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SOIL ENVIRONMENT MONITORING USING SENSORS TO PREDICT MICROBIAL ORGANIC WASTE ASSIMILATION

presented by

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has been accepted towards fulfillment
of the requirements for the

M.S. degree in Biosystems Engineering

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Major Professor's Signature

5/09/2008
Date

SOIL ENVIRONMENT MONITORING USING SENSORS TO PREDICT MICROBIAL ORGANIC WASTE ASSIMILATION

By

Isis L. Fernandez Torres

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ABSTRACT

SOIL ENVIRONMENT MONITORING USING SENSORS TO PREDICT MICROBIAL ORGANIC WASTE ASSIMILATION

By

Isis L. Fernandez Torres

Land application is a means of disposal and treatment for agricultural and food processing waste. The soil's waste assimilation capacity is governed by biological activity. Waste decomposition is attributed to aerobic bacteria; however multiple microbial communities coexist in the same environment. Microorganisms adjust their community structure and biological activity to the surrounding, environmental factors. Individual sensors were used to monitor four environmental soil properties: soil moisture content, oxygen level, oxidation-reduction potential and temperature. The sensors were placed in sand-filled columns at three depths: 4, 12, and 20 inches.

Sensor readings varied with depth, hydraulic load (L/ac/day) applications, organic loading (lbs BOD/ac/day) and nutrient availability. Microbial activity was assessed by measuring iron and manganese concentrations present in soil column leachate. Mobilization of manganese and iron is characteristic of anaerobic microorganisms.

Soil moisture content, oxygen level and temperature sensors were successful in estimating when anaerobic environmental conditions were most favorable. The positive results from this research project provide confidence that a field scale demonstration is warranted.

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The recipe for attaining a Master's Degree is simple. Mix the ingredients below for at least two years or until complete.

1 – Patient Advisor: *Thank you Dr. Steven Safferman for taking a chance on me and guiding me throughout these two years. I've learned so much.*

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1 – Resourceful better half: *Dakujem moja laska for your help and your never-ending, unconditional support.*

1 - Fabulous Department: *Thanks Barb for everything. Thanks Vicki and John for all those leachate analyses. Thanks Lou and Alyse for the helping in the daily tasks to keep the experiment running.*

1 – Bundle of Friends: *Thanks friends for all your support. Becky you'll always be my personal genius. I would have never finished without you.*

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Chapter 1 Background

1.1 Land Application and Microbial Activity

Land application can be traced back as far as 1934 (Crites et. al. 2000). A common form to treat organic, non-hazardous waste is land application which is primarily used by the agriculture and food processing industries due to their rural location. The efficacy to breakdown organic compounds is dictated by the soil's assimilative capacity which depends on biological activity. Microorganisms are essential for organic waste decomposition and nutrient mineralization to simple molecules for roots and plant uptake (Donker et al. 1994, U.S. EPA 1990).

Donker et. Al. (1994) state "microorganisms are suitable to act as a sharp mirror of environmental pollution; they can function as a first warning system due to their ubiquity, size, versatility and importance in foodwebs and recycling of elements."

When land application treatment systems are properly managed the aerobic zones dominate (Crites et al. 2000, Brown and Caldwell 2007). Aerobic decomposition is generally more rapid than anaerobic (U.S. EPA 1990). The maintenance of an aerobic upper soil profile can be managed by controlling organic loading, hydraulic loading and drying time (Brown and Caldwell 2007, Islam and Wright 2006). Brown and Caldwell (2007), McDaniel (2006) and Mokma (2006) report excess organic loading can result in odorous anaerobic conditions, incomplete removal of organics in the soil profile and mobilization of iron, manganese and other compounds.

Land treatment systems remove biodegradable organics primarily through biological oxidation and reduction reactions (Crites et al. 2000). Under aerobic conditions, the carbon source acts as the electron donor and oxygen acts as the electron acceptor resulting in the aerobic-oxidation energy reaction shown below (Tarradellas 1997, Rittmann and McCarty 2001).

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6\,CO_2 + 6\,H_2O$$

Once all the oxygen is consumed, anaerobic and facultative microorganisms have more favorable growth conditions. The carbon source remains the same but electron acceptor order shifts in a step-wise function to nitrate (NO_3^-), manganese (Mn^{4+}), iron (Fe^{3+}), sulfate (SO_4^{2-}) and finally carbon dioxide (CO_2). The corresponding reduction reactions are listed below in metabolic pathway order (Mokma 2006, Paul and Clark 1996, Ricks 2002).

$$2\,NO_3^- + 10\,e^- + 12\,H^+ \rightarrow N_2 + 6\,H_2O \qquad (denitrification)$$

$$MnO_2 + 2\,e^- + 4\,H^+ \rightarrow Mn^{++} + 2\,H_2O$$

$$Fe(OH)_3 + e^- + 3\,H^+ \rightarrow Fe^{++} + 3\,H_2O$$

$$SO_4^{2-} + 8\,e^- + 9\,H^+ \rightarrow HS^- + 4\,H_2O$$

$$CO_2 + 8\,e^- + 8\,H^+ \rightarrow CH_4 + 2\,H_2O$$

The reduced forms of manganese (Mn^{2+}) and ferrous iron (Fe^{2+}) are more soluble and can be mobilized into groundwater (Brown and Caldwell 2007). The

release of dissolved Fe^{2+} into groundwater is one of the most prevalent groundwater problems worldwide (Lovley 2000). Lovley (2000) found ferrous iron in groundwater causes plumbing and staining problems, negatively impacts plant growth, and influences the composition and quality of groundwater.

"A number of food processing facilities in Michigan have been identified as potential causes of groundwater impact (Mokma 2006)." Primarily because their monitoring well water evaluations indicate elevated levels of iron (Fe), manganese (Mn) and other water constituents above recommended values (Mokma 2006).

1.2 Soil Properties

Microorganisms do not exist as single cultures, but rather coexist with many different microbial communities. Microbial communities adjust their structure and activity rate to environmental factors: temperature, soil moisture content, oxygen availability, nutrient concentrations and energy sources (Dilly 2005, Donker et al. 1994, Islam and Wright 2006, U.S. EPA 1990, Tarradellas 1997). The following sections discuss how each soil environmental property affect microbial populations.

1.2.1 *Soil Moisture Content*

Soil moisture content can be controlled via irrigation and drainage (EPA 1990) but is also dependent on precipitation and ground elevation. Moisture content directly affects plant water availability, soil gas permeability, and microbial presence, growth and activity (Leeson and Hinchee 1997, Paul and Clark 1996, Tarradellas 1997). Aerobic microorganisms are usually affected by

soil moisture content and soil compaction (Neilson and Pepper 1990, Tarradellas 1997).

The degradation of organic compounds can be accelerated by maintaining soil moisture content at an optimal level (U.S. EPA 1990). King et al. (1998) found the optimal moisture content for aerobic bioremediation is between 20% to 50% field capacity. This range provides sufficient liquid moisture within soil pore spaces for optimum cellular metabolism, reproduction and growth (King et al. 1998). However microbial population densities can vary greatly under extreme conditions. Under low soil moisture, water may not be available for plant uptake but active organism populations will exist. As moisture content increases, oxygen permeability decreases (Leeson and Hinchee 1997). Neilson and Pepper (1990) reported critical values for aerobic microbial respiration between 77%-97% soil saturation values. Saturated soils lack oxygen in soil pores allowing anaerobic microbial populations to predominate.

1.2.2 *Oxygen Level*

Oxygen is required for aerobic decomposition. Aerobes oxidize the available carbon source and reduce oxygen to water. Tarradellas (1997) reported reduced pore volume restricts oxygen exchange with the atmosphere and creates the presence of water film barriers.

Yaniga and Smith (1986) observed an increase in the number of bacteria with increasing oxygen concentration in soil columns treated with four different oxygen-air mixtures. Smith and Dowdell (1974) monitored ethylene level, oxygen level, moisture content and temperature at different depths in a sandy loam.

Their research showed a decrease in oxygen directly mirrored an increase in residual ethylene because under saturated conditions, oxygen levels were reduced from 20% to 12% - 14%. Hillel (1982) reported microbial respiration was limited at values of 10% air filled porosity.

Bioventing is the process of aerating soils to stimulate in situ biological activity and promote aerobic biodegradation (Leeson and Hinchee 1997). One bioventing site studied by the U.S. EPA (1992) recorded initial oxygen values in the range of 0% to 13%, which indicated oxygen limited biological activity. Oxygen was added to this site to reach atmospheric levels.

1.2.3 *Oxidation Reduction Potential*

Oxidation-Reduction Potential (ORP) characterizes the oxygen status, measures electron activity and the intensity of oxidation-reduction processes (McDaniel 2006, Szogi et al. 2004). In microbial biodegradation studies, reduction-oxidation conditions like aerobic, denitrifying, sulfate reducing, manganese reducing, iron reducing and methanogenic are used to define the electron acceptor involved in the conversion of the organic compound (Donker et al. 1994). Soil reduction-oxidation potential, commonly termed redox potential, ranges between -300mV to +900mV (Brown and Caldwell 2006, McDaniel 2006, Pansu et al. 2001) Figure 1-1 shows a generalized relationship between soil oxygen availability, ORP and pH. When soils remain at a low ORP for prolonged time periods, metal reducing microbes transform iron and manganese to their soluble, mobile valence states. These soluble metal particles are subject to leaching and possible groundwater contamination (Brown and Caldwell 2006).

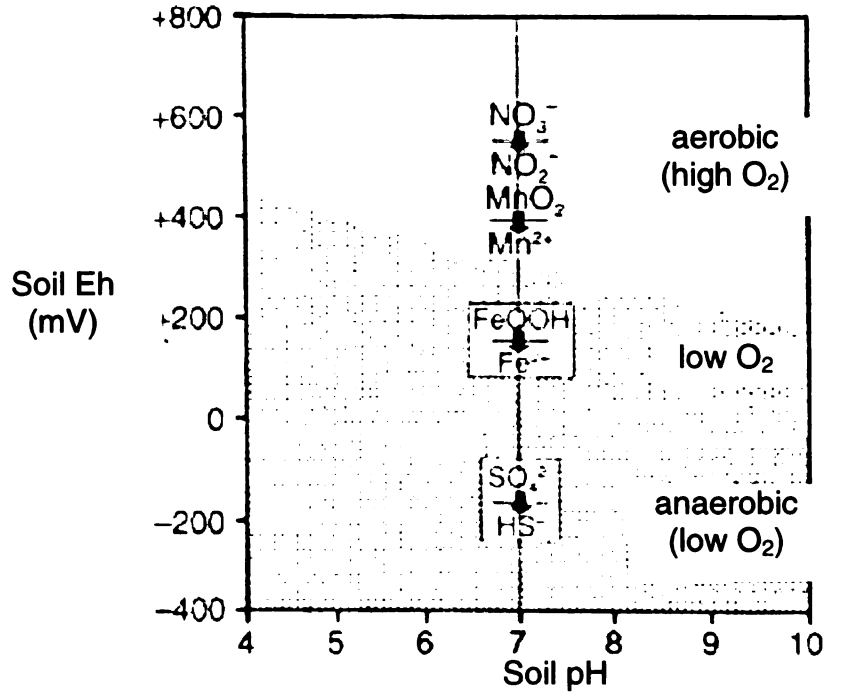

Figure 1-1. ORP relationship to oxygen availability and pH (McDaniel 2006)

Bailey and Beauchamp (1971) measured ORP from soil samples to determine nitrate reduction. His values dropped from an initial 400 mV value to -300 mV range. Quispel (1947), Ricks (2002) and Szogi et al. (2004) used in situ ORP electrodes to monitor redox potentials. All studies were performed on waterlogged conditions. Ricks (2002) monitored ORP at different depths. The most shallow electrode readings varied between 500mV to 700mV, and the deepest electrodes had read between 200mV and 300mV. Szogi et al. (2004) recorded ORP for the treatment of swine wastewater in three different constructed wetlands. ORP values ranged from -100mV to >300mV. Quispel (1947) attained negative readings in anaerobic soils at a 10 cm depth. Bohn (1971) recommends reduction-oxidation potential monitoring should complement and not replace oxygen level monitoring.

1.2.4 *Soil Temperature*

Soil temperature is an important factor that controls microbiological activity and the rate of organic matter decomposition (Paul and Clark 1996, U.S. EPA 1990). Islam and Wright (2006) reported soil microbial activity is greatest between 20°C to 40°C; however Paul and Clark (1996) also state some microbes grow in low temperatures, -12°C, while others under extreme heat, 110°C.

Temperature fluctuations such as cold shock, freezing and thawing cycles, varying cool down and warm up rates, have effects on microbial populations involved (Paul and Clark 1996, Tarradellas 1997).

1.2.5 *Soil pH*

Soil pH affects plant growth and bacterial growth (Crites et al. 2000). Near neutral pH results in the largest and most diverse composition of bacterial populations (Islam and Wright 2006b); specifically most known bacterial species grow within the pH range of 4 to 9 (Paul and Clark 1996). Acidity, pH range of 4 to 6, enhances soil fungi activity (Islam and Wright 2006, Paul and Clark 1996). A change in pH causes negative effects on certain microorganisms while others microorganisms may thrive (Tarradellas 1997).

1.3 Soil Environment Monitoring

The use of sensors for soil environment monitoring is not uncommon. Some irrigation systems are linked to moisture content probes to minimize water use. Weather stations record soil moisture content and temperature. Szogi et. al. (2004) monitored in-situ oxidation-reduction potential in constructed wetlands for swine wastewater treatment using platinum probes. Ricks (2002) also used in-

situ ORP probes to monitor the effects of riparian buffers and vegetation on groundwater nitrate concentration. No literature was found on combined use of available soil property sensors to provide insight on microbial presence and activity which is the main focus for this project. The commercially available sensors used in this project are described in detail in the following chapter.

1.4 Problem Statement

Finding and determining prescriptive, acceptable organic and hydraulic loadings is difficult due to the complexity of soil assimilation. However, by monitoring the soil environment a tool can be developed to prevent over loading the soil and the resulting impacts to groundwater associated with metal mobilization caused by anaerobic microbial communities.

1.5 Project Objective

The project's objective is to determine if changes detected by soil property sensors can be associated to metal mobilization caused by anaerobic microbes. The monitored soil properties were volumetric water content, oxygen level, oxidation reduction potential, and temperature. Sensor response changes were obtained by varying organic waste application.

Chapter 2 Methods and Materials

Soil environmental conditions are theorized to predict microbial activity and in turn, used to estimate soil's assimilative waste capacity. To test, soil columns were assembled and soil property sensors were installed to measure important soil environmental conditions (section 2.1) at various depths (section 2.3).

- A water content reflectometer was used to measure soil moisture content
- Oxygen level was determined by an oxygen sensor
- Oxidation-reduction potential (ORP) was monitored with a platinum electrode
- A thermistor was used to measure soil temperature

Each soil column was equipped with twelve sensors: 3 water content probes, 3 oxygen sensors, 3 ORP electrodes and 3 thermistors. One sensor of each kind was place at three depths (4 in., 12 in. and 20 in. below ground) within the soil column to measure variability by depth.

Columns were operated with various hydraulic and organic loadings and nutrient concentrations varied between columns and research stage (section 2.2).

Images in this thesis chapter are presented in color.

2.1 Sensor Description

2.1.1 *Water Content Reflectometer*

Soil volumetric water content (VWC) was monitored using the Campbell Scientific CS616 water content reflectometer, Figure 2-1. The probe uses time domain measurement methods to calculate volumetric water content.

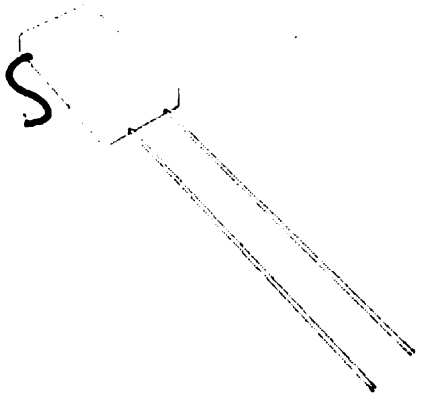

Figure 2-1. Campbell Scientific CS616 Water Content Reflectometer (Campbell Scientific Inc. 2006)

An electromagnetic pulse signal is emitted and received through the stainless steel rods at a velocity dependent on the dielectric permittivity of the surrounding soil (Campbell Scientific Inc. 2006). The soil's dielectric permittivity is dependent on water content and inversely proportional to the pulse velocity. The travel time of the signal along twice the rod length is measured. This time period is used to calculate the volumetric water content using Equation 2-1.

$$VWC = -0.0663 - 0.0063 * period + 0.0007 * period^2 \qquad Eqn\ 2-1$$

The CS616 water content reflectometer measures from 0% water content to saturation. Saturation values for sandy soils, as measured by the CS616 probe, can vary from 0.30 to 0.42 volumetric water content[1]. Other operational

[1] Jason Ritter, Campbell Scientific Inc. Engineer, personal communication, January 14, 2008.

characteristics of the CS616 water content reflectometer are an accuracy of ±2.5% VWC, resolution and precision of 0.1% VWC, and a probe-to-probe variability of ±1.5% VWC in typical saturated soil.

Several factors that can reduce measurement accuracy are high soil electrical conductivity, high soil organic matter and high clay content. Air voids around the rods should be avoided during installation to optimize sensor accuracy.

2.1.2 *Oxygen Sensor*

The amount of oxygen (O_2) gas in air was measured using the O2S-D oxygen sensor designed and manufactured by Apogee Instruments Inc., located in Logan, UT. This sensor measures absolute gas concentration level, but reads out in relative units of oxygen (%) to other gases present in the mixture. Historical atmospheric concentration of O_2 has remained at 20.95%. The absolute O_2 concentration determines the rate of most biological and chemical processes but the relative O_2 is typically reported (Bugbee and Blonquist 2007).

Figure 2-2. Oxygen Sensor (Model O2S-D) by Apogee Instruments Inc. (Bugbee and Blonquist 2007)

Bugbee and Blonquist (2007) describe the Apogee O_2 sensors as "galvanic cell sensors made of a lead anode, gold cathode, acid electrolyte and Teflon membrane, where O_2 diffusion occurs. The current flow between the two electrodes is linearly proportional to the absolute amount of O_2 in the soil environment. A resistor is used to produce a voltage (mV) output instead of a direct current output." The sensors are equipped with a small resistance heater which is designed to warm the sensor slightly above ambient temperature keeping condensation from occurring on the Teflon membrane.

The output of the Apogee O_2 sensor is a linear function of absolute O_2 concentration. A single-point calibration is generally used to derive a calibration factor (CF) (Equation 2-2) used to convert mV output from the sensor to relative O_2 concentration (%). The oxygen sensor can measure between 0 to 100% O_2 however, under anoxic conditions a zero offset was observed, reference section 3.1.2. Additional sensor specifications are accuracy of <0.01% O_2 drift per day and a operational range 0° to 50°C.

$$CF = \frac{20.95\%}{mV_c} \qquad Eqn\ 2-2$$

where, mV_c *is the mV reading at time of calibration*

Under aerobic conditions O_2 level (%) is predicted to remain approximately 20.95%, in contrast to anaerobic conditions where predicted values are below 10% oxygen. Hillel (1982) found that biological activity is restricted at levels of 10% oxygen of air filled porosity.

2.1.3 *Oxygen Reduction Potential Probe*

Oxidation-reduction potential (ORP) is used to measure the electron activity in soil and is commonly measured in millivolts. The Hanna Instruments ORP electrode with PFTE junction, model HI2005-1005, was selected to monitor soil oxidation-reduction (redox) values. This is a flat tip platinum probe with a ground loop matching pin and a BNC connector as shown in figure 2-3. The ORP value is the measured difference between the potential of the reference half-cell and the potential of the platinum electrode.

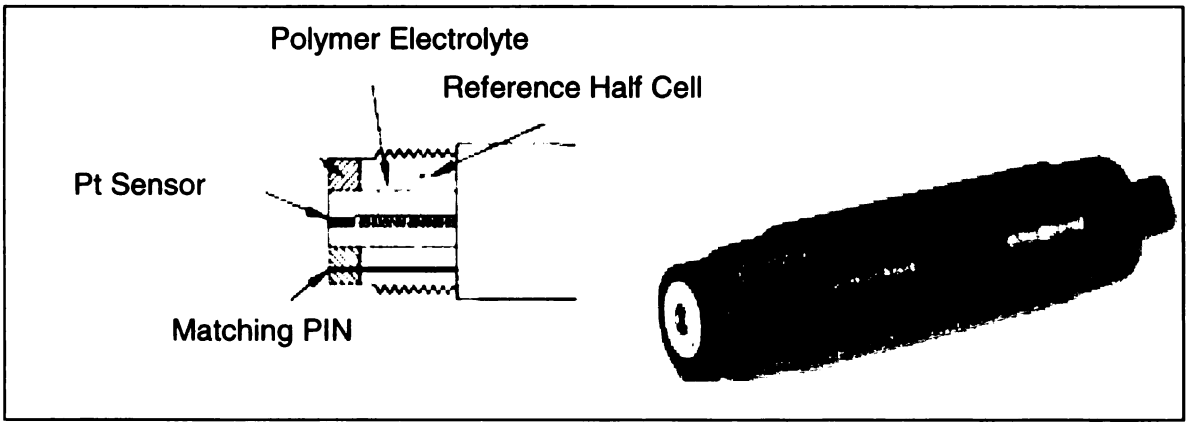

Figure 2-3. HI 2005-1005 ORP Probe and Schematic (Hanna Instruments)

Redox reading errors can be attributed to clogged electrode tips or scratched surfaces. The sensor will become sluggish under these conditions.

Under aerobic conditions, O_2 is the preferred electron acceptor; expected ORP values are greater than +300mV dependent on soil pH (McDaniel 2006, Szogi et al. 2004). Saturated soils are oxygen deficient and preferred electron acceptors shift. Anaerobic microbial respiration electron acceptor preference is nitrate, manganese oxide, iron oxide, sulfate and carbon dioxide (Patrick et al. 1985). Manganese and iron oxidation occurs in the -100mV to 300mV range

(McDaniel 2006, Szogi et al. 2004). A shift from aerobic conditions to anaerobic conditions should result in negative linear trends.

2.1.4 *Temperature Probe*

The Campbell Scientific T108 temperature probe uses a BetaTherm 100K6A thermistor to measure temperature. An excitation voltage of 2.5 V is applied and the voltage drop across an integrated 1 K ohm resistor is measured. The ratio of measured voltage (Vs) to the excitation voltage (Vx) is related to thermistor resistance (Rs) using Equation 2-3 (Campbell Scientific Inc. 2007). A Rs value converts the resistance to temperature in degrees Celsius using the Steinhart-Hart equation (equation 2-4). The Steinhart-Hart equation error is less than ±0.01°C.

$$\frac{Vs}{Vx} = \frac{1000}{(Rs + 40000 + 1000)} \qquad Eqn\ 2-3$$

$$T\ (°C) = \frac{1}{(A + B\ (\ln Rs) + C\ (\ln Rs)^3)} - 273.15 \qquad Eqn\ 2-4$$

where,

$A = 8.271111\,e-4$

$B = 2.088020\,e-4$

$C = 8.059200\,e-8$

The T108 probe can measure air, soil and water temperatures in the range of -5°C to +95°C. Thermistor interchangeability error is less than ±0.2°C. Long lead lengths and electrically noisy environments should be avoided to minimize error.

2.2 Experimental Conditions

The experiment was divided into four stages. Stage 1 only varied hydraulic loading. Hydraulic loading remained constant in the remaining three stages however, biochemical oxygen demand (BOD) loading and nutrient concentrations were varied. During each stage there were duplicates for every condition tested, except during stage 4 where triplicates of each condition existed. Columns 2 & 7 were used as control conditions for stages 2, 3 and 4. Table 2-1 is a summary of each experimental stage and its loading conditions. Each stage's variables are further described in sub-sections below.

Table 2-1 Experimental Stage and Loading Summary

Stage	Stage Dates	Column Number	Hydraulic Loading (L/ac/day)	BOD Loading (LBS/ac/day)	Nutrient Loading (%)
1a	7/24/07 - 8/15/07	2 & 7	2	0	0
		1 & 8	2	0	0
		3 & 6	2	0	0
		4 & 5	2	0	0
1b	8/16/07 - 9/2/07	2 & 7	1	0	0
		1 & 8	0	0	0
		3 & 6	2	0	0
		4 & 5	4	0	0
1c	9/3/07 - 10/2/07 *pump timers installed	2 & 7	2.4	0	0
		1 & 8	2.4	0	0
		3 & 6	2.4	0	0
		4 & 5	2.4	0	0
2	10/3/07 - 12/28/07	2 & 7	2.4	65	0%
		1 & 8	2.4	65	25%
		3 & 6	2.4	65	50%
		4 & 5	2.4	65	100%
3	12/29/07 - 2/5/08	2 & 7	2.4	65	0%
		1 & 8	2.4	65	25%
		3 & 6	2.4	65	50%
		4 & 5	2.4	500	100%
4	2/5/08 - 3/3/08	2 & 7	2.4	65	0%
		1, 3 & 8	2.4	1000	100%
		4, 5 & 6	2.4	500	100%

2.2.1 *Experimental Stage 1*

Hydraulic loading was the only variable during stage 1; no BOD loading or nutrients were introduced to the columns. All columns were watered with dechlorinated tap water. The chlorine was removed from the tap water by adding

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an aquarium water conditioner, Instant Dechlor by WECO Products Inc. Instant Dechlor ingredients are sodium thiosulphate, sodium bicarbonate and water[2].

Stage 1 was further divided into 3 separate phases. All the sensors were connected to the main circuit boar during the first three weeks. Meanwhile 2 L of dechlorinated water were applied manually to the top surface area of each column daily. On August 16, 2007, day 23, the hydraulic loading was varied to test if the volumetric water probes would indicate change. Four hydraulic loads were tested:

- 0 L per day
- 1 L per day
- 2 L per day
- and 4 L per day

On August 21, 2007 column 4 was flooded with approximately 35 L of water to promote sand settling. Seven days later, the remaining seven columns were also flooded. On September 3, 2007, day 41, the pumps were connected to automatic timers to spray 2.4 L per day in four doses of 600 mL each, further described in section 2.3.2. All columns were treated equally until Stage 2.

2.2.2 *Experimental Stage 2*

The first day of stage 2, all columns were seeded with 1L of unchlorinated secondary treatment effluent from the East Lansing Wastewater Treatment Plant in East Lansing, MI, to introduce a variety of microorganisms to each column.

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[2] Robert Brine, WECO employee, personal communication, December 7, 2007

During stage 2, the organic BOD loading remained constant but nutrient concentration was varied. The tested conditions follow.

- 65 lbs BOD/ac/day and 0% nutrient concentration mix
- 65 lbs BOD/ac/day and 25% nutrient concentration mix
- 65 lbs BOD/ac/day and 50% nutrient concentration mix
- 65 lbs BOD/ac/day and 100% nutrient concentration mix

The nutrient solution was introduced to create oxygen limiting conditions only; however 0% and 25% nutrient conditions could also be nutrient limiting. Low nutrient concentrations were introduced to prevent excessive microbial growth which could potentially clog the soil columns. The water irrigated on to each column was composed of dechlorinated tap water, nutrient solution and a carbon source. D-glucose, commonly termed dextrose, was the carbon source. The nutrient solution formulation is described in section 2.3.4 and has a concentration based on oxygen demand.

BOD loading was controlled and fixed for all columns at 65 lbs BOD/acre/day, the median from the data collected by Mokma (2006). Mokma's literature review collected organic loading data presently being land applied by Michigan food processors. Glucose has a theoretical oxygen demand (ThOD) of 533mg/L, calculations shown below.

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$

$$ThOD = \frac{500\ mg\ glucose}{L} \cdot \frac{1\ mmol\ glucose}{180\ mg\ glucose} \cdot \frac{6\ mmol\ O_2}{1\ mmol\ glucose} \cdot \frac{32\ mg\ O_2}{1\ mmol\ O_2} = 533.33\ \frac{mg}{L}$$

The hydraulic loading was calculated based on a concentration of 500 mg BOD/L and a BOD load of 65 lbs BOD/ac/day.

$$Hydraulic\ loading = BOD\ loading \cdot BOD\ concentration \cdot surface\ area$$

$$Hyd.\ loading = 65\frac{lbs\ BOD}{ac \cdot day} \cdot 453592.4\ \frac{\text{mg}}{lb} \cdot \frac{1\ L}{500\ mg} \cdot \pi\ (0.75^2)ft^2 \cdot \frac{1\,\text{ac}}{43560\ \text{ft}^2} = 2.40\ \frac{L}{day}$$

Daily 2.4 L of wastewater was sprayed onto each column in four 600mL doses spaced out every 6 hours. For the following two stages, hydraulic load remained at 2.4 L per day and BOD loads and nutrient concentrations were increased.

2.2.3 *Experimental Stage 3*

In stage 3, only one experimental condition was varied from stage 2. The 100% nutrient condition was increased from 65 lbs BOD/ac/day to 500 lbs BOD/ac/day. The nutrient concentration remained at 100% but proportions were adjusted to the new oxygen demand requirements.

2.2.4 *Experimental Stage 4*

Organic loading was varied in stage 4 to increase the oxygen consumption rate. Conditions tested were included in the following.

- 65 lbs BOD/ac/day at 0% nutrient concentration mix (control)
- 500 lbs BOD/ac/day at 100% nutrient concentration mix
- 1000 lbs BOD/ac/day at 100% nutrient concentration mix

The experiment's control condition was maintained at 65 lbs BOD/ac/day with 0% nutrient concentration for the entire experimental period; stages 2, 3 and 4.

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2.3 Experiment System Set-up

This section discusses the set-up of the columns, sensor electrical wiring, daily soil column operations and experimental maintenance and column deconstruction.

2.3.1 *Column Construction*

All eight columns were constructed using 18 inch inner diameter, single wall, corrugated drainage pipe. The corrugated material wall was selected to prevent water flow short circuits along the edges of the columns.

A split-end cap was placed as the base for each vertical column. The split portion of each end cap was secured with three, industrial strength zip ties and a rectangular piece of hard plastic was caulked at the split to prevent leaks from the base. To allow for drainage, eleven 1/8 inch holes were drilled in each end cap as depicted in Figure 2-4. A clear, plastic container was placed below each soil column to collect leachate.

Each column had doors cut on the sides at each sensor depth to allow for access to the sensors and soil sample collection. Sensor wires exited from the sides of the soil column. Figure 2-4 is a dimensional schematic of each column and depth indication.

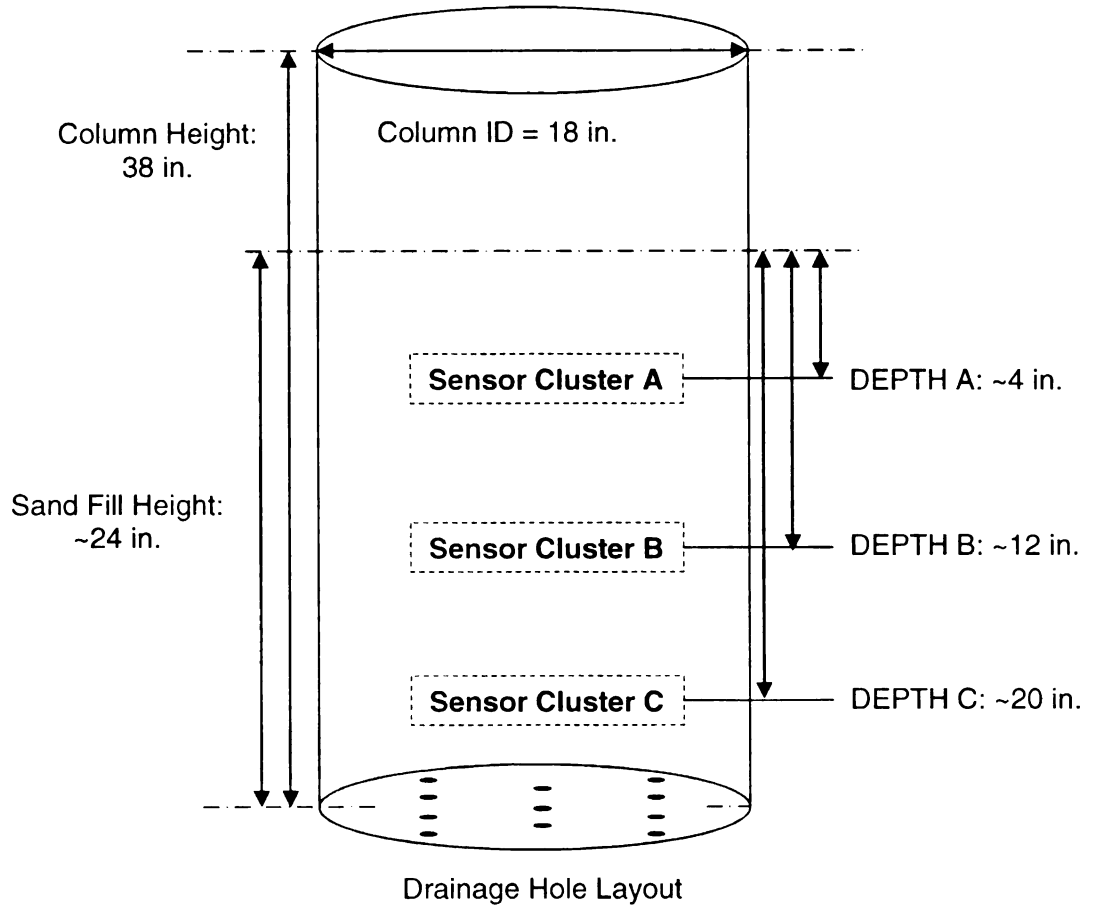

Figure 2-4. Soil Column Dimensional Schematic with Drainage Holes

A layer of pea gravel was placed on the bottom of the column. The gravel particles were sieved to a range of greater than 2.00mm and less than 4.75mm. 2000mL of pea gravel was placed on the bottom of each end cap and spread across the entire end cap surface area.

Each column was filled with play sand purchased from a local hardware store in 50 lbs bags. The sand used in this experiment was characterized by the Michigan State University Soil and Plant Nutrient Laboratory in East Lansing, Michigan, and characterization results are listed in Table 2-2.

Table 2-2. Sand Properties

Play Sand Characteristics	Unit	Test Date 6/12/2007	Test Date 8/1/2007
Soil Nutrient Levels			
Soil pH	ppm	7.8	8.2
Phosphorus (P)	ppm	7	5
Potassium (K)	ppm	12	12
Magnesium (Mg)	ppm	47	53
Calcium (Ca)	ppm	929	795
Micronutrient Level			
Zinc (Zn)	ppm	n/a	2.6
Manganese (Mn)	ppm	n/a	27.3
Copper (Cu)	ppm	n/a	1.6
Iron (Fe)	ppm	n/a	107.5
CEC	meq/100 g	5.1	4.4
% of Exchangable Bases			
Potassium (K)	%	0.6	0.7
Magnesium (Mg)	%	7.7	9.9
Calcium (Ca)	%	91.7	89.4
% Organic Matter	%	n/a	0.20
Nitrate (N)	ppm	n/a	0.2

In step 1, sand was added to each column up to the door at depth C. The sand was compacted by adding water. Then each sensor was carefully placed in its assigned location as shown in Figure 2-5. All sensor wires were threaded through the door opening before it was secured with industrial strength duct tape to prevent leaking. After placing the sensors, sand was carefully placed not to disturb the sensors and then built up to depth B, in the same orientation shown in Figure 2-5. The same steps were repeated for the top sensors. Each column required approximately 450 lbs of sand. All columns were constructed the same day.

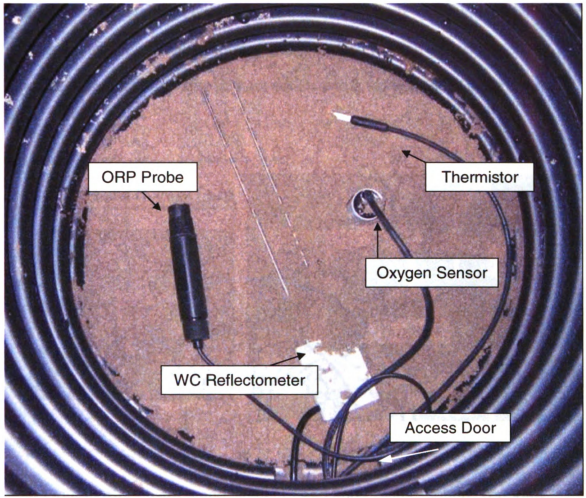

Figure 2-5. Sensor layout

2.3.2 *Irrigation System*

A full-cone spray nozzle was affixed above the soil column so its spray would cover the top surface area. Each experimental condition was assigned to one single influent feed resulting in a total of 4 water feed tanks for stages 1-3, as each condition had duplicated columns. Each influent bucket had two outlets, each to an individual pump assigned to one soil column. The pump used in the experiment is a multipurpose pump, 114 Volts AC with a maximum flow rate of 5 GPM that was purchased at a local hardware store. A standard garden hose was

attached from the influent bucket to the inlet of the pump. The pump outlet was fitted down to a ¼ in ID clear rubber tubing that fed to each individual column. Figure 2-6 are pictures of the influent set-up and a top view of all eight columns.

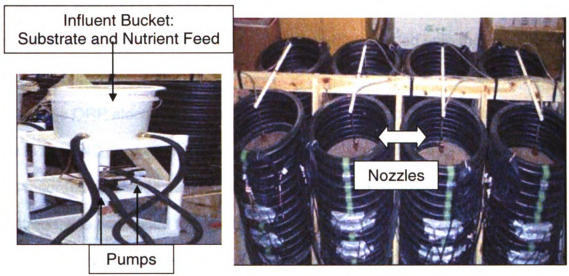

Figure 2-6. Substrate and Nutrient Feed and Column Set-up, Top View

Each pump was connected to an analog, cyclical timer set to activate every 6 hours. The spray for each pump was calibrated to dose 600 mL.

2.3.3 *Sensor Wiring and Program Set-up*

This experiment used a total of 96 sensors, 24 sensors for each soil, environmental property. During column construction after placing each sensor in the indicated location, it was labeled with column number, depth location and environmental property. The sensors were grouped by environmental property for wiring to the main circuit board. Figure 2-7 is a basic representation of the electronic set-up for the sensors to the data acquisition system.

The moisture content sensors where connected to a AM16/32 Campbell Scientific multiplexer, which was connected to the CR1000 Campbell Scientific datalogger. Similarly all the thermistors were connected to a second multiplexer and oxygen sensors required to be connected to two additional multiplexers. The datalogger was connected to a stationary computer and programmed to record measurements every 5 minutes. The data were downloaded onto the computer twice a week.

The ORP electrodes could not be incorporated into the main circuit board due to the BNC connector. Consequently each ORP reading was manually taken and recorded only once daily. In order to take an ORP reading, each ORP electrode was connected to a controller with a read out screen (Hanna Instrument's HI 504 Series pH/ORP controller with Tele-Control and Sensor Check). After approximately 1 minute, the stabilized mV reading was recorded then the controller was connected to the next ORP electrode until all measurements were taken.

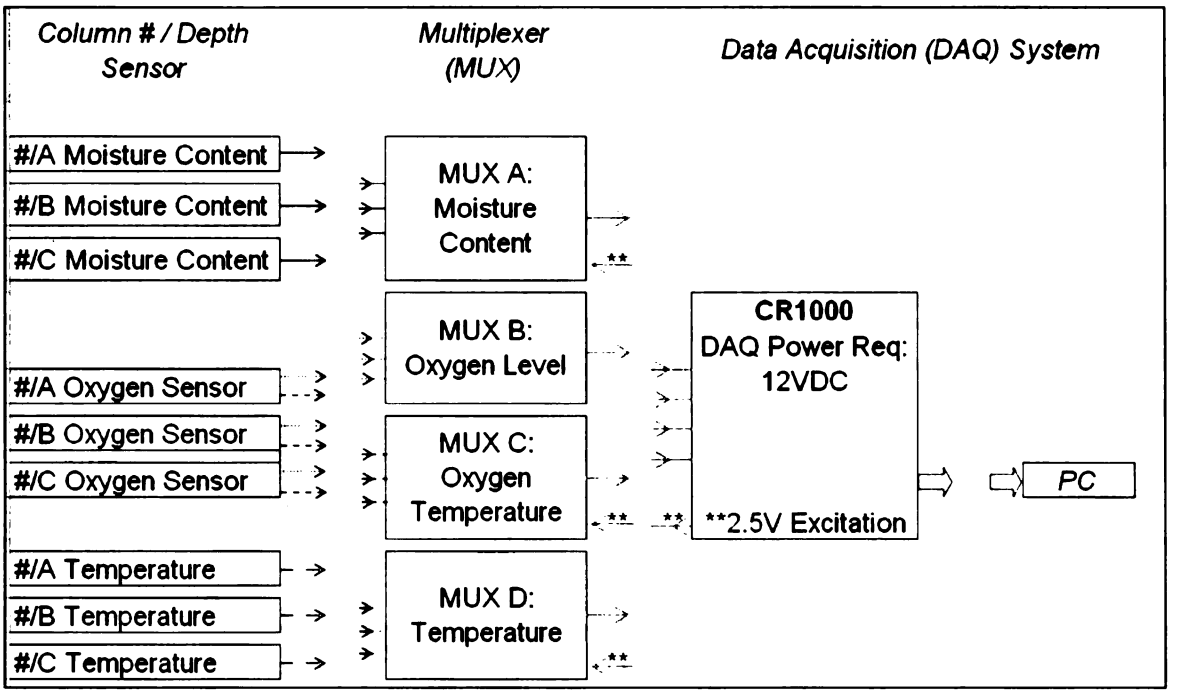

Figure 2-7. Basic Sensor Wiring Diagram

2.3.4 *Column Operations*

The experimental set-up required daily influent wastewater preparation and daily leachate measurements. Influent preparation consisted of mixing unchlorinated tap water, with the appropriate glucose ration (BOD loading) and nutrient solution, if required (Table 2-3).

Table 2-3. Influent Daily Preparation Recipe Summary

			Influent Daily Preparation Recipe		
Stage	**Influent**	**Column Spray**	**Dechlorinated Water (L)**	**Glucose Ration (mg)**	**Nutrient Soln (%)**
1a	n/a	n/a	2	0	0
1b	n/a	n/a	0	0	0
	n/a	n/a	1	0	0
	n/a	n/a	2	0	0
	n/a	n/a	4	0	0
1c	n/a	n/a	2.4	0	0
2	1	1 & 8	8	4000	25%
	2	2 & 7	8	4000	0%
	3	3 & 6	8	4000	50%
	4	4 & 5	8	4000	100%
3	1	1 & 8	8	4000	25%
	2	2 & 7	8	4000	0%
	3	3 & 6	8	4000	50%
	4	4 & 5	8	30800	100%
4	1	1, 3 & 8	8	30800	100%
	2	2 & 7	8	4000	0%
	4	4, 5 & 6	8	61600	100%

The nutrient solution was based on Trulear & Characklis (1982) substrate formulation with concentration oxygen demand (in Table 2-4).

Table 2-4. Composition of Substrate and Nutrient Solution per Stage

		NUTRIENT CONSTITUENT CONCENTRATION **				
	Experimental Stage	2 & 3	2 & 3	2	3 & 4	4
	BOD Loading (LBS/ac/day)	65	65	65	500	1000
	Nutrient Loading (%)	25%	50%	100%	100%	100%
Constituent	*Trulear concentration (mg/L)*	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
$C_6H_{12}O_6$	*10*	500	500	500	3850	7700
$Fe\ Cl_3$	*0.045*	4.331	8.663	17.325	17.325	34.650
$MnCl_2 \cdot 4\ H_2O$	*0.011*	1.059	2.118	4.235	4.235	8.470
$ZnSO_4 \cdot 7H_2O$	*0.008*	1.518	3.037	6.073	6.073	12.147
$CuCl_2 \cdot 2\ H_2O$	*0.005*	0.481	0.963	1.925	1.925	3.850
$CoCl_2 \cdot 6\ H_2O$	*0.007*	0.674	1.348	2.695	2.695	5.390
$(NH_4)_6\ Mo_7\ O_{24} \cdot 4H_2O$	*0.005*	0.481	0.963	1.925	1.925	3.850
$Na_2B_4O_7 \cdot 10H_2O$	*0.003*	0.289	0.578	1.155	1.155	2.310
$Na_3C_6H_5O_7 \cdot 2H_2O$	*0.408*	39.270	78.540	157.080	157.080	314.160
$NaH_2PO_4 \cdot H_2O$	*0.575*	55.344	110.688	221.375	221.375	442.750
$(NH_4)_2\ SO_4$	*0.367*	35.324	70.648	141.295	141.295	282.590
$NH_4\ Cl$	*3.417*	328.886	657.773	1315.545	1315.545	2631.090
$CaCl_2$	*0.308*	29.645	59.290	118.580	118.580	237.160
$MgCl_2 \cdot 6\ H_2O$	*0.565*	54.381	108.763	217.525	217.525	435.050
$KH_2PO_4\ H_2O$ (Buffer)	*.004M*	340.023	680.047	1360.093	1360.093	2720.187
$Na_2HPO_4 \cdot H_2O$(Buffer)	*0.004M*	433.858	867.717	1735.434	1735.434	3470.867

NOTE - Concentration of nutrient constituents was proportionately increased when glucose concentrations were increased.

** Actual nutrient rations were prepared to this value multiplied by 8, since daily influent preparation required 8L of water.

2.3.5 *System Maintenance*

The experimental set-up required biweekly water flow checks for each pump to maintain the hydraulic loading at 2.4 L/day. This loading was dispersed over 4 sprays. Each spray was calibrated to 600 mL as needed during water flow checks. All pumps were inspected daily for proper operation. Pump impellers were replaced as required.

During stages 3 and 4, the nozzles had to be routinely inspected and cleaned as they clogged due to bacteria growth within the influent buckets. At times the influent with added nutrient solutions would present excessive bacterial growth characterized by a rotten egg-like smell and floating, black masses and negative ORP readings. At the first sign of any of the symptoms previously described, the bucket and hoses were flushed with hydrogen peroxide solution and rinsed with water until clean.

2.4 Influent and Leachate Water Monitoring

Influent and soil column leachate was analyzed weekly for chemical oxygen demand (COD), dissolved oxygen (DO), ORP, temperature and pH. Influent samples were collected at each influent tank location before new influent preparation was mixed. The leachate was sampled from each soil column's effluent collection bucket. Samples for COD testing were always collected from fresh effluent.

In addition to water analysis parameters, the influent and effluent was analyzed for concentrations of iron and manganese twice a week. The presence

of manganese and iron in the leachate indicate mobilized metals from anaerobic reduction-oxidation reactions.

2.4.1 *Chemical Oxygen Demand*

COD evaluated the organic load assimilated by the soil column and its microorganisms. BOD is comparable to COD, since COD testing measures chemically oxidizable organic matter. BOD testing relies on bacteria to oxidize organic matter and takes five days to complete, unlike COD which can be completed in two hours.

The EPA approved reactor digestion method, Hach Method #8000, was used. Reagent range 20 to 1500 mg/L was used for the influent samples and 0-150 mg/L for the leachate samples. Each sample volume was separated into two separate samples, allowing for laboratory duplication and an assessment of precision. Each time a COD test was conducted, a blank sample prepared from deionized water and a standard sample were run. The standard sample was prepared from a known quantity of standard solution sample purchased from Hach.

During two occasions COD testing was performed on freshly prepared influent and at each spray nozzle location. Both values were compared to COD values collected the next day directly from the feed container. Significant differences were not found.

2.4.2 *Dissolved Oxygen*

Dissolved oxygen of each sample was determined weekly using a DO probe (Oakton DO300 series Data Meter). DO levels help indicate oxygen

limiting conditions. Influents with added nutrient solution were consistently found to have significantly lower levels of DO, ranging from 0.65 mg/L to 3.00 mg/L, than the leachate. The lower influent DO levels indicate that microbial biological decomposition was occurring in the influent buckets with added nutrient solutions. The control influent location, columns 2 & 7 had no added nutrients and ranged from 5.05 mg/L to 8.77 mg/L.

All leachate samples were consistently saturated with oxygen, ranging from 6.41 mg/L to 10.49 mg/L. Saturated conditions are attributed to the continuous leachate drip into the collector allowing for constant aeration and not thought to be representative of the conditions within the column.

2.4.3 *ORP*

An ORP probe, Oakton Waterproof ORP Testr 10 manufactured by Eutech Instruments, used for water quality testing was submerged in the weekly collected samples to determine ORP values (mV). The influent ORP ranged from 87 to 197 mV except on two occasions when ORP values were -10mV and -167mV. The negative readings indicated anaerobic conditions were present in the influent bucket. The bucket was immediately cleaned with hydrogen peroxide and rinsed with water. The effluent readings ORP values ranged from 53 mV to 225 mV.

2.4.4 *pH*

The pH of each sample was determined weekly using a pH meter, Accumet Excel XL60 pH probe. Before each use the pH meter was tested for accuracy using a 3 point pH calibration buffer solution to a ±0.05 pH units

tolerance. If any of the readings fell out of this range, the pH meter was recalibrated.

Influent with added nutrient solutions yielded a lower pH level than when no nutrient solution was added. All leachate samples had higher pH values than their influent. Table 2- 5 summarizes the pH measurements.

Table 2-5. pH Ranges for Experimental Stage

Stage	pH min	pH max
Influent		
2	6.05	8.44
3	5.24	8.06
4	4.62	7.88
Leachate		
2	6.98	8.81
3	6.22	8.42
4	6.05	8.43

2.4.5 *Manganese and Iron Analysis*

During stages 2, 3 and 4, influent and leachate samples were collected biweekly from each column and analyzed by the Michigan State University Soil and Plant Nutrient Laboratory in East Lansing, Michigan for manganese and iron concentrations.

The laboratory used flame atomic absorption. In this method, as described by ASU (2008), samples are introduced into a hot-flame which atomizes the metal of interest. A lamp of desired wavelength and a detector measure absorbance values based on the amount of the metal present. When compared

to a generated standard curve, the metal of interest can be quantified. Detection limits vary according the metal under consideration, and is reported in ppm units.

2.5 Experimental Shutdown

At the end of the Stage 4, three columns were selected to be disassembled in order to recover all sensors and perform post-calibration procedures. The remaining columns were used for continued testing beyond the scope of this thesis. The dismantled columns were the following: Control Column 7 – exposed to 65 lbs BOD/ac/day during stages 2, 3 and 4, Column 1 – exposed to 1000 lbs BOD/ac/day during stage 4 and Column 5 – exposed to 500 lbs BOD/ac/day during stages 3 and 4.

Prior to dismantling each column, soil samples were collected from each sensor depth for microbiological phospholipid analysis as part of another project. The deconstruction of each column was handled in a systematic manner to minimize damage to sensors. Pictures were recorded throughout the dismantling process as documentation, as discussed below.

First, a picture was taken of the soil column top surface. Secondly sand was removed until the first sensor cluster at depth A was exposed. A sand sample was collected and immediately weighed for soil water content and volatile solids calculations. Also, the color of the sand was matched to Munsell (2000) soil color charts for descriptive purposes. Each sensor was disconnected from its multiplexer connection and carefully inspected for scratches, dents and any other abnormalities. The same steps were followed at each depth. An additional sand sample was collected from the very bottom of each soil column.

Volumetric water content was correlated to the collected sand sampled during column deconstruction. After recording the wet sample weight, the samples were dried in a convection oven at 104°F for 20 hours. Dry sample weight was recorded. Volatile solid content was also determined by weighing the dry samples after placing them a furnace at 550°F for one hour.

After column deconstruction, ORP probes and oxygen sensors were post calibrated. The post-calibration procedures of the oxygen sensor consisted of recording oxygen level measurements at atmospheric and anoxic conditions. The anoxic conditions where created by placing the oxygen sensor in a nitrogen filled glass chamber filled. The oxygen was flushed out of the system while nitrogen was added until the sensor readout reached a stable condition.

The ORP probe was rinsed with deionized water and submerged in a standard ORP solution (YSI ® Zobell solution). Both temperature and ORP reading of the solution were recorded. In addition to standardized solution, each probe was also submerged into anaerobic digester sludge collected from the Swine Laboratory at Michigan State University in East Lansing, MI.

All post-calibration data is located in Appendix A.

2.6 Data Handling

The sensor data was downloaded from the datalogger twice a week. Individual plots were created for each environmental property for each column. At times, due to power source fluctuations data points in all columns would jump out of range, but immediately return to previous reading. In this event, the data point was erased for data continuity.

Chapter 3 Results and Discussion

Each soil environment parameter (volumetric water content, oxygen level, ORP, and temperature) was monitored throughout each experimental stage. In addition to soil parameter testing, soil column influent and leachate were tested for COD, manganese and iron content levels.

Section 3.1 is an overview of the performance of each sensor over the entire experiment. Thereafter, each research stage is discussed in sections 3.2 – 3.5.

3.1 Individual Sensor Evaluation for All Stages

The following sub-sections describe changes observed in the sensor over the entire time span of the experiment. The column sensor readings were averaged as shown in the table 3-1 below. Averaged soil columns were replicates of each organic loading and nutrient concentration.

Table 3-1. Sample and Condition Summary

Stage	BOD Loading	Nutrient Concentration	Soil Columns Averaged
1	0 lbs/ac/day	0%	1 & 8
	0 lbs/ac/day	0%	2 & 7
	0 lbs/ac/day	0%	3 & 6
	0 lbs/ac/day	0 %	4 & 5
2	65 lbs/ac/day	25%	1 & 8
	65 lbs/ac/day	0%	2 & 7
	65 lbs/ac/day	50%	3 & 6
	65 lbs/ac/day	100%	4 & 5
3	65 lbs/ac/day	25%	1 & 8
	65 lbs/ac/day	0%	2 & 7
	65 lbs/ac/day	50%	3 & 6
	500 lbs/ac/day	100%	4 & 5
4	65 lbs/ac/day	0%	2 & 7
	500 lbs/ac/day	100%	4, 5 & 6*
	1000 lbs/ac/day	100%	1, 3 & 8
* Column 6 was not exposed to 500/lbs/ac/day until stage 4 (approximately 30 days after columns 4 &5). The sensor readings were adjusted to the same start date as columns 4 & 5 in order to average values.			

3.1.1 *Soil Water Content*

Figure 3-1 is a historical snap shot of all the volumetric water readings collected during the experiment based on Stage 4 loadings. When the columns were flooded during stage 1, all depth C sensors displayed an increase from approximately 0.12 to 0.28 volumetric water content. The soil moisture content levels showed little variation through out stage 2 for all depths and loading conditions.

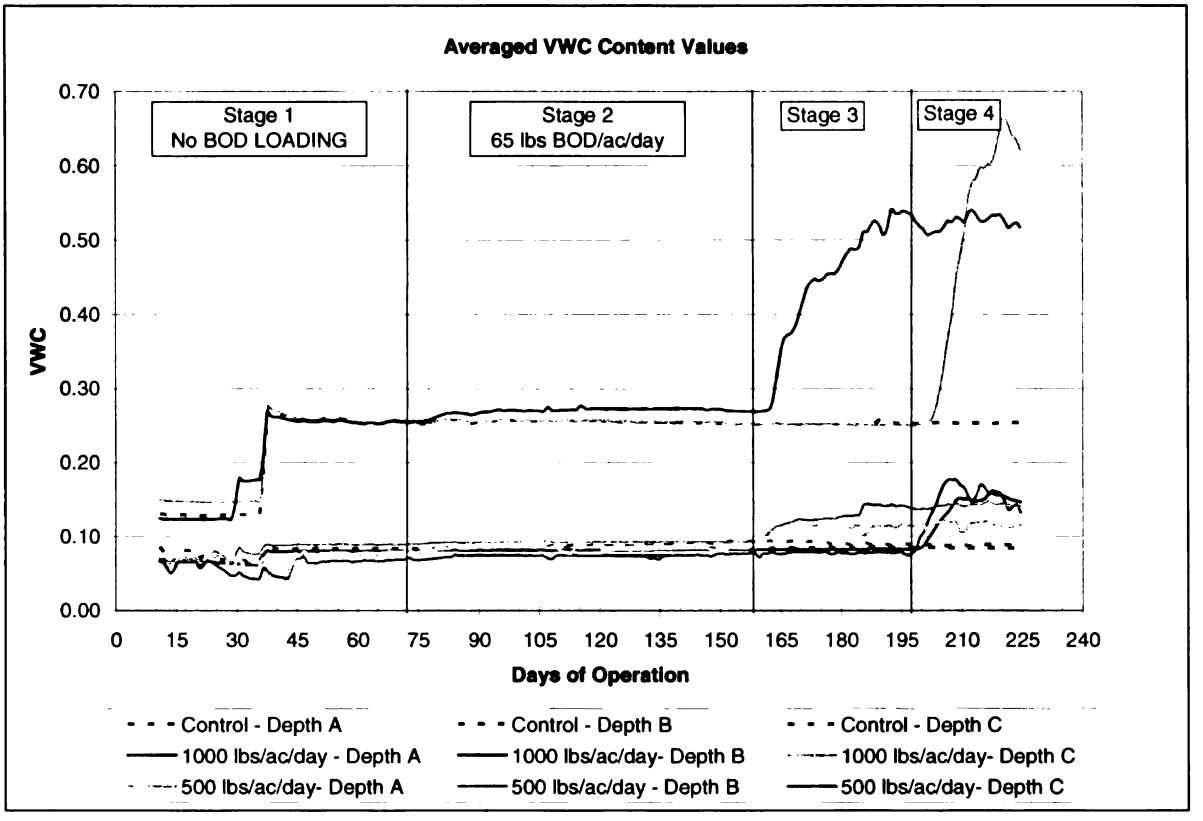

Figure 3-1. All Stages - Volumetric Water Content

In stage 3, the organic load of 500 lbs BOD/ac/day was introduced. This created in increase in VWC for that particular loading at depths A, B and C. Depth C's volumetric water content increased past the upper limit of sand saturation values, 0.42. Higher than expected saturation values are can be caused by high organic matter presence and increased electrical conductivity, which cause a delay in the return of the pulse signal. Similarly, in stage 4 when the organic load of 1000 lbs BOD/ac/day was introduced, the soil moisture content for this condition increases at all depths at a faster rate than the 500 lbs BOD/ac/day trend.

For multivariate statistical analysis, moisture content was treated as the response variable to the explanatory variables time, depth, hydraulic loading, BOD loading and nutrient. Statistical Analysis Software (SAS v 9.1) was used for this analysis and yielded results shown in Table 3-2. In this statistical evaluation, the numerical and denominator degrees of freedom are used to determine a critical F-value. The P-value is calculated by comparing the calculated F-value to the critical F-value; if significantly higher, than the p-value is less than 0.05. The test results determined stage, depth, hydraulic loading, BOD loading and nutrient concentration are significant in measuring soil moisture changes, since all the P-values are below 0.05.

The pair wise comparison test was used to compare stages and depths, shown in Table 3-3. In this test, the standard error between both values is used in conjunction with the degrees of freedom to determine a critical t-value. The absolute value of the calculated t-value is compared to the critical t-value; the comparison provides the listed p-value. All P-values are below 0.05 and show that moisture content is significantly different between depths A, B and C as well as between stages 1, 2, 3 and 4.

Table 3-2. Volumetric Water Content Statistical Analysis Summary

Effect	Numerical Degrees of Freedom	Denominator Degrees of Freedom	Calculated F- Value	P- Value (Critical Limit $\alpha = 0.05$)
Time	3	21	21.39	<.0001
Depth	2	14	5676.10	<.0001
Hydraulic Loading	1	5160	17.27	<.0001
BOD Loading	1	5160	481.40	<.0001
Nutrient Concentration	1	5160	15.34	<.0001
BOD & Depth Interaction	2	5160	1068.09	<.0001

Table 3-3. Depth and Stage Pair Comparison for VWC

Effect	Depth / Stage	Depth / Stage	Standard Error	Degrees of Freedom (DF)	Calculated t-value	P- value
Depth	A	B	0.001458	14	-3.97	0.0014
Depth	A	C	0.001458	14	-129.72	<.0001
Depth	B	C	0.001458	14	-125.75	<.0001
Stage	1	2	0.002265	21	-4.25	0.0004
Stage	1	3	0.002379	21	-6.70	<.0001
Stage	1	4	0.003412	21	-7.29	<.0001
Stage	2	3	0.001775	21	-3.56	0.0018
Stage	2	4	0.002863	21	-5.33	<.0001
Stage	3	4	0.002733	21	-3.27	0.0037

During column deconstruction, sand samples were collected at depths A, B and C. An additional sample was taken from the very bottom (VB) of each column. Wet and dry sample weights were used to calculate water content, sample calculation shown below. These values were compared to the final VWC sensor reading, table 3-4. The sensor readings do not match calculated water content values. The sensors use a generalized quadratic equation to calculate

soil moisture content. The quadratic equation can be individualized to a particular soil type; procedures are dictated in the manual.

$$Water\ Content = \frac{Wet\ weight - Dry\ weight}{Dry\ weight - Container\ weight}$$

$$WC\ (for\ original\ sand) = \frac{47.2889 - 46.1906}{46.1906 - 1.3269} = 0.0245$$

Table 3-4. Water Content calculations after Column Deconstruction

Column	BOD Loading	Depth Label	Calculated Water Content	Final Sensor Reading	% Difference
Original sand	None	-	0.02448	-	-
1	1000 LBS/ac/day	A	0.08567	0.14260	49.9%
		B	0.07657	0.14027	58.8%
		C	0.17260	0.67193	118.2%
		Very Bottom	0.22401	n/a	-
5	500 LBS/ac/day	A	0.09033	0.10879	18.5%
		B	0.10389	0.13093	23.0%
		C	0.22670	0.34586	41.6%
		Very Bottom	0.19722	n/a	-
7	Control - 65 LBS/ac/day	A	0.08037	0.09055	11.9%
		B	0.10305	0.08400	20.4%
		C	0.23788	0.28500	18.0%
		Very Bottom	0.22240	n/a	-

3.1.2 *Oxygen Sensor*

Oxygen level for all stages is plotted in Figure 3-2. Through out stages 1 and 2, oxygen level for depths A and B hovered near atmospheric levels. The level at depth C decreased during the first stage. This is attributed to column 4,

depth C, oxygen levels decreased after the column was flooded and the others were not. This sensor never returned to atmospheric conditions.

In Stage 3, depth A, B and C oxygen sensors for the 500 lbs BOD load began to decrease meanwhile control conditions remained near atmospheric levels. At the introduction of 1000 lbs BOD/ac/day, oxygen levels at all depths decreased at a faster rate to 15% oxygen than the 500 lbs organic loading. The control conditions, 65 lbs BOD/ac/day remained near atmospheric through out the experiment.

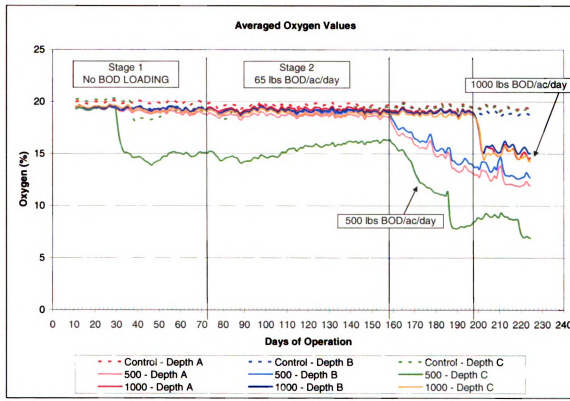

Figure 3-2. All Stages - Oxygen Level

The statistical analysis for oxygen level was treated in the same manner as moisture content. Oxygen level acted as the response variable to the explanatory variables stage, depth, hydraulic loading, BOD loading and nutrient

concentrations. The results from SAS v 9.1 are displayed in Table 3-5. Time, depth, hydraulic loading, BOD loading and nutrient concentration were significant in measuring oxygen level changes, since all the P-values are below 0.05.

Difference of least square means was also tested on oxygen level data; results are shown in Table 3-6. The P-values, determined from the pair-wise comparison, show that oxygen level was not significantly different between depths A and B and stages 1 and 2.

Table 3-5. Oxygen Level Statistical Analysis Summary

Effect	Numerical Degrees of Freedom	Denominator Degrees of Freedom	Calculated F- Value	P Values
Time	3	21	31.19	<.0001
Depth	2	14	154.57	<.0001
Hydraulic Loading	1	5166	4.94	0.0262
BOD Loading	1	5166	216.09	<.0001
Nutrient Concentration	1	5166	4.14	0.0420
BOD & Depth Interaction	2	5166	15.39	<.0001

Table 3-6. Depth and Stage Pair Comparison for Oxygen

Effect	Depth / Stage	Depth / Stage	Standard Error	Degrees of Freedom (DF)	Calculated t-value	P Values
Depth	A	B	0.06645	14	0.27	0.7940
Depth	A	C	0.06645	14	19.81	<.0001
Depth	B	C	0.06645	14	19.54	<.0001
Stage	1	2	0.1038	21	-0.03	0.9740
Stage	1	3	0.1088	21	4.53	0.0002
Stage	1	4	0.1560	21	7.49	<.0001
Stage	2	3	0.0809	21	6.13	<.0001
Stage	2	4	0.1306	21	8.97	<.0001
Stage	3	4	0.1247	21	5.41	<.0001

After column deconstruction, the removed oxygen sensors were tested in atmospheric conditions and also in the absence of oxygen to determine if the sensors presented a zero offset. A zero offset was determined from post-calibration procedures and is depicted in Figure 3-3. The average offset from the nine sensors was 0.88% oxygen. All oxygen sensor graphs were adjusted accordingly.

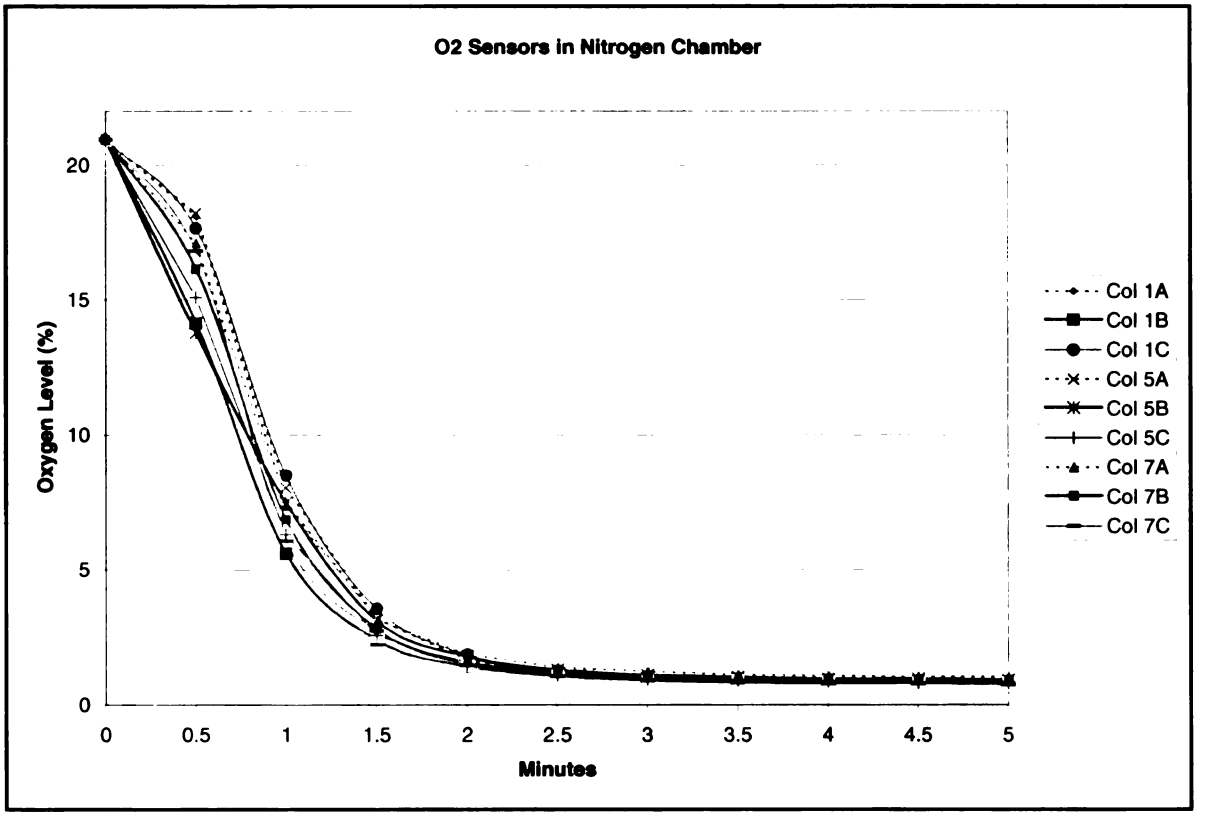

Figure 3-3. Oxygen Sensor Zero Off-set

3.1.3 *ORP Probes*

The ORP probes measurements for each column are plotted in Appendix A. These graphs plot ORP (millivolt reading) vs. time for all four stages. All columns at all depths show increasing linear trends, except column 7, depth c. ORP measurements were expected to decreased when shifting towards

anaerobic conditions as seen in oxygen levels. Figures A-9 and A-10, located in the appendix, compare oxygen levels to ORP linear regression. ORP regression lines either decrease or maintain a neutral slope, contrary to decreasing oxygen levels.

Literature findings define aerobic conditions ORP as greater than 300mV. All the ORP measurements in this experiment began in the 50mV to 200mV range. ORP data was collected at different times each day through out the experiment. On occasion, it was collected twice in one day to confirm time of day had no effect on ORP values. Frequently the influent was sprayed while collecting ORP data; this also did not have an effect on collected ORP values. ORP ranges are further summarized in Table 3-7, per stage and BOD loading.

Table 3-7. ORP Minimum and Maximum Values

Depth	BOD Loading (LBS/ac/day)	ORP Minimum (mV)	ORP Maximum (mV)
A	0	53	209
	65	76	284
	500	118	560
	1000	173	309
B	0	33	234
	65	60	301
	500	190	515
	1000	65	246
C	0	19	348
	65	60	444
	500	156	463
	1000	126	329

The maximum ORP reading, 560 mV, was found at depth A and 500 lbs BOD loading. The minimum reading was only 19 mV, found during stage 1 with no organic loading. These measurements are incongruent with literature findings.

Szogi et al. (2004) measured ORP at three different soil depths 0.02 m, 0.05 m and 0.10 meters. They observed ORP mV readings ranging from >300 mV to below <-100 mV. It was observed that under flooded conditions, soils rapidly became anaerobic and oxygen was depleted as reflected by the ORP readings less than 100 mV. Ricks (2002) monitored ORP values at 1.5 m and 3.0 m depths. Lower ORP values were observed at the lower depth electrodes. Redox values observed in the 1.5 m depth ranged between 500 and 700 mV. At the 3 m depth varied between 200 and 300 mV. Both sources used self-made ORP probes with an external salt bridge reference, unlike in this experiment where the reference point was within the probe.

Since the ORP values did not match those as expected, Hanna Instruments lab technicians were consulted on their protocol to measure soil ORP and to determine possible erroneous readings. Erroneous readings can be caused by scratches on the probe surface, debris clogging the electrode or poor contact.

During column deconstruction, all probes were visually inspected for clogging and scratches and appeared to be clean and scratch free. The ORP probe located in column 5, depth C, did not have any sand touching the surface and appeared extremely clean.

Each ORP probe under went post calibration procedures to prove probe functionality. Each probe was testing in standardized ORP solution and in anaerobic digester sludge.

Table 3-8 displays the ORP readings from the standardized solution and Table 3-9 displays the readings from anaerobic sludge. The standardize ORP solution provides a ±10 mV tolerance, of which all tested probes were slightly out of range. Column 1 depth C was the most deviated.

A negative ORP reading was detected by all probes in the anaerobic digester sludge. The portable ORP sensor used for the influent and effluent testing was also dipped into the anaerobic sludge as a comparison point. All Hanna Instrument ORP probes were within 44mV to 85mV of the portable sensor. The Hanna Instrument ORP probes proved to be operational; however, not in soil applications. Consequently data from ORP readings were found to be of minimal use.

Table 3-8. Post Calibration, ORP Sensor Reading in Standardized ORP Solution

Column	BOD Loading	Depth	Std ORP soln temp (°C)	ORP readout	Standard ORP (±10 mV)	% over Standard ORP value
1	1000 LBS/ac/day	A	14.1	234	245.17	0.49%
		B	14.8	232	244.26	0.95%
		C	14.6	221	244.52	5.81%
5	500 LBS/ac/day	A	13.3	234	246.21	0.92%
		B	14.7	234	244.39	0.16%
		C	14.8	234	244.26	0.11%
7	Control - 65 LBS/ac/day	A	16.3	232	242.31	0.13%
		B	14.2	232	245.04	1.27%
		C	13.8	232	245.56	1.49%

Table 3-9. Post Calibration, ORP Sensor Reading in Anaerobic Digester Sludge

Column	BOD Loading	ORP Readout (mV)
1	1000 LBS/ac/day	-394 -387 -428
5	500 LBS/ac/day	-410 -405 -410
7	Control - 65 LBS/ac/day	-391 -399 -401
Portable ORP Sensor		-343

3.1.4 *Soil Temperature*

Figure 3-4 shows temperatures recorded through out the experiment for all columns and all depths, a total of 24 sensors. There was little variation among sensors during stages 1 and 2. A wider temperature spread was observed towards the end of stage 3 and through out stage 4. The temperature never reached extreme conditions and only varied in the range of 13.5°C to 26°C. Temperatures decreased accordingly to ambient temperatures however, the soil columns were located indoors and were not exposed to the Michigan's winter temperatures.

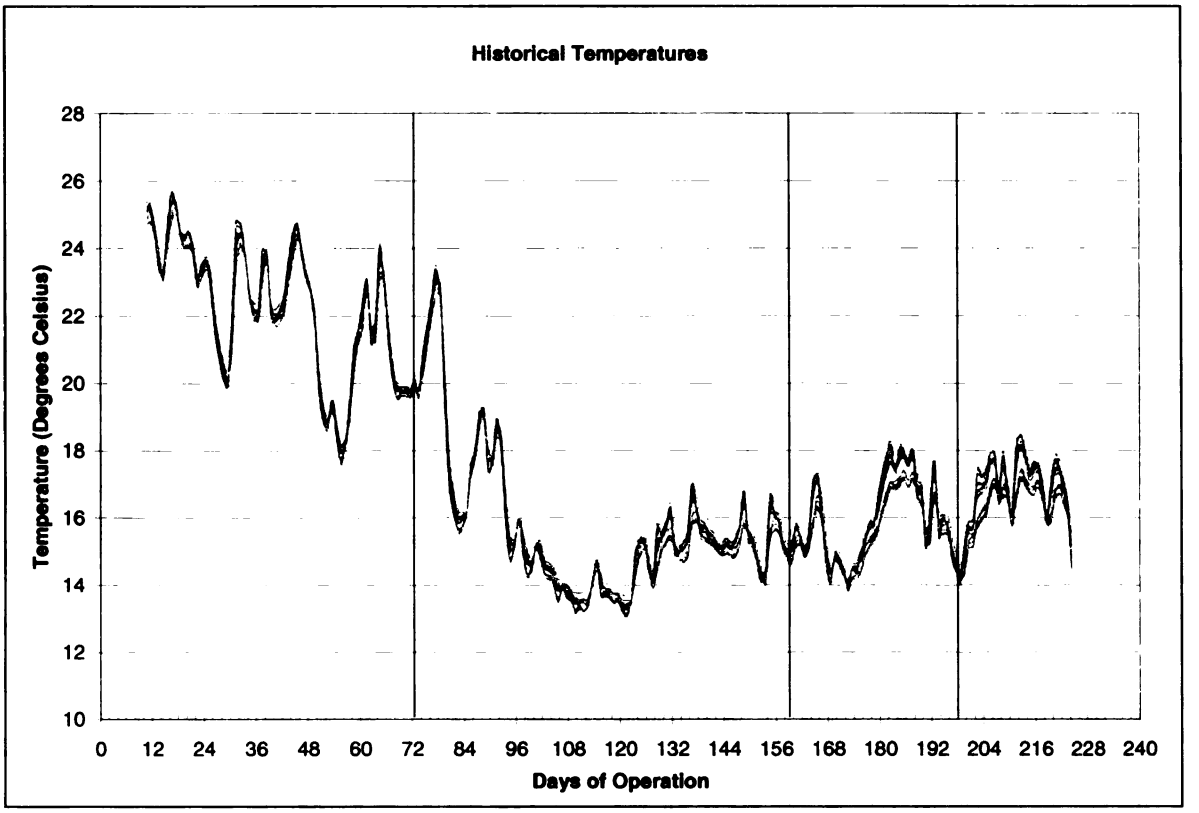

Figure 3-4. All Stages – Temperature, all columns and all depths

Temperature was statistically tested in the same manner as moisture content and oxygen level using SAS v9.1. The fixed effects results are displayed in Table 3-10 and the pair comparison chart in Table 3-11. Temperature was affected by time and hydraulic loading, but it was not affected by depth, BOD loading or nutrient concentration. The pair wise comparison showed no significant difference between depths A, B and C. Among the stages there was a significant difference between stages 2 and 3.

Table 3-10. Temperature Statistical Analysis Summary

Effect	Numerical Degrees of Freedom	Denominator Degrees of Freedom	Calculated F- Value	P-Value
Time	3	21	1904.64	<.0001
Depth	2	14	3.19	0.0720
Hydraulic Loading	1	5166	39.73	<.0001
BOD Loading	1	5166	1.11	0.2929
Nutrient Concentration	1	5166	0.02	0.8828
BOD & Depth Interaction	2	5166	1.87	0.1540

Table 3-11. Depth and Stage Pair Comparison for Temperature

Effect	Depth / Stage	Depth / Stage	Standard Error	Degrees of Freedom (DF)	Calculated t-value	P-value
Depth	A	B	0.06221	14	0.27	0.1666
Depth	A	C	0.06221	14	19.81	0.0950
Depth	B	C	0.06221	14	19.54	0.0058
Stage	1	2	0.08779	21	-0.03	<.0001
Stage	1	3	0.09543	21	4.53	<.0001
Stage	1	4	0.1393	21	7.49	<.0001
Stage	2	3	0.07552	21	6.13	0.3705
Stage	2	4	.1219	21	8.97	<.0001
Stage	3	4	.1163	21	5.41	0.0001

3.2 Stage 1

In the following sections, each experimental stage will be discussed. Generally replicate averages are represented in the plots, unless replicates were substantially different, then it was segregated.

The first stage in this experiment did not apply any organic loading to the columns; only dechlorinated tap water. This experimental stage was subdivided into three separate phases. During the first phase, stage 1a, 2 L of water were applied per day to each column in one dose. In order to provoke changes in the sensors, hydraulic loading was varied in the second phase, 1b. Four hydraulic loading conditions were tested each with duplicate conditions: 0, 1, 2 and 4 L/day applied in a single dose. During this phase all columns were flooded with approximately 33 L. In the final phase, 1c, each column was assigned an individual pump that was connected to a timer to spray 600mL every 6 hours, for a total hydraulic load of 2.4 L/ac/day.

3.2.1 *Sensor Readings*

Volumetric water content and oxygen level for stage one are shown in figures 3-5 and 3-6, respectively. Only depth A sensors during the first phase of stage 1, showed a slight decrease on day 13 and 21. The columns did not receive the hydraulic loading during the weekends, which corresponds to these dates.

In the second phase of Stage 1, depth A and B sensors showed response to the varying hydraulic loadings. The 0 L/day application dropped below 0.05 VWC. The other hydraulic loading conditions only reached a minimum of 0.05 and a maximum of 0.07. Depth C sensors only varied when the columns were flooded, where every depth C sensor increased by 50% volumetric water content. For the remainder of Stage 1 there were minimal changes and depth C soil moisture hovered around 0.27 VWC.

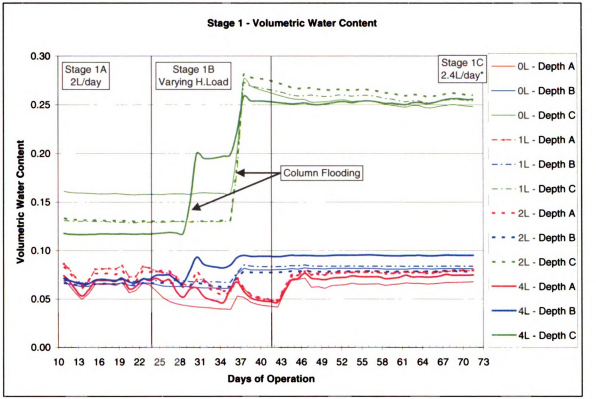

Figure 3-5. Stage 1 - Volumetric Water Content

Figure 3-5 plots the replicate averages. Column 4 was flooded 7 days prior to the other seven columns; this corresponds to the 2 increases shown for depth C in stage 1B. The first increase did not reach the maximum VWC (0.28) reached during flooding. An individual plot for column's 4 and 5 volumetric water content sensors is below, Figure 3-6. Column 4 reached higher saturation values than column 5.

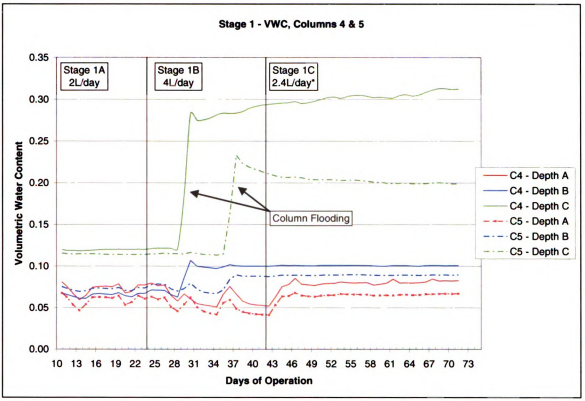

Figure 3-6. Stage 1- VWC for Columns 4 and 5 during Stage 1

Oxygen levels at depths A and B did not fluctuate with the various hydraulic loads as shown in Figure 3-7. Depth C had 2 decreases in two test columns. Individual oxygen responses are plotted for these columns in Figure 3-8 because of the differences in values between the replicates. The cause of this difference is not known and substantial variations between other replicates and parameters were not seen. Oxygen level in column 4, depth C reduced from approximately 20% to 3.8% oxygen over the next 28 days, and never returned to atmospheric levels. Column 7 depth C decreased 4% after flooding.

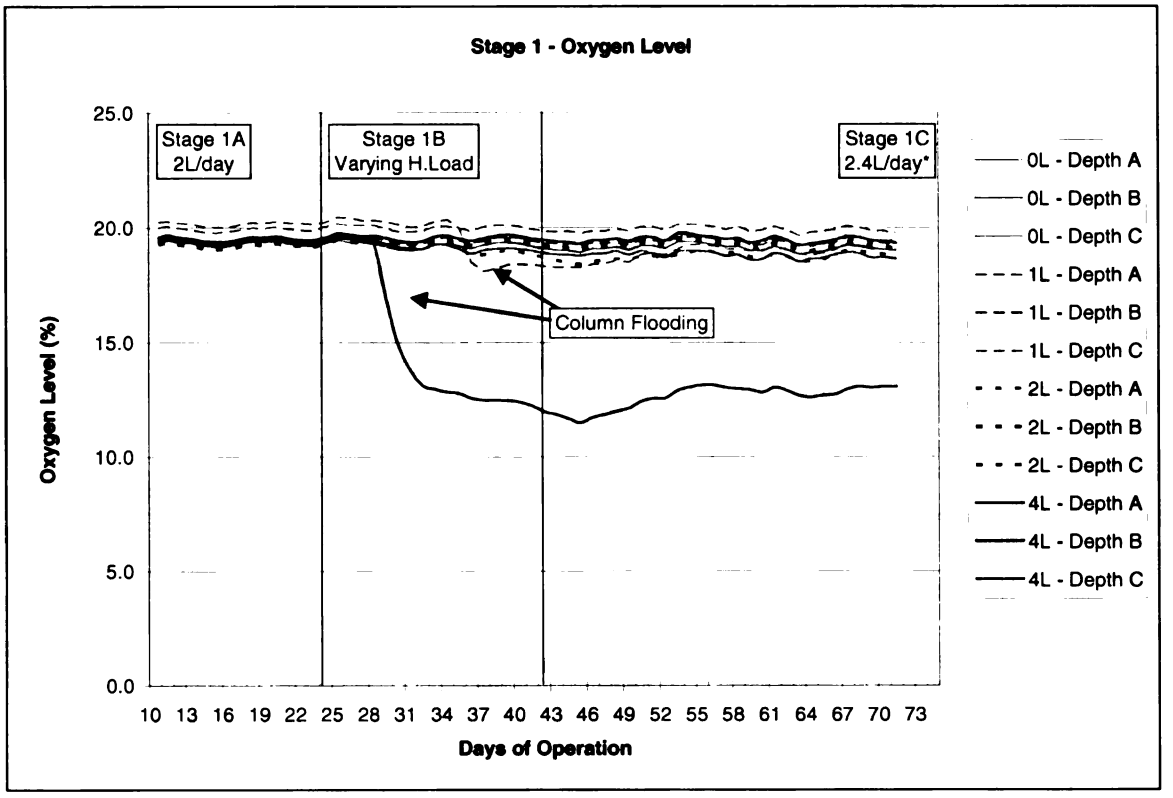

Figure 3-7. Stage 1 - Oxygen Level

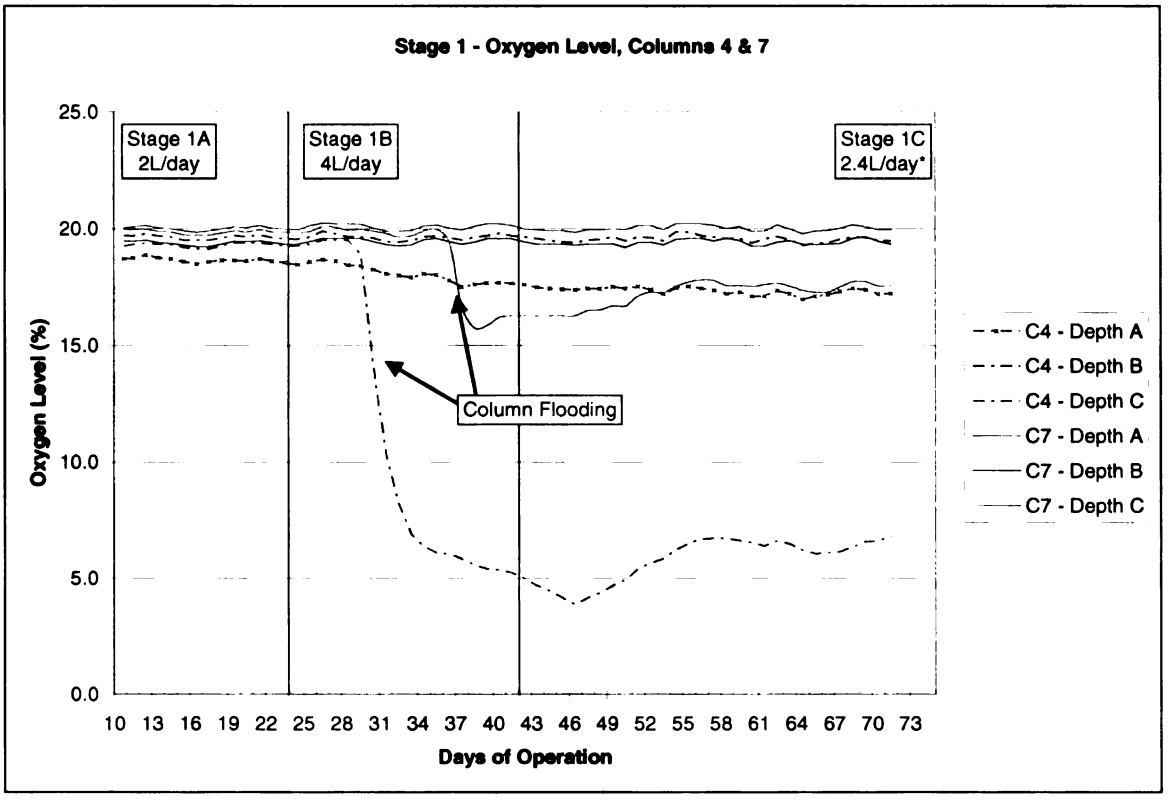

Figure 3-8. Stage 1 - Oxygen Level for Columns 4 and 7

3.2.2 *Leachate Analysis*

The influent, dechlorinated tap water and column leachate samples were collected during stage 1 to measure manganese and iron content while no organic wastewater or nutrients were applied. The tap water at Michigan State University has varying iron content; at times the water wasn't completely clear and had a red tint. Effluent manganese content tested below 0.05 ppm from all columns during stage 1. Iron levels were also below 0.05 ppm and leveled off to 0 ppm near the end of the stage 1 in all columns, Figure 3-9.

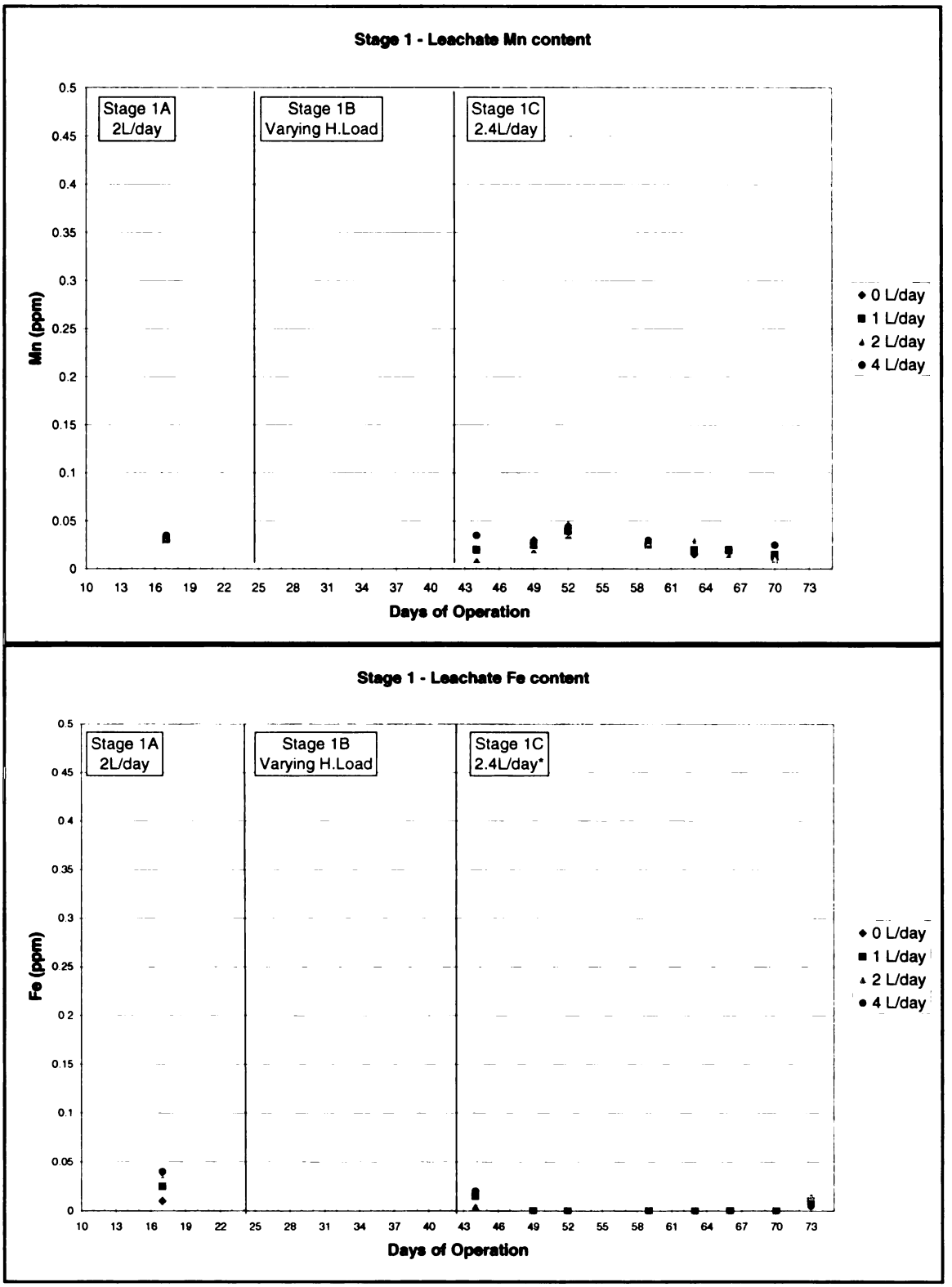

Figure 3-9. Stage 1 - Leachate Manganese and Iron Content

3.3 Stage 2

In stage 2, all columns received the 65 lbs BOD/ac/day of organic loading but nutrient concentrations were varied. The replicates were averaged and plotted against time for all the following figures.

3.3.1 *Sensor Readings*

Soil moisture content had minimal variation throughout stage 2, Figure 3-10. Depth A at 100% nutrient concentration has a notch on day 113; this corresponds to a pump impeller malfunction. Depth C with 100% nutrient had an increase from 0.25 VWC to 0.27VWC; this was due to an increase in one of the replicates, column 4 depth C, which increased 0.05 VWC over the time span of stage 2.

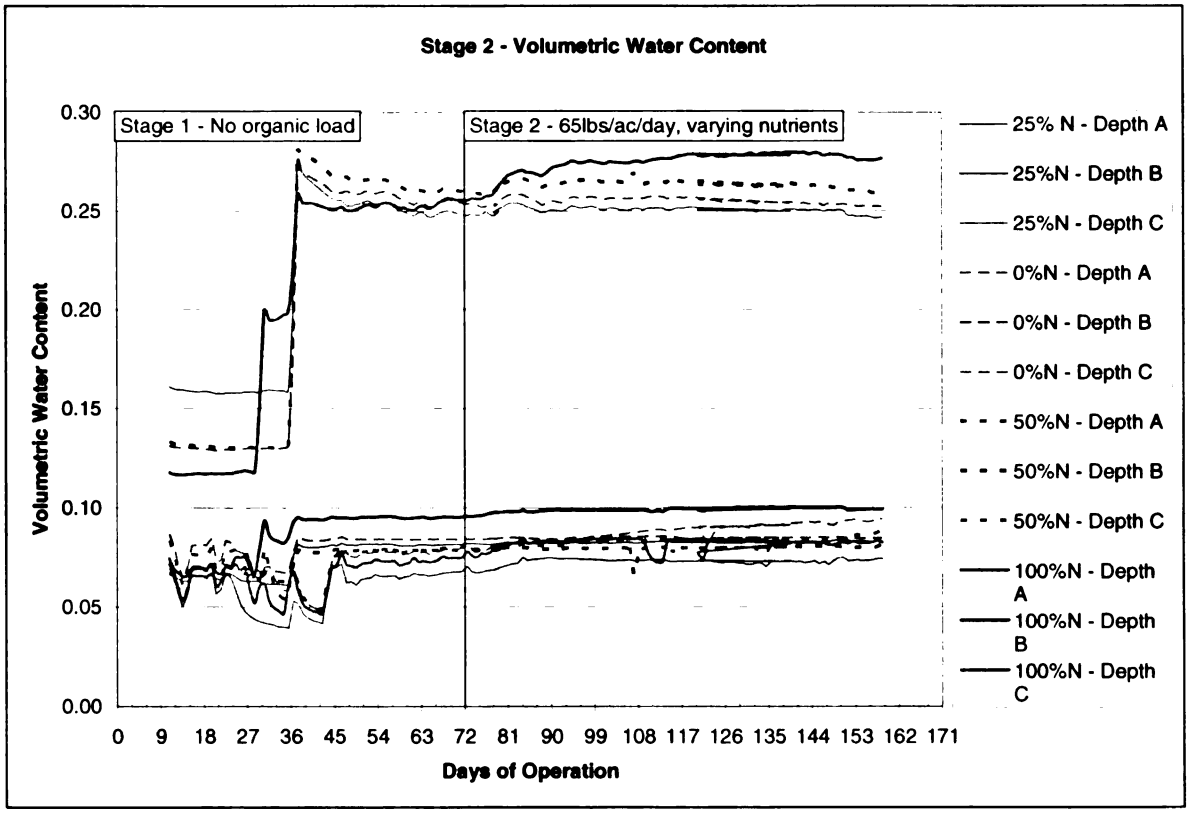

Figure 3-10. Stage 2 – Volumetric Water Content

Oxygen levels also remained near atmospheric conditions with minimal variation to the exception of column 4, depth C. Figure 3-11 does display the not averages for depths C at 100% nutrient concentration to magnify the difference in oxygen sensor readings. Column 4, depth C oxygen level increased 5% during stage 2.

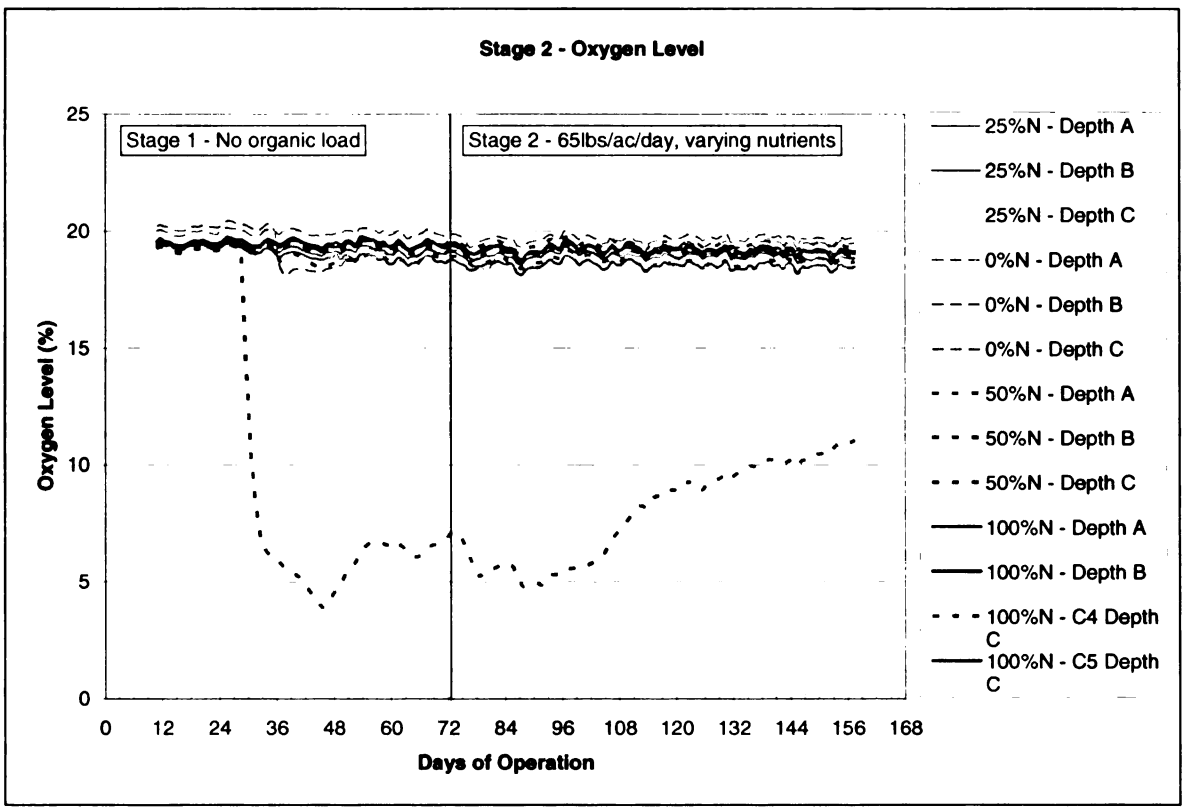

Figure 3-11. Stage 2 – Oxygen Level

3.3.2 *Leachate Analysis*

Influent and leachate was tested for COD and manganese and iron content. COD was reduced by at least 90% from the influent, Figure 3-12, for all nutrient conditions tested.

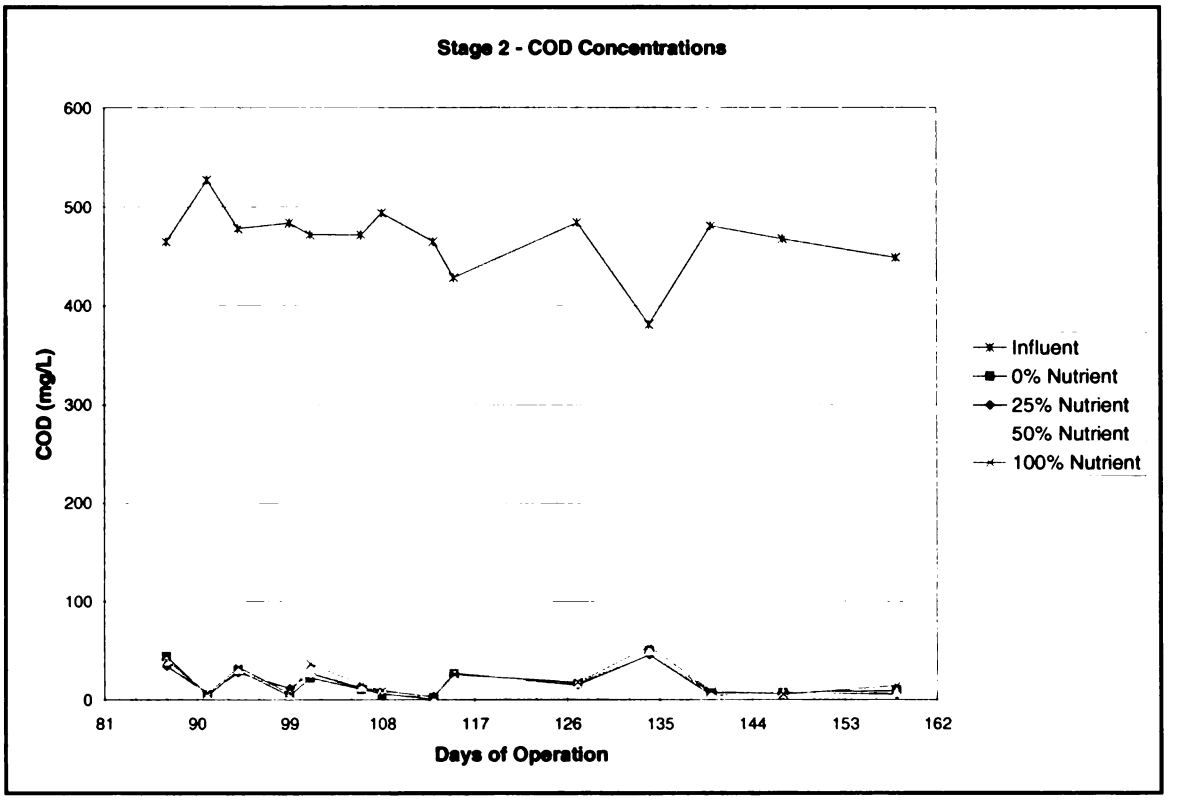

Figure 3-12. Stage 2 – Leachate COD Concentrations

Under the aerobic condition assumption, manganese and iron content were predicted to be below 0.05 ppm. Figure 3-13 plots the manganese and iron content over time for all four varying nutrient conditions. Manganese content has a spike around 10/13 in all four nutrient variables, which could be attributed to the water source variability and not metal reduction processes. All nutrient conditions stabilized to below 0.05 manganese ppm and below 0.10 iron ppm. These manganese and iron content levels are not indicative of metal mobilization or anaerobic reduction reactions.

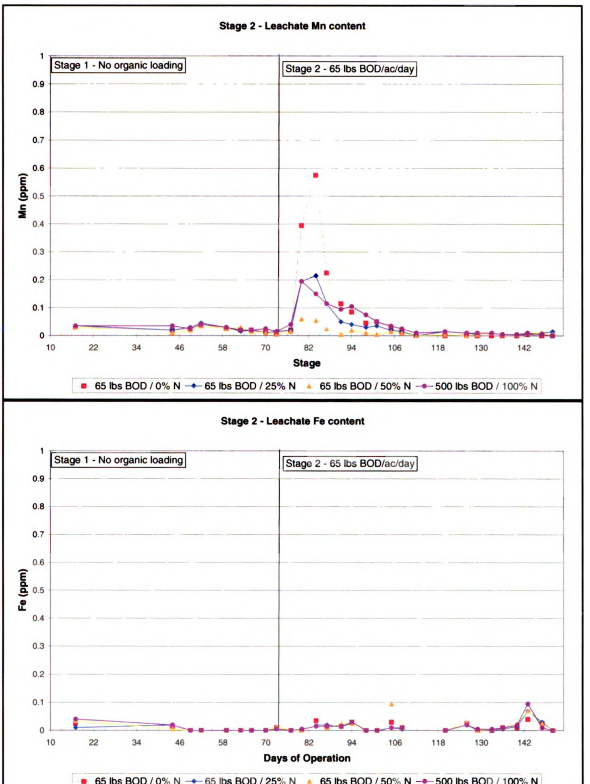

Figure 3-13. Stage 2, Leachate Manganese and Iron Content

3.4 Stage 3

Only one modification occurred during stage 3. The 65 lbs BOD/ac/day with 100% nutrient condition was changed to 500 lbs BOD/ac/day with 100% nutrient concentration. The remaining three testing conditions remained at 65 lbs BOD/ac/day with a varied nutrient concentration.

During this stage the soil columns exposed to the increased organic loading developed a fungal growth at the top of each soil column as seen in Figure 3-14. At times the odor emitted from these columns could be linked to an alcohol, manure-like and sulfur smell.

Figure 3-14. Fungal Growth on Top Surface of Column 4

3.4.1 *Sensor Readings*

The sensors showed no significant variations at the 65 lbs BOD with varying nutrient conditions from stage 2 readings. However figure 3-15 shows for the 500 lbs BOD loading, volumetric water content increased at depths A, B and C approximately four days after the start of stage 3. Water content increased with depth, where depth A values are the lowest and depth C values are the highest. Depth C increased to levels beyond the saturation predicted saturation range 0.30-0.42. The greater than expected reading occurs when high organic matter, high electrical conductivity and high salt content are present.

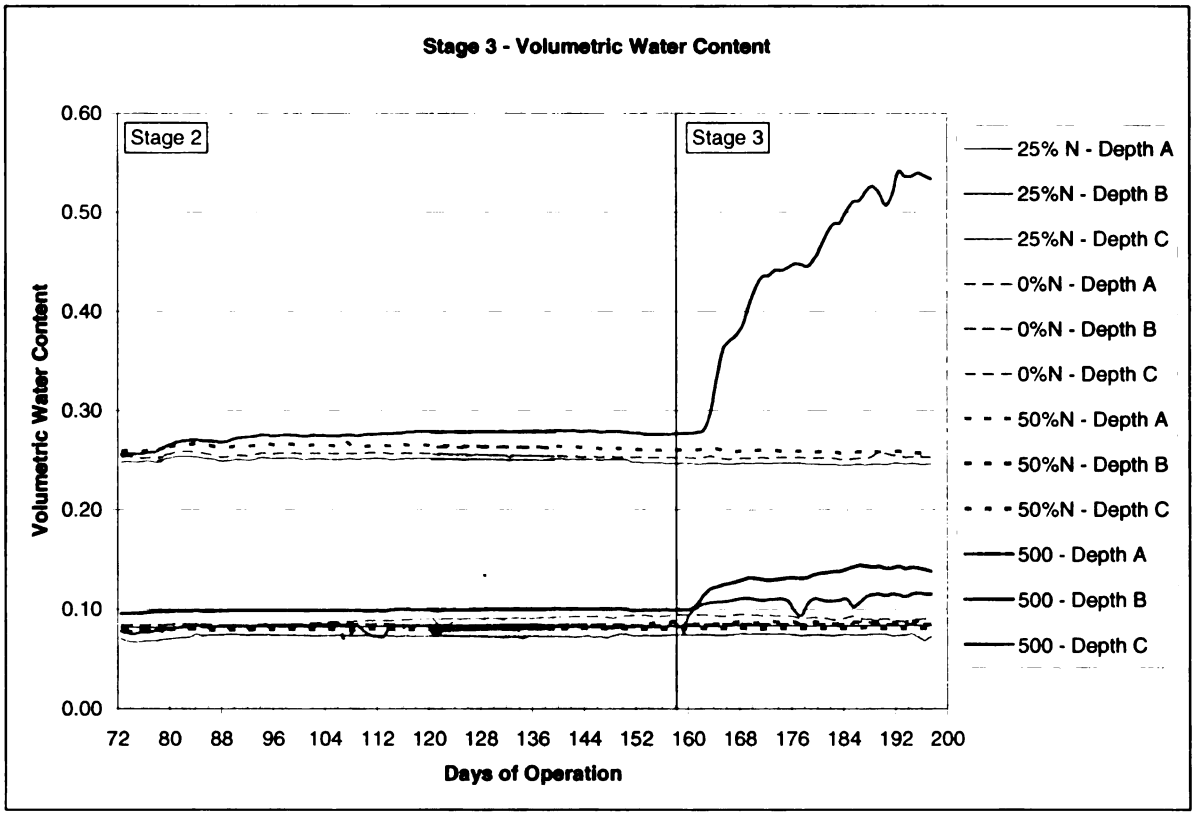

Figure 3-15. Stage 3 – Volumetric Water Content

The oxygen levels remained near atmospheric at 65 lbs BOD/ac/day with varied nutrient concentration, figure 3-16. As soil moisture content increased, oxygen levels decreased accordingly in the 500 lbs BOD/ac/day loading condition. Depth A and B oxygen sensors started to changed approximately one day after initiating stage 3 and depth C started to decrease two days after the start. Depth A had a lower oxygen level than depth B, which could be attributed to the oxygen consumed by the fungal presence. Depth C shows the lowest amount of oxygen which corresponds to the highest soil moisture content levels.

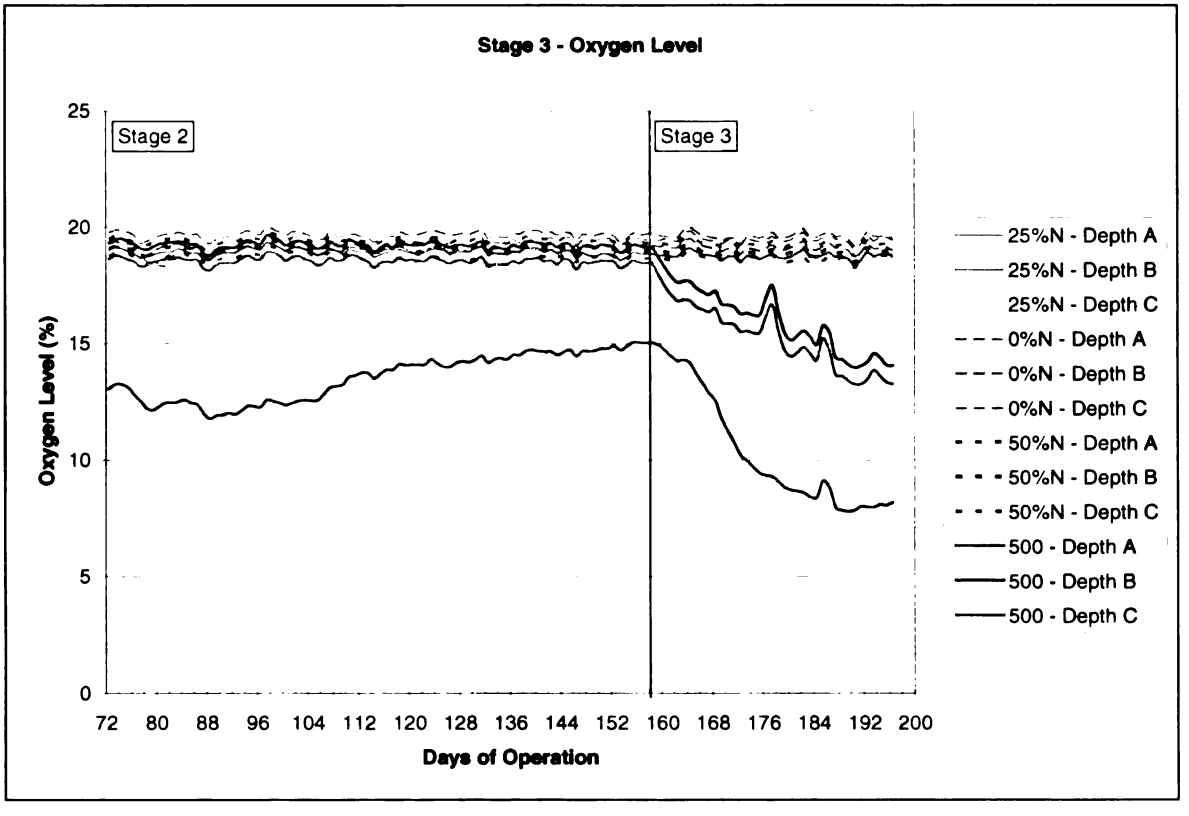

Figure 3-16. Stage 3 – Oxygen Level

3.4.2 *Leachate Analysis*

Approximately two weeks after increasing the organic loading, the leachate changed in color. It cycled through a milky white color to a distinct, clear,

yellow tint. The changes occurred one week prior to manganese mobilization in columns exposed to 500 lbs BOD/ac/day. No correlation was found to other leachate parameters tested: pH, ORP, COD.

Chemical oxygen demand was reduced in all conditions (Figure 3-17). There was a slight increase in COD content for all tested conditions on first day of stage 3 of approximately 25mg/L; thereafter the soil columns treating 65 lbs BOD/ac/day reduced the COD content to levels below 20 mg/L, nutrient concentrations do not appear to have an effect. Fluctuations in COD content could be caused by human error while calibrating pipette tips and COD blank vial preparation. The higher organic load tested, 500 lbs BOD/ac/day, consistently resulted in higher COD content than the 65lbs BOD loading condition. At times the COD concentration was 7 times higher which, indicates soil column was not assimilating organic waste in the same manner as the other columns.

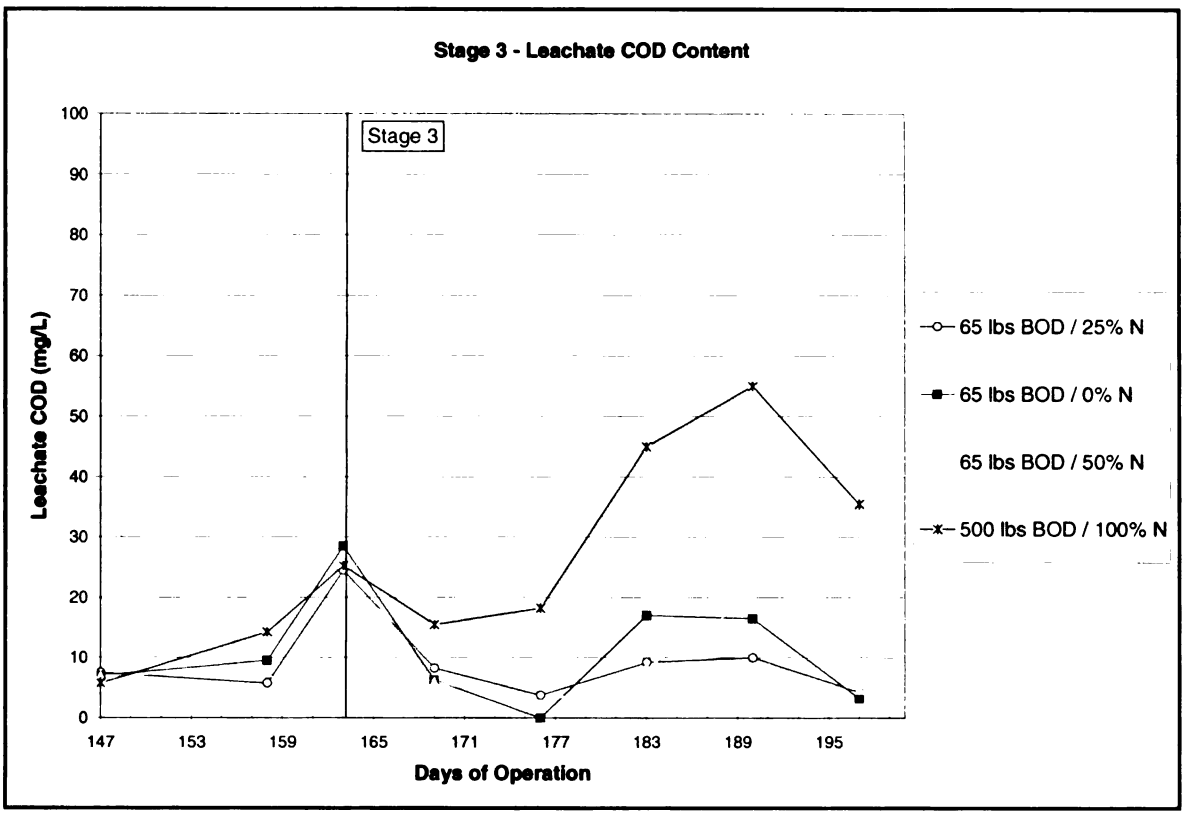

Figure 3-17. Stage 3 – Leachate COD Concentrations

As in stage 2, the 65 lbs BOD/ac/day manganese and iron concentrations remained below 0.05 ppm.

Manganese became mobilized in the other organic loading condition tested, 500 lbs BOD/ac/day, approximately 15 days after initiating stage 3. Figure 3-18 shows the manganese concentration increase during stage 3. The higher manganese concentrations indicate manganese was mobilized through anaerobic oxidation-reduction processes. VWC probes at depths A and B showed a response to the increased organic load within a day, and depth C sensors showed after four days from the start of stage 3. Oxygen sensors at all depths started to decrease one day after initiating stage 3.

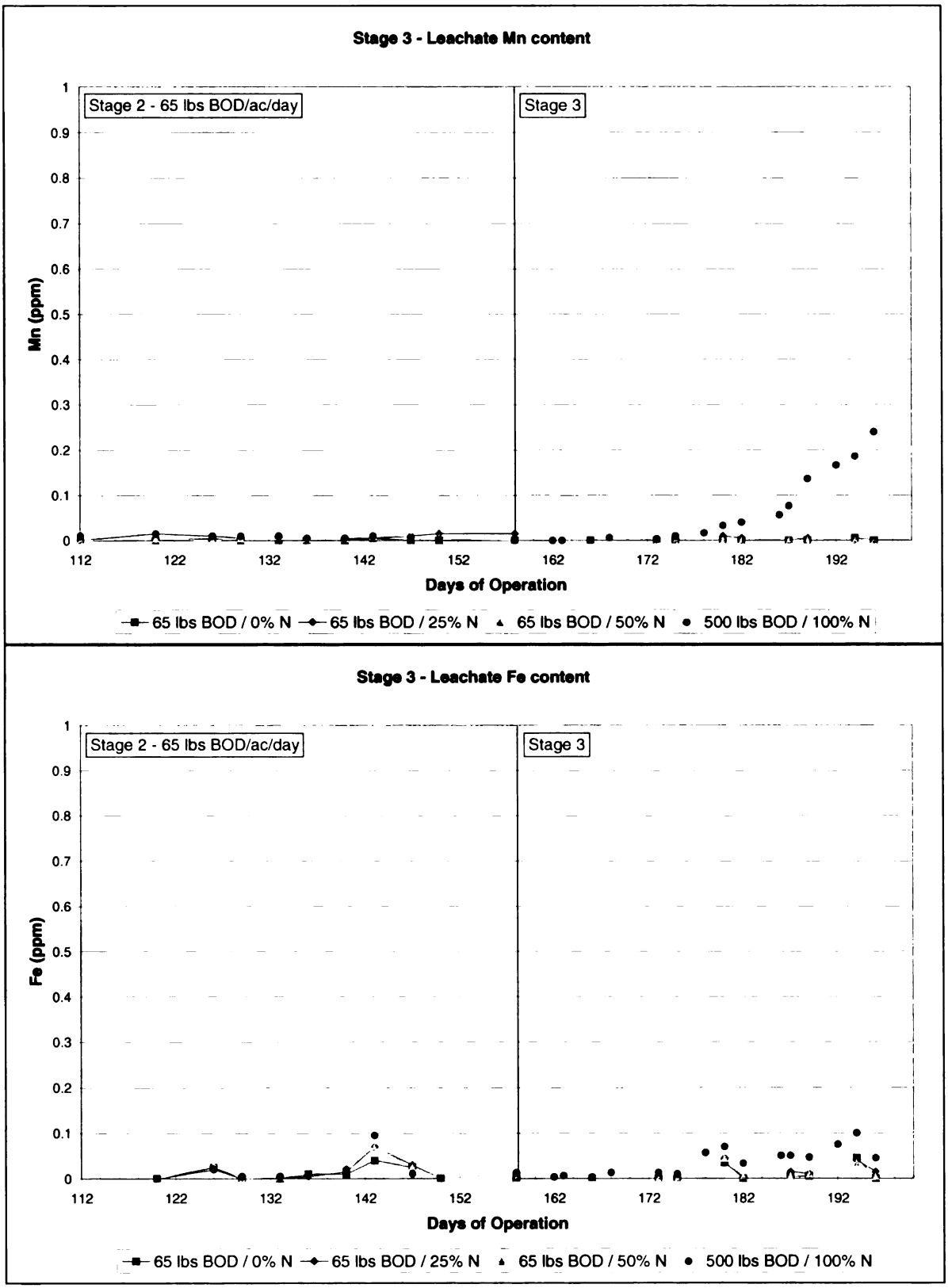

Figure 3-18. Stage 3 - Leachate Manganese and Iron Leachate Content

3.5 Stage 4

For the results evaluation in stage 4, the columns were grouped by the varied organic loading. Three organic loadings were tested: 65 lbs BOD/ac/day with 0% nutrient concentration (designated as the control), 500 lbs BOD/ac/day with 100% nutrient concentration and 1000 lbs BOD/ac/day at 100% nutrient concentration. Triplicates existed for the higher loading conditions and there were duplicates of the control conditions. In order to average triplicates, the start date for the third replicate of 500 lbs/ac/day was adjusted to the same start date as the other two soil columns in stage 3. Only average readings have been plotted.

Fungal growth surfaced much quicker in this stage and covered the top surface area of the remaining soil columns except the control columns. Columns 4 and 5 started to mix black microorganism growth with the present fungi. Near the end of the experiment, infiltration rates on columns 4 and 5 were much slower and puddles of water were formed atop.

Figure 3-19. Column 1 Top Surface, end of Stage 4

3.5.1 *Sensor Readings*

Figure 3-20 is the graphical representation for volumetric water content for stage 4. Control conditions remained constant throughout the experimental stages 2, 3 and 4.

Soil moisture content reached maximum levels during stage 3 for the 500 lbs BOD/ac/day loading condition. For the duration of stage 4, depths A and B remained at 0.12 VWC and 0.15 VWC respectively. Depth C had minor fluctuations yet remained above 0.50 VWC.

For the 1000 lbs BOD/ac/day condition, volumetric water content for all depths increased at a faster rate than 500 lbs BOD/ac/day. Depths A and B reached 0.15 VWC two days after the start of stage 4 and Depth C passed beyond saturation to readings above 0.60 VWC four days after the start of stage

4. At the beginning of stage 4, depth A shows higher water content values than depth B however, both depths leveled at approximately 0.15 VWC.

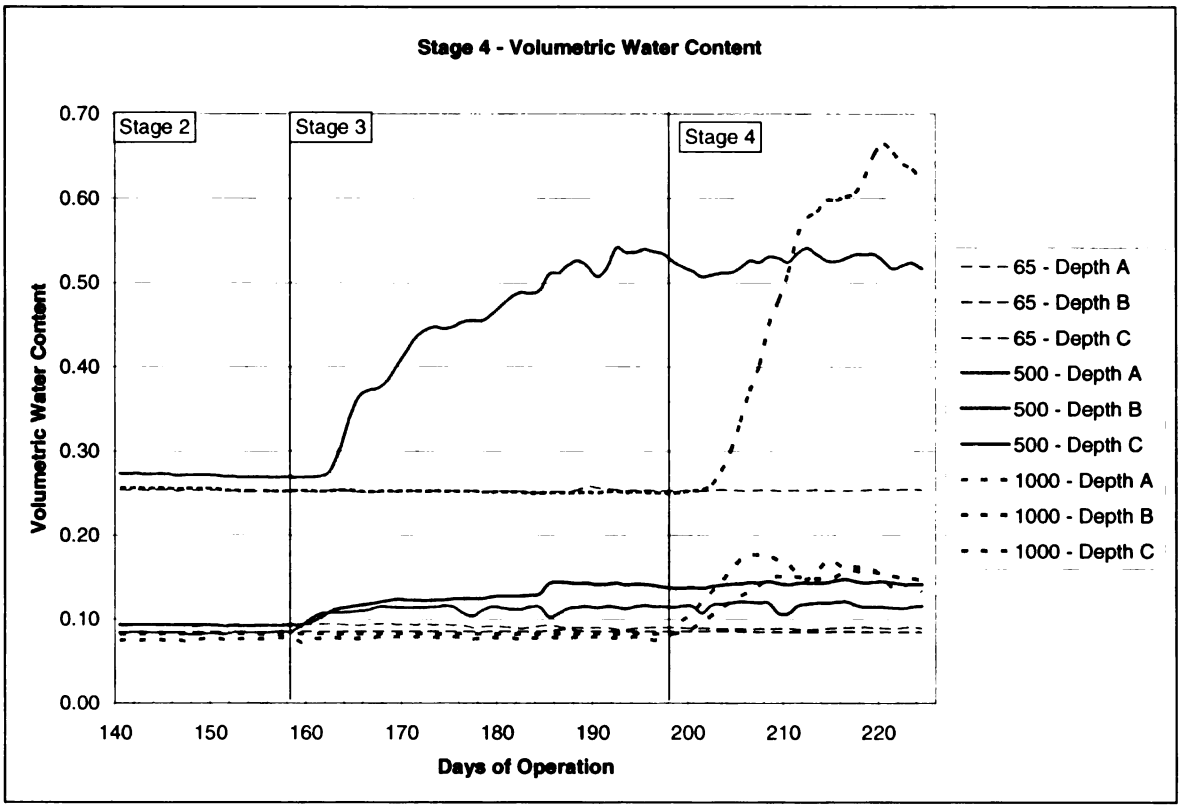

Figure 3-20. Stage 4 – Volumetric Water Content

Figure 3-21 shows oxygen levels continued to decline for 500 lbs BOD/ac/day condition in stage 4. The curve for 500 lbs BOD/ac/day at depth C differs from that shown in Figure 3-16, Stage 3 – Oxygen Level, due to the replicate averages as described in table 3-1. Depth B oxygen level remained higher than depths A and C, most likely due to the added oxygen consumption from the fungi. Oxygen was consumed at a faster rate at the 1000 lbs BOD/ac/day condition, reaching 15% oxygen level by all depths within four days. Although the oxygen sensors showed a decline in oxygen within one day of applying 1000 lbs/ac/day.

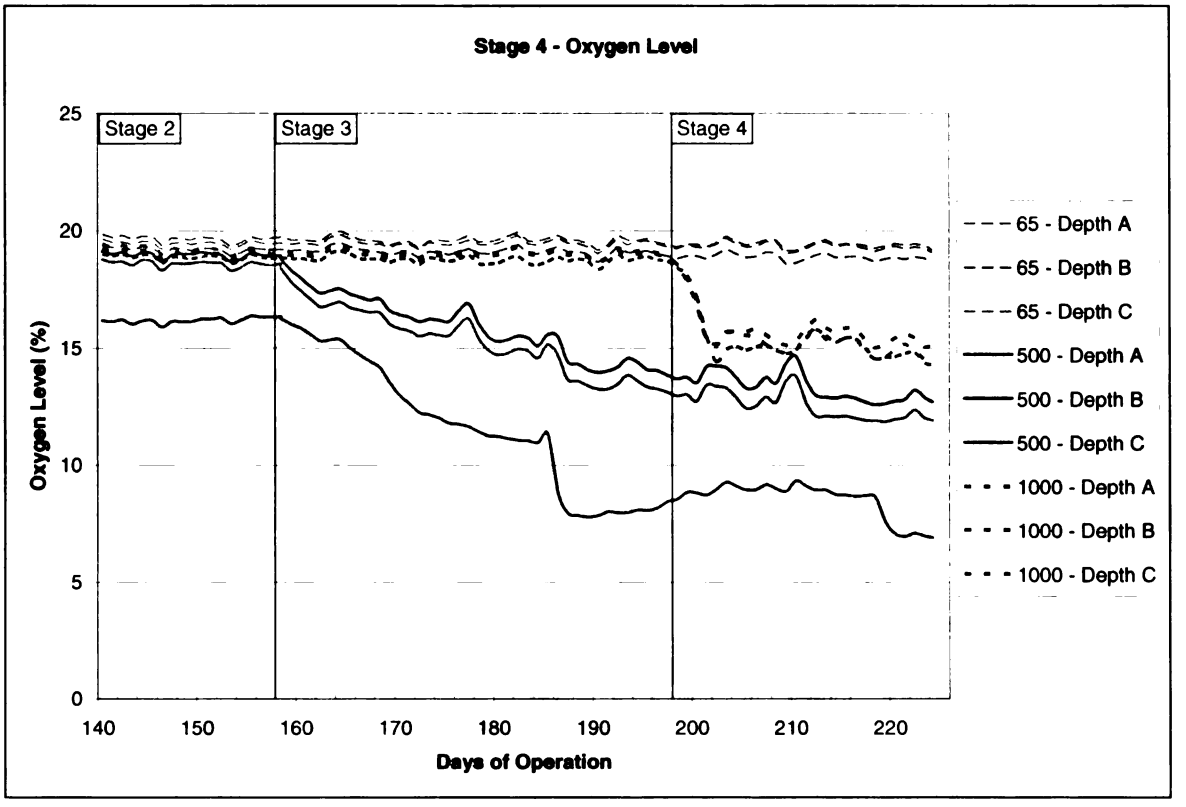

Figure 3-21. Stage 4 – Oxygen Level

Soil moisture content and oxygen levels were compared per organic loading condition in the following graphs for stages 2, 3 and 4. At 65 lbs BOD/ac/day with 0% nutrients, oxygen level remained near atmospheric levels, Figure 3-22. This indicated oxygen was not limited at this organic loading promoting aerobic microorganism growth. Volumetric water content also remained constant and below saturation, maximizing the pore space for oxygen presence.

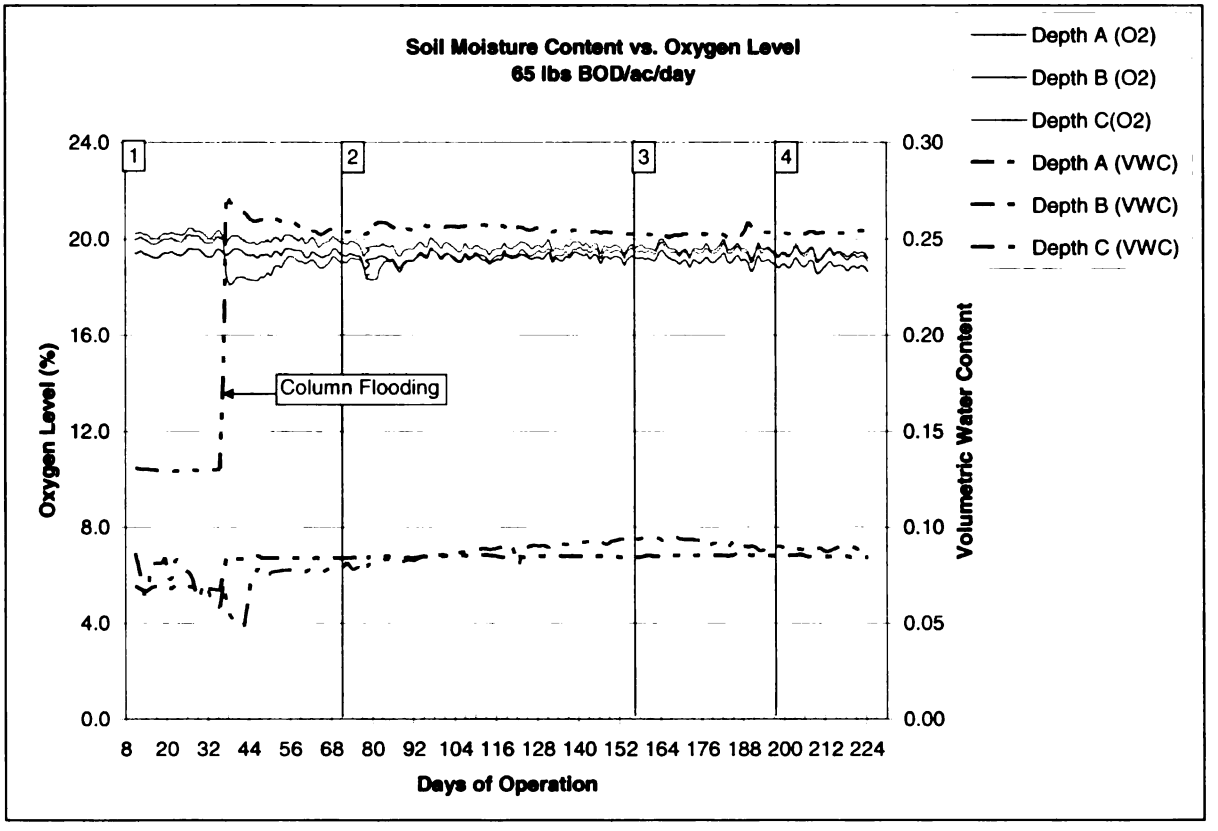

Figure 3-22. 65 lbs BOD/ac/day - Volumetric Water Content vs. Oxygen Level

As seen in Figures 3-23 and 3-24, volumetric water content increased as oxygen levels decreased for 500 and 1000 lbs BOD/ ac/day treatments, the later occurring at faster rates. The decrease in oxygen indicates movement towards anaerobic conditions.

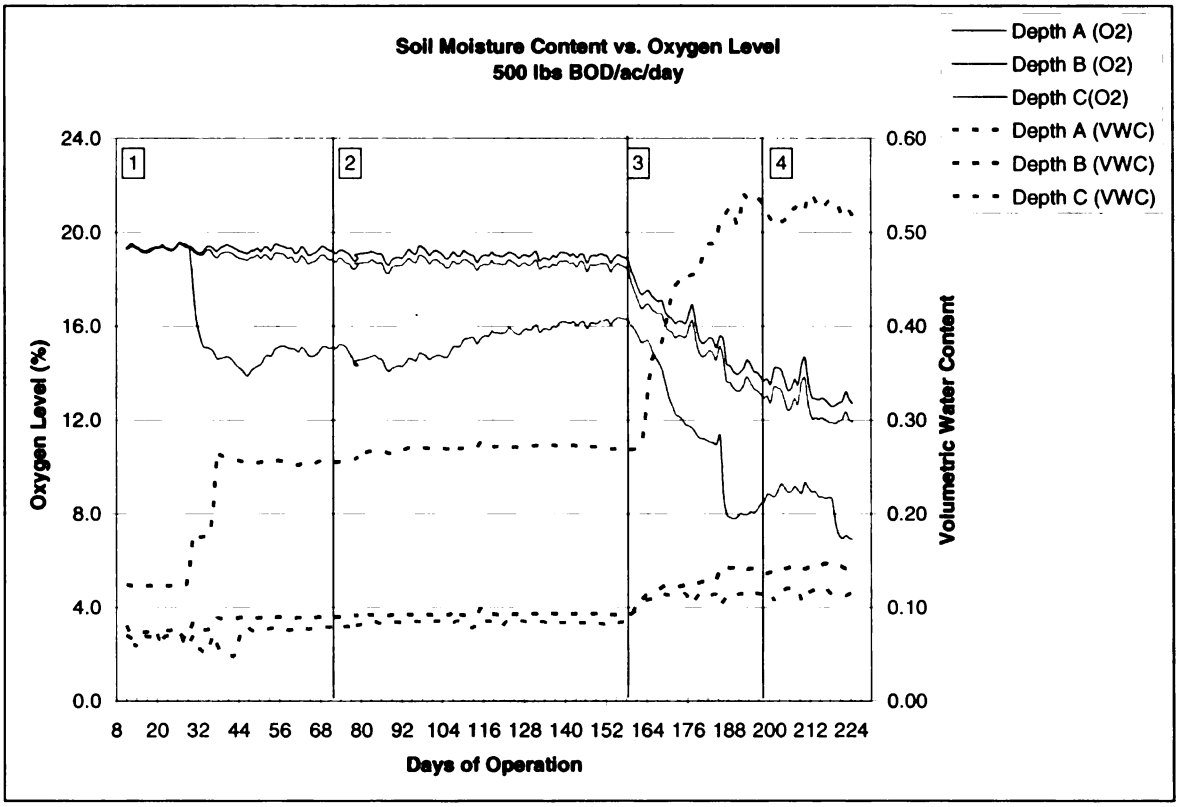

Figure 3-23. 500 lbs BOD/ac/day - Volumetric Water Content vs. Oxygen Level

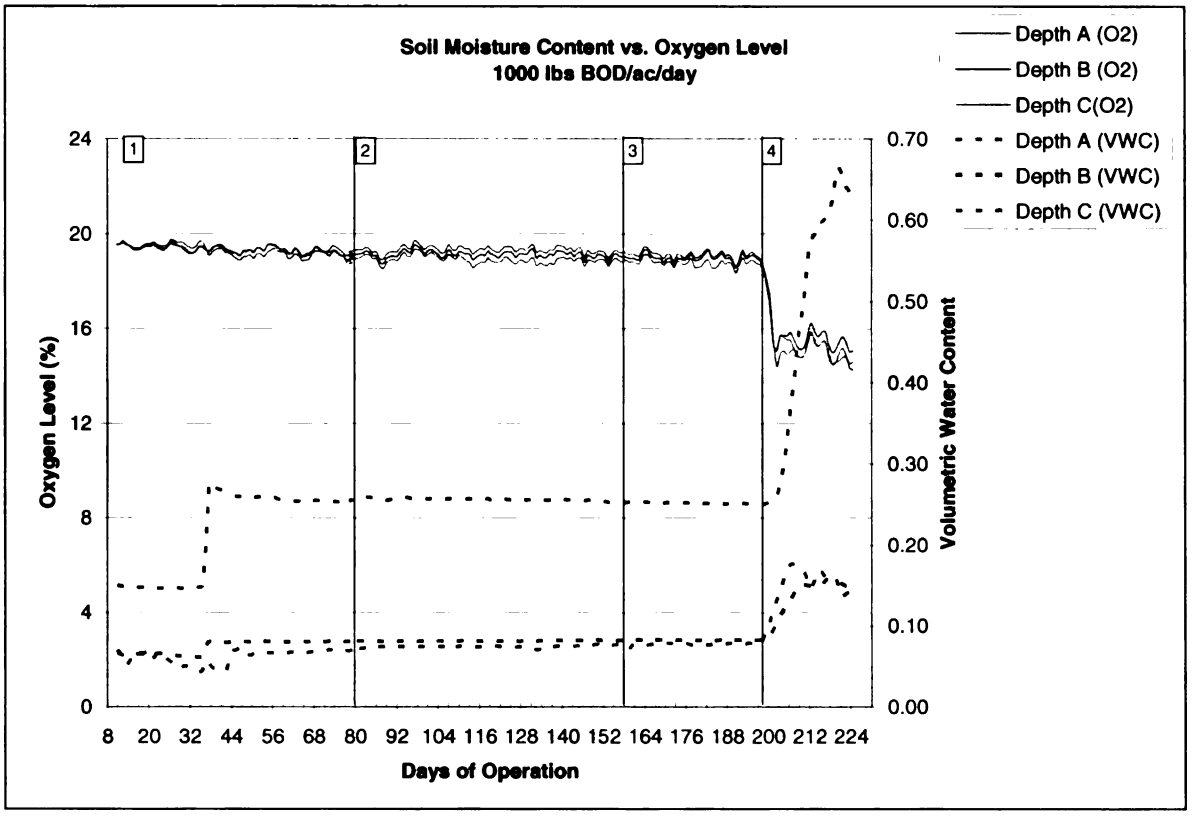

Figure 3-24. 1000 lbs BOD/ac/day - Volumetric Water Content vs. Oxygen Level

3.5.2 *Leachate Analysis*

As previously seen in stage 3, the leachate from the elevated BOD conditions changed colors in the same fashion from milky white to a distinct, clear yellow tint. The leachate color changes occurred within a week of stage 4 start time; stage 3 color changes occurred two weeks after it was initiated. The control leachate remained crystal clear throughout the entire experiment. There was no correlation of leachate color change to the other recorded leachate measurements of COD, pH, iron and manganese content.

The control conditions continued to be reduced to the lowest COD concentrations below 10mg/L. The leachate COD concentration levels tripled at

500 lbs BOD/ac/day and quintupled at 1000 lbs BOD/ac/day from the control values as seen in Figure 3-25.

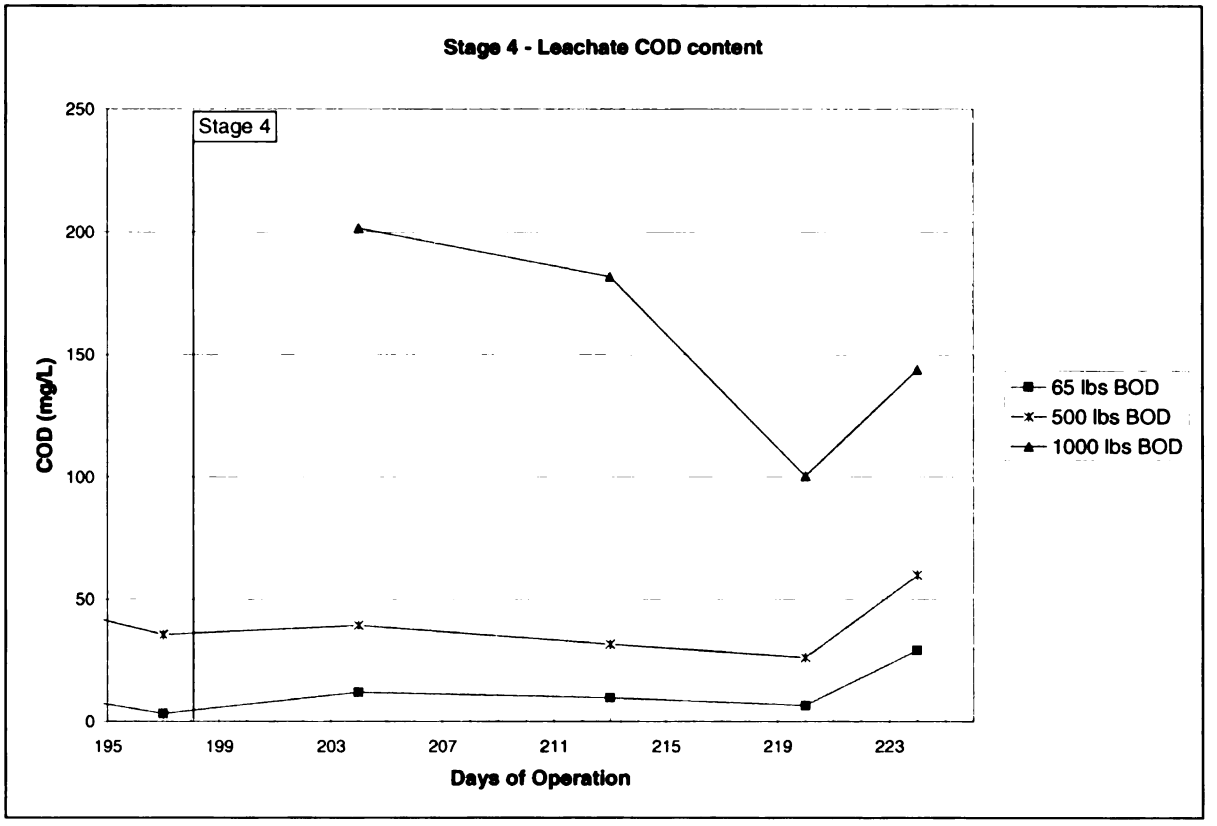

Figure 3-25. Stage 4 – Leachate COD levels

The soil columns with 500 lbs/ac/day organic loading continued to mobilize manganese in stage 4, Figure 3-26. Iron concentrations are higher than those in the control columns, approximately double the values.

Manganese was also mobilized at the higher organic load, 1000 lbs/ac/day approximately 17 days after initiating stage 4. Its maximum peak appears at day 222, which corresponds to the same date as iron starts to increase in the leachate. Both are good indicators that some anaerobic oxidation-reduction processes are occurring within the soil column.

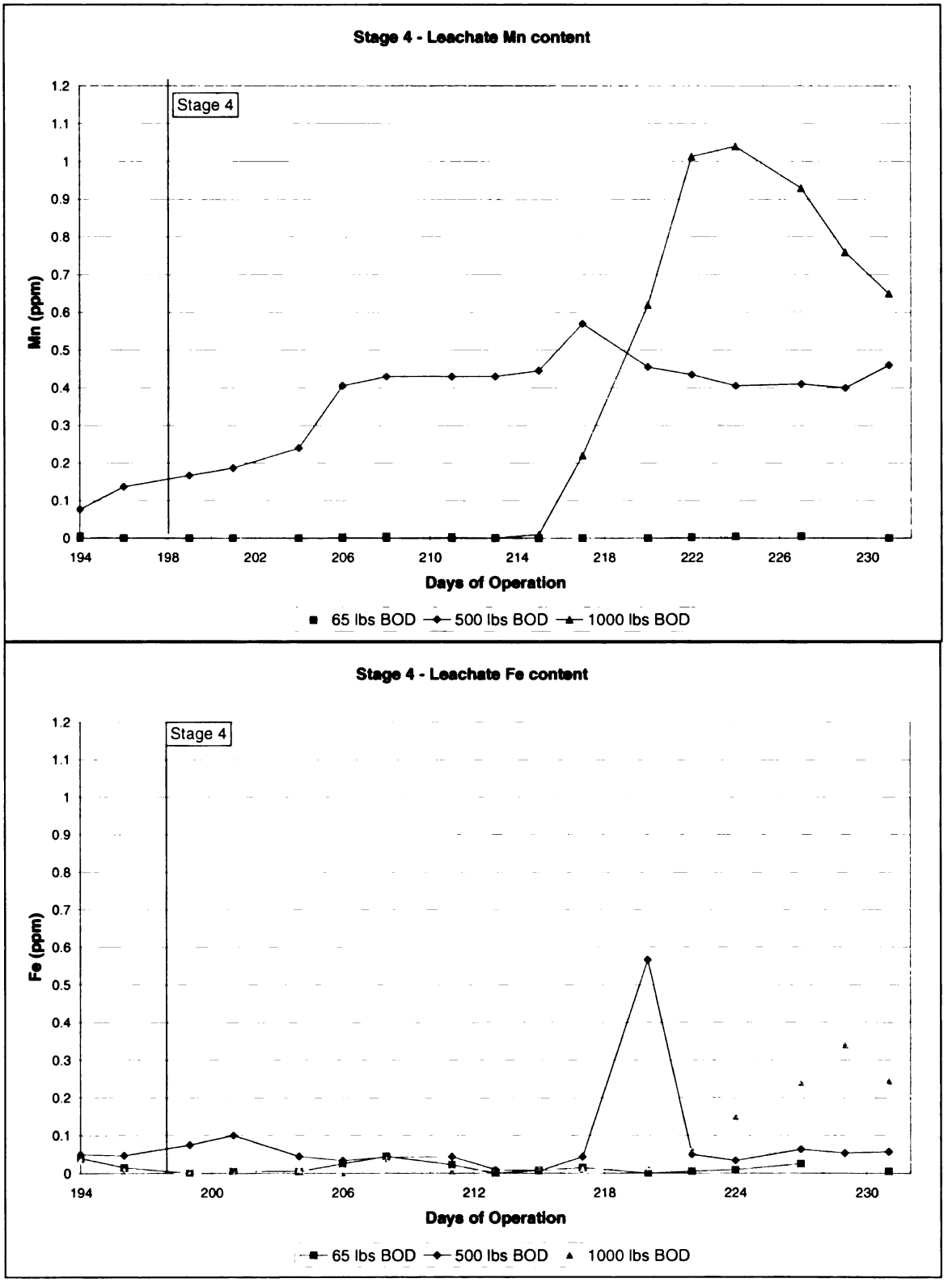

Figure 3-26. Stage 4 – Leachate Manganese and Iron Content

During stages 3 and 4, VWC sensors showed a change in response to the higher organic loading application within four days. The oxygen sensors showed a change in oxygen level within a day of higher organic loading application. The changes detected by these two sensors predicted manganese mobilization, which occurred approximately ten days after a substantial environmental change was measured by either sensor.

Chapter 4 Conclusions and Recommendations

4.1 Conclusions

The functionality of all the sensors was proven in this project; however, the ORP probe cannot be used in-situ soil environment testing. The remaining three sensors are applicable for in-situ soil monitoring. Even small soil moisture content changes were measured by the water content reflectometers by varying hydraulic loading and BOD loading. Oxygen sensors detected changes in oxygen level caused by changes in soil moisture content. Soil temperature was tracked with minimal sensor to sensor variability.

Sensor readings also indicated soil environments can vary by depth. Sensors located closest to the surface, depths A and B, reflect more aerobic favorable conditions, meanwhile the deepest, depth C, sensors reached soil saturation and oxygen limiting conditions.

Microbial oxidation-reduction processes can be characterized by monitoring manganese and iron content in leachate. Manganese was mobilized in this study at high organic loading applications. Manganese mobilization was predicted in this laboratory-scale study by the measured sensor changes. Specifically, there was approximately a 10 day delay after VWC and oxygen sensors detected an environment change.

The positive results from this research project provide confidence that a field-scale demonstration is warranted. Although the combination of moisture content probes, oxygen level sensors and thermistors were suitable in measuring soil microbial environments, only two soil environmental properties are essential.

Soil temperature should be monitored in order to observe temperature effects on microbial oxidation-reduction processes. The recorded temperatures in this study remained in the range of highest microbial activity. Cold shock, freezing and thawing cycle's effects were not studied.

In addition to soil temperature either soil moisture content or soil oxygen level should monitored. Volumetric water content reflected changes inversely proportional to those seen in oxygen level presence, eliminating the need for one sensor. The oxygen sensor detected changes in oxygen level approximately one day sooner than the moisture content probes at depth A. In addition, the oxygen sensor incorporates a thermistor which can also track soil temperature. The oxygen sensor may also be incorporated into a control loop irrigation system to manage the wastewater application rate to maximize oxygen permeability.

4.2 Recommendations

The in-situ functionality of 3 of the 4 sensors was proven in this project. Further testing is required to determine each sensor's sensitivity to changes in soil microbial environments. This can be continued in a laboratory environment or in land applied fields.

In controlled laboratory testing, BOD load can be varied in smaller increments than those tested in this project. This may help determine a maximum, allowable BOD load to sustain aerobic microbial population. Hydraulic loading can also be varied to determine if multiple applications throughout the day versus constant irrigation allows for more oxygen diffusion in soil pores.

Mokma's (2006) research was unable to find congruent, recommended BOD loading values for successful land application. However, a prescriptive value may not be protective of the environment or may be too conservative.

Unfortunately soil columns in a controlled laboratory can not accurately represent the inhabitant microbial population or presence of the groundwater table. Only field testing on current land application fields can provide a true picture of the sensor readings and fluctuations in a natural state.

For accurate determination of the environmental changes, calibration procedures should be performed on the sensors. Water content probes should be fitted to a particular quadratic equation for the soil type and composition being tested. This will help determine the maximum saturation level and alert when high organic matter starts to form in the soil. The oxygen sensors should be calibrated for high humidity environments for most accurate readings. There are no commercially available ORP electrodes to accurately monitor oxidation-reduction potential in soil environments. Methods to build soil ORP probes exist and have successfully been used in literature (Ricks 2002, Szogi et al. 2004). No changes are required for temperature tracking.

APPENDIX A

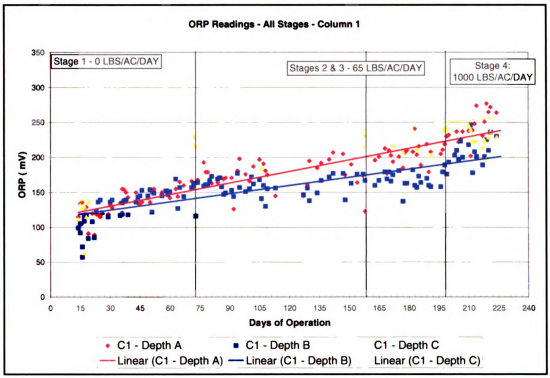

Figure A-1. ORP for Column 1

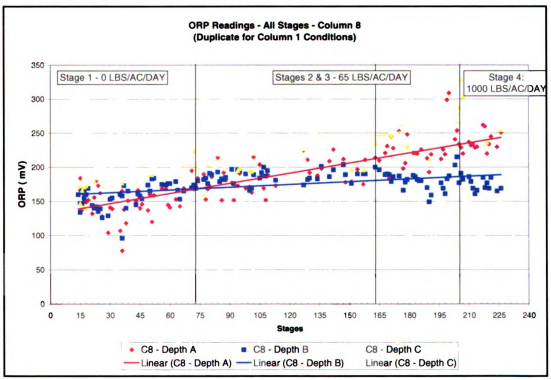

Figure A-2. ORP for Column 8

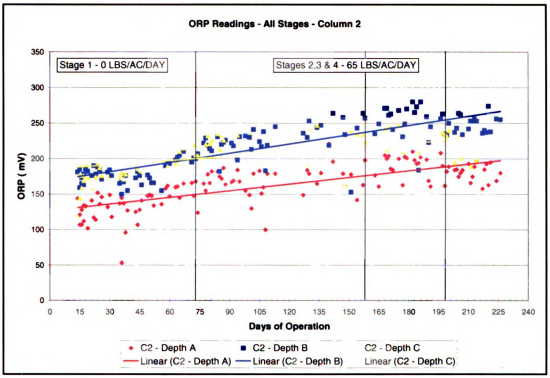

Figure A-3. ORP for column 2

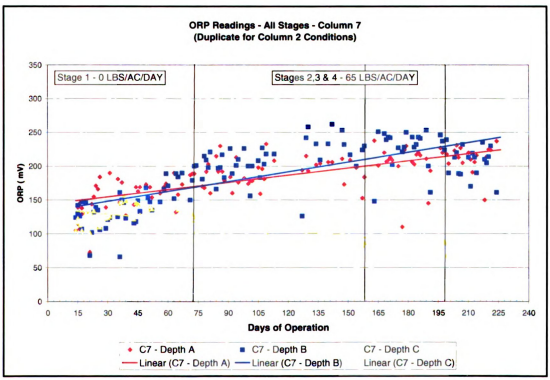

Figure A-4. ORP for Column 7

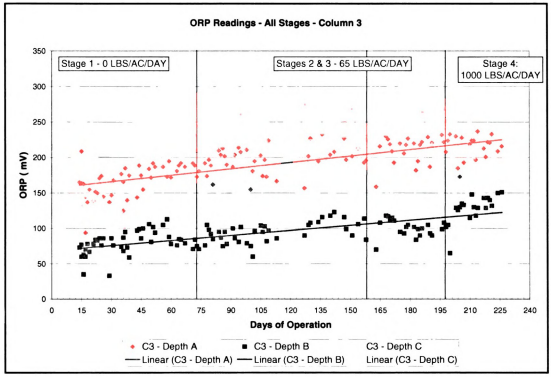

Figure A-5. ORP for Column 3

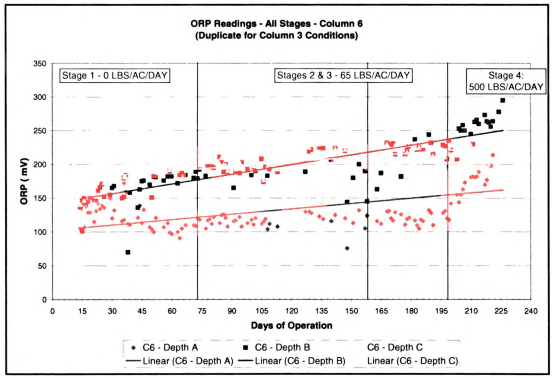

Figure A-6. ORP for Column 6

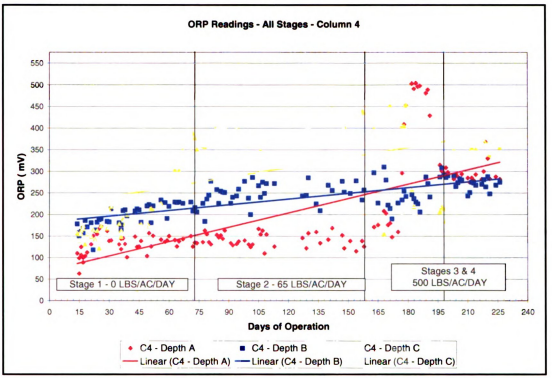

Figure A-7. ORP for Column 4

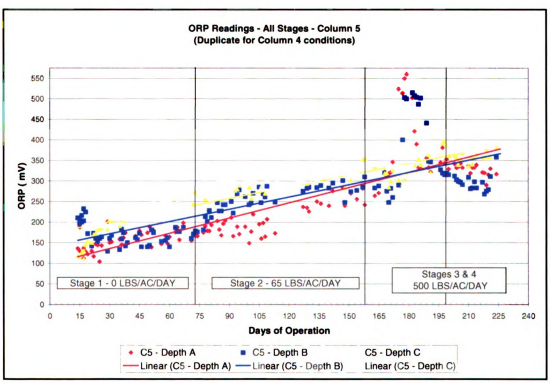

Figure A-8. ORP for Column 5

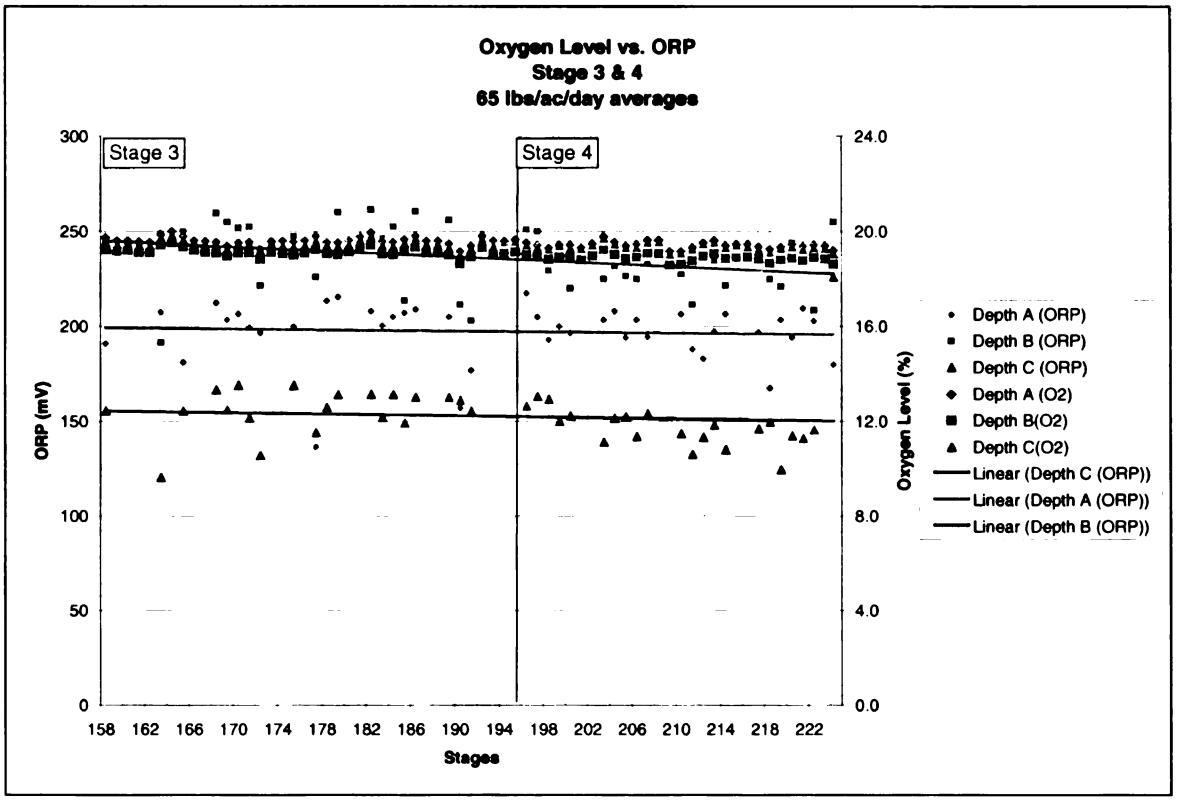

Figure A-9. ORP vs. Oxygen Level for 65 lbs BOD/ac/day

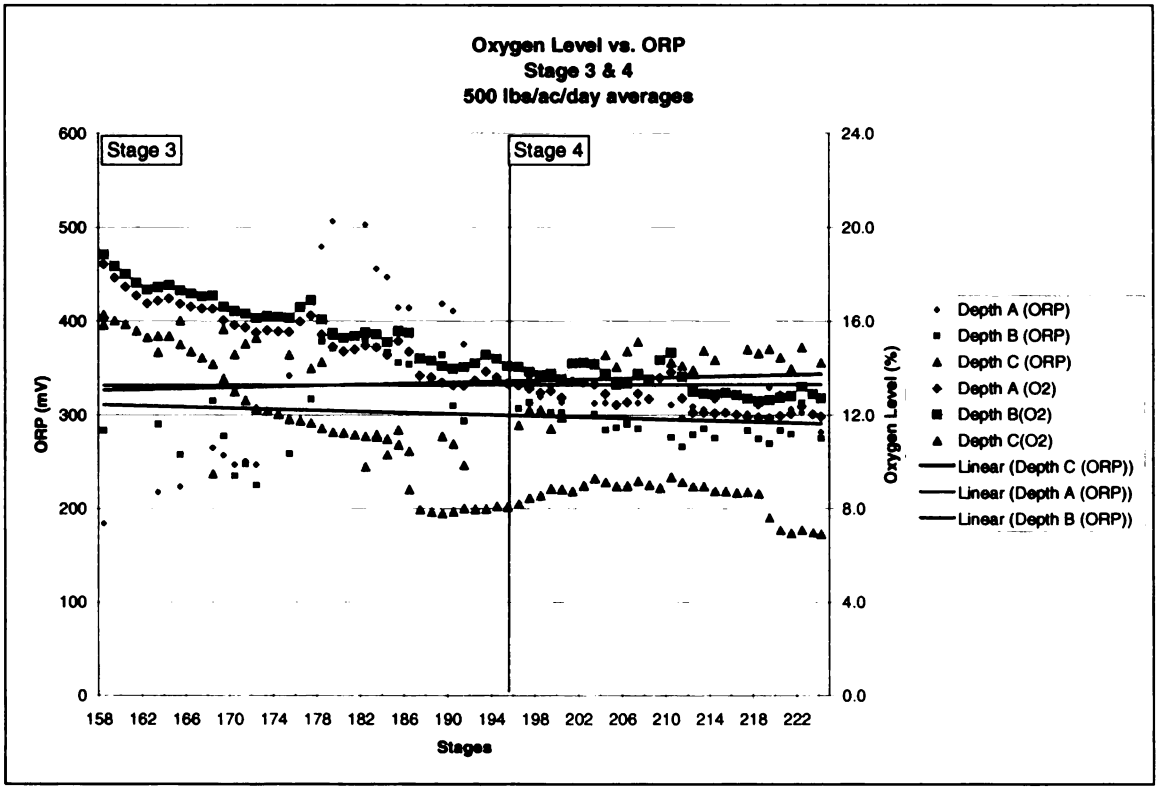

Figure A-10. ORP vs. Oxygen Level for 500 lbs BOD/ac/day

APPENDIX B

Table B-1. Soil Moisture Post-Calibration Weights

Sample							
Column	BOD Loading	Depth Label	Munsell Soil Color Charts Chart:10YR (Value/Chroma)	Depth (inches)	Wet Weight (grams)	Dry Weight (grams)	VSS Weight (grams)
Original	None	-	Pale brown and brown (6/3 & 5/3)	-	47.2889	46.1907	46.00244
1	1000 LBS/ac/day	Depth to top	-	11.875			
		A	Brown (5/3)	5.625	44.2132	40.8292	40.6065
		B	Light brownish gray (6/2)	13.125	56.82335	52.8762	52.578
		C	Dark grayish brown (4/2)	21.125	62.7664	53.7227	53.4671
		VB	Very dark grayish brown (3/2)	23.125	89.6239	73.4644	73.1208
5	500 LBS/ac/day	Depth to top	-	12			
		A	Brown (5/3)	5.625	45.10195	41.4754	41.1959
		B	Brown (5/3)	12.5	48.91101	44.4327	44.1962
		C	Grayish brown (5/2)	20	70.8343	57.989	56.18279
		VB	Dark grayish brown (4/2)	25.125	86.1497	72.1767	71.7027
7	Control - 65 LBS/ac/day	Depth to top	-	11.625			
		A	Brown (5/3)	5.3125	38.80602	36.0178	35.85732
		B	Grayish brown (5/2)	13.75	44.6705	40.6213	40.44046
		C	Dark grayish brown (4/2)	21.5	63.2023	51.3119	51.0794
		VB	Very dark grayish brown (3/2)	26.25	85.30409	70.0254	69.6559

Table B-2. Soil Moisture Post-Calibration Calculations

Sample							
					13269		
Column	BOD Loading	Depth Label	Wet - dry weight (grams)	Volatile Solids = Dry weight - VS (grams)	Dry Mass Content (grams)	Water Content
Original	None	-	1.09825	0.18821	0.97611	0.02448
1	1000 LBS/ac/day	Depth to top				
		A	3.38399	0.22271	0.92109	0.08567
		B	3.94715	0.2982	0.92888	0.07657
		C	9.04373	0.25557	0.85280	0.17260
		VB	16.1595	0.3436	0.81699	0.22401
5	500 LBS/ac/day	Depth to top				
		A	3.62652	0.27953	0.91716	0.09033
		B	4.71481	0.2365	0.90589	0.10389
		C	14.65151	1.80621	0.81520	0.22670
		VB	14.447	0.474	0.83527	0.19722
7	Control - 65 LBS/ac/day	Depth to top				
		A	2.78826	0.16044	0.92560	0.08037
		B	4.04919	0.18085	0.90658	0.10305
		C	11.89039	0.23251	0.80783	0.23788
		VB	15.27869	0.3695	0.81806	0.22240

Table B-3. Oxygen Sensor Post Calibration Data

O2 Sensor Calibration (Date&Location)		7/24/2008	3/3/2008	3/6/2008			
		In barn	In barn	In lab 128			
Column	BOD Loading	Atm (mV)	Atm (mV)	Atm	N2 only	Recup Time	Notes
1	1000 LBS/ac/day	53.7	47.3	53.4	2.2	3'15"	some debris in chamber
		56.1	49.5	55.6	2.2	3'20"	lots of debris in chamber
		53	49.4	51.7	2.2	3'40"	
5	500 LBS/ac/day	53.5	51.5	55.2	2.2	3'22"	lots of debris in chamber
		56.5	52.1	55.1	2.4	2'53"	debris in chamber
		54.4	50.8	53.1	2.1	3'14"	no debris
7	Control - 65 LBS/ac/day	52	49.6	53.7	2.7	3'26"	no debris
		58	53.2	56.4	2.5	2'58"	debris in chamber
		55.1	52.2	55.8	2	3'40"	some debris

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