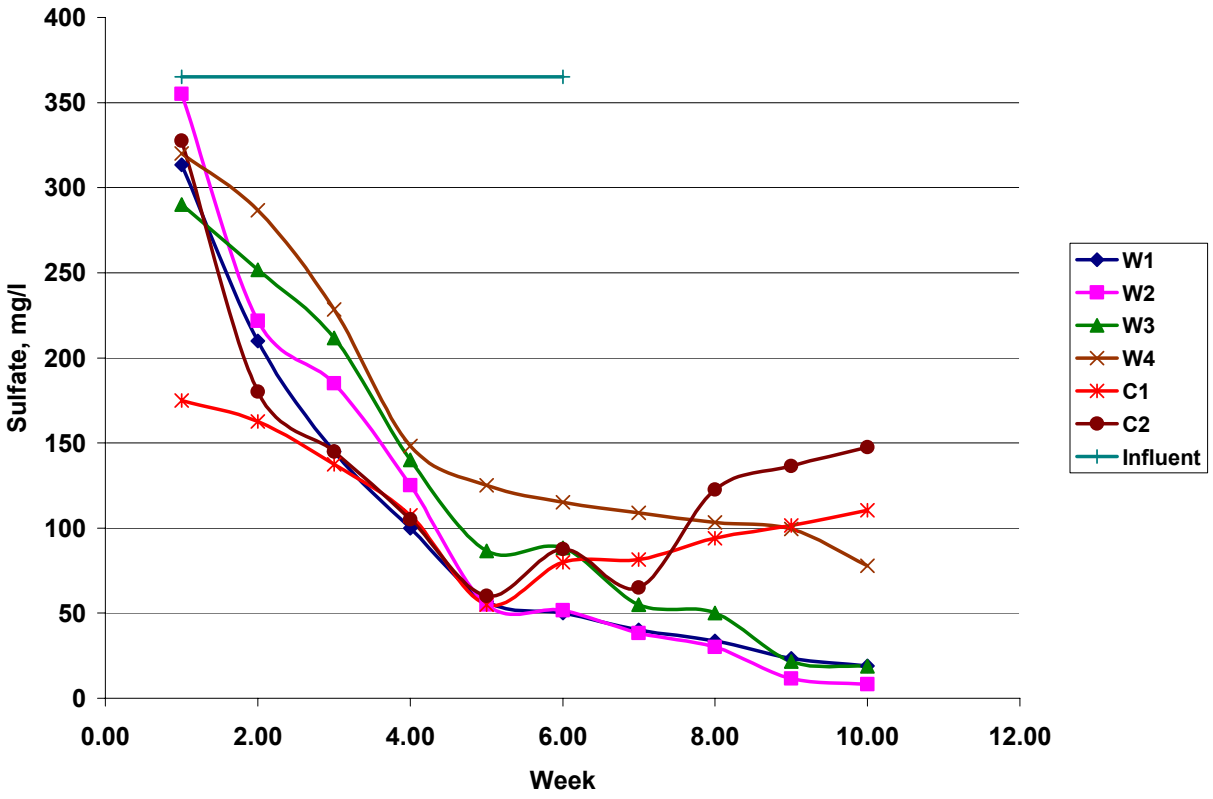


Figure 4 - 10 Average sulfate concentrations over time in the effluent for various treatments

There were no differences observed between worm and control filter beds for chloride and sulfate. However, the results imply that the vermistabilization process gave satisfactory results for the removal of chlorides and sulfate from the wastewater. The removal efficiencies were

approximately 80% for chlorides and 70% for sulfates respectively for the worm filter-beds. The data for chloride and sulfate are shown in Appendix G.

4.2.2.4 Chemical Oxygen Demand (COD)

COD reductions in the effluent over time are shown in Figure 4-11. COD is an indication of the concentration of organic material in wastewater. The COD present in the effluent of all the filter beds was less than the COD in the influent. The control filter beds had lower COD in the effluent for the first three weeks than the worm filter beds, but as the experiment continued, solids started accumulating on the surface of both the worm and control filter beds. Again, as time proceeded, the COD levels in the effluent from the worm filter beds decreased, as the worms had decomposed the organic material available in wastewater. Raymond (1984) reported that the vermistabilization process reduces COD in the effluent by 75% and COD probably will not add an extraordinary load to waste treatment facilities. In the control filter beds, COD level in the effluent increased after the third week for both loading rates.

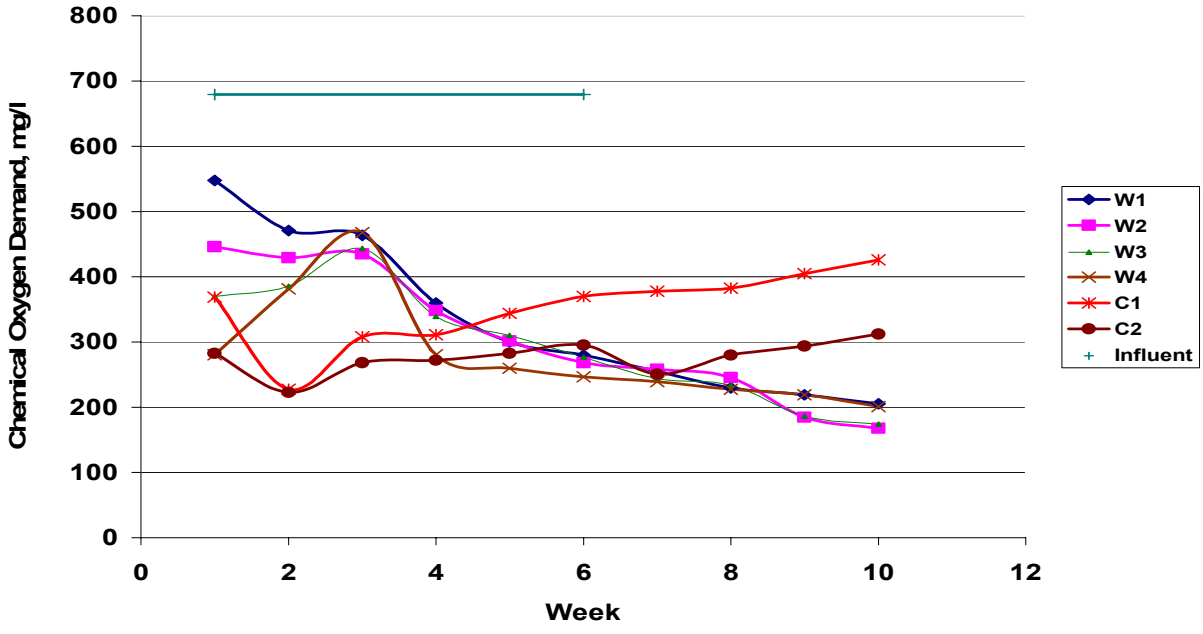


Figure 4 - 11 Average COD concentration over time in the effluent for various treatments

There were statistical differences in the concentration of COD level in the effluent between the worm filter-beds, implying that within the loading rates tested, filter efficiency was a function of loading rate. The removal for COD concentration was approximately 70% for the worm filter beds. However, there were no statistical differences observed for COD between worm and control filter beds. The data for COD are shown in Appendix G.

4.2.2.5 Total Phosphorus (TP) and Soluble Phosphorus (SP)

The total phosphorus (TP) present in the influent applied to the filter beds was similar throughout the experiment. It can be seen from Figure 4-12 that the worm filter beds had higher concentrations of TP in the effluent at the beginning of the experiment; but as the experiment continued, the TP concentration in the effluent started decreasing after the second week. As time proceeded, solids started accumulating on the surface of both the worm and control filter beds. However, the worms present on the surface of the filter beds converted the TP into SP. In control filter beds, the solids that accumulated on the surface restricted the movement of wastewater, ponding occurred, and TP leached into the effluent. After the second week, the TP concentration in the effluent from the worm filter beds decreased by approximately 82%, whereas the concentration of TP in the effluent from the control filter beds increased by approximately 90% compared to the concentration of TP from the second week of the experiment until the end of the experiment. Kristiansen (1996) noted in his experiments that “sand filter TP concentration increased with time”.

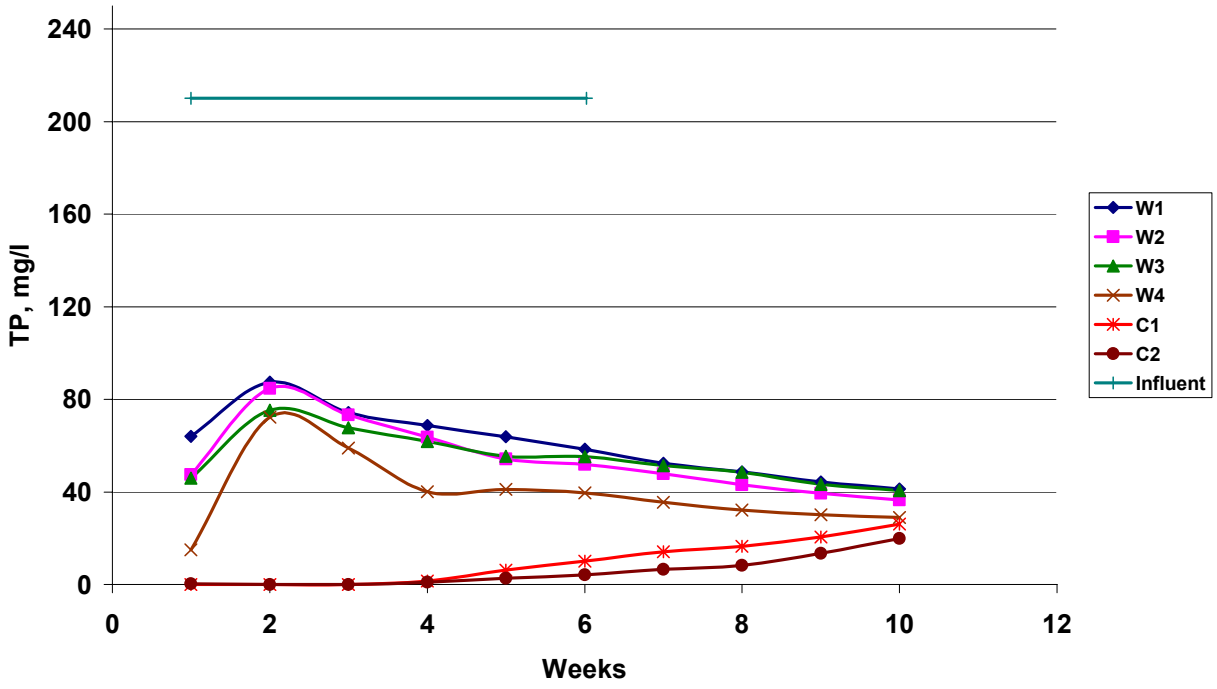


Figure 4 - 12 Average TP concentration over time in the effluent for various treatments

The SP concentration in the effluent over time is illustrated in Figure 4-13. The SP concentration in the effluent increased in worm filter beds by approximately 80%. This was due to the conversion of TP into SP. In the control filter beds, SP in the effluent also increased, but the worm filter beds had higher concentration of SP in the effluent than the control filter beds. Only a limited removal of SP was achieved for control filter beds because of the quartzite nature of the sand (Nilsson 1990). Most of the binding capacity is concentrated within the organic clogging layer of the filters. During operation, mineralization in these layers occurs. Timmons (2001) reported that "There was a slight downward trend in percent SP removal in the sand columns after three applications, but the trend was not significant".

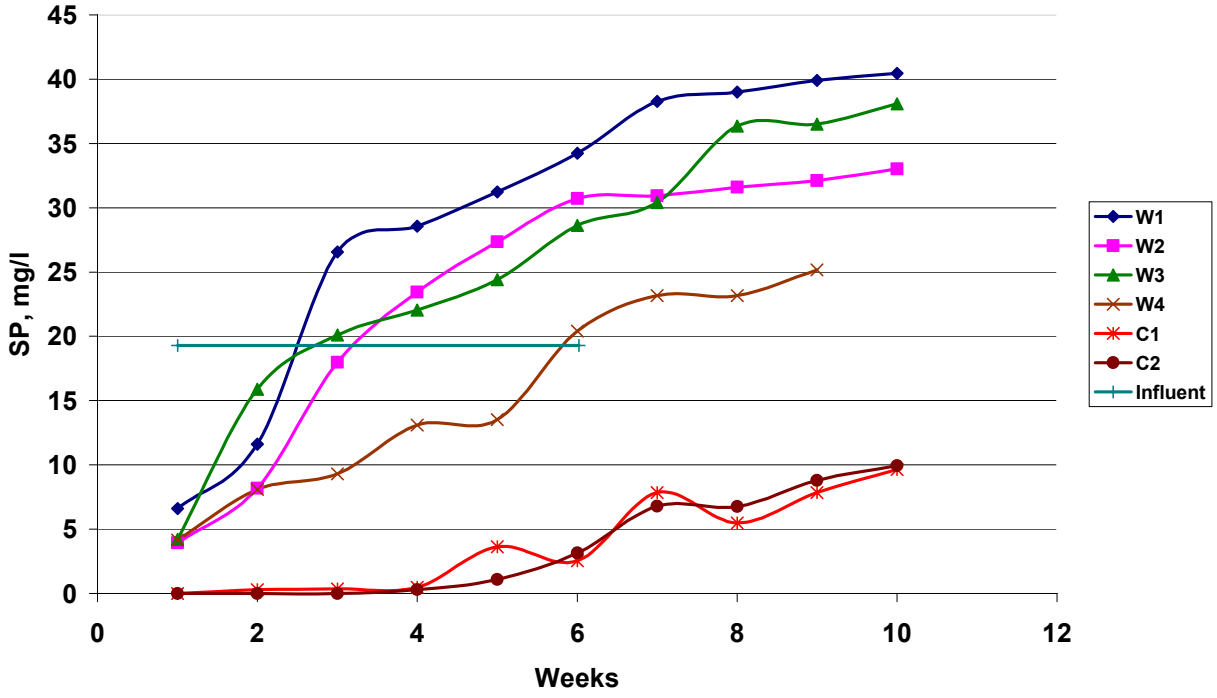


Figure 4 - 13 Average SP concentration over time in the effluent for various treatments

Assessing similar results, Stuanes (1984) stated that "Phosphorus removal efficiency using sand as a renovating medium may be substantially improved by using suitable sand types with high contents of metals such as calcium, aluminum or iron".

There were significant statistical differences between worm and control filter beds for both parameters TP and SP. There were also statistical differences in the concentration of SP in the effluent between the worm filter-beds, implying that within the loading rates tested, filter efficiency was a function of loading rate. It can also be concluded that the filter-beds with worms were statistically more effective than filter-beds without worms in reducing TP and less effective in reducing SP concentrations in effluent. The data for TP and SP are shown in Appendix G.

4.2.2.6 Dissolved Oxygen (DO)

Dissolved Oxygen (DO) is an important parameter in determining water quality. The minimum objectionable odor potential, maximum treatment efficiency and stabilization of wastewater are

dependent on maintenance of adequate dissolved oxygen (Hach, 1982). Harrison, 2002 reports that "oxygen levels in wastewater must remain sufficiently high in order to avoid odor problems and to optimize the treatment efficiency of a system".

Figure 4-14 shows the DO in the effluent over time. It was observed that worm filter-beds had higher DO contents in the effluent for the first four weeks but DO levels started reducing after that. By contrast, DO levels started increasing in the control filter-beds after week 3. The reduction in DO in the worm filter beds may have resulted due to the presence of worms. The oxygen might be taken by the bacteria available in either the worms guts or in the bedding material. There were significant differences in DO levels in effluent between control and worm filter-beds. Since dissolved oxygen is an essential for fish survival, and the effluent coming out from the worm filter beds had lower dissolved oxygen content than the control filter-beds, this effluent is not feasible for reuse in facility without oxygenation. It appears that the denitrification process occurred in the control filter beds, although the DO concentrations were higher. Discrepancies may have occurred between application periods, since nitrate may get trapped on the surface of the control filter-beds after each application of wastewater. It then leached out during subsequent application periods, while the DO concentration increased in the effluent.

It can be concluded that the filter beds with worms were statistically less effective than filter beds without worms in retaining DO concentrations in effluent. Also, there were differences in the concentrations of DO in the effluent between the worm filter beds, implying that within the loading rates tested, filter efficiency was a function of loading rate. The data for DO are shown in Appendix G.

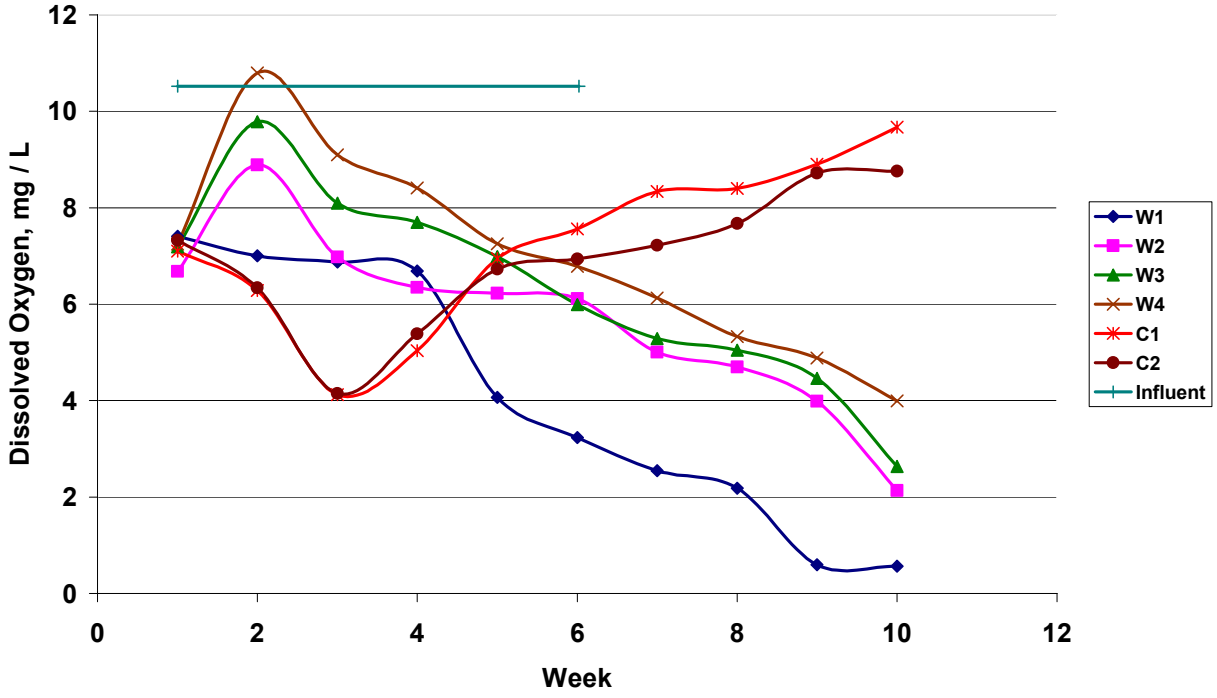


Figure 4 - 14 Average Dissolved Oxygen Concentration over Time in the Effluent for Various Treatments

4.2.2.7 pH and Alkalinity

Figure 4-15 shows the pH in the effluent over time. The pH of the influent was monitored throughout the experiment. The pH in the influent of all the filter-beds was less than the pH in the effluent. It was observed that worm filter beds had a lower pH value (more acidic) than the control filter beds. The pH in the effluent of all the worm filter beds was stable throughout the experiment, whereas the pH in the effluent of the control filter beds increased over the period of experiment. The reduction in pH can be explained by the mineralization of nitrogenous compounds into nitrates. By the end of ten weeks, a favorable pH (pH = 7.2) was attained in the worm bed filters. Similarly, Loehr (1984) observed the pH level in the influent municipal sludge at 6.7 and 7.6 in the effluent wastewater. The pH is also suitable for the survival of fish. It appears that the high pH in the control filter beds was possibly due to high ammonia-N concentrations in the effluent.

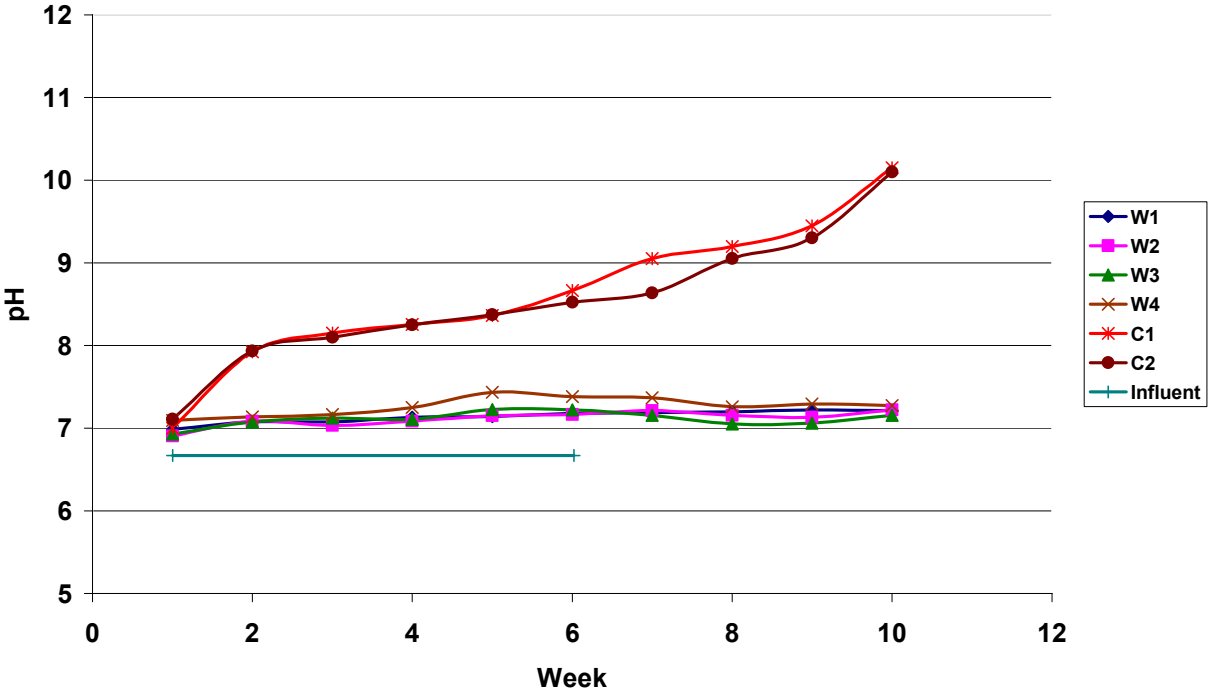


Figure 4 - 15 Average pH over Time in the Effluent for Various Treatments

The alkalinity (Figure 4-16) present in the influent was identical throughout the experiment. The worm filter beds had lower alkalinity in the effluent at the beginning of the experiment, but as the experiment continued, the alkalinity in the effluent increased after the fifth week. As time proceeded, solids accumulated on the surface of both the worm and control filter beds, resulting in higher alkalinity. However, the presence of the worms resulted in lower alkalinity than the control filter beds. This implies that the presence of carbonate, bicarbonate, and hydroxide are greater in control filter beds than in worm filter beds. As observed from the effluent pH, all of the worm filter beds were around 7.0. Therefore, the carbonate content in the effluent was more than that of the influent wastewater, which created beneficial conditions for aquatic life.

Filter beds with worms and without worms were different for both pH and alkalinity concentration in the effluent. However, there were no differences in the concentrations of alkalinity and pH levels in the effluent between the worm filter beds, implying that within the

loading rates tested, filter efficiency was not a function of loading rate. The data for pH and alkalinity are shown in Appendix G.

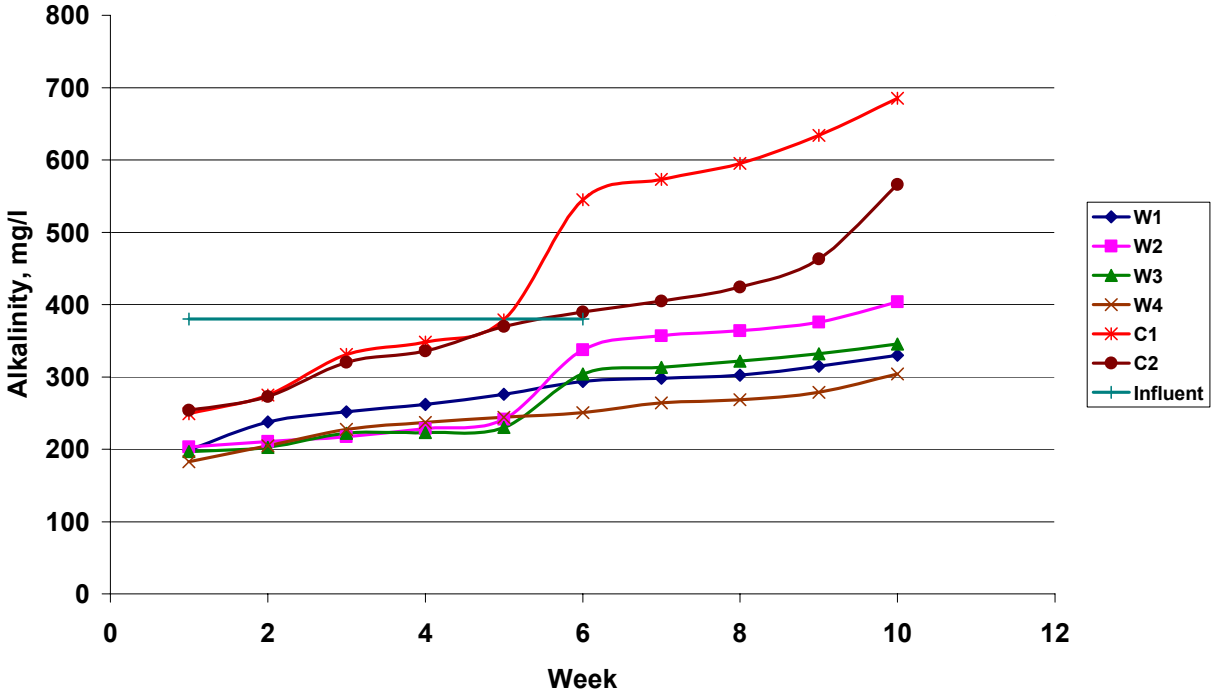


Figure 4 - 16 Average alkalinity concentration over time in the effluent for various treatments

4.2.2.8 Hydraulic Conductivity

The hydraulic conductivity of all the treatments was calculated at the end of the experiment. Table 4-7 shows that maximum hydraulic conductivity was achieved for maximum application rates. The hydraulic conductivity of all the worm filter beds was greater than the control filter beds. This is possible because the worms present in the filter beds digested the accumulated solids and increased the pore size of the medium, whereas the solids that accumulated on the control filter beds decreased pore size of the medium and clogged the filter bed.

Hydraulic conductivity generally decreased with the length of filter operations, and at a much faster rate as filter failure approached. All four control filter beds failed during the fourth and fifth week. Filter failure was defined as ponding of wastewater for 10 min after application. One possible explanation for this was that, while the filter approached failure due to

increasingly longer ponding times, anaerobic conditions developed in the filter and the aerobic nitrifying bacteria were significantly stressed and killed. Nitrification became less efficient as the filter approached failure. Other studies have demonstrated that clogging proceeds more rapidly under anaerobic conditions because the rates of both biodegradation of entrapped organic material and endogenous destruction of biomass are reduced (Jones and Taylor, 1964; Miller et al., 1994). Timmons (2001) stated that "Once the fish manure settles, which is much before the water drains from the sand column, the newly settled fish manure increases resistance to flow for the current application event". Timmons (2001) demonstrated that from visual inspection of his experiment, the accumulation of biosolids from the wastewater was 10% by volume of the influent wastewater. Kristiansen (1996) likewise reported that a large reduction in hydraulic conductivity was caused by the establishment of a clogging mat of fish solids on the sand filter surface.

Table 4 - 7 Average hydraulic conductivity for various treatments

Treatment	Hydraulic Conductivity (cm/day)
1	35
2	31
3	32
4	30
C1	22
C2	20

5 Summary and Conclusion

5.1 Filter Bed Test

The results of this study provided useful information on the performance of the vermistabilization process and related design and operational characteristics.

- Earthworms (*Eisenia fetida*) were key components of the vermistabilization process. Control filter beds that did not contain worms failed in a short period of time; 24 days for 0.5 L of wastewater per application and 35 days for 0.4 L of wastewater per application.
- All of the worm filter beds functioned satisfactorily for the entire study period (70 days).
- Aerobic conditions must be maintained in the worm filter beds.
- Cocoon production was noticed after 24 days for the highest wastewater application rate and after 65 days for the lowest application rate, indicating that the worms did best in the high-application rate treatment.
- Nitrate nitrogen in the drainage from the worm filter beds indicated that aerobic conditions were being maintained in the filter beds. A decrease or lack of nitrate in the drainage may indicate possible process failure.
- Liquid wastewater may be treated by the worm filter beds. The vermistabilization process increases the concentration of volatile solids destruction rate.
- Denitrification unit is essential to remove nitrate nitrogen from the sludge in the wastewater treatment plant.
- The greatest loading rate tested was 1000 grams of VS/m^2/week. The vermistabilization process functioned satisfactorily at this rate. It is not known if higher rates could be tolerated.
- For worm filter beds, TS, VS, TSS, and ammonia in effluent were reduced to virtually zero. Reductions in other parameters were as follows: total phosphorus 90%, chlorides 50%, sulfate 80%, and COD 70 %.

- The drainage from the worm filter beds had a pH range of 7 to 7.5 (neutral).
- The vermistabilization process reduced oxygen from the wastewater. So, some oxygen source is necessary if the water will be recycled for fish production.
- Hydraulic conductivity of the worm filter beds remained high throughout the study; hydraulic conductivity of control filter bed declined as solids built up. Ponding was observed in the third week of operation.

This comparative study clearly indicates that the vermistabilization process has greater potential to treat aquaculture wastewater than the control filter beds. Aquaculture facilities can design a worm bed to reduce costs and mitigate pollution by eliminating the need to directly discharge wastewater to municipal sewage systems. Furthermore, as an endproduct of the vermicomposting process, compost can be sold to increase economic returns to aquaculture farmers.

Engineering Perspective:

A main purpose of this research was to develop a basis for the design and operation of vermistabilization processes. As identified in previous sections, vermistabilization can be effective in treating aquaculture wastewater. An appropriate loading rate for the wastewater is about 1000 grams VS/m^2 of surface area per week.

This section attempts to put the vermistabilization process in perspective by identifying the relative economics and size of the process if it were to be used as an aquaculture wastewater treatment plant in place of more conventional sludge management processes, i.e., thickening, aerobic, or anaerobic digestion. All of these processes are unnecessary if vermistabilization is used. Figure 5-1 shows the schematics of wastewater treatment process using a vermistabilization unit.

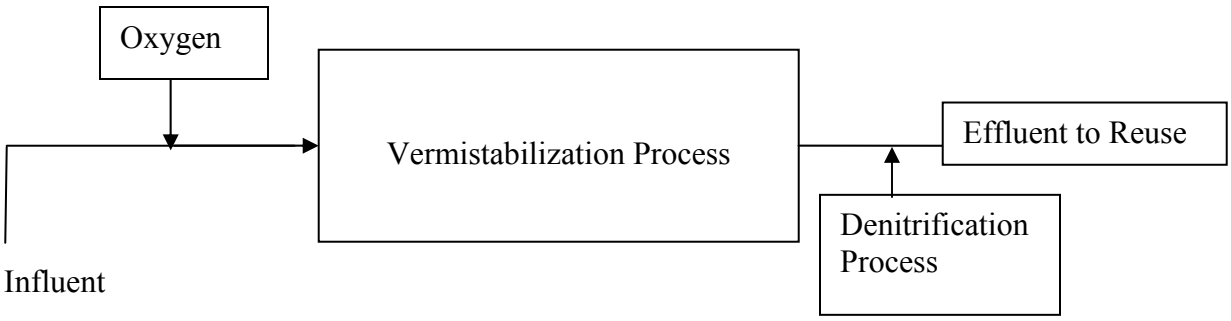


Figure 5- 1 Schematics of wastewater treatment plant with vermistabilization process

The size of the worm filter bed was determined using a loading rate of 1000 grams VS/m^2 of surface area per week to treat the 1670 m^3 of wastewater per day generated by Blue Ridge Aquaculture (BRA). The calculation for the surface area used to treat 1670 m^3 wastewater is shown below.

Maximum application rate for worm filter-bed = 1.2 L per application;
Thus, the maximum amount of wastewater applied to each worm filter bed = 1.2 * 3 = 3.6 L per week.

Maximum wastewater applied per day = 0.51 L/day (≈ 0.00051 m^3 per day)
Surface area of worm filter-bed = πR^2 (R = radius of worm filter-bed = 13 cm)
= π * $(13/100)^2$m = 0.053 m^2
To treat 0.00051 m^3 wastewater, surface area of the worm filter-bed is 0.053 m^2;
therefore, (0.00051 m^3 / day) / (0.053 m^2) = 0.01 (m^3 /day) /m^2
to treat 1670 m^3 wastewater, the surface area of the worm filter bed must be 167,000 m^2 (≈ 202,064 square yard).

Finally, a large surface area (≈ 202,064 square yard) would be needed for treating the 1670 m^3 of wastewater generated by BRA facility. It is not feasible to treat all the facility's wastewater. The size indicates that vermistabilization may not be an economically feasible wastewater treatment process. More detailed size and cost estimates will have to await larger scale studies and evaluation.

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Appendix

Appendix A: Statistical Analyses Code

Feedstock Acceptability Test

Experiment 1

- **SAS Code:**

```
*file is f:\clients\marsh\worms\experiment1;

libname marsh 'f:\clients\marsh\worms';
run;
*Just looking at worm weight;
options pageno=1 formdlim='*';

title 'Lori Marsh - May 2003 - Worm composting data - expt 1';
*analyze the trend across time for each box of worms, output the slopes and test if the slopes
 are different for the different fish solids concentrations;
*10 worms/box were fed 0, 5, 10, 15 or 20 ppm fish solids and then were re-weighed
 every week for 13 weeks to see if they gained weight;
*develop a model for each box and replicate combination, and then take the
 estimates from those linear models and run an ANOVA to determine if rate of
 increase in worm weight is different for the different levels of fish solids;
proc sort data=marsh.worm1;
  by rep fishsolid;
proc reg data=marsh.worm1 outest=estimates noprint;
  by rep fishsolid;
  model wtperworm=datenum;
run;
proc sort data=estimates;
by fishsolid rep;
proc print data=estimates;
title2 'Regression parameter estimates from each box and fish solids combination';
run;
proc glm data=estimates;
  class rep fishsolid;
  model intercept datenum=rep fishsolid;
  title2 'Test if rates (in particular) are different for different levels of solids';
  means fishsolid / tukey;
run;
proc sort data=estimates;
  by fishsolid;
proc means data=estimates noprint mean;
```

```
  by fishsolid;
  var datenum;
  output out=meanslopes mean=m_slope;
run;
proc plot data=meanslopes;
  plot m_slope*fishsolid;
  title2 'Graphical representation of growth rates vs fish solids';
run;
symbol1 i=rl v=star;
proc gplot data=meanslopes;
  plot m_slope*fishsolid;
  run;
data meanslopes;
  set meanslopes;
  f2=fishsolid*fishsolid;
run;
proc plot data=meanslopes;
  plot m_slope*fishsolid;
run;
proc glm data=meanslopes;
  model m_slope=fishsolid f2;
run;
proc sort data=marsh.worm1;
  by datenum fishsolid;
proc means data=marsh.worm1 noprint;
   by datenum fishsolid;
   var wtperworm worms;
   output out=meandata mean=m_wt m_num;
run;
proc print data=meandata;
title2 'Mean response across the 6 reps, at each date in time';
run;
proc plot data=meandata;
  where datenum=13;
  plot m_wt*fishsolid;
  title2 'Looking at week 13, is there an effect of fishsolid on mean worm weight';
run;

proc glm data=marsh.worm1;  where datenum=13;
   model wtperworm worms = fishsolid;
run;

proc glm data=meandata;  where datenum=13;
   model m_wt M_num = fishsolid;
   title3 'Only looking at the means at week 13, for r-square purposes';
run;
```

```
*multivariate repeated measures;
proc sort data=marsh.worm1;
  by fishsolid rep datenum;
proc transpose data=marsh.worm1 out=transout(rename=(col1=wt1 col2=wt2 col3=wt3
col4=wt4 col5=wt5
            col6=wt6 col7=wt7 col8=wt8 col9=wt9 col10=wt10 col11=wt11 col12=wt12
col13=wt13));
by fishsolid rep;
var wtperworm;
run;

proc print data=transout;
title2 'Transposed data, for multivariate analysis';
title3 ' ';
run;

proc glm data=transout;
  class fishsolid;
  model wt1-wt13=fishsolid / nouni;
  repeated weeks 13 profile / summary printe;
  title2 'All the weeks, is there a time effect across 13 weeks?';
run;

*just looking at last 6 weeks, where worms are acclimated, and none have died;
proc glm data=transout;
  class fishsolid;
  model wt8-wt13=fishsolid / nouni;
  repeated weeks 6 profile / summary printe;
  title2 'Only last 6 weeks of experiment';
run;

*survival of worms;
data marsh.worm1; set marsh.worm1;
  worms=worms*10;
run;
proc sort data=marsh.worm1;
  by fishsolid rep datenum;
proc transpose data=marsh.worm1 out=transout(rename=(col1=s1 col2=s2 col3=s3 col4=s4
col5=s5
            col6=s6 col7=s7 col8=s8 col9=s9 col10=s10 col11=s11 col12=s12 col13=s13));
by fishsolid rep;
var worms;
title2 'Survival of worms over the course of the experiment';
run;
```

```
proc print; run;

proc glm data=transout;
  class fishsolid;
  model s1-s13=fishsolid / nouni;
  repeated weeks 13 profile / summary printe;
  title2 'All the weeks, is there a time effect across 13 weeks on worm survival?';
run;
proc glm data=transout;
  class fishsolid;
  model s13=fishsolid;
  title2 'At week 13 is there an effect of fishsolids on worm survival?';
run;
quit;
```

Experiment 2

- **SAS Code:**

```
*file is f:\clients\marsh\worms\experiment2;

libname marsh 'f:\clients\marsh\worms';
run;
options pageno=1 formdlim='*' ls=75;
title 'Lori Marsh - May 2003 - Worm composting data - expt 2';
*10 worms/box were fed 15 25 or 50 ppm fish solids and then were re-weighed
 every week for 13 weeks to see if they gained weight;
*develop a model for each box and replicate combination, and then take the
 estimates from those linear models and run an ANOVA to determine if rate of
 increase in worm weight is different for the different levels of fish solids;
proc sort data=marsh.worm2;
  by replicate fish_solid;
*generate the parameter estimates and store in another data set, suppress the
 individual printing of the regression analyses;
proc reg data=marsh.worm2 outest=estimates noprint;
  by replicate fish_solid;
  model mean_w=datenum;
run;
proc sort data=estimates;
  by fish_solid replicate;
proc print data=estimates;
title2 'Regression parameter estimates from each box and fish solids combination';
run;
proc glm data=estimates;
  class replicate fish_solid;
  model intercept datenum=replicate fish_solid;
  means fish_solid / tukey;
  title2 'Test if rates (in particular) are different for different levels of solids';
```

```
run;
proc sort data=estimates;
  by fish_solid;
proc means data=estimates noprint mean;
  by fish_solid;
  var datenum;
  output out=meanslopes mean=m_slope;
run;
proc plot data=meanslopes;
  plot m_slope*fish_solid;
  title2 'Graphical representation of growth rates vs fish solids';
run;
symbol1 i=rl v=star;
proc gplot data=meanslopes;
  plot m_slope*fish_solid;
  run;
proc plot data=marsh.worm2;
  plot mean_w*datenum=fish_solid;
run;
*Just looking at worm weight;
options pageno=1 formdlim='*';
**********************************************************************
***************;
*beginning of repeated measures approach - was abandoned in favor of previous
 approach;
proc sort data=marsh.worm2;
  by datenum fish_solid;
proc means data=marsh.worm2 noprint;
   by datenum fish_solid;
   var Mean_w n_worms;
   output out=meandata mean=m_wt m_num;
run;
proc print data=meandata;
title2 'Mean response across the 6 reps, at each date in time';
run;
proc plot data=meandata;
  plot m_wt*datenum=fish_solid;
  title2 'Mean response graphed over time';
run;
proc plot data=meandata;
  where datenum=11;
  plot m_wt*fish_solid;
  title2 'Looking at week 11, is there an effect of fishsolid on mean worm weight';
run;

proc glm data=marsh.worm2;  where datenum=11;
```

```
  class fish_solid;
  model Mean_w = fish_solid;
run;

proc glm data=meandata;  where datenum=11;
  model m_wt = fish_solid;
  title3 'Only looking at the means at week 11, for r-square purposes';
run;

*multivariate repeated measures;
proc sort data=marsh.worm2;
 by fish_solid replicate datenum;
proc transpose data=marsh.worm2 out=transout(rename=(col1=wt1 col2=wt2 col3=wt3
col4=wt4 col5=wt5
            col6=wt6 col7=wt7 col8=wt8 col9=wt9 col10=wt10 col11=wt11));
by fish_solid replicate;
var mean_w;
run;
proc print data=transout;
title2 'Transposed data, for multivariate analysis';
title3 ' ';
run;
proc glm data=transout;
 class fish_solid;
 model wt3-wt8=fish_solid / nouni;
 repeated weeks 6 profile / summary printe;
 title2 'Just the first eight weeks, is there a time effect across 8 weeks?';
run;

quit;
```

Appendix B: SAS Output for both the Experiments of the Feedstock Acceptability Test

Experiment 1

```
**************************************************************************
*****

           Lori Marsh - May 2003 - Worm composting data - expt 1
1
  Regression parameter estimates from each box and fish solids
combination
                                                    11:37 Tuesday, May 27,
2003

           f                                        I
w
           i                  _                     n
t
           s    _             D                     t         d
p
           h    M        _    E          _          e         a
e
           s    O        T    P          R          r         t
r
           o    D        Y    V          M          c         e
w
    O  r   l    E        P    A          S          e         n
o
    b  e   i    L        E    R          E          p         u
r
    s  p   d    _        _    _          _          t         m
m

    1  1   0  MODEL1  PARMS  wtperworm  0.035791  0.37399  0.001118  -
1
    2  2   0  MODEL1  PARMS  wtperworm  0.038958  0.30923  0.008341  -
1
    3  3   0  MODEL1  PARMS  wtperworm  0.028881  0.35669  0.000868  -
1
    4  4   0  MODEL1  PARMS  wtperworm  0.034688  0.32642  0.004575  -
1
    5  5   0  MODEL1  PARMS  wtperworm  0.029929  0.29196  0.006379  -
1
    6  6   0  MODEL1  PARMS  wtperworm  0.042800  0.36971  0.006238  -
1
    7  1   5  MODEL1  PARMS  wtperworm  0.028067  0.36103  0.004644  -
1
    8  2   5  MODEL1  PARMS  wtperworm  0.019988  0.37908  0.009505  -
1
```

9	3	5	MODEL1	PARMS	wtperworm	0.033739	0.34031	0.004352	-1
10	4	5	MODEL1	PARMS	wtperworm	0.029418	0.34697	0.007788	-1
11	5	5	MODEL1	PARMS	wtperworm	0.028727	0.36733	0.008875	-1
12	6	5	MODEL1	PARMS	wtperworm	0.037587	0.36432	0.003533	-1
13	1	10	MODEL1	PARMS	wtperworm	0.034530	0.28412	0.023808	-1
14	2	10	MODEL1	PARMS	wtperworm	0.031988	0.35485	0.010440	-1
15	3	10	MODEL1	PARMS	wtperworm	0.035846	0.36466	0.014502	-1
16	4	10	MODEL1	PARMS	wtperworm	0.054358	0.42215	0.015500	-1
17	5	10	MODEL1	PARMS	wtperworm	0.049629	0.34192	0.011751	-1
18	6	10	MODEL1	PARMS	wtperworm	0.032537	0.27766	0.011817	-1
19	1	15	MODEL1	PARMS	wtperworm	0.041397	0.33883	0.015175	-1
20	2	15	MODEL1	PARMS	wtperworm	0.031205	0.34427	0.014165	-1
21	3	15	MODEL1	PARMS	wtperworm	0.039679	0.36469	0.015516	-1
22	4	15	MODEL1	PARMS	wtperworm	0.034844	0.30876	0.015707	-1
23	5	15	MODEL1	PARMS	wtperworm	0.055060	0.30089	0.015221	-1
24	6	15	MODEL1	PARMS	wtperworm	0.023602	0.33069	0.017275	-1
25	1	20	MODEL1	PARMS	wtperworm	0.026052	0.34888	0.013049	-1
26	2	20	MODEL1	PARMS	wtperworm	0.040442	0.31651	0.022543	-1
27	3	20	MODEL1	PARMS	wtperworm	0.028044	0.36876	0.007619	-1

****

Lori Marsh - May 2003 - Worm composting data - expt 12

Regression parameter estimates from each box and fish solids combination

11:37 Tuesday, May 27, 2003

f I w

i _ n

```
t
           s    _              D               t         d
p
           h    M       _      E        _      e         a
e
           s    O       T      P        R      r         t
r
           o    D       Y      V        M      c         e
w
    O  r   l    E       P      A        S      e         n
o
    b  e   i    L       E      R        E      p         u
r
    s  p   d    _       _      _        _      t         m
m

   28  4  20  MODEL1  PARMS  wtperworm  0.022542  0.26784  0.021455  -
1
   29  5  20  MODEL1  PARMS  wtperworm  0.024866  0.29094  0.008907  -
1
   30  6  20  MODEL1  PARMS  wtperworm  0.035635  0.30919  0.023240  -
1
```

****

```
           Lori Marsh - May 2003 - Worm composting data - expt 1
3
Test if rates (in particular) are different for different levels of
solids
                                                11:37 Tuesday, May 27,
2003

                               The GLM Procedure

                           Class Level Information

                  Class           Levels    Values

                  rep                  6    1 2 3 4 5 6

                  fishsolid            5    0 5 10 15 20


                        Number of observations    30
```

****

```
           Lori Marsh - May 2003 - Worm composting data - expt 1
4
Test if rates (in particular) are different for different levels of
```

solids
11:37 Tuesday, May 27, 2003

The GLM Procedure

Dependent Variable: Intercept Intercept

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	0.01034200	0.00114911	0.87	0.5657
Error	20	0.02640417	0.00132021		
Corrected Total	29	0.03674616			

R-Square	Coeff Var	Root MSE	Intercept Mean
0.281444	10.76832	0.036335	0.337422

Source	DF	Type I SS	Mean Square	F Value	Pr > F
rep	5	0.00453445	0.00090689	0.69	0.6389
fishsolid	4	0.00580754	0.00145189	1.10	0.3839

Source	DF	Type III SS	Mean Square	F Value	Pr > F
rep	5	0.00453445	0.00090689	0.69	0.6389
fishsolid	4	0.00580754	0.00145189	1.10	0.3839

****

Lori Marsh - May 2003 - Worm composting data - expt 1 5
Test if rates (in particular) are different for different levels of solids
11:37 Tuesday, May 27, 2003

The GLM Procedure

Dependent Variable: datenum datenum

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	0.00080195	0.00008911	4.70	0.0019
Error	20	0.00037937	0.00001897		
Corrected Total	29	0.00118132			

R-Square	Coeff Var	Root MSE	datenum Mean
0.678860	37.99239	0.004355	0.011464

Source	DF	Type I SS	Mean Square	F Value	Pr > F
rep	5	0.00007776	0.00001555	0.82	0.5499
fishsolid	4	0.00072419	0.00018105	9.54	0.0002

Source	DF	Type III SS	Mean Square	F Value	Pr > F
rep	5	0.00007776	0.00001555	0.82	0.5499
fishsolid	4	0.00072419	0.00018105	9.54	0.0002

****

Lori Marsh - May 2003 - Worm composting data - expt 16
Test if rates (in particular) are different for different levels of solids
11:37 Tuesday, May 27, 2003

The GLM Procedure

Tukey's Studentized Range (HSD) Test for Intercept

NOTE: This test controls the Type I experimentwise error rate, but it
generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.00132
Critical Value of Studentized Range	4.23186
Minimum Significant Difference	0.0628

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	fishsolid
A	0.35984	6	5
A			
A	0.34089	6	10
A			
A	0.33800	6	0
A			
A	0.33136	6	15
A			
A	0.31702	6	20

****

Lori Marsh - May 2003 - Worm composting data - expt 17
Test if rates (in particular) are different for different levels of solids
11:37 Tuesday, May 27, 2003

The GLM Procedure

Tukey's Studentized Range (HSD) Test for datenum

NOTE: This test controls the Type I experimentwise error rate, but it
generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.000019
Critical Value of Studentized Range	4.23186
Minimum Significant Difference	0.0075

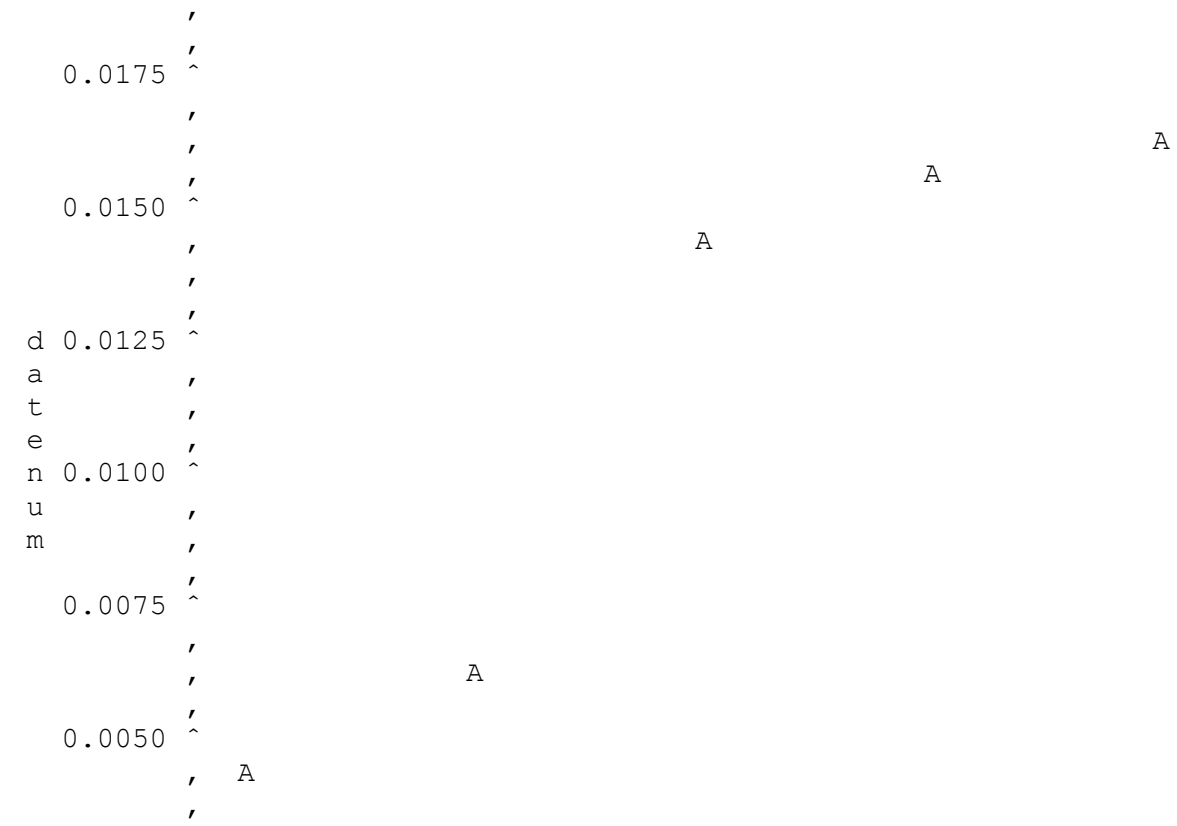
Means with the same letter are not significantly different.

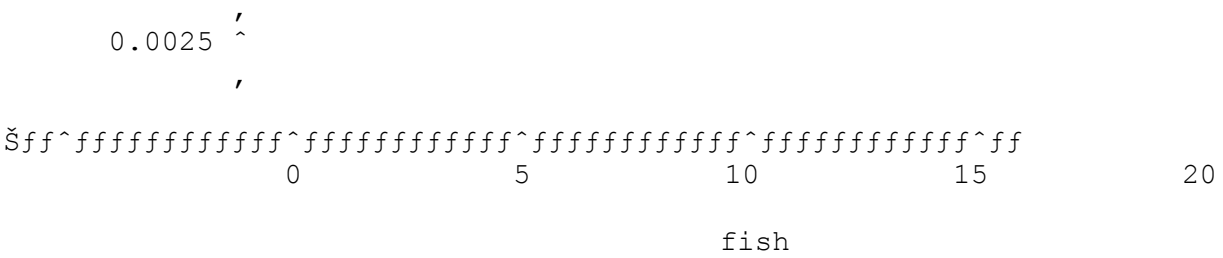
Tukey Grouping	Mean	N	fishsolid
A	0.016135	6	20
A			
A	0.015510	6	15
A			
A	0.014636	6	10
B	0.006450	6	5
B			
B	0.004587	6	0

****

Lori Marsh - May 2003 - Worm composting data - expt 1
8
Graphical representation of growth rates vs fish solids
11:37 Tuesday, May 27,
2003

Plot of m_slope*fishsolid. Legend: A = 1 obs, B = 2 obs, etc.





Experiment 2

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************************************************************************
*****

           Lori Marsh - May 2003 - Worm composting data - expt 2
1
  Regression parameter estimates from each box and fish solids
combination
                                                  11:37 Tuesday, May 27,
2003

     Replicate   Fish_Solids   MODEL   TYPE   DEPVAR   PARMS   Intercept   Mean_Weight
 1   1           15           MODEL1  PARMS  Mean_W   0.052173   0.46904    0.004873
-1
 2   2           15           MODEL1  PARMS  Mean_W   0.058383   0.41344   -0.000209
-1
 3   3           15           MODEL1  PARMS  Mean_W   0.056346   0.45291    0.000909
-1
 4   4           15           MODEL1  PARMS  Mean_W   0.049044   0.44298    0.004382
```

```
-1
 5   5   15   MODEL1   PARMS   Mean_W   0.058546   0.38544    0.003336
-1
 6   6   15   MODEL1   PARMS   Mean_W   0.056594   0.39949    0.001873
-1
 7   1   25   MODEL1   PARMS   Mean_W   0.058683   0.39858    0.014555
-1
 8   2   25   MODEL1   PARMS   Mean_W   0.044781   0.42000    0.013909
-1
 9   3   25   MODEL1   PARMS   Mean_W   0.048105   0.39673    0.010773
-1
10   4   25   MODEL1   PARMS   Mean_W   0.042779   0.48635    0.014791
-1
11   5   25   MODEL1   PARMS   Mean_W   0.053666   0.43036    0.003864
-1
12   6   25   MODEL1   PARMS   Mean_W   0.054190   0.38791    0.014545
-1
13   1   50   MODEL1   PARMS   Mean_W   0.033536   0.46337    0.014625
-1
14   2   50   MODEL1   PARMS   Mean_W   0.029483   0.45868    0.015155
-1
15   3   50   MODEL1   PARMS   Mean_W   0.037238   0.52197    0.019025
-1
16   4   50   MODEL1   PARMS   Mean_W   0.037526   0.43998    0.013916
-1
17   5   50   MODEL1   PARMS   Mean_W   0.039687   0.47138    0.020289
-1
18   6   50   MODEL1   PARMS   Mean_W   0.037753   0.44437    0.018035
-1

*************************************************************************
*****

           Lori Marsh - May 2003 - Worm composting data - expt 2
2
Test if rates (in particular) are different for different levels of
solids
                                                  11:37 Tuesday, May 27,
2003

                              The GLM Procedure

                         Class Level Information

                  Class           Levels    Values

                  Replicate            6    1 2 3 4 5 6

                  Fish_Solid           3    15 25 50


                       Number of observations    18
```

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********************************************************************
*****

           Lori Marsh - May 2003 - Worm composting data - expt 2
3
Test if rates (in particular) are different for different levels of
solids
                                                  11:37 Tuesday, May 27,
2003

                              The GLM Procedure

Dependent Variable: Intercept   Intercept

                                      Sum of
 Source                     DF       Squares   Mean Square  F Value  Pr
> F

 Model                       7    0.01243574    0.00177653     1.56
0.2526

 Error                      10    0.01138990    0.00113899

 Corrected Total            17    0.02382564


          R-Square     Coeff Var      Root MSE    Intercept Mean

          0.521948      7.706249      0.033749          0.437942


 Source                     DF     Type I SS   Mean Square  F Value  Pr
> F

 Replicate                   5    0.00487536    0.00097507     0.86
0.5416
 Fish_Solid                  2    0.00756038    0.00378019     3.32
0.0784


 Source                     DF   Type III SS   Mean Square  F Value  Pr
> F

 Replicate                   5    0.00487536    0.00097507     0.86
0.5416
 Fish_Solid                  2    0.00756038    0.00378019     3.32
0.0784

********************************************************************
*****
```

Lori Marsh - May 2003 - Worm composting data - expt 2
4
Test if rates (in particular) are different for different levels of solids
11:37 Tuesday, May 27, 2003

The GLM Procedure

Dependent Variable: datenum datenum

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	0.00065129	0.00009304	7.01	0.0034
Error	10	0.00013282	0.00001328		
Corrected Total	17	0.00078411			

R-Square	Coeff Var	Root MSE	datenum Mean
0.830614	34.77362	0.003644	0.010480

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Replicate	5	0.00001382	0.00000276	0.21	0.9515
Fish_Solid	2	0.00063747	0.00031874	24.00	0.0002

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Replicate	5	0.00001382	0.00000276	0.21	0.9515
Fish_Solid	2	0.00063747	0.00031874	24.00	0.0002

****

Lori Marsh - May 2003 - Worm composting data - expt 2
5
Test if rates (in particular) are different for different levels of solids

11:37 Tuesday, May 27, 2003

The GLM Procedure

Tukey's Studentized Range (HSD) Test for Intercept

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.001139
Critical Value of Studentized Range	3.87676
Minimum Significant Difference	0.0534

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Fish_Solid
A	0.46662	6	50
A			
A	0.42722	6	15
A			
A	0.41999	6	25

****

Lori Marsh - May 2003 - Worm composting data - expt 2 6
Test if rates (in particular) are different for different levels of solids

11:37 Tuesday, May 27, 2003

The GLM Procedure

Tukey's Studentized Range (HSD) Test for datenum

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	10

Error Mean Square	0.000013
Critical Value of Studentized Range	3.87676
Minimum Significant Difference	0.0058

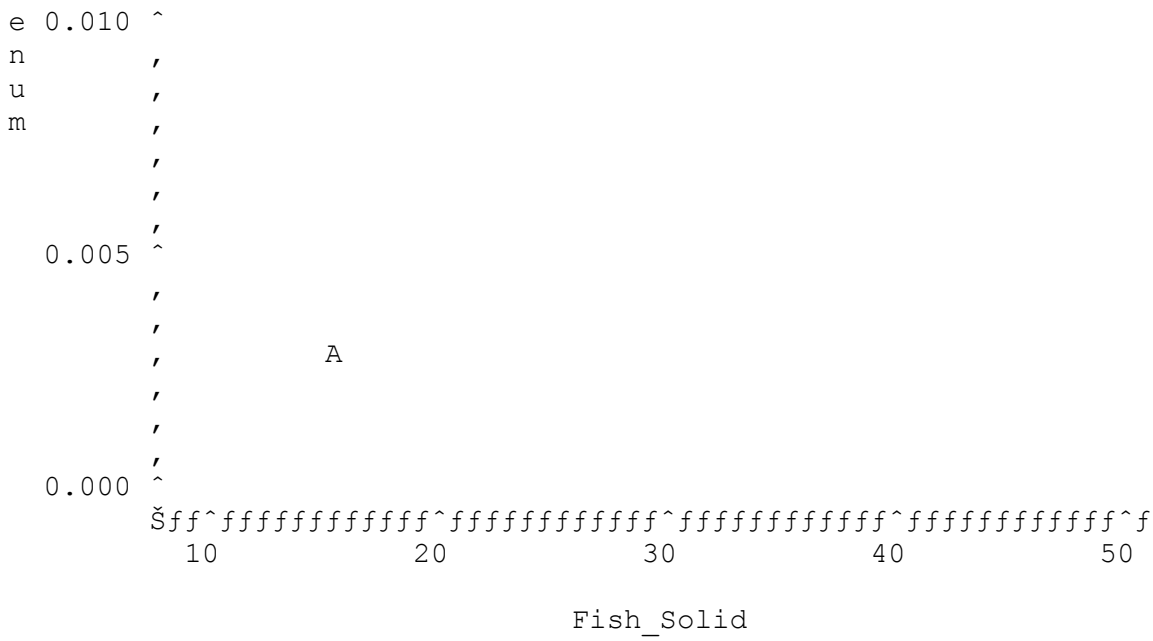
Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Fish_ Solid
A	0.016841	6	50
B	0.012073	6	25
C	0.002527	6	15

****

Lori Marsh - May 2003 - Worm composting data - expt 2
7
Graphical representation of growth rates vs fish solids
11:37 Tuesday, May 27,
2003

Plot of m_slope*Fish_Solid. Legend: A = 1 obs, B = 2 obs, etc.


e 0.010
n
u
m
0.005
A
0.000
10
20
30
40
50
Fish_Solid

Appendix C: Procedures for Variables

Total and Soluble Phosphorus:

Initially, the COD reactor was turned on and heated to 150^{o}C. Program 541 was selected from the menu of the HACH Spectrophotometers. 5.0 ml of deionized water was added to total phosphorous Test 'N Tube with a TenSette® Pipette (the control). Similarly 5.0 ml of sample was added to another Test 'N Tube (this was the sample). A funnel was used to add the contents of one potassium Persulfate powder pillow for phosphate to each vial. The tube was then capped tightly and shaken to dissolve the powder. Next, the vial was placed on the COD reactor to heat for 30 minutes while the timer icon was on. After 30 minutes, the vial was removed carefully, and the tubes were kept on a test tube rack to cool to room temperature (18-25^{o}C). 2.0 ml of 1.54 N sodium hydroxide was added to the vial with a TenSette® Pipette. The vial was closed and inverted to mix. A polyethylene dropper was used to add 0.5 ml of molybdovanadate reagent to each vial. The vial was capped and inverted to mix the solution. The vial was held in place for seven minutes while the timer icon was on. Then, the vial was wiped with a damp towel followed by a dry one to remove fingerprints or other marks. After seven minutes, the control was placed into the cell holder and zeroed out to display 0.0 mg/l PO_4^{3-}. The prepared sample was placed into the cell holder and readings were recorded in mg/l PO_4^{3-}.

Sulfate:

The sample was placed into the cell holder and the readings were recorded. The readings were over the readable range; so to get the appropriate value, the sample was diluted with deionized water until the apparatus could read a value. The exact dilution was two ml of samples with eight ml of deionized water, at which the readings were in range. Results were multiplied by five to get the exact readings in mg/L SO_4^{2-}.

Ammonia-N:

A 0.1 mL sample was added to one AmVer™ diluent reagent Test 'N Tube for high range ammonia nitrogen. Another sample of 0.1 mL of ammonia-free water was added to one AmVer™ diluent reagent Test 'N Tube for high range ammonia nitrogen, which served as the control. The contents of one ammonia salicylate and the contents of one ammonia cyanurate reagent powder pillow for 5 mL sample to each vial were added. The vial was tightly capped

and thoroughly mixed to dissolve the powder. The vial was held in place for 20 minutes while the timer icon was on; when the timer beeped, the control was placed the blank in the cell holder and zeroed out to display 0.0 mg/l NH_3–N. Then the sample was placed in the cell holder and the readings were recorded in mg/l NH_3-N"] mg/l NH_3-N.

Chemical Oxygen Demand:

The COD reactor was turned on and heated to 150^{o}C. The caps of the COD digestion reagent vials were removed. One vial was held at a 45-degree angle and 2.0 ml of deionized water was added to the vial with a clean volumetric pipette. This served as the control. The other vials were also held at 45-degree angle and 2.0 ml of sample was added to the vial. The vials were tightly caped, rinsed with deionized water, and wiped with a clean paper towel. Before being placed in the preheated COD reactor, the vials were inverted gently several times to mix the sample and the vial gets heated during mixing. The vials were placed into the COD reactor for two hrs. After 2 hrs the reactor was turned off and the vials were kept for 20 minutes to get cool down. Program 435 HR was selected from the menu of the HACH Spectrophotometers. Then vials were wiped with a damp towel followed by a dry one to remove the fingerprints or other marks. The blank was placed into the cell holder and touched zero to display 0.0 mg/l COD. Then sample was placed into the cell holder and recorded the readings in mg/l COD. But because the influent wastewater the readings were over range, to get the appropriate value, the sample was diluted with deionized water till the apparatus reads a value in the range. The exact dilution was 1.0 ml of samples with 9.0 ml of deionized water, at which point the readings were in range. The result would be multiplied by 10 to get the exact readings in mg/L COD.

Chloride:

Volume sample and standard titrant were selected from Table 4-3 [pp – 34] that was corresponded to the expected chloride (Cl^-) concentration. The volume sample 25 mL was measured with the graduated cylinder or pipette and transferred into a 250 ml Erlenmeyer flask. The final samples were prepared by adding the contents of one chloride 2 indicator powder pillows and mix the sample by swirling the flask. The other part was to get the 25 ml burette filled with silver nitrate standard solution. Sample was titrated while swirling the flask until the color changes from yellow to red-brown. The formula used to calculate the Cl^- concentration was:

mL Titrant * Multiplier Used = mg/l Chloride as Cl^-

Total Suspended Solids:

Procedure- Filter paper was placed on the aluminum plate and weighed on a weighing machine. Filter paper with wrinkled side was placed up in filtration apparatus. A measuring cylinder was used to measure the 20 mL of sample and poured it into filtration apparatus. Vacuum was applied and continued suction to remove all traces of wastewater, and discarded washings. Filter paper was removed from filtration apparatus and transferred to an aluminum plate. Aluminum plate with filter sample was placed in an oven at 103 to 105^o C for 1 h. Cool in desiccator to balance temperature and weighed. The cycle of drying, cooling, desiccating and weighing was repeated until a constant weight was obtained or until the weight change was less than 4 % of the previous or 0.5 mg, whichever was less.

Calculation:

mg of total suspended solids/L = {(A-B) * 1000}/sample volume, mL

Where,

A = weight of filter + dried residue, mg and B = weight of filter, mg

Volatile Solids:

The sample was kept in a preheated oven and evaporates at 103 to 105^{o}C for approximately six hours. After six hours, the dish was taken out from the oven and allowed it to cool to room temperature in a desiccator. Again dish was weighed with the sample, transferred the aluminum dish into a pre-heated muffle furnace at 550^{o}C and the sample was kept in the furnace for 30 minutes. The dish was taken out from the furnace with the thongs and cooled to room temperature in a desiccator. Again the dish was weighed, with the loss of weight measuring the total volatile solid. The formula to calculate the total solids is as follows:

mg/l Volatile Solids = {(A - B) * 100} / Sample in volume in mL

Where:

A = Weight (mg) of solids +dish before ignition

B = Weight (mg) of solids + dish after ignition.

Total Solids:

The sample was kept in a preheated oven and evaporated at 103 to 105^{o}C for approximately six hours. After six hours, the dish was taken out from the oven and allowed it to cool to room temperature in a desiccator. Again dish was weighed with sample. The procedure was repeated until results did not differ by more than 0.4 mg. Formula used to calculate the total solids as follows:

mg/l Total Solids = {(A - B) * 100} / Sample in volume in mL

Where:

A = Weight (mg) of solids +dish

B = Weight (mg) of dish.

Alkalinity:

Volume sample and standard titrant were selected from Table 4-4 [pp – 35] that was corresponded to the expected carbonate concentration. The volume sample 25 mL from Table 4-4 [pp – 35] was measured with the graduated cylinder or pipette and transferred into a 250 ml Erlenmeyer flask. The final sample was prepared by adding the contents of one Phenolphthalein indicator powder pillow and mixed the sample by swirling the flask. The other part was to get the 25 ml burette filled with 0.020 N sulfuric acid standard solutions. Sample was titrated while swirling the flask until the color changes from pink to colorless. But as the solution was colorless before titrating with sulfuric acid, so the phenolphthalein alkanity was zero. Again the content of one Bromcresol Green-Methyl Red indicator powder pillow was added and mixes the sample by swirling the flask. The titration was continued until a light pink end point was reached. Formula used to calculate the alkanity was:

(mL Titrant) * (Multiplier Used) = mg/l Total Alkanity as $CaCO_3$

Dissolved Oxygen:
Extensive testing of the YSI Model 55 suggests the following typical performance:

Temperature

Sensor type……Thermistor

Range……-5 to +45^{o}C

Accuracy……± 0.2^{o}C

Resolution……..0.1^{o}C

Dissolved Oxygen, mg/l

Sensor type…….Calibrated from % air saturation, temperature and salinity.

Range……..0 to 20 mg/l

Accuracy……...± 0.3 mg/l

Resolution…….0.01 mg/l

Calibration-

Step 1: Ensured that the sponge inside the instrument's calibration chamber was wet. The probe was inserted into the calibration chamber.

Step 2: The instrument was turn ON by pressing the ON/OFF button on the front of the instrument. It took 15 minutes usually after turning on the instrument to get stabilized the dissolved oxygen and temperature readings.

Step 3: Both the keys UP ARROW and DOWN ARROW was pressed and released at the same time to enter the calibration menu.

Step 4: Local altitudes in hundreds of feet were entered when the LCD prompt.

Step 5: After the proper altitude appears on the LCD, ENTER button was pressed. The Model 55 displayed the CAL in the lower left on the display, the calibration value was displayed in the lower right of the display and the current DO reading (before calibration) was on the main display.

Step 6: Make sure that DO reading (large display) was stable, then ENTER key was pressed. Once the calibration process was completed, the only MODE, LIGHT and ON/OFF key was used. The probe was dipped into the sample, moved up and down and the reading was recorded when the value on the display gets stable.

pH:

Two Point Calibration-

Step 1: Black cover from tip of electrode was removed.

Step 2: Electrode was rinsed with distilled water and blotted with a kimwipe.

Step 3: Electrode was immersed in fresh pH 7.0 buffer.

Step 4: "ON/OFF" button was pressed.

Step 5: Waiting until pH was displayed or the numbers 1 and 2 flash alternately.

Step 6: "MODE" was pressed until 1 and 2 flash alternately, when pH value was displayed.

Step 7: "2PT" was pressed. A buffer value began flashing.

Step 8: "SET" was pressed until 7.0 began flashing.

Step 9: A "READ" button was pressed.

Step 10: "CAL" began flashing.

Step 11: Electrode was gently stirred in buffer. "2^{nd}" started flashing when "CAL" stopped.

Step 12: Again the electrode was rinsed and blotted.

Step 13: Electrode was placed in pH 4.0 buffer and a pH value began flashing.

Step 14: "SET" was pressed until 4.0 began flashing.

Step 15: A "READ" button was pressed.

Step 16: "CAL" began flashing.

Step 17: Electrode was gently stirred in buffer. pH and Temperature started flashing when "CAL" stopped.

Sample Analysis-

Step 1: Samples were allowed to reach at room temperature.

Step 2: Rinsed electrode was immersed in sample; value was recorded on bench sheet.

Step 3: Again the electrode was rinsed and blotted.

Step 4: Step 2 was continued for next sample.

Step 5: Finally, electrode was rinsed and blotted dry and the black cover was placed on tip of electrode.

Step 6: The pH meter was turn off.

Hydraulic Conductivity:

The volume of waste collected at the bottom of each filter bed over a specific period of time defined the flow rate. The hydraulic conductivity of each treatment was calculated by dividing the flow rate with the product of area (A) and hydraulic gradient ($I = \Delta H/L$), whereas ΔH is the change in head and L is the length of sample.

$$K = (V * L) / (A * t * \Delta H)$$

Where; K = Hydraulic conductivity (cm/day), V_{out} = Volume of effluent (cm^3), L = Length of filter bed (cm), A = Cross-Sectional area of the filter bed (cm^2), t = Time taken to drain water (day), ΔH = Change in hydraulic head (cm) = V_{in} / Surface area (A), V_{in} = Influent volume

Appendix D: Calculation for Application Rates

Calculation for application rate:

As was mentioned by Raymond et al., 1988 that the maximum loading rate of volatile solid at which the worm filter-bed not failed was 1000 gm/m^2/week and the maximum loading rate of volatile solid for control filter-bed was 400 gm/m^2/week. On the basis of Raymond et al., (1988) study, the application rate for present experiment was calculated:

Stepwise calculation:

Step 1: Total and Volatile solid of collected fish sludge were measured.

Total Solid (TS) - 85,500 mg/L

Volatile Solid (VS) - 70,000 mg/L

Step 2: Ratio of the two were calculated.

VS/TS = 70,000/85,500 = 0.82

Step 3: Amount of VS present in 100 gm total sample at 1.5 gm of total solid.

1.5 gm TS X 0.82 = 1.23 gm of VS per 100 gm of sample

Step 4: Area of each filter-bed.

0.0488 m^2 of each pipe

Step 5: Frequency of application per week.

3 times per week

Step 6: Multiplied the recommended value by Raymond et al., 1988 with area of filter-bed and divided by frequency of application to get the amount of VS per feeding per pipe.

Maximum loading rate of VS for worm filter-bed

{(1000 gm/m^2/week) X 0.0488} / 3

= 16 gm of VS/feeding/pipe

Step 7: Total amount of samples needed to achieve the calculated amount of VS per feeding per week.

As, 1.23 gm of VS present in 100 gm of total sample

Therefore, 16 gm of VS

{(100 gm total sample X 16 gm of VS) / 1.23 gm of VS = 1300 gm total Sample

It was assumed that the density of fish wastewater at 1.5 % total solid was equal to the density of water (1gm/ml).
So on volume basis the maximum loading rate for worm filter-bed was 1300 gm / (1 gm/mL) = 1300 mL or 1.3 L.

Similarly, the calculations for the rest of the three worm filter-beds and two control filter-beds were same.

Appendix E: Calculation for Feed Preparation

Sample calculation for the feed preparation:

Suppose the 1200 mL sample has total solid content before freezing was 16 % and to convert it into 1.5 % of total solid, amount of water needed:

$$(1.5/16) = (1200\ mL/X)$$

$$X = (1200\ mL * 16)/1.5 = 12{,}800\ mL$$

Amount of water needed = 12, 800-1200 = 11,600 mL of water needed with 1200 mL of fish sludge at 16 % total solid to make it at 1.5 % total solid.

Appendix F: SAS Code for Pilot Study

Pilot Study

- **SAS Code: Repeated Measure-**

Total Solids:

```
proc print data=ts;
run;
*multivariate repeated measures analysis of variance;
/*proc glm data=ts;
  class sample rep;
  model ts1-ts10 = sample rep / nouni;
  repeated time 10 profile / summary nom;
  title2 'Multivariate repeated measures analysis of variance with contrasts';
  run;*/
*rearrange the data for proc mixed repeated measures analysis of variance;
proc transpose data=ts out=transp(rename=(col1=TS)) name=time;
  by sample rep;
run;
data transp(drop=time);
  set transp;
*change the 4 to a 3 in the line below for the variable ts;
  week=(substr(time,3,2)) + 0;
run;
proc print data=transp;
title2 'Transposed data, set up for use with proc mixed';
run;
proc mixed data=transp;
  class sample rep week;
  model ts=sample week sample*week;
  repeated week / subject=sample*rep(week) type=ar(1);
  lsmeans sample sample*week / adjust=tukey;
  title2 'Repeated measures using proc mixed, with contrasts';
run;
quit;
```

- **SAS Code: Contrast Statement-**

Total Solids

```
proc print data=ts;
run;
*multivariate repeated measures analysis of variance;
/*proc glm data=ts;
  class sample rep;
  model ts1-ts10 = sample rep / nouni;
  repeated time 10 profile / summary nom;
```

```
  title2 'Multivariate repeated measures analysis of variance with contrasts';
  run;*/
*rearrange the data for proc mixed repeated measures analysis of variance;
proc transpose data=ts out=transp(rename=(col1=TS)) name=time;
  by sample rep;
run;
data transp(drop=time);
  set transp;
  week=(substr(time,3,2)) + 0;
run;
proc print data=transp;
title2 'Transposed data, set up for use with proc mixed';
run;
proc mixed data=transp;
  class sample;
  model ts=sample;
  lsmeans sample / adjust=tukey;
  Contrast ‘worm filter-bed Vs control filter-bed’ sample -2  -2  -2  -2  4  4;
run;
quit;
```

Appendix G: Weekly Data for Different Parameters

Total Solids, mg/l

Sample #	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10
1.1	3000	2000	1000	1000	1000	1000	1000	0	0	0
1.2	5000	2000	3000	2000	0	1000	2000	0	0	0
1.3	3000	4000	3000	1000	2000	1000	0	0	0	0
2.1	2000	5000	3000	2000	1000	2000	1000	0	0	0
2.2	3000	7000	3000	3000	2000	0	3000	0	0	0
2.3	5000	3000	3000	1000	1000	2000	0	0	0	0
3.1	4000	3000	4000	4000	2000	1000	2000	0	0	0
3.2	4000	4000	3000	1000	3000	2000	1000	0	0	0
3.3	6000	4000	3000	2000	0	1000	0	0	1000	0
4.1	3000	5000	4000	2000	0	1000	2000	1000	0	0
4.2	6000	3000	3000	4000	1000	1000	1000	0	0	0
4.3	5000	5000	2000	2000	7000	2000	0	0	0	0
C1.1	3000	4000	5000	7000	7000	8000	8000	8000	8000	13000
C1.2	4000	4000	5000	6000	7000	7000	8000	11000	12000	9000
C2.1	4000	4000	3000	4000	6000	6000	6000	7000	7000	11000
C2.2	2000	3000	5000	6000	6000	7000	9000	10000	11000	9000
Influen	7000	7000	7000	7000	7000	7000	7000	7000	7000	7000

Volatile Solids, mg/l

Sample #	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10
1.1	2000	2000	1000	0	0	0	0	0	0	0
1.2	5000	2000	2000	0	0	0	0	0	0	0
1.3	3000	4000	0	0	0	0	0	0	0	0
2.1	2000	5000	0	0	0	0	0	0	0	0
2.2	4000	5000	0	0	0	0	0	0	0	0
2.3	5000	3000	0	0	0	0	0	0	0	0
3.1	4000	3000	0	0	0	0	0	0	0	0
3.2	3000	4000	0	0	0	0	0	0	0	0
3.3	5000	4000	0	0	0	0	0	0	0	0
4.1	3000	5000	0	0	0	0	0	0	0	0
4.2	4000	3000	0	0	0	0	0	0	0	0
4.3	3000	5000	0	0	0	0	0	0	0	0
C1.1	2000	2000	2000	3000	5000	5000	5000	6000	10000	11000
C1.2	4000	2000	2000	4000	5000	6000	7000	11000	9000	9000
C2.1	4000	2000	2000	5000	4000	6000	5000	7000	10000	12000
C2.2	3000	2000	3000	3000	5000	6000	8000	8000	11000	11000
Influent	6000	6000	6000	6000	6000	6000	6000	6000	6000	6000

Total Suspended Solids, mg/l

Sample #	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10
1.1	0.00	135.00	0.00	105.00	0.00	20.00	0.00	0.00	0.00	0.00
1.2	0.00	150.00	0.00	90.00	0.00	15.00	25.00	0.00	0.00	0.00
1.3	0.00	130.00	0.00	95.00	0.00	40.00	50.00	0.00	0.00	0.00
2.1	23.33	230.00	140.00	100.00	115.00	15.00	50.00	0.00	0.00	0.00
2.2	23.33	310.00	30.00	115.00	60.00	15.00	5.00	0.00	0.00	0.00
2.3	86.67	170.00	0.00	110.00	75.00	35.00	0.00	0.00	0.00	0.00
3.1	100.00	130.00	120.00	200.00	115.00	45.00	50.00	0.00	0.00	0.00
3.2	43.33	75.00	50.00	100.00	75.00	20.00	40.00	0.00	0.00	0.00
3.3	200.00	185.00	80.00	100.00	65.00	35.00	35.00	0.00	0.00	10.00
4.1	66.67	225.00	50.00	100.00	120.00	5.00	40.00	10.00	0.00	0.00
4.2	160.00	210.00	315.00	145.00	65.00	25.00	15.00	35.00	0.00	10.00
4.3	206.67	90.00	280.00	170.00	35.00	10.00	10.00	105.00	110.00	0.00
C1.1	0.00	0.00	35.00	40.00	150.00	155.00	165.00	240.00	255.00	280.00
C1.2	0.00	0.00	170.00	85.00	120.00	135.00	155.00	220.00	225.00	310.00
C2.1	133.33	0.00	25.00	60.00	128.00	132.00	150.00	200.00	205.00	280.00
C2.2	30.00	0.00	25.00	85.00	120.00	128.00	154.00	180.00	215.00	295.00
Influen	8850					6920				

Ammonia-N, mg/l

Sample #	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10
1.1	31.23	10.23	9.88	10.40	3.90	1.70	1.30	0.20	1.10	0.10
1.2	12.20	11.20	10.45	5.70	9.80	7.60	3.70	1.50	1.40	0.00
1.3	10.60	10.00	9.00	8.00	5.10	2.90	5.20	6.00	0.90	0.00
2.1	28.65	12.20	11.10	7.50	5.30	2.80	2.30	2.60	0.60	0.00
2.2	11.90	9.00	8.70	7.90	5.70	1.00	2.00	0.20	1.10	0.00
2.3	14.54	12.32	10.00	6.30	4.10	1.40	0.10	0.00	1.00	0.00
3.1	31.23	12.21	11.34	7.90	5.00	5.70	1.70	1.70	0.00	0.00
3.2	29.23	11.32	10.12	4.50	4.70	2.30	1.00	0.10	0.10	0.00
3.3	36.34	13.00	10.00	2.10	4.10	0.00	2.20	2.70	0.00	0.00
4.1	34.10	12.23	11.00	4.30	2.10	0.90	3.90	0.00	0.70	0.00
4.2	25.60	11.12	10.45	4.50	2.30	1.50	0.90	1.20	0.70	0.00
4.3	38.23	11.25	9.00	11.60	9.40	3.70	0.70	1.50	0.20	0.00
C1.1	15.32	5.30	5.10	9.80	8.65	8.80	21.90	24.76	26.42	28.08
C1.2	13.80	6.60	7.80	8.88	12.00	12.40	21.40	22.36	24.02	25.68
C2.1	14.32	5.20	6.00	8.88	8.65	10.88	19.80	23.76	25.10	26.44
C2.2	13.98	6.50	6.00	8.00	7.98	11.65	20.70	18.76	20.10	21.44
Influent	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00

Nitrate-N, mg/l

Sample #	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10
1.1	628	726	243	210	180	175	165	154	148	134
1.2	404	684	618	254	235	210	190	178	160	145
1.3	760	574	488	284	180	165	155	138	130	125
2.1	780	546	548	220	180	167	152	154	150	138
2.2	676	712	530	428	225	180	165	155	143	125
2.3	632	728	580	210	175	165	148	140	140	135
3.1	572	622	516	276	210	198	168	160	130	120
3.2	536	646	536	288	188	180	160	145	155	142
3.3	628	712	508	176	152	145	140	138	125	110
4.1	500	530	426	212	150	138	128	120	118	114
4.2	402	446	326	82	106	98.67	90	93	91	85
4.3	380	564	512	126	164	154	148	142	140	135
C1.1	24	22	26	96	50	32	18	14	12.5	8.9
C1.2	18	18	8	32	24	108	15	13	11.4	7.5
C2.1	20	24	2	26	104	76	16	14	8	6.5
C2.2	14	34	14	64	78	32	13	10	9.65	5.45
Influent	130	130	130	130	130	130	130	130	130	130

Chloride, mg/l

Sample #	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10
1.1	184	170	130	114	106	90	86	80	74	70
1.2	190	136	136	120	100	96	84	76	78	72
1.3	210	136	120	108	102	84	84	90	86	70
2.1	176	140	130	120	110	94	86	80	80	64
2.2	200	156	124	100	90	90	82	92	84	70
2.3	176	176	130	112	96	90	92	80	80	70
3.1	176	144	140	120	100	90	82	90	82	74
3.2	176	156	136	110	92	96	88	86	82	72
3.3	176	166	144	104	90	90	84	80	80	72
4.1	200	170	150	120	110	90	92	92	82	76
4.2	244	168	156	116	96	92	86	80	76	70
4.3	216	190	140	112	98	86	80	80	80	78
C1.1	144	94	88	100	108	112	120	124	134	140
C1.2	128	156	104	110	124	128	126	130	138	142
C2.1	130	110	100	112	116	124	132	132	136	142
C2.2	132	124	90	106	124	130	130	136	140	148
Influent	156	156	156	156	156	156	156	156	156	156

Sulfate, mg/l

Sample #	Week1	Week2	Week3	Week4	Week5	Week6	Week7	Week8	Week9	Week10
1.1	300.00	230.00	95.00	70.00	55.00	50.00	35.00	32.00	20.00	15.66
1.2	340.00	195.00	170.00	105.00	55.00	45.00	45.00	35.00	25.00	20.66
1.3	300.00	205.00	170.00	125.00	60.00	55.00	40.00	34.00	25.00	20.66
2.1	315.00	205.00	200.00	130.00	60.00	45.00	35.00	36.98	15.00	10.77
2.2	375.00	225.00	165.00	135.00	60.00	65.00	40.00	28.76	10.00	6.13
2.3	375.00	235.00	190.00	110.00	45.00	45.00	40.00	24.78	10.00	7.66
3.1	380.00	235.00	245.00	135.00	120.00	120.00	95.00	85.00	20.00	16.33
3.2	240.00	250.00	190.00	155.00	50.00	60.00	35.00	37.00	20.00	17.85
3.3	250.00	270.00	200.00	130.00	90.00	85.00	35.00	28.00	25.00	22.00
4.1	280.00	330.00	210.00	155.00	145.00	125.00	120.00	115.00	108.00	88.20
4.2	340.00	265.00	245.00	125.00	100.00	110.00	102.00	75.00	87.00	66.00
4.3	340.00	265.00	230.00	165.00	130.00	110.00	105.00	120.00	103.00	79.00
C1.1	85.00	140.00	115.00	115.00	90.00	120.00	98.00	110.00	113.00	121.67
C1.2	265.00	185.00	160.00	100.00	20.00	40.00	65.00	78.00	90.00	99.18
C2.1	280.00	160.00	130.00	110.00	45.00	90.00	95.00	160.00	176.00	188.34
C2.2	375.00	200.00	160.00	100.00	75.00	85.00	35.00	85.00	97.00	106.34
Influent	365.00	365.00	365.00	365.00	365.00	365.00	365.00	365.00	365.00	365.00

Chemical Oxygen Demand, mg/l

Sample #	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10
1.1	565	467	460	350	310	285	265	234	209	185.5
1.2	492	477	465	380	305	280	255	235	203	179.5
1.3	586	468	465	350	285	275	245	220	245	251.5
2.1	491	381	450	365	322	280	275	280	183	166.15
2.2	419	461	425	355	307	265	255	234	211	194.15
2.3	428	446	430	325	275	259	245	223	160	143.15
3.1	435	351	435	340	310	285	244	235	190	177.66
3.2	347	395	455	356	315	265	256	255	214	201.66
3.3	327	411	440	322	305	280	231	212	155	142.66
4.1	248	396	450	295	224	234	234	210	220	202.24
4.2	286	347	465	257	269	261	245	216	228	210.24
4.3	307	401	488	290	287	244	239	256	209	191.24
C1.1	366	230	228	275	373	420	526	430	445	466
C1.2	372	225	388	347	315	320	230	335	365	386
C2.1	120	210	319	290	297	310	210	320	357	375.9
C2.2	445	235	217	254	268	280	292	240	230	248.9
Influent	679.5	679.5	679.5	679.5	679.5	679.5	679.5	679.5	679.5	679.5

Total Phosphorus, mg/l

Sample #	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10
1.1	71.9	106.8	73.45	68.89	65.12	58.78	52.78	48.88	46.23	42.19
1.2	63.2	73.6	73.34	67.34	62.23	57.23	51.19	49.43	43.23	40.04
1.3	56.7	82.1	75.86	70.1	64.21	59.43	53.08	47.9	44	41.45
2.1	64.1	92.3	80.1	76.6	55.1	50.8	48.89	43.56	40.21	38.98
2.2	37.1	81.5	63.4	58.7	56	55	46.98	43.87	38.76	36.8
2.3	41.5	80.6	76.3	55.6	51.4	50.1	47.23	42.12	39	34
3.1	57.3	67.1	66.5	62.34	55.34	56.48	52.21	50.12	43.45	41.2
3.2	38.9	78.6	72.2	61.87	56.45	54.54	51.56	45.67	44.03	39.8
3.3	41.8	80.3	64.4	60.89	54.87	54.89	50.65	49.87	42.45	40
4.1	13.9	59.6	53.9	41	39.8	35.7	25	16.9	12.4	9.5
4.2	10	74.8	58.3	43.2	37.4	36.1	26.9	18.4	15.4	11.43
4.3	21.1	82.5	64.7	36.1	46.1	46.8	38.8	26.7	19.6	14
C1.1	0	0	0	0	6.9	7.4	13.8	15.6	19.4	24.4
C1.2	0.1	0	0	3.2	5.7	12.8	14.6	17.5	21.6	27.8
C2.1	0.6	0	0	0.1	3	4.9	6.4	7.8	11.4	18.4
C2.2	0	0	0	2	2.4	3.5	6.9	8.6	15.4	21.3
Influent	210	210	210	210	210	210	210	210	210	210

Soluble Phosphorus, mg/l

Sample #	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10
1.1	11	10.7	28.45	30.14	32.23	35	42	41.4	43.2	44.76
1.2	0.3	12.9	24.56	28.32	30.56	34.23	38.2	39.2	40.1	39.1
1.3	8.5	11.2	26.8	26.45	31.2	33.67	34.6	36.4	36.4	37.5
2.1	2.6	8.4	16.88	28.58	32.3	33.6	33.76	33.21	33.56	33.87
2.2	4.1	9.9	19.34	27.44	28.5	28.65	28.89	30.32	30.56	32.23
2.3	5.1	6.3	17.23	26.67	30.8	29.89	30.12	31.23	32.2	33
3.1	5.7	23.4	20	23.8	25.2	25.7	27.9	36.5	33.2	25.2
3.2	3	9.5	22.3	23.7	28.7	32.8	36.1	39.4	46.3	63.5
3.3	3.9	14.8	18	18.6	19.3	27.4	27.2	33.2	30	25.6
4.1	5.7	8	8.3	10.6	13.7	16.9	16.9	19.6	25.1	25.1
4.2	2.6	8.5	9.9	9.7	14.2	20.2	23.9	25.3	25.8	25.8
4.3	4.2	7.8	9.7	19	12.7	24.1	28.7	24.6	24.6	28.7
C1.1	0	0.6	0.7	0	2.3	2.3	8.4	6	8.4	10.8
C1.2	0	0	0	1	5	2.8	7.3	5	7.3	8.5
C2.1	0	0	0	0.6	-0.1	2.4	6.2	6.2	9.8	10.4
C2.2	0	0	0	0	2.3	3.9	7.4	7.3	7.8	9.5
Influent	19.3	19.3	19.3	19.3	19.3	19.3	19.3	19.3	19.3	19.3

Dissolved Oxygen, mg/l

Sample #	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10
1.1	7.88	7.28	6.88	6.41	4.3	3.5	2.1	2.43	0.54	0.56
1.2	7	5.27	6.89	6.77	3.78	3.23	2.87	2	0.72	0.65
1.3	7.34	8.45	6.86	6.88	4.12	2.98	2.66	2.12	0.54	0.48
2.1	6.78	7.66	6.72	5	5.06	6.2	4.98	4.88	4	2
2.2	6.88	9.77	6.83	6.9	7.65	6.15	5	4.34	3.98	2.04
2.3	6.38	9.24	7.38	7.14	5.96	6	5.04	4.87	3.99	2.36
3.1	7.39	9.77	8.8	6.62	7.19	5.8	5.55	5.03	4.88	2.81
3.2	7.34	9.88	7.25	7.85	7.04	5.8	5.34	5.1	4.13	2.5
3.3	6.84	9.71	8.23	8.62	6.74	6.38	4.98	5	4.37	2.61
4.1	7.22	10.84	8.98	7.22	7.2	6.37	5.98	5.09	5.1	4.01
4.2	6.78	11.14	9.15	9.14	6.74	7.18	6.3	5	4.67	4
4.3	7.73	10.4	9.17	8.88	7.83	6.81	6.1	5.89	4.89	3.98
C1.1	7	6.34	4	5.02	6.94	7.45	8	8	9.57	10.09
C1.2	7.2	6.23	4.24	5.05	6.95	7.67	8.68	8.8	8.24	9.25
C2.1	7.27	6	4	5.56	6.45	6.89	7.17	7.78	8.04	9.18
C2.2	7.38	6.68	4.3	5.21	7	6.99	7.28	7.56	9.4	8.33
Influent	10.52	10.52	10.52	10.52	10.52	10.52	10.52	10.52	10.52	10.52

pH

Sample #	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10
1.1	6.66	6.88	7.12	7.23	7.32	7.56	7.45	7.22	7.15	7.22
1.2	7.44	7.35	7.2	7.16	7.08	6.98	7.12	7.2	7.3	7.23
1.3	6.86	7	6.9	7	7.03	7	7	7.18	7.21	7.18
2.1	6.85	7.1	6.9	7.1	7.15	6.8	7.09	7	7.2	7.22
2.2	6.93	7.16	7.1	6.96	7	7.1	7.2	7.34	6.88	7.21
2.3	6.93	7	7.1	7.2	7.3	7.6	7.34	7.13	7.32	7.23
3.1	6.78	7	7.12	7.15	7.2	7.2	7.3	7.1	7	7.13
3.2	7.07	7.23	7.15	7.03	7.2	7.26	7	7.2	6.84	7.1
3.3	6.94	7	7.1	7.14	7.28	7.2	7.17	6.86	7.34	7.24
4.1	7.25	7.3	7.2	7.35	7.5	7.38	7.26	6.88	6.88	7
4.2	6.93	7	7.2	7.3	7.4	7.34	7.3	7.1	7	7
4.3	7.1	7.11	7.09	7.1	7.4	7.43	7.55	7.8	8	7.82
C1.1	7	8.15	8.3	8.43	8.6	9.1	9.6	9.4	9.8	10
C1.2	7	7.7	8	8.08	8.13	8.23	8.5	9	9.1	10.3
C2.1	7.11	7.87	8	8.15	8.21	8.27	8.3	9	8.9	10.2
C2.2	7.11	8	8.2	8.35	8.54	8.78	8.97	9.1	9.7	10
Influent	6.67	6.67	6.67	6.67	6.67	6.67	6.67	6.67	6.67	6.67

Alkalinity as $CaCo_3$, mg/l

Sample #	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10
1.1	186	240	250	270	284	284	292	300	314	320
1.2	224	230	250	256	270	288	290	296	310	324
1.3	186	244	256	260	274	310	312	312	320	346
2.1	210	216	226	236	250	346	366	380	396	420
2.2	210	216	210	216	228	330	350	354	360	398
2.3	190	200	216	234	246	336	354	358	372	394
3.1	200	204	224	230	236	292	308	316	324	340
3.2	200	200	216	224	234	304	316	326	336	346
3.3	190	204	226	216	220	316	316	324	336	350
4.1	180	208	228	240	252	260	286	290	300	320
4.2	180	206	234	236	240	244	248	252	260	292
4.3	188	200	220	236	240	248	258	264	276	300
C1.1	248	286	328	340	394	560	590	620	656	690
C1.2	250	264	334	356	364	530	556	570	612	680
C2.1	252	270	324	338	384	400	416	428	454	560
C2.2	256	276	316	334	356	380	394	420	472	572
Influent	380	380	380	380	380	380	380	380	380	380

Curriculum Vitae

Sudhanshu Mishra was born in Rewa, India in 1976. He graduated from St. Joseph's College, India in 1995. Sudhanshu graduated with a Bachelor in Technology degree in Agricultural Engineering from the University of Allahabad, Allahabad, India in 1999. He started his work towards his Masters degree in Land and Water Resources program in Biological Systems Engineering Department at Virginia Polytechnic and State University in the fall of 2001. Sudhanshu will start his work with Blue: Land, Water, and Infrastructure at Raleigh, North Carolina from August, 2003.