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hereby submit this as part of the requirements for the degree of:
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in:
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It is entitled:
Investigation of Physical and Biological Properties of a Full Scale and a Pilot Scale Biofilter

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Investigation of Physical and Biological Properties
of a Full Scale and a Pilot Scale Biofilter

Master’s Thesis

Presented to

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ABSTRACT

The United States Environmental Protection Agency does not regulate the air emissions of volatile compounds from municipal waste treatment plants as of this writing. There have been proposals for changes in this requirement. California has already enacted legislation to limit the emissions from wastewater treatment plants, and many municipalities recognize that the regulations to limit these emissions are inevitable.

In order to be prepared for new regulations, The Metropolitan Sewer District of Greater Cincinnati has taken a proactive approach to limiting their volatile emissions. This approach will make it easier to comply with the proposed regulations. The purpose of this research project was to determine the emission of volatile organic compounds (VOC's) and other hazardous materials from a biofilter operating at a representative municipal wastewater treatment plant.

The research involved the monitoring of a biofilter that controls the gas emissions from a sludge holding tank. A correlation was developed between an operating biofilter and the physical and biological properties of the biofilter.

The study was performed at the Polk Run Wastewater Treatment Plant. This plant treats primarily residential sewage. The average daily flow is 3.5 million gallons. This plant serves the residential community of Loveland, Ohio and the surrounding suburban area.

This study confirmed that a biofilter is an effective method for controlling odors and emission of volatile compounds from a sludge holding tank at a municipal wastewater treatment plant. The main gaseous emission identified and the source of the predominant odors was determined to be hydrogen sulfide gas. The bacteria and fungi found in the biofilter controlled this odor by reducing the concentration of hydrogen sulfide gas. The bacteria found in the biofilter
were identified as *Pseudomonas aeruginosa* and *Salmonella*. These bacteria are commonly found in wastewater.

The wood bark in the biofilter provided the medium for bacterial and fungal growth. The optimum conditions for this biofilter were found to include temperatures from 20 to 35 degrees C and relative humidity of 90 to 95%. Under these conditions, an approximately 95% reduction in the concentration of hydrogen sulfide gas was achieved.

The project was funded by The Metropolitan Sewer District of Greater Cincinnati.
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INTRODUCTION

This project involved the monitoring and optimization of a biofilter that controls the gas emissions from a sludge holding tank. A correlation will be developed between an operating biofilter performance and the physical and biological properties of the biofilter.

The study was performed at the Polk Run Wastewater Treatment Plant. This plant treats primarily residential sewage. The average daily flow is 3.5 million gallons. This plant serves the residential community of Loveland, Ohio and the surrounding suburban area. The treatment process consists of the following: (MSD of Greater Cincinnati, 1993)

<table>
<thead>
<tr>
<th>TREATMENT PROCESS</th>
<th># UNITS</th>
<th>SIZE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bar Screen</td>
<td>1 unit</td>
<td></td>
</tr>
<tr>
<td>Aerated Grit Chamber</td>
<td>1 unit</td>
<td>60' L x 8.5' W x 16.5' D</td>
</tr>
<tr>
<td>Primary Settling Tanks</td>
<td>4 units</td>
<td>100' L x 20' W x 12' D</td>
</tr>
<tr>
<td>Aeration Tanks</td>
<td>4 units</td>
<td>100' L x 32' W x 16' D</td>
</tr>
<tr>
<td>Secondary Settling Tanks</td>
<td>4 units</td>
<td>70' dia x 12' D</td>
</tr>
<tr>
<td>Chlorine Contact Tank</td>
<td>1 unit</td>
<td>55' L x 19.5' W x 16' D</td>
</tr>
</tbody>
</table>

(Chlorination with gaseous chlorine; dechlorination with sulfur dioxide)

<table>
<thead>
<tr>
<th>Process</th>
<th>Comment</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post Aeration</td>
<td>Cascade Fall</td>
<td>7.5' Drop</td>
</tr>
<tr>
<td>Sludge Thickening Tanks</td>
<td>2 units</td>
<td>35' dia x 8' D</td>
</tr>
<tr>
<td>Sludge Holding Tanks</td>
<td>2 units</td>
<td>32' L x 27' W x 13' D</td>
</tr>
<tr>
<td>Biofilter (for stored sludge)</td>
<td>1 unit</td>
<td>30' L x 30' W x 16' D</td>
</tr>
</tbody>
</table>

(With approximately 5’ depth of wood bark)
The plant was designed to handle a design flow of 6 million gallons per day (MGD), with a peak flow of 12 MGD. The design of the plant allows for an influent biochemical oxygen demand (BOD$_5$) of 220 mg/L, an influent suspended solids (SS) of 220 mg/L, and an influent ammonia nitrogen concentration of 20 mg/L as N. The current average daily flow is 3.5 MGD. The plant effluent has an average BOD$_5$ content of 2.5 mg/L, a SS content of 2.8 mg/L and 1.4 mg/L of NH$_3$-N. A schematic of the treatment plant is shown in Figure 1.

There is no on-site drying or treatment of the primary or secondary sludge. The sludge is pumped from the storage tanks into a tanker truck for off-site disposal. The primary source of the odors from the sludge storage tank area is hydrogen sulfide gas. Most of the volatile organic carbon compounds (VOCs) are believed to be liberated from the wastewater prior to the sludge holding tanks.

The hydrogen sulfide gas is liberated from the sludge by two separate processes. The first is a steady state emission of the gas during storage. The second source occurs during the transfer of the sludge from either the thickening tanks into the storage tank or from the storage tank to the tanker truck. The transfer mechanism causes abrupt increases in the hydrogen sulfide concentration at the inlet of the biofilter.

Hydrogen sulfide, (H$_2$S), can be dangerous or fatal at very high levels. The lethal level is 800 parts per million (ppm), or 600 ppm for a 30 minute exposure (Verschueren, 1983). The H$_2$S levels measured above the biofilter are much lower than this (0.01 ppm to 2 ppm). Unfortunately, the odor threshold (0.0047 ppm) is much lower than the H$_2$S measured (Cox, 1975).
Figure 1

Layout of Polk Run Wastewater Treatment Plant
The biofilter is approximately 30 feet long x 30 feet wide x 16 feet deep, and constructed of concrete on three sides and wood on the fourth side. The air from the sludge holding tanks is pumped into the biofilter by two 1300 cubic foot per minute blowers. These blowers are each powered by 7.5 HP electric motors. The air is humidified as it passes through the inlet pipes by a small tube that supplies a constant drip of water. The exhaust air from the sludge holding tanks is distributed throughout the biofilter by a grid of air distribution blocks. These blocks are constructed of concrete and are manufactured by ROTTAER, Leichlingen, Germany. The blocks have large passages on all four sides for distribution of the air throughout the base of the biofilter, and small slots on the upper surface to allow the air to pass upward into the filter media. A picture of the distribution blocks is included on page XI-10 in the appendix of this document.

The biofilter is filled with hardwood bark to a depth of approximately 5 feet. The bark is designed to be maintained at a moisture content of approximately 50% by weight to maintain a medium for bacterial growth. The presence of this bacterial growth is the mechanism by which the hydrogen sulfide is absorbed and decomposed. A diagram of the biofilter is shown in Figure 2.

The hydrogen sulfide gas originates from the reduction of the sulfate ions in the wastewater by bacteria under anaerobic conditions (Grady and Lim, 1980).

\[
\text{SO}_4^{\text{-2}} + \text{organic matter (C,H,O)} \Rightarrow \text{S}^{\text{-2}} + \text{H}_2\text{O} + \text{CO}_2 \\
\text{S}^{\text{-2}} + 2\text{H}^+ \Rightarrow \text{H}_2\text{S}
\]
The source of the sulfur in the wastewater is primarily from the breakdown of plant and animal proteins by the human body to form urine, which is high in SO$_4^{-2}$. 

Figure 2

Layout of Biofilters
The concentration of hydrogen sulfide at the inlet to the biofilter was measured using grab sample tubes and a hand pump manufactured by Sensidyne. The hydrogen sulfide level at the access port to the sludge holding tank is typically approximately 0.06 ppm. This access port is at the top of the covered tank. The hydrogen sulfide concentration near the surface of the sludge is normally much higher. The \( \text{H}_2\text{S} \) concentration at a sampling port at the inlet to the biofilter was below 0.01 ppm with 2 blowers operating and approximately 0.10 ppm with only one blower operating. These samples were taken with no sludge flowing into or out of the sludge holding tanks. The air temperature above the sludge was 24° C, with a relative humidity of 40%. The air temperature of the air at the inlet to the biofilter was 22° C, with a relative humidity of 50%. When two blowers were operating, the airflow rate was 2750 cubic feet per minute. When only one blower was operating, the air temperature was 17° C, with a relative humidity of 79% and a flow rate of 2500 cubic feet per minute. The sampling was done near sunset on a clear day, which explains the drop in temperature between the two samplings.

After the initial air readings were made, sludge was added from the thickener to the sludge holding tanks. The hydrogen sulfide peaked almost immediately at over 2 ppm (the limit on these sampling tubes for one pull of the sampler) at the inlet to the biofilter. After the sludge transfer was complete, the concentration of hydrogen sulfide was 1 ppm in the holding tank and 0.625 ppm at the inlet to the biofilter. This level decreased steadily to the steady-state levels reported earlier.

These observations lead to two major conclusions. The first is that the liberation of the hydrogen sulfide is greatest when turbulence in the sludge exists, as during the transfer of sludge. Second, the hydrogen sulfide continuously released from the sludge is greatly diluted when two blowers are operating.
The Sensidyne sampling pump uses a colorometric analysis. The hand-operated pump delivers a known quantity of the sampling gas to the analysis tube (100 ml/stroke). A reagent is present in the analysis tube. The reagent will change color if the gas is present. For hydrogen sulfide in the 0.2 to 2.0 ppm range, mercuric chloride reacts with the hydrogen sulfide and liberates hydrogen chloride, which is reddish brown. For hydrogen sulfide in the 2.5 to 60 ppm range, lead acetate reacts with the hydrogen sulfide to form lead sulfide, which is brown.

\[
H_2S + HgCl_2 \Rightarrow HSHgCl_2 + H^+
\]

\[
H_2S + Pb(Ch_3COO)_2 \Rightarrow PbS + 2Ch_3COOH
\]

For a complete description of the experimental method for the gas sampling, see Appendix II, beginning on page II-1.

The second part of the research concentrated on the biological properties of an operating biofilter. To do this, a pilot scale biofilter was constructed adjacent to the main biofilter. This pilot biofilter is constructed of cedar wood, and uses the same distribution blocks as the main biofilter. It is filled with 5 feet of the wood bark collected off the top of the main biofilter. This ensures that the same organisms are present in both biofilters at startup. The portion of the inlet air to the main biofilter was diverted by a tee in the main supply tube. PVC pipe transported this air to the pilot biofilter.

The pilot biofilter has two chambers, side by side, each measuring 30 inches x 30 inches. The contaminated air feed is tapped off the inlet to the main biofilter inlet. The flow rate was measured at the inlet of the main biofilter and the inlets to the pilot biofilters to ensure that the flow rates were equal. This was achievable because the resistance of the PVC tubing is much lower than the wood bark of the biofilter. A diagram of the pilot biofilter is shown in Figure 3. The piping for the air into the pilot biofilter is shown along with the main biofilter in Figure 2.
The physical properties of the pilot biofilter were different from the main biofilter over time. This allowed some comparison of biological properties and physical properties, with no change to the influent air.
RESEARCH OBJECTIVE

The emission of odorous gases can be both dangerous and an annoyance. As stated earlier, the United States Environmental Protection Agency does not currently regulate the emissions of gases from municipal wastewater treatment plants, but there are indications that there will be controls in the near future. The use of a biofilter is an inexpensive way to control odorous emissions.

The objective of this research was to determine the optimum operating conditions for the biofilter located at the Polk Run Wastewater Treatment Plant in Loveland, Ohio. The optimum conditions at other biofilters used for treating odorous emissions from municipal wastewater treatment plants should be similar to conditions determined in the study.

An operating manual for the wood bark biofilter was developed, and is included in Appendix XI.
LITERATURE REVIEW

There is very little published work available on operating biofilters. Soil biofilters can be very effective to first absorb and then oxidize volatile organics, sulfur dioxide, nitrous oxides and hydrogen sulfide (Vatavuk, 1990). The theory and operation of soil and wood bark biofilters are similar. The biofilter absorbs the compound, and then the bacteria in the soil or wood oxidize the pollutant. Vatavuk reported that 90 to 99+ percent efficiency is possible, but did not report any details regarding what factors control the efficiency of the biofilter.

The removal efficiency of the biofilter is strongly affected by the choice of biofilter media (Sorial et al, 1997). Ideal biofilter media contains a high specific surface area for the microorganisms to attach to. This attachment media can be made from an organic material (wood bark, peat, etc.), an inert material or a combination of the two. The microorganisms require a nutrient supply and water for growth. Natural organic materials are a nutrient source and the humidified air is the source of water. Inert materials require the addition of a nutrient and humidified air. The Polk Run biofilter is constructed of wood bark. This media has a high specific surface area. The surface area was estimated by the BET analysis performed during this study. Also, wood bark is a natural organic material, and therefore does not require the addition of nutrients.

As stated above, a biofilter uses a fixed biofilm to absorb the pollutant. Fixed biofilms have definite advantages over suspended bacterial cultures. Lazarova and Manem (1995) report that the biofilm is less strongly affected by changes in environmental conditions than suspended cultures. Also, the biofilm activity is not proportional to the amount of fixed biomass, but increases with the
thickness up to a level termed the active thickness. Above this level, diffusion becomes the limiting factor. A stable thin and active biofilm is preferred. The biofilter used in this study uses wood bark as the substrate for the biofilm. The thickness of the biofilm would be very difficult to measure. An estimate of total biomass was made by using an analysis of the bacterial lipids. A lipid analysis is reported by Lazarova and Manem to have precision within ±10%.

There are advantages and disadvantages to using a biofilter (Wittorf, 1993). The advantages include simple operation, low investment cost, low operating costs, satisfactory degradation of less water soluble components, and suitability for reduction of odorous pollutants present in low concentrations. The disadvantages include low waste-air volumetric flow rate, effectiveness only for low pollutant concentrations, difficult process control and limited service life for the filter medium. The advantages of the Polk Run biofilter outweigh the disadvantages, as it has a low airflow rate and the wood bark, if properly rotated, lasts in excess of five years.

Biofilter Optimal Operating Conditions

Much of the previous research has concentrated on laboratory analysis of biofilters. Corsi and Seed (1995) published a good article on the effects of different operating conditions on the performance of the biofilter. The following is a summary of their conclusions. Following each observation by Corsi and Seed is a description of how the particular operating condition affected the performance of the wood bark biofilter located at the Polk Run Wastewater Treatment Plant studied in this research.

Research has shown that the density of organisms is greatest where volatile organic compound removal is highest. This is usually at the inlet point to the biofilter. Because of the high concentration of microorganisms at the air inlet, the biofilter is usually under-utilized. The
concentration of microorganisms in the Polk Run biofilter should also be highest at the bottom of the filter near the distribution blocks. Therefore, sampling of the wood bark from the biofilter was done at several different depths.

At startup of the biofilter, Corsi and Seed found that inoculation of organisms might be required. Caution must be exercised when inoculating. This is because the bacteria that are added could dominate all the other bacteria, and therefore kill the bacteria present. The other more likely condition is to have the added bacteria be dominated by the bacteria that were present prior to the inoculation, and die. This can be expensive if the biofilter needs to be inoculated at periodic intervals. Regardless of whether the biofilter is inoculated or not, 1-3 weeks are typical biofilter acclimation times. For this reason, the pilot biofilter constructed at Polk Run was supplied with wood bark from the main biofilter. The acclimation time was reduced to nearly zero since the microorganisms were already present in the wood bark.

Nutrient availability can be the limiting factor for degradation of the inlet gas. This can also work in favor of the biofilter, for short periods, since cell death can occur if insufficient nutrients are available and cell death can provide nutrients. The Polk Run wood bark biofilter studied should have no problem with the availability of nutrients. The sludge is the natural source of food for the bacteria found in the filter. The inlet air should provide plenty of hydrogen sulfide, carbon, oxygen and nitrogen as food to the biofilter. Table 1 shows a partial list of the elemental composition of four typical sewage sludges (Cheremisinoff, 1994). The source of the sludge at Polk Run is from the activated sludge process treating residential waste. Sludges 2, 3 or 4 would be typical compositions expected at Polk Run.
Table 1

Elemental Composition of Typical Sewage Sludges

<table>
<thead>
<tr>
<th>ELEMENTAL COMPOSITION</th>
<th>SLUDGE 1</th>
<th>SLUDGE 2</th>
<th>SLUDGE 3</th>
<th>SLUDGE 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>CARBON %</td>
<td>5.18</td>
<td>55</td>
<td>65.6</td>
<td>63.4</td>
</tr>
<tr>
<td>OXYGEN %</td>
<td>38</td>
<td>33.4</td>
<td>20.9</td>
<td>21</td>
</tr>
<tr>
<td>HYDROGEN %</td>
<td>7.2</td>
<td>7.4</td>
<td>9</td>
<td>8.2</td>
</tr>
<tr>
<td>NITROGEN %</td>
<td>3</td>
<td>3.1</td>
<td>3.4</td>
<td>4.3</td>
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<tr>
<td>SULFUR %</td>
<td>TRACE</td>
<td>1.1</td>
<td>1.1</td>
<td>2.2</td>
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Inlet gases that consist of multiple substrates can limit the performance of the biofilter. There is very little published work on mixed gas biofilter performance. The inlet gases for the Polk Run wood bark biofilter is relatively constant over time. The bacteria present in the biofilter have acclimated to the existence of the gas mixture.

Moisture control is crucial for maintaining performance of the biofilter. At moisture levels below 30% there appears to be very little removal of VOCs. Humidification of the incoming gas stream is the preferred method for maintaining moisture in the biofilter. Additional water may need to be added, but this is usually added from the top of the biofilter, and may not reach the bottom where it is needed for the biological activity. Also, the optimum temperature appears to be between 25°C and 30°C. The effect of moisture and temperature has been observed with the Polk Run wood bark biofilter. During the hot dry summer months, the biofilter periodically dried out. When this happened, the odors originating from the biofilter increased. Bacterial analysis also showed a decrease in population with the dry bark. During the winter months, the decrease in temperature usually did not decrease the number of organisms, but slowed the rate of growth of these organisms.
Degradation of hydrogen sulfide in biofilters can decrease the pH and affect the products that are formed. The optimum pH for VOC removal appears to be between 6-8. The pH of the Polk Run biofilter was determined to be approximately 7. Biofilters with concentrations of hydrogen sulfide in excess of 100 ppm can operate more efficiently with a two-stage system. The first stage uses an acid resistant inorganic medium, which operates at a low pH. Water is used to wash away the acid and humidify the air for the second stage (Devinny, 1998). The acidic water is further treated before release to the environment. Another two-stage biofilter uses the first stage of the biofilter to regulate the pH by removing hydrogen sulfide with activated carbon (Park et al, 1993). The second stage of both biofilters contains an organic medium for the biofiltration of the remaining gases. The Polk Run biofilter had an average inlet concentration of approximately 0.01 ppm, and therefore does not require a two-stage system.

The gas flow rate affects loading rates and elimination capacities. Increasing the flow rate can have a negative impact on the biofilter by causing bed drying or cracking. Also, if the gas is diluted, the biofilter can be under-utilized and the residence time decreased. Dynamic mass loading can have a negative effect on the biofilter. This can cause a shock to the microorganisms. The concentration of the inlet gas can exceed the elimination capacity of the biofilter. After this shock loading, the biofilter may require a substantial amount of time to recover. The Polk Run wood bark biofilter did not seem to be affected by changes in loading. The concentration of hydrogen sulfide in the sludge holding tank air increased dramatically during sludge transfer into the sludge holding tanks, but no appreciable difference in the odor emanating from the biofilter was noticed. The dilution of the influent air by the blowers and the large size of the biofilter provide a buffer to the increase in concentration of the hydrogen sulfide.
The ability of a biofilter to remove odorous and sulfur-containing compounds was investigated by Amirhor (1995). The study was performed at a biosolids composting facility in Dartmouth, Massachusetts. The biofilter is somewhat similar to the one at the Polk Run facility, except instead of using only wood bark for the biofilter, a combination of one part bark to two parts wood chips to one part peat compost was used. The inlet and outlet concentrations of the various sulfur-containing compounds were measured. Dimethyl sulfide, dimethyl disulfide, and methyl mercaptan gases were measured. The study also used an odor panel to determine the amount of odor removal. An odor panel refers to the use of people to determine the difference between samples using the sense of smell. This is especially useful since the odor thresholds for dimethyl sulfide, dimethyl disulfide, and methyl mercaptan are very low.

The average removal efficiency of these sulfur-containing compounds was 17% for the dimethyl disulfide, 55% for dimethyl sulfide, and 90% for methyl mercaptan. The odor removal efficiency varied from 80% to over 90%. The higher removal efficiencies correlated to longer retention times in the biofilter. This time was approximately 36 seconds for the 80% removal, and 60 seconds for the 90% removal. The study concludes that the percent removal is inversely proportional to the airflow rate (assuming a constant concentration of the odorous compounds in the inlet).

The Operation of Municipal Wastewater Treatment Plants Manual of Practice (Water Pollution Control Federation, 1990) has an entire section devoted to odor control. The odor thresholds of various compounds are reported, with hydrogen sulfide being perceptible at 0.47 parts per billion (ppb). As a comparison, methyl mercaptan is noticed at a level of 1.1 ppb. The manual states that the main source of hydrogen sulfide is from the bacterial decomposition of
organic material. Hydrogen sulfide can reach dangerous levels in confined areas such as stagnant sewers.

**Biofilter Operating Costs**

The Operation of Municipal Wastewater Treatment Plants Manual of Practice (WPCF, 1990) also reports a cost comparison of different odor control technologies. For a flow of 10,000 cubic feet/minute, the following is the annual amortized cost for a ten-year period (in 1988 dollars). The comparison assumes an inlet hydrogen sulfide concentration of 20 parts per million (ppm) and an outlet concentration of less than 1 ppm.

<table>
<thead>
<tr>
<th>Amortized Cost</th>
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<tr>
<td>Biofilter</td>
<td>$25,750/yr</td>
</tr>
<tr>
<td>Ozonation</td>
<td>$38,780/yr</td>
</tr>
<tr>
<td>Wet Scrubber</td>
<td>$45,850/yr</td>
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<tr>
<td>Catalytic Incinerator</td>
<td>$51,900/yr</td>
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<tr>
<td>Thermal Incinerator</td>
<td>$69,720/yr</td>
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<tr>
<td>Activated Carbon</td>
<td>$97,690/yr</td>
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</tbody>
</table>

The manual also states that if odor is the only problem, masking agents can be used to counteract the smell. These should only be used for short periods, as they can be costly and disguise the odor of more dangerous emissions.

The cost of another biofilter subject to the same conditions was reported to have a capital cost of $97,300 and a yearly operating and maintenance cost of $7,870 (in 1990 dollars) (Water Environment Federation, 1995). Distributing the capital cost over a ten-year period and not including the cost of interest expenses on the capital cost, this equals $17,600 per year. This cost
is comparable to the cost of the biofilter reported by The Operation of Municipal Wastewater Treatment Plants Manual of Practice, and well below the cost of other methods.

The cost of the biofilter constructed at Polk Run was much less than reported above. The biofilter was constructed using an existing concrete tank previously used as a settling tank. The biofilter used existing concrete walls on three sides, and the fourth side was constructed of wood. The major capital expenses included the cost of the blowers, piping, air distribution blocks and the wood bark.

The influent concentration of hydrogen sulfide to the Polk Run wood bark biofilter was usually between 0.01 and 0.02 ppm, and the exhaust concentration was below measurable levels, and less than the perceptible odor threshold of 0.47 parts per billion (ppb).

H₂S Measurement

There are two basic methods for measuring hydrogen sulfide (Lue-Hing et al., 1992). These methods include the use of hydrogen sulfide meters and Draeger tubes.

There are two different types of hydrogen sulfide meters. The first type has a detector that is sensitive to the presence of H₂S molecules. This detector can measure concentrations of between 1.0 and 100 ppm H₂S in air. The second type of instrument uses a gold film. H₂S reacts with the gold film. Concentrations of between 1.0 and 500 ppb of H₂S in air can be detected.

The other method of measuring H₂S is to use Draeger Tubes. These glass tubes have a chemical present in a packing material. By pumping the sampler, air passes through the packing, which chemically reacts with the H₂S in the air. As the reaction takes place, the color of the packing changes. The side of the tube is graduated, and the amount of color change indicates the concentration of H₂S. These tubes are much less expensive than the H₂S meters, and provide
quick results. The detection range is usually 0.5 to 30.0 ppm for a single pull on the sampler pump.

Draeger tubes manufactured by Sensidyne were used for the study at Polk Run. These tubes contain a fine grain silica gel. Absorbed on this gel is a reagent that reacts with the hydrogen sulfide as it enters the tube. Pulling the pump multiple times allows more hydrogen sulfide to enter the tube, and more of the reagent will react with the hydrogen sulfide. The larger the reaction quantitatively, the more color change in the tube. The hydrogen sulfide levels were usually below the 0.5 level, but by taking multiple pulls on the sampler, lower detection levels were possible. The Sensidyne tubes have a minimum detection concentration for hydrogen sulfide of 0.05 ppm for 2 pulls of the pump. The manufacturer states that up to 5 pulls on the sampler can be used without decreasing the accuracy of the results by more than 25% of the actual value (NIOSH Standard). The minimum detection limit for 5 pulls of the pump would therefore be 0.02 ppm. More than 5 pumps on the sampler can measure lower concentrations, but the accuracy of the measurement will be decreased.

**H₂S Removal in Biofilter**

The necessary size of a biofilter is a function of the flow rate of the gas. A guideline used states that the biofilter should be sized to have a face velocity of approximately two to eight feet per minute (Shen et al., 1989). The Polk Run biofilter has a flow rate of 2800 cfm and a surface area of 900 square feet. This gives a face velocity of 3.1 feet per minute.

Most sulfur is found in the form of sulfate, SO₄²⁻ (Grady and Lim, 1980). In the sulfate form, the sulfur has a valence of +6, but in most organic compounds, sulfur has a -2 charge. Therefore, when H₂S is formed from organic compounds, no reduction of the sulfur is required.
Under aerobic conditions, the sulfur is oxidized to form the sulfate ion. If sulfate ions are present in an anaerobic environment, sulfate-reducing bacteria can also enable the sulfur to be reduced to form H$_2$S.

It is reported that 80% of the sulfur containing malodorants can be removed through an aerated biofilter (Hwang et al, 1994). The compounds studied included dimethyl sulfide and dimethyl disulfide. The inlet concentrations were 3.3 mg/l for dimethyl sulfide and 4.6 mg/l for dimethyl disulfide. The retention time for dimethyl sulfide was approximately 10 minutes, and the retention time for dimethyl disulfide was approximately 25 minutes. It should be noted that these biofilters used either activated carbon or anthracite as an absorption medium, and the inlet concentrations were much higher than even from raw wastewater.

Hwang also reports that odorous compounds can be removed by ozonation. The retention time required for 80% removal was 5 minutes for dimethyl sulfide, and 25 minutes for dimethyl disulfide. These measurements were taken with a blank ozone residual of 2.6 mg/l.

Solids-attached biofilms are crucial in avoiding air stripping during the treatment of toxic volatile organic compound-containing wastewaters (Santos and Livingston, 1995). They concentrated their study on measuring the thickness of a biofilm using a nondestructive testing technique. They called this technique the projection technique. A light source with a measured illumination of 28,000 lux at 0.5 m from the projector was used.

The biofilm was grown on a silicone rubber tube that measured 3 mm in diameter. The reactor was seeded with *Xanthobacter autotrophicus* GJ10, and 1,2 dichloroethane was the nutrient. The biofilm, along with a calibrated scale, was projected onto a screen. The maximum resolution obtained with the 1-mm markings led to an estimated error of ± 10 μm. This method allowed continuous monitoring of the biofilm thickness, with minimal disruption to the biofilm.
The biofilm thickness after 10 days measured 1200 µm for run 1 and 750 µm for run 2. The terminal biofilm average density was measured to be approximately 60 Kg m$^{-3}$.

Ponte and Miller (1995) studied the use of trickling filters to control odorous emissions from a wastewater treatment plant. The influent wastewater is trickled over a medium. Bacteria are present on the medium, which consume the biodegradable substances in the wastewater. The effluent flows out a drain located under the filter. The bacteria in trickling filters are predominately aerobic and rely on natural draft ventilation to provide the oxygen source.

The driving force for the ventilation relies on the difference in weight of the air inside the filter versus the air outside the filter. The cooler the air, the higher the density. On a cold day, the air will flow upward through the filter. The opposite is true for a hot day. When the temperature inside the filter is the same as the temperature outside the filter, no air will flow through the filter. It has been estimated that when the air difference is 7 to 9 degrees C, there will not be enough airflow to support the oxygen requirements of the trickling filter. During these periods and immediately after the air flow resumes, there may be releases of odorous gases to the atmosphere. Some wastewater treatment plants have used forced air ventilation to overcome this problem.

The trickling filters studied were under the control of the South Monmouth Regional Sewerage Authority in New Jersey. Two filters operated in parallel and four fans were connected to each filter. The four fans were capable of withdrawing 25,000 to 40,000 cfm of air through each filter. The residence time of the air was approximately 10 to 16 seconds in the trickling filters. The exhaust from the two filters was passed through a 50-foot stack with scrubbers installed. This combined system reduced the concentration of hydrogen sulfide from 42.8 parts per billion at the inlet to 19.6 parts per billion at the outlet. The odor units (ED50) were also measured at the inlet.
and outlet to the filters. The odor units were measured using an odor panel, which is subject to
human error, but were shown to be reduced by 25 to 64% using the filter/scrubber system.

The trickling filter with forced ventilation acts similar to the biofilter used at the Polk Run
Wastewater Treatment Plant. The effect of the forced ventilation on the trickling filter was not
measured since the scrubber provides an additional treatment to the air.

The effect of using a high rate trickle bed biofilter to control the removal of volatile organic
compounds was reported by Smith, et al (1995). Three different media were used to study the
removal efficiency of toluene. One biofilter used a compost medium and studied the effect of
temperature and loading on toluene removal efficiency. A second biofilter used a synthetic
channelized medium and evaluated the effect of temperature and nutrient feed rate on removal
efficiency. The third biofilter used a synthetic pelletized medium and evaluated the temperature
and pressure drop on removal efficiency. The study concluded that the removal efficiency
increased with increasing temperature. Smith further stated that much less medium would be
required for a biofilter operating at 32 degrees C than a biofilter operating at -15 degrees C.
Further, there may be an economic incentive to preheat the incoming air rather than building a
larger biofilter. Smith reported modest improvement in the operation of the compost biofilter with
increased loading. He reported that this effect was probably the result of increased bed moisture
content, with an optimum range from 50 to 60 percent. The best removal efficiency was obtained
by the pelletized medium. This biofilter did experience increased pressure drop caused by an
excessive accumulation of biomass. Backwashing the bed to full fluidation reduced the biomass
and allowed efficiencies consistently in excess of 99 percent.

The organisms responsible for oxidation of VOCs to CO\textsubscript{2} are the same heterotrophic
bacteria and fungi that are found naturally in nature (Bohn, 1992). The Polk Run biofilter is
continuously seeded with the microorganisms from the sludge holding tank effluent air. This study identified the major microbes to be *Pseudomonas aeruginosa*, *Salmonella*, and an unidentified fungus. The *Pseudomonas aeruginosa* is a denitrifying bacteria and is commonly found in wastewater sludge (Drysdale, 1999). *Salmonella* is a producer of hydrogen sulfide (Bergey, et al 1994).

Several groups of microorganisms are known to be involved in the degradation of hydrogen sulfide in biofilters, including bacteria, actinomycetes and fungi (Singhal et al, 1996). Also, fungi are known to degrade more complicated molecules by excreting enzymes to break down the polymers (Bohn, 1992). Although the exact microorganisms responsible for the decomposition of hydrogen sulfide in the biofilter is difficult to determine, the unidentified fungi found in the Polk Run biofilter may be responsible for the decomposition of hydrogen sulfide.
EXPERIMENTAL METHODS

This chapter will be divided into two sections.

- Sampling
- Bacterial analysis

**Sampling**

In general, the biofilter should normally operate satisfactorily with little human intervention. It is, however, important to periodically sample the wood bark and the influent air to determine the general health of the biofilter. The wood bark requires an influent air to contain enough water to maintain nearly 100% relative humidity. This will ensure that the wood bark maintains approximately 50% water by weight. A moisture content of 50% or greater is necessary for the bacteria to maintain growth in the wood bark.

There is no sampling procedure for wood bark described in Standard Methods or any other archived publication. Therefore, a method was established that provides good sampling repeatability. This procedure is described below.

**Wood Bark Sampling and Analysis**

The sampling of the wood bark was performed at two depths in the biofilter. Wood bark samples were collected and placed in Ziploc style bags for moisture content analysis. A Temperature/Humidity probe was used to measure the temperature and humidity at the locations where the samples were taken. A Cole Parmer Tri Sense Meter, Model 37000-00 with air...
velocity/temperature probe Model 37000-60 and relative humidity/temperature probe Model 37000-50 was used in this study.

The ambient temperature and relative humidity were also recorded along with the temperature and relative humidity of the air inlet to the biofilter.

For the pilot biofilter, samples of wood bark were taken from the three sampling doors located on the side of the biofilter. The temperature and relative humidity at the three sample locations were also measured.

The samples were taken immediately to the lab to determine the weight percentage of moisture in the wood bark.

\[
\text{% Moisture of the Wood Bark, by Weight} = 1 - \frac{(\text{Weight of Wood Bark} - \text{Weight of Empty Beaker})}{\text{Original Weight of Wood Bark}} \times 100\%
\]

The complete method for this procedure is found in Appendix I.

**Gas Sampling and Analysis**

The gas was sampled at the entrance to the main biofilter at the sampling port in the PVC tubing. The sampling port consists of a 0.25 inch (6 mm) diameter hole drilled in the 4 inch (10 cm) diameter tubing. The air velocity was checked first to make sure the blowers were running properly. If there was a problem with the blowers, the biofilter would not operate properly.
To analyze the gas, take the portable gas sampler and one of the grab tubes. Break off both ends of the grab tube and insert the proper end into the sampler, as directed by the sampler operating manual. The H₂S concentration during normal operation is approximately 0.01 to 0.02 ppm. If the range of the sampling tube is 0.1 ppm and up, start by taking 5 pulls on the sampler. The tube will change colors at the tip to indicate how much H₂S is present. Wait the number of minutes required by the manufacturer between each pull. When complete, record the reading displayed on the tube. This time period between pulls allows the chemical reaction to reach completion. If no color change is visible, take additional pulls until a color change appears. The accuracy of the reading will decrease with greater than 5 pulls.

Take the reading and divide by the number of pulls to determine the H₂S concentration. If the reading is greater than the maximum value on the tube, the measurement was probably taken during the transfer of sludge from the thickeners into the sludge holding tank (the reading can reach over 2 ppm). Wait a few minutes, then take a new grab tube and repeat the measurement. Record the H₂S reading, along with the air velocity reading.

Measure and record the temperature and humidity of the gas. Also measure the ambient temperature and humidity. During summer months, the gas temperature is usually lower than the ambient temperature. The opposite is true in the winter. This is because the sludge tends to be at a temperature of 65 - 70°F (18 - 22°C) year-round, and the sludge will cool the gas in the summer, and warm the gas in the winter.

The relative humidity of the gas should be 95% to 100% during most times. This will enable the wood bark to maintain sufficient moisture to promote bacterial growth. If the relative humidity is lower than 95%, the amount of water dripped into the air inlet pipes should be
increased. Do not add too much water by dripping, as it may scrub the H$_2$S from the gas (the H$_2$S gas will become dissolved in the water) and short-circuit the biofilter, exiting with the water at the drain. If the wood bark has dried out to below 40% moisture by weight (see wood bark sampling procedure), water may need to be sprayed onto the top of the biofilter. The spraying should be done in such a manner as to create a light mist. A garden sprinkler set on a diffused spray or a soaker hose works well. Do not over-water, as it may clog the filter.

The complete procedure for gas sampling of the biofilter is described in Appendix II.

Gas Sampling from Stored Sludge

A sample of the sludge from the sludge holding tank was analyzed for hydrogen sulfide gas. This procedure attempts to simulate the conditions for hydrogen sulfide production in the sludge holding tanks.

The complete procedure for gas sampling of stored sludge is described in Appendix III.

Bacterial Analysis

The bacterial analyses were performed using a portion of the sample of the wood bark obtained for moisture analysis from the pilot biofilters and the main biofilter. The bark was analyzed for bacterial enumeration, identification and biomass content.

Bacterial Enumeration

The bacterial enumeration was done by taking 25 grams of the wood bark sample and mixing with 100 ml of Super Q water in a kitchen blender. The bark was chopped up until only
small pieces of bark remained. The liquid was then decanted off and diluted in increments of $10^{-2}$, $10^{-3}$ and $10^{-4}$ with autoclaved Super Q water. A 0.5 ml sample of each of these concentrations was placed onto agar enriched plates. Samples were analyzed in triplicate, and the standard method for heterotrophic plate count was used (Bergey et al, 1994).

The complete procedure for bacterial enumeration is described in Appendix IV.

**Biomass Content by Phospholipid Analysis**

The biomass content was analyzed by a phospholipid method (Findley et al., 1989). There were no published methods for analyzing bacterial biomass in wood bark. The method below is a modification of Findley’s method that was developed to analyze the wood bark. The modified procedure follows.

The blended sample represents the background phospholipid present in the wood bark. The unblended sample contains both the biofilm and the background phospholipid. The difference between the two is the biofilm phospholipid. The bacterial biomass can be compared between samples and for different sampling dates.

The actual biomass can not be determined without further study. This study would require the use of a medium other than wood. This medium would allow measurement of the biomass without a background phospholipid reading.

The complete procedure for measuring biomass content using the modified phospholipid analysis method is described in Appendix V.

**BET Analysis**

The BET analysis was used to determine the surface area per gram of dry wood bark. The surface area of the wood bark is important for the quantification of the biomass determined by the
phospholipid analysis. The BET method uses molecular nitrogen to adhere to the surface and pores of the wood bark sample. The Monosorb Instrument measures the change in thermal conductivity of a flowing mixture of an absorbate, the bark sample and the inert carrier gas. The carrier gas used is a mixture of nitrogen and helium. A single layer of molecules is assumed to adhere to the surface of the sample.

The correlation between surface area of the sample and volume of gas absorbed was determined to be 2.84 square meters per cubic centimeter of nitrogen absorbed.

The BET equation that is the basis for this method is:

\[
\frac{1}{X[(P_0/P) - 1]} = \frac{1}{X_mC} + \left(\frac{C - 1}{X_mC}\right) \times \left(\frac{P}{P_0}\right)
\]

Where

\( P = \) the partial pressure of the adsorbate.

\( P_0 = \) the saturation equilibrium vapor pressure of the adsorbate at the temperature of the coolant bath.

\( X = \) weight of adsorbate at a given relative pressure, \( P/P_0 \).

\( X_m = \) Weight of adsorbate required to cover the surface with one molecular layer.

\( C = \) a constant which is a function of the adsorbate-adsorbent interaction energy.

Using Nitrogen as adsorbate at 22° C and 1.0 atmosphere, the equation simplifies to give a resulting 2.84 m² of surface area for each cm³ of gas adsorbed.
The complete procedure for determining the surface area of the wood bark using the BET method is described in Appendix VI. For more information on the BET theory, see the Monosorb Owner’s Manual.

**Bacterial Identification Using Biolog Plates**

Biolog plates are used to identify individual bacterial colonies. This procedure requires a fresh, isolated bacterial colony of the sample to be analyzed. There are different Biolog plates for gram positive and gram negative types of bacteria. Therefore, the bacteria must first be identified as gram positive or gram negative. The complete procedure for identifying bacteria using Biolog Plates is described in Appendix VII.

**The Streak Plate Method**

The Biolog plate method requires a homogenous colony of a single bacterial species. The streak plate method produces individual homogenous bacterial colonies. The complete streak plate procedure for isolating bacterial colonies is described in Appendix VIII.

**Gram’s Test**

The Biolog plates used for gram-positive bacteria are different from the Biolog plates used for gram negative bacteria. Gram’s Test is used to determine whether a bacterium is gram positive or gram negative. The complete procedure for performing Gram’s test is described in Appendix IX.
EXPERIMENTAL RESULTS

The complete step by step experimental methods for the following results are found in Appendix I through Appendix IX.

Biological Analysis

The biological analysis was performed on samples of the wood bark from the Polk Run pilot biofilters and the main biofilter. Sample ports were constructed in the sides of the pilot biofilter to allow for the sampling at different levels: bottom (inlet), middle, and top (outlet). Samples from the pilot biofilter were taken at all three locations over a period of 5 months. The samples from the main biofilter were taken at the top, and approximately two feet below the surface. This was done by digging into the biofilter with a pitchfork.

The bark was sampled for moisture content, bacterial enumeration, and biomass content.

The moisture content was performed by weighing a sample of the wood bark. The bark was then placed in a 100° C oven for a minimum of 24 hours and then reweighed. The result is the % moisture by weight of the wood bark. The results are presented in Figure 4. The moisture content of the biofilter was in the desired range of 40% to 60% for most of the samples shown.

The chart shows that there were a few isolated incidents where the wood bark moisture content dropped below desired levels. On September 11, 1995, the ambient temperature was near 35 degrees C with low humidity. The top layers of the biofilter dried out and resulted in a wood
bark moisture content of 10% for the main biofilter and 25% for the pilot biofilter, and the odor near the biofilter increased. Water was added to the influent gas stream. On September 22, 1995, the wood bark moisture content increased to 51% for the main biofilter top area and 36% for the pilot biofilter top area. The water flow was reduced again on October 2, 1995; the moisture content decreased to 21% for the main biofilter top layer and 16% for the pilot biofilter top layer, and the odor near the biofilter increased. The water flow was adjusted and the ambient temperatures decreased. On October 9, 1995, the moisture content of the wood bark stabilized to 48 to 66% throughout both biofilters.

The bacterial enumeration was done by taking a separate wood bark sample and mixing it with 100 ml of Super Q water in a blender. The bark was chopped up until only small pieces of bark remained. The liquid was then decanted off and diluted in increments of $10^{-2}$, $10^{-3}$ and $10^{-4}$. The standard method for heterotrophic plate count using plate count agar was used (Bergey et al, 1994). The results for the bacterial sampling are presented in Figure 7. As the figures show, there does not seem to be any correlation between the bacterial count and the moisture content of the wood bark. This data is suspect, though, since fungal growth could be causing interference with the true bacterial counts.

The data shown in Figure 7 shows that the bacterial count did not vary by height in the biofilter. Also, when compared to the data shown in Figure 4, the bacterial count was not affected by the moisture content, as long as there was enough moisture present to maintain survivability.

Figure 7 also shows that there does seem to be a seasonal variation in the bacterial count. A variation might be expected since warmer temperatures would normally result in a higher bacterial population. The large bacterial counts on November 6 correlated with the samples taken from the pilot biofilter. This was one of the first hard frosts after several days of very cold
weather. The high counts were near the inlet from the gas stream (bottom and middle). There was frost present on the surface of the main biofilter, but not on the pilot biofilter. This is because the main biofilter is somewhat more exposed due to its larger surface area. It is possible that the frost on the main biofilter may have caused more resistance to airflow, causing an increase in airflow to the pilot biofilter. Further sampling may either prove or disprove this theory.

Figure 4
Moisture Content of Wood Bark in the Biofilter
Figure 5
Moisture Content of Wood Bark in the Biofilter for Top of Biofilter Sampling Locations

Figure 6
Moisture Content of Wood Bark in the Biofilter for Middle of Biofilter Sampling Locations

Moisture Content of Wood Bark for Top Biofilter Samples Only

Moisture Content of Wood Bark for Middle Biofilter Sampling Locations Only
The biomass content was analyzed by a lipid phosphate method. The portion of the solid bark left in the blender after decanting the liquid off was used for the analysis. The method was developed by Findly \textit{et al.} (1989) and was modified to analyze the wood bark. The modified procedure is detailed in the Experimental Methods section. The results of the lipid phosphate analysis are shown in Figure 8. The blended sample represents the background phospholipid

\textbf{Figure 7}

\textit{Bacterial Content of Wood Bark in the Biofilter}
present in the wood bark. The unblended sample contains both the biofilm and the background phospholipid. The difference between the two is the biofilm phospholipid.

Figures 9 shows the input screen for the UV-VIS spectrophotometry analysis for the phospholipid standard. The wavelength used for calibration was 610 nm. Figure 10 shows the absorbance spectrum for the different standards. Note the family of curves at the 610 nm wavelength. Figure 11 shows the resulting plotted calibration curve of absorbance at 610 nm for lipid phosphate concentrations of 10 nmol to 100 nmol. Figure 12 is included to show the last page of the calibration output file.

Figures 13 and 14 show the absorbance of the samples from the lipid phosphate derived from samples from the wood bark. The following is the identification of the samples:

- **MM1A**: Main biofilter middle location, Sample 1A, wood bark background
- **MM1B**: Main biofilter middle location, Sample 1B, wood bark background
- **MM2A**: Main biofilter middle location, Sample 2A, wood bark background
- **MM2B**: Main biofilter middle location, Sample 2B, wood bark background
- **MMD1A**: Main biofilter middle location, Sample 1A, wood bark background with lipid absorbance
- **MMD1B**: Main biofilter middle location, Sample 1B, wood bark background with lipid absorbance
- **MMD2A**: Main biofilter middle location, Sample 2A, wood bark background with lipid absorbance
- **MMD2B**: Main biofilter middle location, Sample 2B, wood bark background with lipid absorbance
The identification of the samples from the pilot biofilter have a similar identification, except the MM is replaced with PM. The difference between the wood background with lipid absorbance and the wood background absorbance is the lipid absorbance. These results of the phospholipid analysis for the samples from the biofilter are shown in Figure 8.

Figure 8
Phospholipid Results
Figure 9
Page 1 of Output From UV-VIS Spectrophotometry of Phospholipid Standard
Figure 10
Page 2 of Output From UV-VIS Spectrophotometry of Phospholipid Standard
Figure 11
Page 3 of Output From UV-VIS Spectrophotometry of Phospholipid Standard

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Figure 12
Page 4 of Output From UV-VIS Spectrophotometry of Phospholipid Standard
Figure 13

Page 1 of Output From UV-VIS Spectrophotometry of Phospholipid Sample

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Figure 14
Page 2 of Output From UV-VIS Spectrophotometry of Phospholipid Sample

BET Analysis to Determine Surface Area of Wood Bark

The chart shown in Table 2 shows the results of the BET analysis for the wood bark. The analysis was performed using the procedure outlined in the Methods Chapter. The results of the phospholipid analysis from Figure 8 are combined with the results from Table 2 to produce Figure 15. This figure illustrates that the amount of biofilm per square meter of surface area of wood bark
was much higher in the pilot biofilter than the main biofilter. This result is in agreement with the results of the bacterial enumeration. Also, the moisture content of the pilot biofilter was usually higher than the moisture content in the main biofilter. The best conditions for bacterial growth are achieved at higher moisture content.

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<td>SAMPLE TUBE USED</td>
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<td>WEIGHT OF EMPTY TUBE (GRAMS)</td>
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<td>WEIGHT OF TUBE AND SAMPLE (GRAMS)</td>
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<td>WEIGHT OF SAMPLE</td>
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<td>DESORB READING</td>
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Table 2
Results of BET Analysis for Wood Bark
**Bacterial Identification Using Biolog Plates**

**Bacteria**

The bacterial samples were incubated and the resulting colonies had two distinct appearances. These bacterial samples were then isolated. Samples of each colony were transferred to microscope slides and Gram’s test was performed. The bacteria were determined to be Gram Negative. Biolog analysis was performed to identify the colonies. The plates were analyzed in duplicate and the species were identified as *Pseudomonas aeruginosa* and *Salmonella*. *Salmonella* is a bacteria that is known to be a producer of hydrogen sulfide gas (Bergey et al, 1994).

The results of the Biolog analysis are shown in Figures 16 through 19. The species identified by the arrow is the most likely specie
MICROLOG (TM) 3N RELEASE 3.51

Date : 10/09/97
Hour : 24
Plate Type : GN
Media Type : TSA/BUGM
Plate # : 1
Strain Name : 3A
Strain # : ?
Other Info : ?
Input Mode : Reader : MOLECULAR DEVICES Vmax, Uvmax, Tmax
Data Base : MicroLog GN

POSITIVE/NEGATIVE DATA

XXX = percent change in optical density versus A1 control well
<XXX> = positive, {XXX} = borderline, XXX = negative
-XXX = percent change negative
XXX+ = data negative or borderline, “⇒“ ID choice positive > 90% of time
XXX- = data positive or borderline, “⇒“ ID choice positive < 10% of time

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BIO-NUMBER : 3777-7777-7777-7777-7777-7777-7777-7777

SPECIES IDENTIFICATION : SALMONELLA SUBSPECIES 1 G
*confirm by serology*

CLOSEST SPECIES : ..........................................................SIM......DIST.....AVG..... MAX
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1) SALMONELLA SUBSPECIES 1 G 0.527 6.311 1.646 5.494
2) ENTEROBACTER GEROVIAE 0.035 7.205 0.979 4.494
3) BURKHOLDERIA CEPACIA 0.027 7.292 1.375 3.713
4) SERRATIA MARCESCENS 0.000 8.641 1.781 5.031
5) SERRATIA FONTICOLA 0.000 10.962 0.938 5.381
6) BURKHOLDERIA GLADIOLI 0.000 11.023 0.750 4.963
7) SERRATIA FICARIA 0.000 11.376 0.625 2.088
8) PSEUDOMONAS PYRRACINIA 0.000 12.241 0.063 0.119
9) KLEBSIELLA PLANTICOLA/ORNITHINOLYTICA 0.000 13.335 0.738 3.306
10) SERRATIA RUBIDAŒA 0.000 13.517 1.219 3.619
other : --- --- --- ---

Figure 16

Plate #1 Strain #3A Identified as Salmonella
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Hour: 24  
Plate Type: GN  
Media Type: TSA/BUGM  
Plate #: 2  
Strain Name: 3A  
Strain #: ?  
Other Info: ?  
Input Mode: Reader: MOLECULAR DEVICES  
Data Base: MicroLog GN

**POSITIVE/NEGATIVE DATA**

- ***XXX*** = percent change in optical density versus A1 control well  
- <***XXX***> = positive, {***XXX***} = borderline, ***XXX*** = negative  
- -***XXX*** = percent change negative

- ***XXX+*** = data negative or borderline, “⇒” ID choice positive > 90% of time
- ***XXX-*** = data positive or borderline, “⇒” ID choice positive < 10% of time

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**BIO-NUMBER**: 3777-7777-7777-7777-7777-7777-7777-7777

**SPECIES IDENTIFICATION**: SALMONELLA SUBSPECIES 1 G  
*confirm by serology*

**CLOSEST SPECIES**

1. SALMONELLA SUBSPECIES 1 G  
2. ENTEROBACTER GERGOVIAE  
3. BURKHOLDERIA CEPAICIA  
4. SERRATIA MARCESCENS  
5. SERRATIA FONTICOLA  
6. BURKHOLDERIA GLADIOLI  
7. SERRATIA FICARIA  
8. PSEUDOMONAS PYRRACINIA  
9. KLEBSIELLA PLANTICOLA/ORNITHINOLYTIKA  
10. SERRATIA RUBIDAEA  

**Figure 17**

**Plate #2 Strain #3A Identified as Salmonella**
Date : 10/09/97
Hour : 24
Plate Type : GN
Media Type : TSA/BUGM
Plate # : 1
Strain Name : 3B
Strain # : ?
Other Info : ?
Input Mode : Reader : MOLECULAR DEVICES Vmax, U vmax, Tmax
Data Base : MicroLog GN

POSITIVE/NEGATIVE DATA
XXX = percent change in optical density versus A1 control well
<XXX> = positive, {XXX} = borderline, XXX = negative
-XXX = percent change negative
XXX+ = data negative or borderline, “⇒” ID choice positive > 90% of time
XXX- = data positive or borderline, “⇒” ID choice positive < 10% of time

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BIO-NUMBER : 0326-2103-0023-7442-6573-6735-7553-7150

SPECIES IDENTIFICATION : PSEUDOMONAS AERUGINOSA

CLOSEST SPECIES : ........................................................................SIM.....DIST.....AVG..... MAX
⇒ 1) PSEUDOMONAS AERUGINOSA 0.754 3.719 0.797 3.456
2) PSEUDOMONAS FLUORENSCENS TYPE C 0.000 6.897 0.875 4.137
3) PSEUDOMONAS PUTIDA TYPE B1 0.000 9.544 0.604 1.319
4) PSEUDOMONAS CHLORORAPHIS (FLUOR. TYPE D) 0.000 9.589 0.081 1.419
5) PSEUDOMONAS VIRIDILIVIDA 0.000 9.706 0.083 0.950
6) PSEUDOMONAS CITRONELLOLIS 0.000 9.725 0.076 1.306
7) PSEUDOMONAS FUSCOVAGINAE 0.000 10.064 0.438 2.162
8) PSEUDOMONAS PUTIDA TYPE A2 0.000 10.249 0.109 0.076
9) PSEUDOMONAS CORRUGATA 0.000 10.404 0.211 2.747
10) PSEUDOMONAS AURANTIACA 0.000 10.887 0.438 2.894
other :

Figure 18
Plate #1 Strain #3A Identified as Pseudomonas Aeruginosa
Date : 10/09/97
Hour : 24
Plate Type : GN
Media Type : TSA/BUGM
Plate # : 2
Strain Name : 3B
Strain # : 
Other Info : 
Input Mode : Reader : MOLECULAR DEVICES Vmax, Uvmax, Tmax
Data Base : MicroLog GN

POSITIVE/NEGATIVE DATA
XXX = percent change in optical density versus A1 control well
<XXX> = positive, [XXX] = borderline, XXX = negative
-XXX = percent change negative
XXX+ = data negative or borderline, “⇒” ID choice positive > 90% of time
XXX- = data positive or borderline, “⇒” ID choice positive < 10% of time

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BIO-NUMBER : 0326-2103-0023-7442-6573-6737-7553-7151

SPECIES IDENTIFICATION : PSEUDOMONAS AERUGINOSA

CLOSEST SPECIES :..................................................SIM.....DIST.....AVG..... MAX
⇒ 1) PSEUDOMONAS AERUGINOSA 0.629 5.719 0.797 3.456
  2) PSEUDOMONAS FLUORENSCENS TYPE C 0.001 7.922 0.875 4.137
  3) PSEUDOMONAS FUSCOVAGINAE 0.000 9.848 0.438 2.162
  4) PSEUDOMONAS SYNXANTHA 0.000 11.228 0.116 0.723
  5) PSEUDOMONAS AURANTIACA 0.000 11.481 0.508 2.894
  6) PSEUDOMONAS CITRONELLOLIS 0.000 11.511 0.076 1.306
  7) PSEUDOMONAS CHLORORAPHIS (FLUOR. TYPE D) 0.000 11.597 0.081 1.419
  8) PSEUDOMONAS VIRIDILIVIDA 0.000 11.706 0.096 0.950
  9) PSEUDOMONAS AUREOFACIENS (FLUOR. TYPE E) 0.000 12.180 0.081 2.419
10) PSEUDOMONAS PUTIDA TYPE B1 0.000 12.254 0.604 1.319
other :

![Figure 19](Plate #2 Strain #3A Identified as Pseudomonas Aeruginosa)

Figure 19

Plate #2 Strain #3A Identified as Pseudomonas Aeruginosa
DISCUSSION AND CONCLUSIONS

Air emission of volatile compounds originating from wastewater treatment plants are currently not currently regulated, but the odors caused by these emissions can result in complaints from neighbors. High concentrations of hydrogen sulfide gas are usually responsible for these odors. This gas is commonly found near sludge handling and storage areas at wastewater treatment plants. The source of the sulfur in the wastewater is primarily from the breakdown of plant and animal proteins by the human body to form urine, which contains a high concentration of the sulfate ion ($\text{SO}_4^{2-}$). The hydrogen sulfide gas originates from the reduction of the sulfate ions in the wastewater by bacteria under anaerobic conditions. At the Polk Run wastewater treatment plant, the sludge holding tanks are the main source of the hydrogen sulfide gas. A biofilter can be an efficient method to control odors caused by hydrogen sulfide gas.

Sampling of the gas at the inlet and the outlet of the biofilter was performed when the biofilter was operating efficiently and no perceptible odor was detected at the exit of the biofilter. The concentration of hydrogen sulfide gas was measured using Draeger tubes. This method confirmed that the biofilter reduced the concentration of hydrogen sulfide from 0.01 to 0.02 parts per million (ppm) at the inlet to less than the perceptible odor threshold. This threshold is recognized to be approximately 0.47 parts per billion, (ppb). The measurements of the hydrogen sulfide gas at the inlet and exit of the biofilter confirm that an efficiently operating biofilter is capable of an approximately 95% reduction in the concentration of hydrogen sulfide.

The theory of the operation of a wood bark biofilter is that the wood bark absorbs the compound, and then the bacteria in the wood oxidize the pollutant. These bacteria control the odor by reducing the concentration of the hydrogen sulfide gas.
Wood bark samples were taken from the Polk Run biofilter for bacterial analyses. The bacteria were transferred to petri dishes containing a growth medium using the hot wire method. This process was used to identify and enumerate the bacteria. After incubating these samples, the bacteria were overgrown by a fungus. This fungus inhibited the growth of the bacteria, so a fungicide was added to the agar. The bacterial identification and enumeration was then performed.

The samples indicated that the bacteria were approximately evenly distributed throughout the biofilter. The most prevalent bacteria found during the investigation of the biofilter were identified to be *Pseudomonas aeruginosa* and *Salmonella*. These bacteria are naturally occurring and are commonly found in wastewater sludge. The *Pseudomonas aeruginosa* is a denitrifying bacteria and is commonly found in wastewater sludge (Drysdale, 1999). *Salmonella* is a producer of hydrogen sulfide (Bergey, et al 1994). Further study indicates that the fungus may be responsible for breaking down hydrogen sulfide (Singhal et al, 1996).

The bacteria found in the biofilter grew best under conditions that included high humidity and warm temperatures.

Phospholipid analysis was performed on the wood bark sample from the biofilter. This analysis was done to determine the biomass of the bacteria in the biofilter. Biomass is an indication of the viability of the bacteria in the biofilter. The same procedure was also performed on the same wood bark sample from the biofilter that was placed in an oven at 100 to 110 degrees C for a minimum of 24 hours. The heating of the wood bark was done to kill the bacteria present in the wood bark.

Both wood bark samples indicated positive phospholipid content. This analysis determined that the wood bark interfered with the phospholipid analysis of the bacteria. Although further
analysis is required, it is believed that the estimated biomass of the bacteria is equal to the total biomass minus the biomass measured from the wood bark that was heated for a minimum of 24 hours. This hypothesis can be confirmed by further study using an inert medium for the biofilter other than wood bark.

This study confirmed that a biofilter is an effective method for controlling odors and emission of volatile compounds from a sludge holding tank at a municipal wastewater treatment plant. The main gaseous emission identified and the source of the predominant odors was determined to be hydrogen sulfide gas. The bacteria and fungi found in the biofilter controlled this odor by reducing the concentration of hydrogen sulfide gas. The bacteria found in the biofilter were identified as *Pseudomonas aeruginosa* and *Salmonella*. These bacteria are commonly found in wastewater.

The wood bark in the biofilter provided the medium for bacterial and fungal growth. The optimum conditions for this biofilter were found to include temperatures from 20 to 35 degrees C and relative humidity of 90 to 95%. Under these conditions, an approximately 95% reduction in the concentration of hydrogen sulfide gas was achieved.
REFERENCES


Cox, J. P. *Odor Control and Olfaction*, 1975, Pollution Sciences. Lynden, WA


Vatavuk, W. M. *Estimating Costs of Air Pollution Control*, 1990, Lewis. Chelsea, MI


Water Pollution Control Federation (1990) *The Operation of Municipal Wastewater Treatment Plants, Manual of Practice No. 11*. Water Pollution Control Federation, Alexandria, VA
APPENDIX I

Wood Bark Sampling and Analysis

Tools Required:

(1) Pitchfork

(2) Quart Size Ziploc Bags

(1) Portable Temperature/Humidity Sampler (a Cole Parmer Tri Sense Meter Model 37000-00 with Air Velocity/Temperature Probe Model 37000-60 and Relative Humidity/Temperature Probe Model 37000-50 was used in this study)

(1) Laboratory Oven (a Fischer Scientific Isotemp Model 615G was used in this study)

(4) 200 ml Beakers

(1) Laboratory Scale (a Fischer Scientific Model S-300D was used in this study)

(1) Pair of Sterile Gloves

The sampling of the wood bark was performed at two depths in the biofilter. Start by randomly picking a location in the biofilter. With a pitchfork, dig down approximately three feet (90 cm). Take a sample at approximately six inches (15 cm) below the surface, and another sample three feet (90 cm) below the surface. Put each sample in a Ziploc style bag for moisture content analysis. Using the Temperature/Humidity probe, measure the temperature and humidity at the locations where the samples were taken. Record the readings on the sample bags. Measure the ambient temperature and relative humidity and record the readings. Measure the temperature and relative humidity of the air inlet to the biofilter and record the readings.
For the pilot biofilter, take samples of wood bark from the filter by opening the three sampling doors located on the side of the biofilter. With the pair of gloves on, reach in and take a handful of wood bark. Measure the temperature and relative humidity at the three sample locations, as explained for the main biofilter. Record all measurements.

Take the samples immediately to the lab. Do not allow the wood bark to dry out during transportation. This can be accomplished by ensuring that the Ziploc bags are sealed and that the bags remain at a temperature close to the temperature of the biofilter. If this is not possible, heat (or cool) the bags to the temperature of the biofilter in the lab by using an oven (or refrigerator). Leave the bags at this temperature for a minimum of 1 hour.

Tare a 200 ml beaker on the scale. Open the bag and add approximately half of the sample wood bark to the beaker. Weigh the bark. Repeat with the other half of the sample, and then repeat the procedure for the other samples. Place the beakers into a laboratory oven that has been set at 100 - 110° C. Leave the samples in the oven for at least 24 hours, and remove the beakers. Be careful, the beakers will be hot! Allow the beakers to return to room temperature. Weigh the beaker, record the value, then empty the wood bark, and reweigh the beaker.

\[
\text{% Moisture of the Wood Bark, by Weight} = \frac{1 - \frac{(\text{Weight of Wood Bark} - \text{Weight of Empty Beaker})}{\text{Original Weight of Wood Bark}}}{1} \times 100\%
\]
Figure 20

Sampling Hole Dug in the Biofilter

Figure 21

Bark Sample Placed in a Plastic Bag.
Figure 22

Temperature/Humidity Measurement Using Probe

Figure 23

View of a Typical Bark Sample
Figure 24

Tare the Scale with the Beaker.

Figure 25

Weight Measurement of Wood Bark Sample.
Figure 26

Oven Used to Dry Wood Bark
APPENDIX II

Gas Sampling of Biofilter

Tools Required:

Portable “Draeger (Grab) Tube Type” Sampler (a Sensidyne Model 800 was used for this study)

H₂S Grab Tubes, 0.1 - 4 ppm range (Sensidyne Model 4LT were used in this study)

Portable Temperature/Humidity/Velocity Sampler (a Cole Parmer Tri Sense Meter Model 37000-00 with Air Velocity/Temperature Probe Model 37000-60 and Relative Humidity/Temperature Probe Model 37000-50 was used in this study)

The gas is sampled at the entrance to the main biofilter at the sampling port in the PVC tubing. The sampling port consists of a 0.25 inch (6 mm) diameter hole drilled in the 4 inch (10 cm) diameter tubing. The hole is plugged by a pressure gauge. Remove the pressure gauge by turning the gauge counterclockwise. The biofilter gas will exit the port at a low pressure, but high velocity. Insert the velocity probe into the hole. Make sure the probe is oriented in the direction of gas flow. Record the reading. With two blowers operating, the air velocity should be approximately 2750 cubic feet per minute (70 cubic meters per minute). With only one of the blowers running, the air velocity should be approximately 2500 cubic feet per minute (70 cubic meters per minute), but the air pressure will be less than with two blowers running.

If the velocity is within 25% of this speed, the blowers are probably operating properly. If the air velocity is more than 25% lower than these speeds, check the blowers and valves. First check the electrical panel located by the sludge holding tank to ensure the
blowers are turned on. There is one switch for each blower. If one of the switches is locked out, do not restart it without determining why it has been locked out. A good rule of thumb is to only allow the person that locked a switch out to remove the lock.

If the switches are on and there is a low air velocity reading, there is probably a problem with the blowers or valves. Turn off both blowers before checking their operation. Check the valves. There is an indicator on top of the valve that indicates whether it is open or closed. To open the valve, turn it clockwise. To close the valve, turn it counterclockwise. If both blowers are operating, as recommended during pumping of the sludge into the tanker truck, both valves should be open. If only one blower is operating, as recommended during normal operation, only the operating blower’s valve should be open, and the other blower’s valve should be closed.

If a visual inspection of the valves does not indicate a problem, take the cover off the blowers and inspect the blower assembly. Refer to the blower maintenance manual for possible problems. If a problem with one of the blowers is discovered, lock out the switch for that blower, and close the valve. Open the valve for the other blower, and turn it on. Return to the biofilter and measure the velocity of the gas. Allow the blower to run for several minutes before beginning the sampling of the gas.

Take the portable gas sampler and one of the grab tubes. Break off both ends of the grab tube and insert the proper end into the sampler, as directed by the sampler operating manual. The sampler used was a Sensidyne Gastec Precision Gas Detector.

The H₂S concentration during normal operation is approximately 0.01 to 0.02 ppm. If the range of the sampling tube is 0.1 ppm and up, start by taking 5 pulls on the sampler.
The tube will change colors at the tip to indicate how much H$_2$S is present. Wait the number of minutes required by the manufacturer between each pull. When complete, record the reading displayed on the tube. This time period between pulls allows the chemical reaction to reach completion. If no color change is visible, take additional pulls until a color change appears. The accuracy of the reading will decrease with greater than 5 pulls.

Take the reading and divide by the number of pulls to determine the H$_2$S concentration. If the reading is greater than the maximum value on the tube, the measurement was probably taken during the transfer of sludge from the thickeners into the sludge holding tank (the reading can reach over 2 ppm). Wait a few minutes, then take a new grab tube and repeat the measurement. Record the H$_2$S reading, along with the air velocity reading.

![Figure 27](image.png)

**Figure 27**

*Electrical Control Box for the Blowers.*
Figure 28

Valve for Opening/Closing Air Supply to the Blowers.

Figure 29

View of Biofilter.

Sample Port Location For Air Inlet Behind Vertical Pipes.
Figure 30

Sample Port Location For Air Inlet to Biofilter.

Removing Pressure Gauge to Gain Access to Port.

Figure 31

Sample Port. Make Sure The Valve Is In The Open Position As Shown.
Figure 32

Air Flow Measurement of the Sample Gas.

Make Sure the Arrow on the Probe is Aligned with the Direction of Flow of the Gas.

Figure 33

Measuring H₂S using the Sampler As Specified.
Figure 34

**Color Change of Draeger Tube.**

Take the Portable Temperature/Humidity/Velocity Sampler and disconnect the velocity probe (the Temperature/Humidity probe is different from the velocity probe). After connecting the Temperature/Humidity probe, measure and record the temperature and humidity of the gas. Also measure the ambient temperature and humidity. During summer months, the gas temperature is usually lower than the ambient temperature. The opposite is true in the winter. This is because the sludge tends to be at a temperature of 65 - 70°F (18 - 22°C) year-round, and the sludge will cool the gas in the summer, and warm the gas in the winter.

The relative humidity of the gas should be 95% to 100% during most times. This will enable the wood bark to maintain sufficient moisture to promote bacterial growth. If the relative humidity is lower than 95%, the amount of water dripped into the air inlet pipes
should be increased. Do not add too much water by dripping, as it may scrub the H$_2$S from the gas (the H$_2$S gas will become dissolved in the water) and short-circuit the biofilter, exiting with the water at the drain. If the wood bark has dried out to below 40% moisture by weight (see wood bark sampling procedure), water may need to be sprayed onto the top of the biofilter. The spraying should be done in such a manner as to create a light mist. A garden sprinkler set on a diffused spray or a soaker hose works well. Do not over-water, as it may clog the filter.

![Image of measurement equipment]

**Figure 35**

Measurement of Temperature and Humidity of the Influent Air.
Figure 36

Closing the Sampling Port After Measurement.
A sample of the sludge from the sludge holding tank was analyzed for hydrogen sulfide gas. The procedure explained below attempts to simulate the conditions for hydrogen sulfide production in the sludge holding tanks.

Tools Required:

- Portable “Draeger (Grab) Tube Type” Sampler (a Sensidyne Model 800 was used in this study)
- (4) H₂S Grab Tubes, 0.1 - 4 ppm range (a Sensidyne Model 4LT was used in this study)
- One Gallon Plastic Jar with Lid

1. Obtain a fresh sample of sludge from the sludge holding tank and place in the one-gallon plastic jar. This liquid sample should fill approximately half of the container.

2. Cover the container.

3. Open the jar and leave the lid off for two minutes.

4. Take a gas sample with the grab tube sampler as described previously for the inlet gas sampling. This sample should represent the steady state liberation of hydrogen sulfide from the sludge holding tanks that are the source of the influent air to the biofilter.

5. Swirl the sludge around in the jar for approximately ten seconds. Take a gas sample with the grab tube sampler. This sample should represent the liberation of hydrogen sulfide from the sludge holding tanks during transfer of the sludge from the thickeners to the sludge holding tanks.
6. Let the jar sit undisturbed for two minutes. Take a gas sample with the grab tube sampler. This sample reading should decrease with time until the reading reaches the steady state value.

This gas sampling should be performed in a well ventilated area or under a hood. Shaking the jar for one minute can produce a level of hydrogen sulfide in excess of 120 parts per million. These levels can cause nausea, and light headed sensations.
APPENDIX IV

Bacterial Enumeration

The bacterial analyses were performed by taking a portion of the sample of the wood bark obtained for moisture analysis from the pilot biofilters and the main biofilter.

The bark was analyzed for bacterial enumeration, identification and biomass content.

Tools Required:

(1) Incubating oven set at 35°C (a Boekel Industries Inc. Model 133000 was used in this study)

(40) Plastic Petri Dishes

(25) Grams Plate Count Agar Mix

1 mg Cyclohexamide per gram solution used as an Antifungal Agent

Approximately 3 Liters Super Q Water

(1) Hot Plate with Magnetic Stirrer (a Thermodyne Model SP46925 was used in this study)

(1) Blender (a Osterizer Model Cycle Blend was used in this study)

(1) Autoclave (a Amsco Model 3021 was used in this study)

(1) 3 Liter Flask

(1) Scale (a Fischer Scientific Model S-300d was used in this study)

(1) Pair Heat Resistant Gloves

(1) Bunsen Burner

(1) Bent Glass Stir Stick (“Hockey Stick”)

(25) ml Methanol
Prepare one liter of Plate Count Agar per the manufacturer’s instructions. This is done by adding approximately 25 grams of the agar mix to one liter of Super Q water in the three-liter flask. Heat the flask on the hot plate until it just reaches boiling while continuously stirring the mixture with a magnetic stirrer. Be very careful, as the solution has a tendency to boil over. Add approximately 1µg cyclohexamine for each ml of solution. Continue to stir the solution for a minimum of one minute. Place the flask in an autoclave and heat for a minimum of 30 minutes at 121°C and 17 psi. Remove the flask and allow it to cool only to the point where the flask can be handled safely with the pair of heat resistant gloves. Pour the agar mixture into the forty plates. Allow the plates to cool (and the agar to solidify) before continuing onto the next step of the bacterial analysis.

The bacterial enumeration was done by taking a 25 grams of the wood bark sample and mixing with 100 ml of Super Q water in a kitchen blender. The bark was chopped up until only small pieces of bark remained. The liquid was then decanted off and diluted in increments of $10^{-2}$, $10^{-3}$ and $10^{-4}$ with autoclaved Super Q water. A 0.5 ml sample was placed into each plate. Samples were analyzed in triplicate, and the standard method for heterotrophic plate count was used (Bergey et al, 1994).
Figure 37
Placement of Wood Bark in Blender.

Figure 38
Using Blender to Reduce Bark Pieces to Less Than 1/4” Long.
Figure 39
Decant Off the Liquid.

Figure 40
Bacterial Growth After Incubation for 24 Hours.
APPENDIX V

Biomass Content by Phospholipid Analysis

Tools Required:

(1) Blender (an Osterizer Model Cycle Blend was used in this study)
Approximately 0.2 Liters Super Q Water
(1) 3 Liter Flask
(1) Laboratory Scale (a Fischer Scientific Model S-300D was used in this study)
(5) ml Chloroform
(5) ml Methanol
(2.5) ml 0.0306 M H$_2$SO$_4$
(10) Disposable pipettes
(20) 5 ml Wheaton Ampoules
Ultra Pure Nitrogen gas
(20) ml Persulfate Digestion Reagent (5% potassium persulfate in 0.36 M H$_2$SO$_4$)
(1) Fischer Burner
(1) Glass Pulling Pliers
(1) Oven set at 105° C (a Fischer Scientific Isotemp Model 615G was used in this study)
(5) ml Ammonium Molybdate Reagent (2.5% Ammonium Molybdate in 5.72 N H$_2$SO$_4$)
(18) ml Malachite Green Reagent (0.111% Polyvinyl Alcohol and 0.011% Malachite Green)
(520) micro liters 0.05 M K$_2$HPO$_4$
(1) UV-VIS Spectrophotometer capable of analysis at 610 nm (a Hewlett Packard Model 8453 Spectrophotometer with Hewlett Packard VL Series 3 Computer was used in this study)

The biomass content was analyzed by a phospholipid method (Findley et al., 1989). There are no published methods for analyzing bacterial biomass in wood bark. The method below is a modification of Findley’s method that was developed to analyze the wood bark. The modified procedure follows.

1. Take samples of wood bark from the pilot biofilter. Try to get small pieces to facilitate the extraction.

2. Add approximately 25 g of wood bark to 100 ml of Super Q water.

3. Use a blender to thoroughly mix the wood and water, and to grind up the wood pieces.

4. Decant off the liquid.

5. Add approximately 0.5 g of the blended solid to 2 ml Super Q water. In a separate container, add approximately 0.5 g of the unblended wood bark to 2 ml Super Q water.

The following steps are performed separately on each sample.

6. Add 2.5 ml chloroform and 5 ml methanol.

7. Shake bottles to thoroughly mix and let sit for at least 2 hours.

8. Add 2.5 ml chloroform and 2.5 ml 0.0306 M H₂SO₄.

9. Shake solution and let sit for at least 18 hours.

10. Using a disposable pipette, remove the chloroform layer and place it in a 5 ml wheaton ampoule.
11. Dry off the chloroform with nitrogen gas.

12. Add 0.9 ml persulfate digestion reagent (5% potassium persulfate in 0.36 M H₂SO₄).

13. Heat the tip of the ampoule in the flame of a Fischer Burner. When the glass is glowing red, pull the tip with the glass pulling pliers. Twist and fold over the tip to seal the ampoule. Heat the sealed ampoule for at least 18 hours at 105° C.

14. Let the ampoules cool to room temperature. Break open the ampoule and add 0.2 ml ammonium molybdate reagent (2.5% ammonium molybdate in 5.72 N H₂SO₄).

15. Let the ampoules sit for 10 minutes then add 0.9 ml malachite green reagent (0.111% polyvinyl alcohol and 0.011% malachite green). Wait 30 minutes before analyzing samples.

16. Analyze samples spectrophotometrically at 610 nm using a 6 point standard curve in duplicate. The standard curve is produced using ampoules of the following amounts of K₂HPO₄: 0 nmol, 5 nmol, 10 nmol, 25 nmol, 40 nmol and 50 nmol (this is 10, 20, 50, 80, 100 micro liters of the standard stock solution).

The blended sample represents the background phospholipid present in the wood bark. The unblended sample contains both the biofilm and the background phospholipid. The difference between the two is the biofilm phospholipid. The bacterial biomass can be compared between samples and for different sampling dates.

The actual biomass can not be determined without further study. This study would require the use of a medium other than wood. This medium would allow measurement of the biomass without a background phospholipid reading.
Figure 41

Wood Bark Sample and the Decanted Liquid After Blending.

Figure 42

Resulting Wheaton Ampoule After Addition of Malichite Green.

The Darker the Sample, The Higher Concentration of Phospholipids
APPENDIX VI

Wood Bark Surface Area Measurement Using BET Method

Tools Required:

BET Analyzer (a Monosorb Model MS-12 Manufactured by Quantachrome was used in this study)

30 Mole % Nitrogen in Helium

Scale with capability of reading to 0.0001 g (a Mettler Model AE 200 was used in this study)

Dewer Flask

Stainless Steel Thermos Container for Liquid Nitrogen

Approximately 500 ml Liquid Nitrogen

(1) Pair of Protective Gloves

(1) 250 ml Beaker filled with tap water.

The BET analysis was used to determine the surface area per gram of dry wood bark. The surface area of the wood bark is important for the quantification of the biomass determined by the phospholipid analysis. The BET method uses molecular nitrogen to adhere to the surface and pores of the wood bark sample. The Monosorb Instrument measures the change in thermal conductivity of a flowing mixture of an absorbate, the bark
sample and the inert carrier gas. The carrier gas used is a mixture of nitrogen and helium. A single layer of molecules adheres to the surface of the sample.

The correlation between surface area of the sample and volume of gas absorbed was determined to be 2.84 square meters per cubic centimeter of nitrogen absorbed. The following is the procedure used to determine the surface area.

The BET equation that is the basis for this method is:

\[
\frac{1}{X [(P_0/P) - 1]} = \frac{1}{X_m C} + \left( \frac{C - 1}{X_m C} \right) \left( \frac{P}{P_0} \right)
\]

where

\( P = \) the partial pressure of the adsorbate.

\( P_0 = \) the saturation equilibrium vapor pressure of the adsorbate at the temperature of the coolant bath.

\( X = \) weight of adsorbate at a given relative pressure, \( P/P_0 \).

\( X_m = \) Weight of adsorbate required to cover the surface with one molecular layer.

\( C = \) a constant which is a function of the adsorbate-adsorbent interaction energy.

Using Nitrogen as adsorbate at 22°C and 1.0 atmosphere, the equation simplifies to give a resulting 2.84 m² of surface area for each cm³ of gas adsorbed.

See the Monosorb Owner’s Manual for more information.
1. Calibrate the instrument per the manufacturer’s instructions. After the instrument is calibrated, do not adjust the gas flow, threshold sensitivity, or count switch. Adjusting them will give inaccurate results.

2. Weigh the empty glass tube that will be used for sampling.

3. Load the sample into the glass tube. The bulk solid sample cell works best. The largest particle size must be less than 7 mm. To fit in the glass tube, the sample must be broken into very small pieces. The wood bark must be thoroughly dried. This can be done by heating in an oven set to 105°C for 24 hours or longer.

4. Place the glass tube in the “OUTGAS” station. Clamp the heating mantle around the tube and plug the leads and the thermocouple. Set the temperature control to 150°C. Heat the sample for a minimum of 15-30 minutes.

5. Fill the Dewer flask with liquid nitrogen.

6. Press ADS (adsorb) and zero the signal meter to ±0.000 and zero the surface area meter.

7. Raise the Dewer flask until the liquid nitrogen is within 0.5” of the top of the arms of the glass tube cell. Clamp the Dewer flask into place.

8. Wait for the Signal Meter to return to zero. Record the SA count and the ADS #.

9. Press DES (desorb) reset the Signal Meter to zero and reset the SA counter.

10. Remove the Dewer flask and cover it to minimize the evaporation of the liquid nitrogen.
11. Raise the beaker of room temperature water so that the water is within 0.5” of the top of the glass cell.

12. Wait for the signal meter to return to zero. Record the SA count and the DES #.

13. Remove the beaker and repeat the procedure starting with step 6. Continue repeating the procedure until two consecutive DES #’s are the same. Three to six cycles are usually required to complete this procedure.

14. Carefully dry off the glass tube. Weigh the glass tube with the sample. The difference between this weight and the weight from step 2 is the sample weight.

15. Divide the SA count by the sample weight. This is the surface area measurement.

![Figure 43](image)

**Figure 43**

**Monosorb Model MS-12 BET Analyzer**
Figure 44
U-Shaped Sample Cell
APPENDIX VII

Bacterial Identification Using Biolog Plates

Biolog plates are used to identify individual bacterial colonies. This procedure requires a fresh, isolated bacterial colony of the sample to be analyzed. There are different Biolog plates for gram positive and gram negative types of bacteria. Therefore, the bacteria must also be identified as gram positive or gram negative. The following procedures outline the methods used to isolate the bacteria into homogenous colonies, determine whether the colonies are gram positive or gram negative, and perform the Biolog analysis.

Tools Required: (The following quantities are for identification of one unique colony.)

(1) Incubating oven set at 35°C (a Boekel Industries Inc. Model 133000 was used in this study)

(1) Bunsen Burner

(1) 5 ml Pipette with Autoclaved Disposable Tips

(2) Autoclaved 25 ml Capped Test Tubes

Approximately 50 ml Autoclaved Super Q Water with .85% by Weight NaCl

Clean Wipes

Turbidometer (a Bausch & Lomb Spectronic 70 was used in this study)

Biolog Standards for Turbidity

Approximately 5 Autoclaved Cotton Tipped Swabs

Petri Dishes with Isolated Bacterial Colonies from the Streak Plate Method

(1) Test Tube Vortex Mixer (a LabLine Model 1290 was used in this study)

(1) Eight Channel Pipetter with Autoclaved Disposable Tips
(1) Autoclaved Reservoir for the Eight Channel Pipette

(2) Biolog Plates

Note: During this procedure, pass all containers through the Bunsen burner flame prior to opening or closing. This is considered good sterile technique, and helps prevent contamination of the sample.

1. Fill the test tubes with 20 ml of the autoclaved saline solution. One of the test tubes will be the blank, while the other will be used for the bacterial sample.

2. Turn on the turbidometer and allow to warm up for approximately 15 minutes. Set the meter at approximately 490 nm.

3. Place the blank in the turbidometer and zero it to 100% transmittance.

4. Remove the blank, and place one of the Biolog standards in the turbidometer. Record the reading and place the other Biolog standard in the turbidometer. Record the reading and replace the blank in the turbidometer. The reading should return to 100% transmittance. If the meter does not measure 100% transmittance, repeat the procedure. The bacterial sample required for the Biolog analysis must have a turbidity reading between the two Biolog standards.

5. Take the petri dish with isolated bacterial colonies obtained from the streak plate method. Take a cotton swab and wet it with the saline solution from the open sample test tube.

6. Gently roll the swab on a bacterial colony on the petri dish. Do not get any of the agar on the tip of the swab.
7. Place the swab in the test tube just above the water line and press the tip of the swab against the side of the test tube. This helps break up the clumps of the bacterial colony. Swish the swab around in the solution to remove all of the bacteria.

8. Remove the swab and discard.

9. Cap the test tube, and place in a vortex mixer to stir up the solution.

10. Allow the liquid to stop rotating and place the test tube in the turbidometer. If the transmittance reading is between the two Biolog standards, the solution is ready for analysis. If the transmittance is too high, repeat the procedure above beginning with step 5 to add more bacteria until the transmittance reading is within the acceptable range. If the transmittance is too low, dilute the solution until the transmittance reading is within the acceptable range.

11. Take the test tube with the bacterial sample and pour it into the multi-channel pipette reservoir.

12. Place the autoclaved tips on the six channel pipette. Transfer 150 µl of the sample into each reservoir of the Biolog plate.

13. After filling all the reservoirs of the Biolog plate, incubate the plate at room temperature for 24 hours. Inspect the plate every two hours for color change. The color of the individual reservoirs will turn purple if the nutrient contained in the reservoir is used by the bacteria.

14. Compare the resulting array of purple reservoirs with the Biolog database to identify the bacteria. For quicker analysis, the plates can be read automatically by a Biolog Plate reader.
Figure 45
Sampling of Bacteria from the Isolated Colony.

Figure 46
Analyze the Bacterial Suspension using a Turbidometer
Figure 47
Loading the Eight Channel Pipetter with the Suspension.

Figure 48
Dispensing the Bacterial Suspension into the Biolog Plate.
APPENDIX VIII

The Streak Plate Method

The streak plate method is used to isolate colonies of bacteria. A homogenous colony is required to make an identification using Biolog plates.

Tools Required:

Approximately 5 Petri Dishes filled with Agar and Cyclohexamide from the Bacterial Enumeration Study

Petri Dishes with Bacterial Colonies from the Bacterial Enumeration Study

(1) Bunsen Burner

Wire Loop

Incubating Oven Set at 35°C (a Boekel Industries Inc. Model 133000 was used in this study)

1. Take the petri dishes with growing colonies from the bacterial enumeration study. Isolate individual colonies by taking the wire loop and passing it through the flame of the Bunsen burner until the wire glows red. Allow the wire to cool for approximately 15 seconds.

2. Carefully obtain a sample of the bacteria from the petri dish by picking up a small amount with the wire loop.

3. Take the wire loop and gently brush it back and forth on one side of a new petri dish, (this new dish has the agar in it but no bacteria).

4. Then take the wire loop and pass it through the flame of the Bunsen burner again.
5. After cooling, take the wire loop and gently place it in the same petri dish where the last streak of was made. Streak in a motion perpendicular to the first streak was made.

6. Repeat the heating of the wire loop and streaking until a box shape is formed.

7. Incubate the petri dish at 35°C for 24 hours in an oven.

8. Take the petri dish out of the oven and allow to cool to room temperature. If there appears to be individual colonies, go on to the Gram’s Test or the Biolog Identification Test. If the bacteria needs to be isolated more, repeat the entire plate streaking method until bacterial colonies are isolated.

![Image of plate streaking process]

**Figure 49**

Plate Streaking of Bacterial Sample to Isolate Colony
APPENDIX IX

Gram’s Test

Gram’s Test determines if the bacteria is gram positive or gram negative. Gram positive bacteria require different Biolog plates than gram negative bacteria.

Tools Required:

Petri Dishes with Isolated Bacterial Colonies from the Streak Plate Method

(1) Bunsen Burner

Wire Loop

Approximately 1 Drop Autoclaved Super Q Water

Crystal Violet Stain

Gram’s Iodine Stain

Approximately 40 ml Ethanol

Saffranin Stain

Clean Wipes

(1) Glass Microscope Slide

(1) 100X Power or Greater Microscope (a Nikon Labophot was used in this study)

Microscope Oil

The following method will determine whether the bacteria are gram positive or gram negative.

1. Clean the slide so that no dust or other contaminants are present.

2. Place a small drop of the Super Q water on a microscope slide.

3. Take the petri dishes with growing isolated colonies from the streak plate method.
4. Take the wire loop and passing it through the flame of the Bunsen burner until the wire glows red. Allow the wire to cool for approximately 15 seconds.

5. Carefully obtain a sample of the bacteria from the petri dish by picking up a small amount with the wire loop.

6. Place the wire loop on the drop of water on the slide. Swirl the drop around the slide with the wire loop until the drop is spread as much as possible. This should create a single layer of bacteria, which will make it easier to focus with the microscope later. Allow the slide to dry.

7. After the slide has dried, fix the bacteria by passing the slide quickly through the flame of the Bunsen burner two or three times. The bacteria side should be on the side opposite the flame.

8. Flood the slide with crystal violet stain. Allow the stain to set for one minute.

9. Rinse the slide with water.

10. Flood the slide with Gram’s iodine stain. Allow the stain to set for one minute.

11. Rinse the slide with ethanol until it runs clear. Rinse the slide with water.

12. Counter stain the slide with safranine stain. Allow the stain to set for one minute.

13. Rinse the slide with water. Blot dry the slide with clean wipes.

14. Examine the slide under the microscope. If the bacteria appear purple, they are gram positive. If the bacteria appear pink, they are gram negative.
Figure 50
Insertion of Wire Loop into Flame

Figure 51
Getting a Small Sample of the Isolated Bacteria With Wire Loop.
(See Figure 49 for Location of the Isolated Colony)
Figure 52
Spreading the Bacteria on the Microscope Slide

Figure 53
Examining the Slide Under a Microscope
APPENDIX X

Supporting Data for Charts

Data for Figure 4, Moisture Content of Wood Bark in the Biofilter

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Data for sample calculation for moisture content.

1/30/96

PILOT BIOFILTER

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Data for Figure 7, Bacterial Content of Wood Bark in the Biofilter

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Data for Figure 46 Bacterial Content of Wood Bark in the Biofilter

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Data for Figure 49 Phospholipid Results

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<td>0.430</td>
<td>0.086</td>
<td>0.718</td>
<td>6.171</td>
<td>21.920</td>
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D=NOT BLENDED
APPENDIX XI

Biofilter Operating Manual
Polk Run Wastewater Treatment Plant
Loveland, Ohio
BIOFILTER OPERATING MANUAL
FOR
SLUDGE HOLDING TANK AT
POLK RUN WASTEWATER TREATMENT PLANT
LOVELAND, OHIO
Special Thanks To:

Ross Geiger        Plant Supervisor
Mike Gilliland     Maintenance Worker
Kaniz Siddiqui     Chemist
Paul Bishop        Professor of Engineering

This project was funded by the Metropolitan Sewer District of Greater Cincinnati. The completion of this manual was a part of the masters degree requirements for Mark D. Smith at the Department of Civil and Environmental Engineering, of the University of Cincinnati, Cincinnati, Ohio.
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INTRODUCTION

As of the date of this manual, November 1996, the United States Environmental Protection Agency does not regulate the emissions of volatile compounds from municipal waste treatment plants. There have been proposals for changes in this requirement. California has already enacted legislation to limit the emissions from waste water treatment plants, and it is recognized by many municipalities that the implementation of regulations to limit these emissions is inevitable.

In order to be prepared for new regulations, The Metropolitan Sewer District of Greater Cincinnati (MSD) has taken a proactive approach to limiting their volatile emissions. In order to comply with the proposed regulations, The MSD has constructed a biofilter that controls the gaseous emissions from a sludge holding tank at the Polk Run Wastewater Treatment Plant. This plant treats primarily residential sewage. The average daily flow is 4 million gallons. This plant serves the residential community of Loveland, Ohio and the surrounding suburban area.

The treatment consists of the following:

<table>
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<th>TYPE</th>
<th># UNITS</th>
<th>SIZE</th>
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<tr>
<td>Bar Screen</td>
<td>1 unit</td>
<td>60' L x 8.5' W x 16.5' D</td>
</tr>
<tr>
<td>Aerated Grit Chamber</td>
<td>1 unit</td>
<td>60' L x 8.5' W x 16.5' D</td>
</tr>
<tr>
<td>Primary Settling Tanks</td>
<td>4 units</td>
<td>100' L x 20' W x 12' D</td>
</tr>
<tr>
<td>Aeration Tanks</td>
<td>4 units</td>
<td>100' L x 32' W x 16' D</td>
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<tr>
<td>Secondary Settling Tanks</td>
<td>4 units</td>
<td>70' dia x 12' D</td>
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The plant was designed to handle a design flow of 6 million gallons per day (MGD) with a peak flow of 12 MGD, and a peak hydraulic flow of 18 MGD. The design of the plant allows for an influent biological oxygen demand (BOD) of 220 mg/L, an influent suspended solids (SS) of 220 mg/L, and an influent ammonia nitrogen concentration of 20 mg/L. The effluent has an average BOD content of 2.5 mg/L, a SS content of 2.8 mg/L and 1.4 mg/L of ammonia nitrogen.

A map of the treatment plant is shown on page 6.

The biofilter was constructed to control the emissions of odorous compounds from the sludge holding tanks. There is no on-site drying or treatment of the sludge. The sludge is pumped from the storage tank into a tanker truck for off site disposal. The primary source of the odors is Hydrogen Sulfide gas. Most of the volatile organic compounds (VOC's) are believed to be liberated from the wastewater prior to the sludge holding tanks.
MAP OF POLK RUN WASTEWATER TREATMENT PLANT
OVERVIEW OF BIOFILTER SYSTEM

The wood bark biofilter controls the gaseous emissions from a sludge holding tank. Hydrogen sulfide is the primary gas emitted. The biofilter is approximately 30 ft. long x 30 ft. wide x 16 ft. deep, and constructed of concrete on 3 sides and wood on the fourth side. The air from the sludge holding tanks is pumped into the biofilter by two blowers. The air is humidified as it passes through the inlet pipes by a small pipe that supplies a constant drip of water. The exhaust air from the sludge holding tanks is distributed throughout the biofilter by a grid of distribution blocks. The biofilter is filled to approximately a depth of 5 ft. with hardwood bark. The bark is maintained at a moisture content of approximately 50% by weight to maintain a medium for bacterial growth. This bacteria growth is the mechanism for which the hydrogen sulfide is absorbed and decomposed. The hydrogen sulfide gas is liberated from the sludge by the steady state emission of the gas during storage, and the transfer of the sludge from the sludge thickeners into the sludge holding tank.

Side View of Biofilter
Front View of Biofilter

View of Blowers
Note: The Cover is Off One Blower Motor While it is Being Repaired
Side View of Blowers
Air Shut Off Valve is to The Right Of Blower

View of Air Diffuser Blocks. The Large Holes in the Sides of the Blocks Allow Air Flow Between Blocks and the Small Holes in the Top Allow Air Flow Upward Through the Bark
View of Top of Sludge Holding Tank with Tanker Truck in Background

Pumping of Sludge into Tanker Truck
View Looking Down Into Sludge Holding Tank. Fumes Are Dangerous, So Wear Proper Breathing Apparatus When Entering Confined Spaces.

Inspection Hole in Top of Sludge Tank.
Cover Should Be Over Hole During Operation.
SAFETY

Know and understand the safety rules for your workplace, and follow them. The following list includes a partial listing of the more important rules.

- Know the location of first aid equipment.
- Know the location of the nearest telephone, and the emergency phone numbers.
- Know the procedures for emergencies including fire, chlorine leaks and tornadoes.
- Always work with another person when working in dangerous areas.
- If working alone, make sure someone knows where you are, and how long you plan to work in that area. If you are delayed, let someone know.
- Do not enter confined areas without proper breathing equipment.
- Always wear an approved pair of steel-toed shoes.
- Always wear an approved hard hat.
- Do not work on any electrical equipment without first disconnecting the main power source.
- Be especially careful working in wet areas. It can be slippery.
**OPERATION OF BIOFILTER**

As stated earlier, the Hydrogen Sulfide gas is liberated from the sludge by two separate processes. The first is a steady state emission of the gas during storage. The second source is from the transfer of the sludge from either the thickening tanks into the storage tank or from the storage tank to the tanker truck. The latter transfer mechanism causes abrupt increases in the hydrogen sulfide concentration at the inlet of the biofilter.

The biofilter is approximately 30 ft. long x 30 ft. wide x 16 ft. deep, and constructed of concrete on three sides and wood on the fourth side. The air from the sludge holding tanks is pumped into the biofilter by two 1300 cubic foot per minute blowers. These blowers are each powered by a 7.5 HP motor. The air is humidified as it passes through the inlet pipes by a constant drip of water. The exhaust air from the sludge holding tanks is distributed throughout the biofilter by a grid of distribution blocks. These blocks are manufactured by ROTTAER, and are constructed of concrete. The blocks have large passages on all four sides for distribution of the air throughout the biofilter, and small slots on the upper surface to allow the air to pass upward into the filter media. The biofilter is filled to approximately a depth of 5 ft. with hardwood bark. The bark is maintained at a moisture content of approximately 50% by weight to maintain a medium for bacterial growth. This bacteria growth is the mechanism for which the hydrogen sulfide is absorbed and decomposed.

The mechanism for the liberation of the Hydrogen Sulfide gas is the reduction of the sulfate ion by bacteria under anaerobic conditions.

\[
\begin{align*}
\text{SO}_4^{2-} + \text{organic matter (C, H, O)} & \Rightarrow S^{2-} + \text{H}_2\text{O} + \text{CO}_2 \\
S^{2-} + 2\text{H}^+ & \Rightarrow \text{H}_2\text{S}
\end{align*}
\]
The source of the sulfur in wastewater is the breakdown of plant and animal proteins by the human body to form urine, which is high in SO$_4^{2-}$.

The concentration of hydrogen sulfide at the inlet to the biofilter has been measured using grab tubes and a pump manufactured by Sensidyne. The following sample was taken at sunset on a clear day, which explains the drop in temperature between the two samplings.

The level of hydrogen sulfide at the sludge tank prior to the blowers was approximately 0.06 ppm. The level from a sampling port at the inlet to the biofilter was below 0.01 ppm with two blowers operating, and approximately 0.05 ppm with only one blower operating. These readings were with no sludge flowing into or out of the sludge holding tanks. The temperature above the sludge was 76° F with a relative humidity of 40%. The temperature of the air at the inlet to the biofilter was 72° F with a relative humidity of 50%. Fifteen minutes later, as the sun went down, the temperature was 63° F with a relative humidity of 79%. This example shows how the time of day can drastically affect the temperature and humidity readings.

When sludge was then added from the thickener to the sludge holding tanks, the hydrogen sulfide level peaked almost immediately at over 2 ppm (the limit on these sampling tubes for one pull of the sampler) at the inlet to the biofilter. After the sludge transfer was complete, the concentration of hydrogen sulfide was 1 ppm in the holding tank, and 0.63 ppm at the inlet to the biofilter. This level then decreased steadily to the levels reported earlier.

These observations lead to two major conclusions. The first is that the generation of the hydrogen sulfide is greatest when turbulence in the sludge exists, as during the transfer of sludge. Second, the hydrogen sulfide is greatly diluted when two blowers are operating.
MAINTENANCE

The biofilter will require very little maintenance if operated properly. A cursory inspection is usually all that is necessary to determine the whether the biofilter needs attention.

A quick inspection each day will take less than five minutes.

• Look at the biofilter. Make sure no weeds are growing in the wood bark. Weeds are an indication that there is no air flow through the bark. Remove the weeds and turn the bark with a pitchfork.

• Look at the bark. If it appears dry, add water by increasing the drip through the influent air or by adding water directly to the bark. If it appears too moist, reduce the water added to prevent the filter from clogging. See the sampling section for the method to test the wood bark for moisture content.

• Smell the biofilter. If the odor is stronger than normal, there could be a problem with the biofilter. The problem could be with the storage tank, the blowers, or the wood bark.

• Listen to the blowers. If they are louder than normal, a valve may be closed. If there is a squealing noise, the blower may need to be inspected more closely.

• Look down the access covers. Make sure the sludge level is not too high. Turn off the sludge transfer pumps if necessary.

• Listen for any air leaks in the PVC piping. The piping should withstand cold weather unless water backs up and freezes inside the pipes.

If any of the pumps, valves or blowers need any maintenance, refer to their manufacturer’s manual.

If wood bark needs to be added, use bark of the same size and type as originally used in the biofilter.
SAMPLING

In general, the biofilter will operate satisfactorily with little human intervention. It is, however, important to periodically sample the wood bark and the influent air to determine the general health of the biofilter. The wood bark requires an influent air to contain enough water to maintain nearly 100% relative humidity. This will ensure that the wood bark maintains approximately 50% water by weight.

Wood Bark Sampling

Tools Required:

(1) Pitchfork
(2) Quart Size Ziplock Bags
(1) Portable Temperature/Humidity Sampler
(1) Laboratory Oven
(4) 200 ml beakers
(1) Laboratory Scale

The sampling of the wood bark is performed at two depths of the biofilter. Start by randomly picking a location in the biofilter. With a pitchfork, dig down approximately three feet. Take sample at approximately six inches below the surface, and another sample three feet below the surface. Put each sample in a ziplock style bag for moisture content analysis. Using the Temperature/Humidity probe, measure the temperature and humidity at the locations where the samples were taken. Record the readings on the sample bags. Also measure the ambient temperature and relative humidity and record the readings.
Take the samples into the lab. Tare a 200 ml beaker. Add approximately half of the sample wood bark to the beaker. Weigh the bark. Repeat with the other half of the sample, then repeat the procedure for the other sample. Place the four beakers into the laboratory oven that has been set at 100 - 110 °C. Leave the samples in the oven for at least 24 hours, and remove the beakers. Be careful, the beakers will be hot! Allow the beakers to return to room temperature. Weigh the beaker, record the value, then empty the wood bark, and reweigh the beaker.

The % Moisture of the Wood Bark by Weight =

\[
1 - \frac{(Final\ Weight\ of\ Wood\ Bark - Weight\ of\ Empty\ Beaker)}{Original\ Weight\ of\ Wood\ Bark} \times 100\%
\]

Dig a Hole Three Feet Deep into the Biofilter
Take a Bark Sample Six Inches Below the Surface, and Another Sample Three Feet Below the Surface of the Biofilter. Put Each Sample in a Separate Bag.

Insert the Portable Temperature/Humidity Probe into the Wood Bark and Take Readings at the Sample Locations
View of a Typical Bark Sample

Tare the Scale with the Beaker
Add the Wood Bark to the Beaker and Reweigh

Place the Beaker in an Oven Set at 100 - 110°C For 24 Hours. Then Reweigh the Beaker With the Bark, and Weigh the Empty Beaker. Calculate The Moisture Content of the Wood Bark using the Equation on Page 18.
Gas Sampling

Tools Required:

(1) Portable “Grab Tube Type” Sampler

(1) (Or more) H₂S Grab Tubes 0.1 -- 4 ppm range

(1) Portable Temperature/Humidity/Velocity Sampler

The gas is sampled at the sampling port in the PVC tubing. Start by removing the pressure gauge. Turn the gauge counterclockwise. Insert the velocity probe into the hole. Make sure the probe is oriented in the direction of gas flow. Record the reading. With two blowers operating, the air velocity should be approximately 2750 feet per minute. With only one of the blowers running, the air velocity should be approximately 2500 feet per minute.

If the velocity is within 25% of this speed, the blowers are probably operating properly. If the air velocity is more than 25% lower than these speeds, check the blowers and valves. First check the electrical panel located by the sludge holding tank to ensure the blowers are turned on. There is one switch for each blower. If one of the switches is locked out, do not restart it without determining why it has been locked out. A good rule of thumb is only allow the person that locked a switch out to remove the lock.

If the switches are on, there is probably a problem with the blowers or valves. Turn off both blowers before checking their operation. Check the valves. There is an indicator on top of the valve that indicates whether it is open or closed. To open the valve, turn it clockwise. To close the valve, turn it counterclockwise. If both blowers are operating, as recommended during pumping of the sludge into the tanker truck, both valves should be open. If only one blower is operating, as recommended during normal operation, only the operating blower’s valve should be open, and the other blower’s valve should be closed.
If a visual inspection of the valves does not indicate a problem, take the cover off the blowers and inspect the blower assembly. Refer to the blower maintenance manual for possible problems. If a problem with one of the blowers is discovered, lock out the switch for that blower, and close the valve. Open the valve for the other blower, and turn it on. Return to the biofilter and measure the velocity of the gas. Allow the blower to run for several minutes before beginning the sampling of the gas.

Take the portable gas sampler and one of the grab tubes. Break off both ends of the grab tube and insert the proper end into the sampler, as directed by the sampler operating manual. The H$_2$S concentration during normal operation is approximately 0.01 to 0.02 ppm. If the range of the sampling tube is 0.1 ppm and up, take 10 pulls on the sampler. The tube will change colors at the tip to indicate how much H$_2$S is present. Take the reading and divide by the number of pulls to determine the H$_2$S concentration. If the reading is greater than the maximum value on the tube, the measurement was probably taken during the transfer of sludge from the thickeners into the sludge holding tank (the reading can reach over 2 ppm). Wait a few minutes, then take a new grab tube and repeat the measurement. Record the H$_2$S reading, along with the velocity reading.

**Electrical Control Box for The Blowers.**

Sludge Holding Tank Biofilter Manual
Polk Run Wastewater Treatment Plant
Loveland, Ohio
Part of the Metropolitan Sewer District of Greater Cincinnati
Valve for Opening/Closing Air Supply to the Blowers

Sample Port is Located Behind the Pipes Entering the Biofilter.
Water Drips From the Top of These Tubes to Add Moisture to the Gas.
Removing Pressure Gauge at Sample Port

Sample Port
Measuring the Air Flow of the Gas. Make Sure the Arrow on the Probe is Aligned with the Direction of Flow of the Gas

Measuring H₂S By Inserting into the Port and Pulling the Sampler Handle As Specified
Observe Reading Indicated by Color Change in Tube

Take the Portable Temperature/Humidity/Velocity Sampler and disconnect the velocity probe, (the Temperature/Humidity probe is different from the velocity probe). After connecting the Temperature/Humidity probe, measure and record the temperature and humidity of the gas. Also measure the ambient temperature and humidity. During summer months, the gas temperature is usually lower than the ambient temperature. The opposite is true in the winter. This is because the sludge tends to be at a temperature of 65 - 70°F year-round, and the sludge will cool the gas in the summer, and warm the gas in the winter.

The relative humidity of the gas should be 95% to 100% during most times. This will enable the wood bark to maintain sufficient moisture to promote bacterial growth. If the relative humidity is lower than 95%, the amount of water dripped into the air inlet pipes should be increased. Do not add too much water by dripping, as it may scrub the H₂S from the gas, (the H₂S gas will become part of the water), and short circuit the biofilter, exiting with the water at the drain. If the wood bark has dried out to below 40% moisture by weight, (see wood bark sampling on page 17), water may need to be sprayed onto the top of the biofilter. The spraying should be done in a
manner as to create a light mist. A garden sprinkler set on a diffused spray or soaker hose works well. Do not overwater, as it may clog the filter.

Measure the Temperature and Humidity of the Influent Air

Return the Pressure Gauge to the Sampling Port
WITH EVERYONE'S HELP, POLK RUN WASTEWATER TREATMENT PLANT CAN CONTINUE TO BE AN AWARD WINNING PLANT!