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# Influence of Bioaugmentation in the Sewer to Improve the Economics of Wastewater Treatment in a Full-Scale System

Rich Schici, Senior Project Engineer
In-Pipe Technology Company, Inc., 100 Bridge Street, Wheaton, Il 60187

## ABSTRACT

The high cost of upgrading a municipal wastewater treatment plant for increased organic capacity or stringent effluent requirements has urged many investigators to examine bioaugmentation as a means to improve the economics of wastewater treatment. A full-scale wastewater treatment plant selected an external bioaugmentation process to continuously add high concentrations of select, facultative, non-pathogenic bacteria. Bacteria are added to the outer reaches of the wastewater collection system in order to grow throughout the surface of the sewer pipes and thereby modify the sewer biofilm; improve the ability of the sewer biofilm to degrade the organic material; and take advantage of the residence time of the wastewater within the sewer to degrade the waste. A comparative study illustrates a correlation of improved operating data to increased metabolism of wastewater characteristics that result in operational performance at the plant by transforming the collection system into an active part of the wastewater treatment process. This report describes the performance metrics, the history and discussion of the plant, and the environmental impacts and plant performance improvements resulting from bioaugmentation.

## KEYWORDS

Bacteria, Bioaugmentation, Biofilm, Facultative, Sewer, Treatment Improvement, Wastewater.

## INTRODUCTION

In the collection system, microorganisms act to catalyze the oxidation of biodegradable organics and other contaminants generating by-products such as carbon dioxide, water, and biomass. Bacteria grow and divide producing biosolids and clean water. This metabolism occurs in wastewater treatment plants around the world. However, the limits of size, retention time, processing capacity, and operating budgets create economic challenges for every municipality.

Sewers are large biological reactors, whether we like it or not. They are often seen as a means to an end for the wastewater treatment plant, but actually provide similar treatment capabilities as a trickling filter if the microbiology is properly harnessed. The concept of bioaugmentation is a technique to improve the degradability of a specific area by introduction of competent microorganisms (Morgenroth, 2009). In an effort to utilize the miles of existing pipe and convert the passive sewer system into a meaningful treatment step, bioaugmentation in the sewer offers sustainable solutions through utilization of existing infrastructure with a natural, biological process.

Bioaugmentation using *Bacillus* bacteria was introduced at strategic locations throughout the sewer collection system in accordance with an engineered plan. Continual addition twenty four hours per day, seven days a week (24/7) added to the outer reaches of the wastewater collection system grow throughout the surface of the sewer pipes and thereby modify the sewer biofilm; improve the ability of the sewer biofilm to degrade the organic material; and take advantage of the residence time of the wastewater within the sewer to degrade the waste. By extending wastewater treatment from the plant into the sewer collection system, bioaugmentation enhances the microbial community such that more reactions occur in the sewer biofilm that contribute to increased metabolism of wastewater compounds within the sewer.

The objectives of this study are as follows:

- Analyze the impacts of *Bacillus* bacteria on wastewater characteristics and take advantage of the residence time of the wastewater within the sewer to degrade the waste.

- Investigate the effects of sewer bioaugmentation to reduce influent load and improve effluent quality at the wastewater treatment plant with zero capital cost and no additional energy requirements.

- Measure biomass of the organisms containing the *catalase* enzyme using the HMB IV instrument to determine the increase in catalase positive *Bacillus* bacteria and the rate of metabolism.

This paper tracks the progress of reducing influent load to provide additional capacity and improved effluent quality through bioaugmentation with *Bacillus* in the collection system at a full-scale wastewater treatment plant. A comparative study illustrates a correlation of improved operating data to increased metabolism of wastewater characteristics using external bioaugmentation in the sewer.

## DESCRIPTION OF HMB IV ANALYSIS

The HMB IV measures the activity of *catalase*, a peroxidase enzyme (Untitled, 2010). This enzyme catalyzes the breakdown of hydrogen peroxide which can oxidize transition metals and form free hydroxide radicals which are toxic to all organisms. Catalase is not present in strict anaerobes, but is present in facultative anaerobes or strict aerobes. Thus, the instrument measures only the biomass of organisms containing the catalase enzyme and not the total biomass of a sample. *Bacillus* organisms all are catalase positive. By measuring biomass minus strict anaerobic biomass, the instrument provides a good indication of the increase in numbers of *Bacillus* facultative anaerobes dosed in the sewer.

**Figure 1 – HMB IV Instrument**


Aerobic and facultative organisms use the *catalase* enzyme for all metabolic activity. When oxygen enters the bacterial cell, it is converted to the highly toxic compounds superoxide ($O_2$-) and hydrogen peroxide ($H_2O_2$). The enzyme *superoxide dismutase* converts superoxide to hydrogen peroxide and oxygen.

$2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2$

The enzyme *catalase* converts hydrogen peroxide to water and oxygen.

$2H_2O_2 \rightarrow 2H_2O + O_2$

Obligate anaerobic bacteria do not have the *catalase* enzyme and do not survive in aerobic conditions. The production of the enzymes is proportional to the rate of metabolism. As metabolism increases, so too must the production of *catalase* enzyme (Hosetti, 1994). The HMB test measures the amount of *catalase* in the sample and thus measures the metabolic activity of the bacteria in the sample.

The HMB test is unit less, not a count of bacteria present, and not a measurement of the water's pollutant level. The HMB results are valuable measurements when a baseline is established and used as a comparison. For example, operators at the wastewater treatment plant may set a mixed liquor HMB baseline for "ideal biological treatment". The plant can then measure HMB periodically in the mixed liquor to determine metabolic activity. Changes that would normally show up after the 5-day BOD test may show up in 15 minutes with the HMB test. This is useful to evaluate impacts from toxic events, filamentous outbreaks, or impacts to effluent quality.

## REVIEW OF COLLECTION SYSTEM MICROBIOLOGY AND BIOFILM

*Bacillus* bacteria are common soil bacteria that naturally become introduced into the wastewater treatment system because of their presence in the air, on food, on dust, and in dirt that enters the system. When isolated from the human gastrointestinal tract, they are present only in low numbers and are usually not metabolically active. Thus, they are present within the wastewater but at far smaller proportions than the intestinal bacteria that are introduced continually into the collection system.

Soil bacteria utilize the excretions and dead tissues of plants and animals. Many of these soil bacteria, when found in wastewater, do not get a chance under normal conditions to become the dominant organisms in the system. The soil bacteria have many desirable qualities that can enhance biodegradation of sewage including excretion of enzymes to break down recalcitrant nutrient sources (cellulose, starch, protein, etc) thereby making them more bio-available to other organisms (Gray, 1968).

**Influence of Sewer Biofilms on Wastewater Characteristics**

Sewer biofilm is composed of EPS, Extracellular Polymeric Substance (Exopolysaccharide), which is a sticky organic matter (slime) produced by microbes and responsible for cells adhesion in biofilms. Three distinct layers of aerobic, anoxic, and anaerobic conditions exist within the sewer biofilm. Within each layer, separate conversion processes occur. Hydrolysis, fermentation, and oxidation of organics result in decomposition within the sewer to influence sewer biofilms and modify wastewater characteristics (Morgenroth, 2008).

There is a delicate balance between organic carbon, nitrogen, and phosphorus of wastewater. The amount of organic carbon is only meaningful when it is expressed in terms of different mechanisms and rates of biodegradability. Dr. Huismen (2004), et al, studied oxidation of organic matter in the sewer and found that up to 30% of COD is oxidized aerobically. In addition, up to 8% of COD was oxidized using nitrate as an electron acceptor (anoxic). The study concluded that both the suspended bacteria and biofilm contributed to COD oxidation.

Is the conversion of 30% COD relevant to wastewater treatment? Dr. Henze (1992) and Dr. Kobylinski (2008) determined up to 60% of COD was readily and slowly biodegradable. Dr. A. Randal (2006) determined that adding facultative bacteria into the sewer increased influent RBCOD and heterotrophic plate counts. By increasing influent RBCOD, added bacteria improve conversion or organics within the sewer, which can improve biological phosphorus and nitrogen removal.

**Biological Process Description**

*Bacillus* can grow under anaerobic conditions, either with nitrate or nitrite as the electron acceptor or by fermentation (Ye, 2000). The added bacteria have a competitive advantage over the wild bacteria, but only if they are added in a manner and at selected locations that grow to a higher population level than bacteria that occurs in normally untreated conditions.

Heterotrophic bacteria were shown to have symbiotic, and sometimes communal, interactions with nitrifying bacteria. The heterotrophs provide the autotrophic nitrifiers with a carbon source as a byproduct of their nitrification and denitrification activities. The heterotrophs also were shown to reduce the amount of excretion products that can inhibit the growth of *Nitrosomonas* bacteria while they make use of the organic excretion products for their own energy in carbon depleted environments. In addition, increasing the influent heterotrophic plate counts beneficially impacts the rates of metabolism within the wastewater treatment plant for synthesizing organic matter (Kuenen and Gottschal, 1982).

The addition of *Bacillus* allows a gradual repopulation of the sewer biofilm by bacteria that are more efficient at degradation of organics than the bacteria that are present in natural, untreated conditions (Gerardi, 2006). As the added bacteria grow, multiply, and reach the wastewater treatment facility, they assist in further degradation of the remaining COD in all phases of treatment. The newly diversified bacterial population is able to assist the bacteria primarily responsible for nitrification and denitrification than the indigenous population present in untreated wastewater treatment facilities by increasing the oxidation of soluble organic matter and reducing influent nitrates (Ye, 2000).

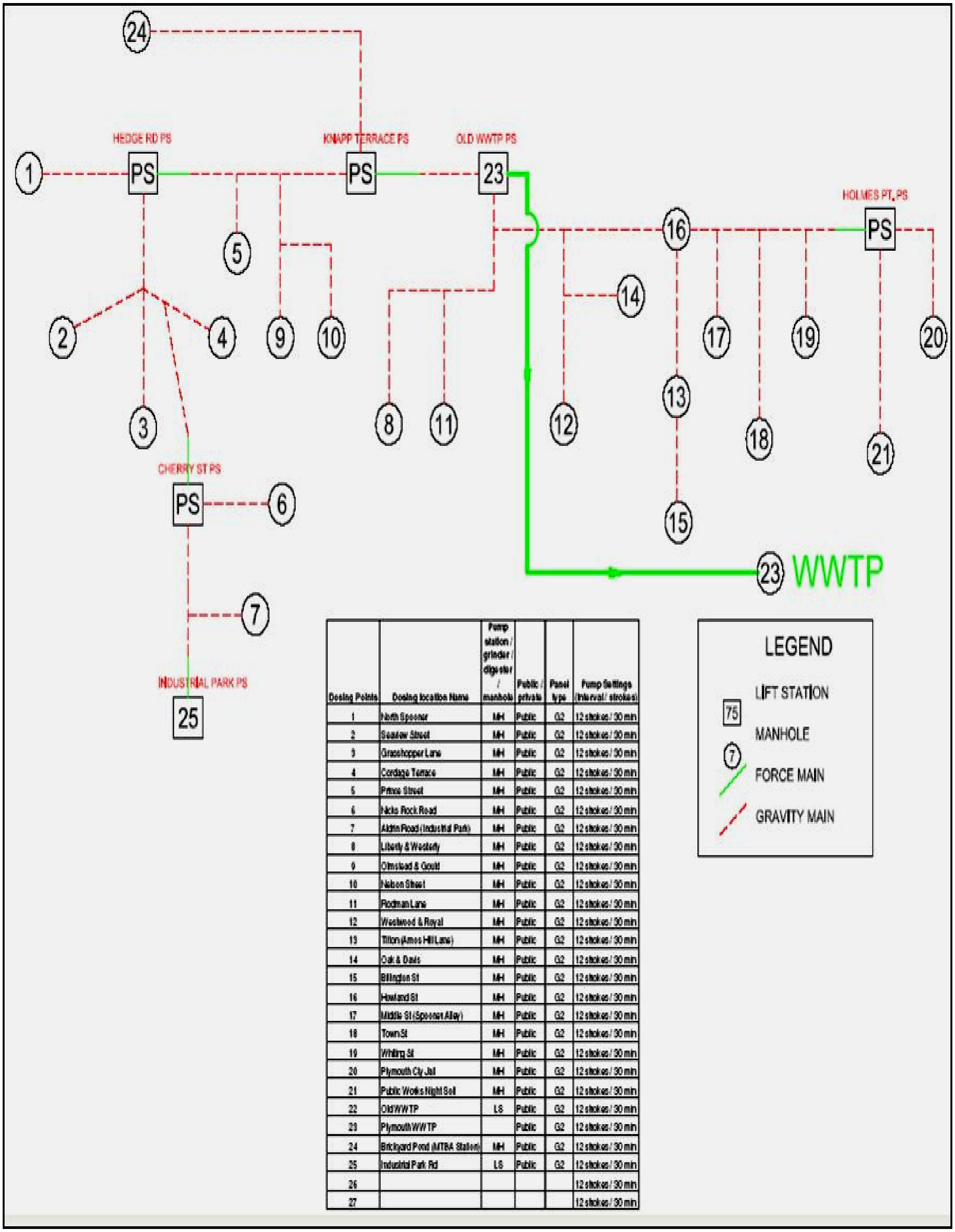
Within a period of time, the added bacteria convert the biofilm on the surface area of the infrastructure into a controlled, beneficial biological population. They also metabolize fats, oil, and grease (FOG) in the collection system and at the treatment plant. This collection system reactor provides beneficial treatment in the sewer by accelerating metabolic conversions that reduce organic material entering the WWTP.

## IMPLEMENTATION OF BIOAUGMENTATION IN THE SEWER

The microbiological treatment for a system is carefully engineered and driven by such factors as organic loads, distribution, collection system layout, and treatment objectives, among others. Battery-powered dosing units consist of a panel slightly larger than a shoe box containing a solenoid pulse pump operated by a small circuit board. The dosing panel holds a one (1) liter replaceable reservoir with a 30 day supply that can provide time controlled treatment for as long as 90 days.

**Figure 2 – Bioaugmentaion Dosing Panel**

**Figure 3 – Schematic of Collection System Layout with Panels**




| Dosing Points | Dosing location Name | Pump station / grinder / digester / manhole | Public / private | Panel type | Pump Settings (interval / strokes) |
|---|---|---|---|---|---|
| 1 | North Spooner | MH | Public | G2 | 12 strokes / 30 min |
| 2 | Seaview Street | MH | Public | G2 | 12 strokes / 30 min |
| 3 | Grasshopper Lane | MH | Public | G2 | 12 strokes / 30 min |
| 4 | Cordage Terrace | MH | Public | G2 | 12 strokes / 30 min |
| 5 | Prince Street | MH | Public | G2 | 12 strokes / 30 min |
| 6 | Nicks Rock Road | MH | Public | G2 | 12 strokes / 30 min |
| 7 | Aldrin Road (Industrial Park) | MH | Public | G2 | 12 strokes / 30 min |
| 8 | Liberty & Westerly | MH | Public | G2 | 12 strokes / 30 min |
| 9 | Olmstead & Gould | MH | Public | G2 | 12 strokes / 30 min |
| 10 | Nelson Street | MH | Public | G2 | 12 strokes / 30 min |
| 11 | Rodman Lane | MH | Public | G2 | 12 strokes / 30 min |
| 12 | Westwood & Royal | MH | Public | G2 | 12 strokes / 30 min |
| 13 | Tilton (Ames Hill Lane) | MH | Public | G2 | 12 strokes / 30 min |
| 14 | Oak & Davis | MH | Public | G2 | 12 strokes / 30 min |
| 15 | Billington St | MH | Public | G2 | 12 strokes / 30 min |
| 16 | Howland St | MH | Public | G2 | 12 strokes / 30 min |
| 17 | Middle St (Spooner Alley) | MH | Public | G2 | 12 strokes / 30 min |
| 18 | Town St | MH | Public | G2 | 12 strokes / 30 min |
| 19 | Whiting St | MH | Public | G2 | 12 strokes / 30 min |
| 20 | Plymouth Cty Jail | MH | Public | G2 | 12 strokes / 30 min |
| 21 | Public Works Night Soil | MH | Public | G2 | 12 strokes / 30 min |
| 22 | Old WWTP | LS | Public | G2 | 12 strokes / 30 min |
| 23 | Plymouth WWTP | | Public | G2 | 12 strokes / 30 min |
| 24 | Brickyard Pond (MTBA Station) | MH | Public | G2 | 12 strokes / 30 min |
| 25 | Industrial Park Rd | LS | Public | G2 | 12 strokes / 30 min |
| 26 | | | | | 12 strokes / 30 min |
| 27 | | | | | 12 strokes / 30 min |

Bioaugmentation goals are to reduce the cost of treating the wastewater by reducing the influent organic loads, controlling fats, oils and grease (FOG) in the collection system, and improving effluent quality. This requires zero capital cost and no additional energy requirement. Performance in the collection system provides increased additional capacity within the plant, forestalls costly upgrades, and extends the life of existing infrastructure.

To utilize the collection system as an active part of the wastewater treatment process, significant biofilm coverage of the interior surface area of the infrastructure is required. Influent wastewater quality changes and thus operational changes at the plant are expected. Changes typically develop between 30 and 60 days after implementation because of the time required displacing the wild biofilm in the collection system.

## HISTORY AND DISCUSSION

Bacteria added to the outer reaches of the wastewater collection system in order to grow throughout the surface of the sewer pipes and thereby modify the sewer biofilm to convert compounds in the bulk liquid, which is used in biological wastewater treatment (Morgenroth, 2008). Since bioaugmentation engages the biofilm inside the piping, unit loading in critical for removal of organics in the sewer. Specific loading rates are calculated as pounds of flow for every square foot per cubic feet of surface area (Metcalf & Eddy, 2003).

General design criteria for various loading rates depend on desired removal rates or permit limits of the facility. Based on general BOD and TSS limits of 30 mg/l, the loading rates for different types of processes are calculated as follows (Steichen, 2010):

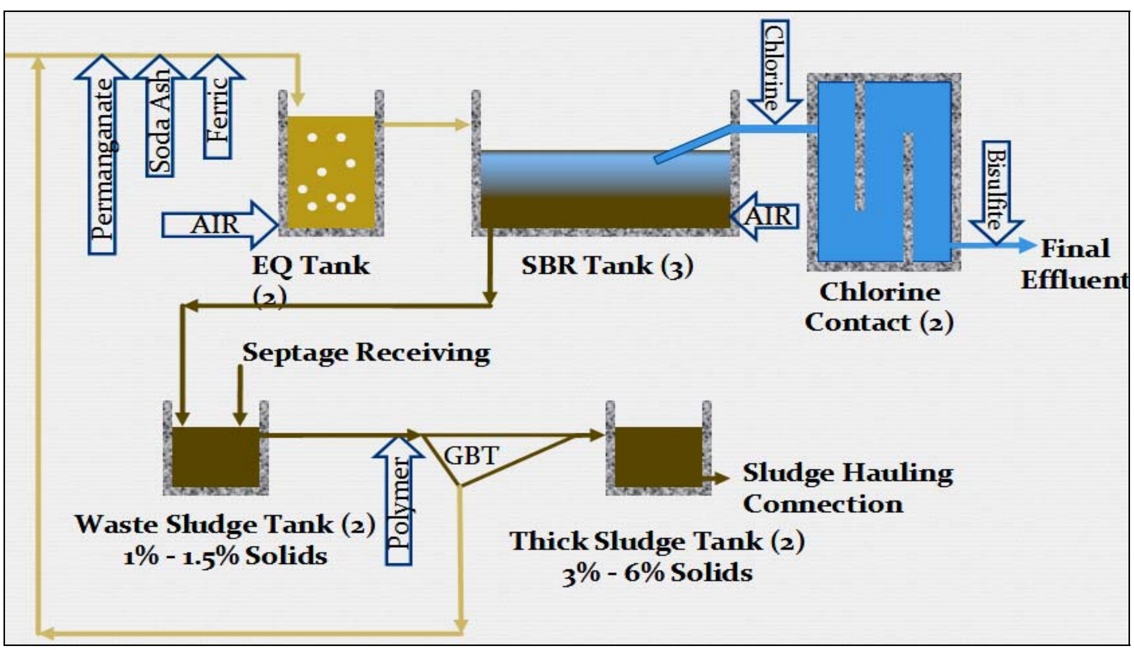
- Rock trickling filter media: 15 $ft^2/ft^3$
- Plastic trickling filter media: 30 $ft^2/ft^3$
- IFAS and MBBR: 152 $ft^2/ft^3$

A municipal full-scale wastewater treatment plant in Massachusetts 40 miles south of Boston selected the external bioaugmentation process to continuously add high concentrations of select, facultative, non-pathogenic bacteria to the sewer system. The original wastewater collection system was comprised of mostly gravity sewer which collected at the old treatment plant near the downtown area adjacent to a harbor. When the new wastewater treatment plant was built on the outside of town, a 4.5 mile 30 inch diameter ductile iron force main was built to transport the wastewater from the Water Street location to the new wastewater treatment plant (WWTP).

The WWTP is a Sequencing Batch Reactor (SBR) with a design capacity of 2.5 MGD. The annual average flow is 1.7 MGD with typical domestic strength wastewater of influent BOD at 200 mg/l and TSS at 180 mg/l. The SBR system treats wastewater in a batch process, going through each step by changing the conditions in the reactor according to the desired treatment. Each step of the process occurs in the same tank and is limited by influent flow, but allows for some control over the process. The process flow diagram is shown in Figure 3.

**Figure 4 – Arial Photo of the WWTP**

**Figure 5 – WWTP Process Flow Diagram**



Calculating the loading rate of the gravity system and force main required a complete inspection of the system; however, this was not accurately completed. As a result, the approximate loading rate was estimated using the number of lineal feet of the gravity system with pipes flowing half full. The sewer biofilm was estimated to cover approximately 57,500 $ft^2$ with a loading rate approximately equal to a rock trickling filter at 15 $ft^2/ft^3$.

The project consisted of 24 dosing panels in three separate phases. The first phase included the installation of the 8 treatment locations closest to the Water Street pump station. Both subsequent phases added 8 additional locations each, with the dosing locations extending farther out in the collection system from the plant with each phase. December 2009 was the first full month of microbial treatment in the collection system.

## RESULTS

Performance evaluations compare operating data for the same seasonal time periods in consecutive years before and with the technology in place. After 3 months of treatment, influent BOD load decreased 16% from 2565 lbs/day to 2143 lbs/day and influent TSS load decreased 16% from 2739 lbs/day to 2296 lbs/day. The combination of BOD and TSS pounds converted in the sewer provided 78,500 lbs of additional capacity to the treatment plant. Effluent BOD load decreased 21% from 126 lbs/day to 99 lbs/day and effluent TSS load decreased 6% from 153 lbs/day to 143 lbs/day.

**Figure 6 – Impact of Bioaugmentation on Influent Load**

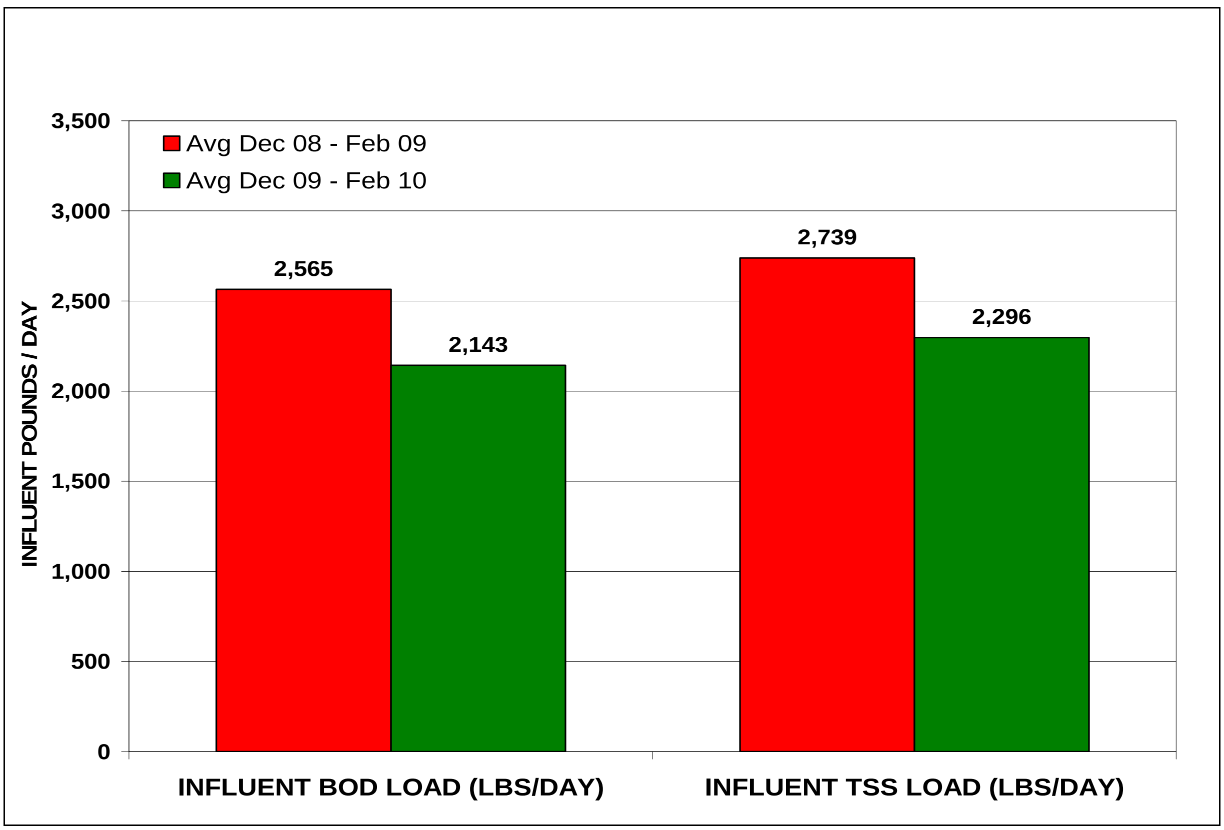
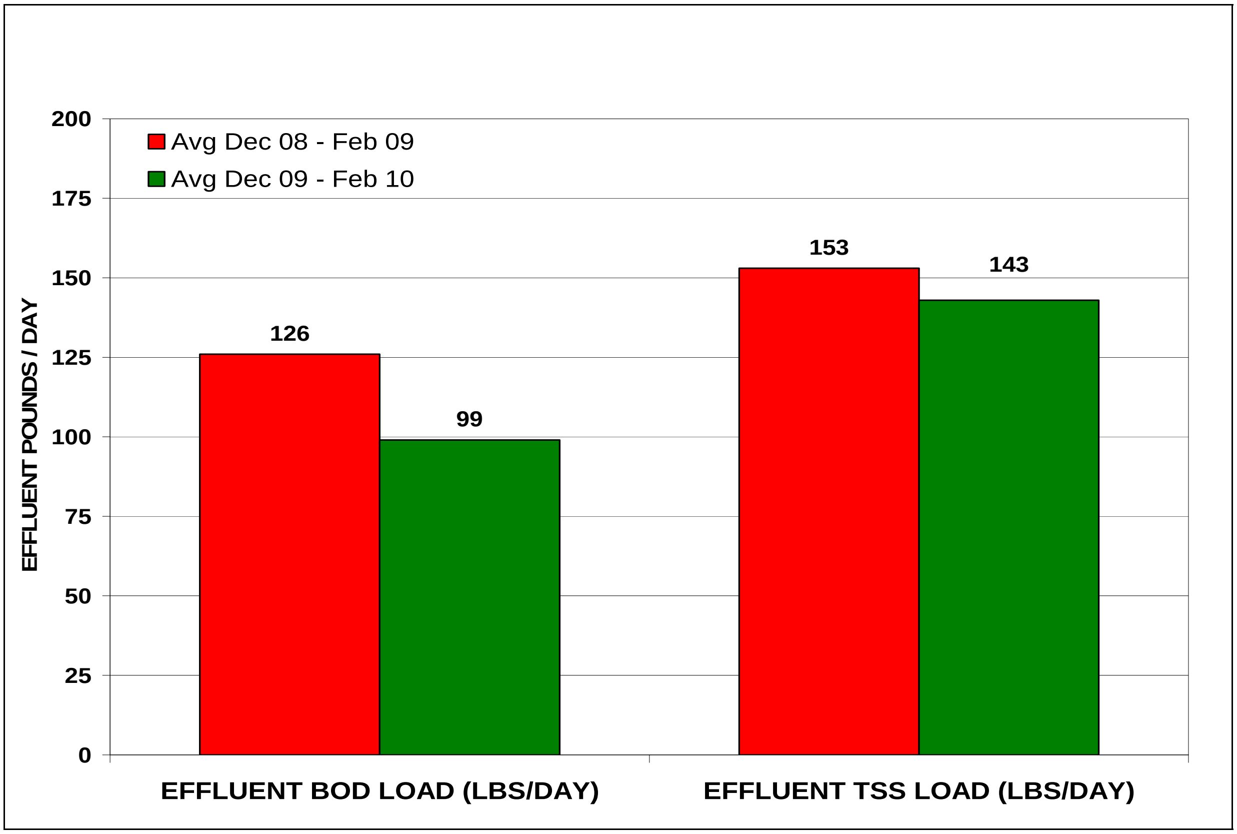


**Figure 7 – Impact of Bioaugmentation on Effluent Load**

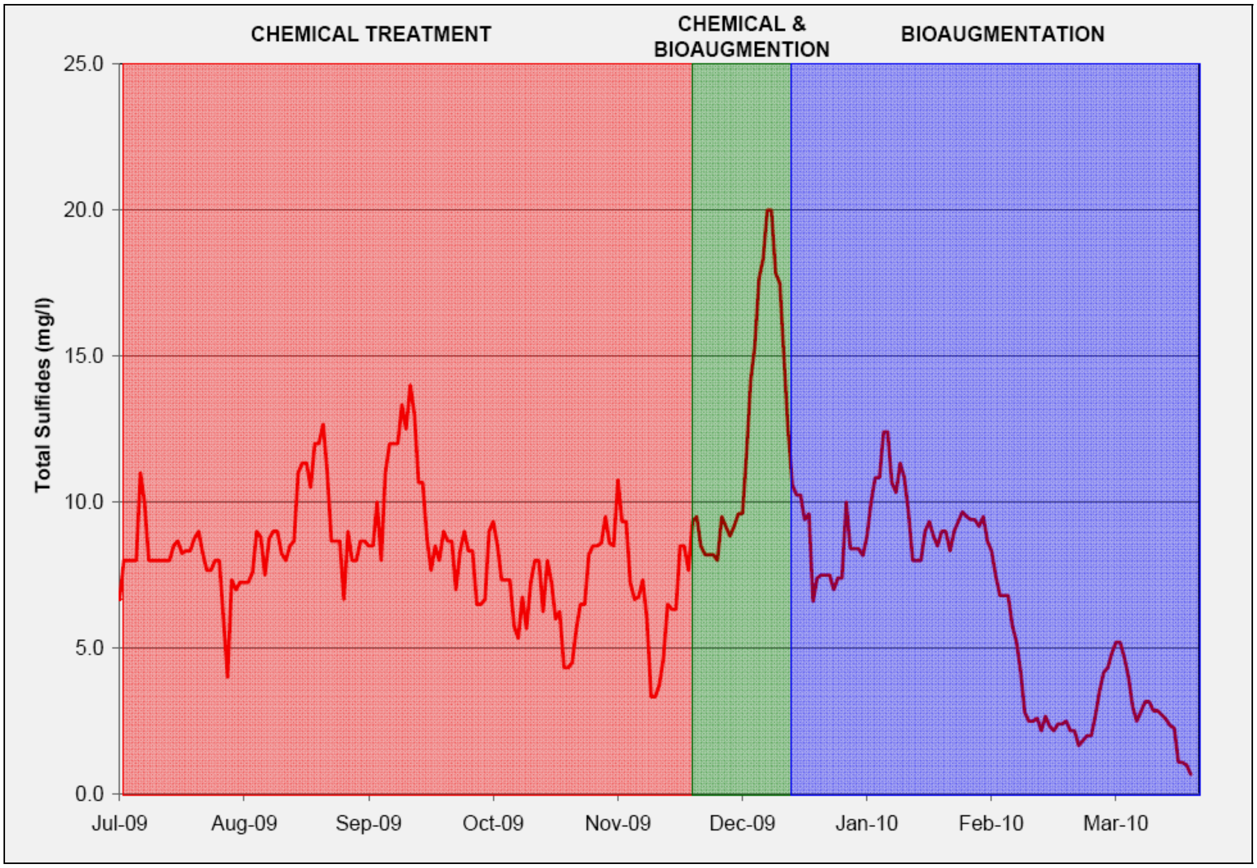



Force mains in a wastewater collection system cause anaerobic, oxygen-deficient environments. Under conditions with very low or nonexistent free molecular oxygen available, anaerobic bacteria use nitrate-bound oxygen (NO3) as a final electron acceptor. If there is no nitrate available they will use sulfate-bound oxygen (SO4) as a final electron acceptor which releases sulfide ions into the wastewater (Gerardi, 2006). The sulfide ions associate with free hydrogen ions in the water and form hydrogen sulfide gas which occupies the headspace of the piping. The hydrogen sulfide gas in the pipe headspace is oxidized to sulfuric acid by hydrogen sulfide-oxidizing bacteria existing on the inner surface of the headspace piping. This sulfuric acid is created constantly in the presence of hydrogen sulfide gas and is highly corrosive to many common piping materials, including steel, ductile iron and concrete (Metcalf & Eddy, 2003).

The WWTP personnel monitor solution sulfides in the influent wastewater to determine the corrosion potential. When the solution sulfide concentrations are low, the amount of hydrogen sulfide gas is proportionately low and the potential for corrosion due to the production of sulfuric acid is lower.

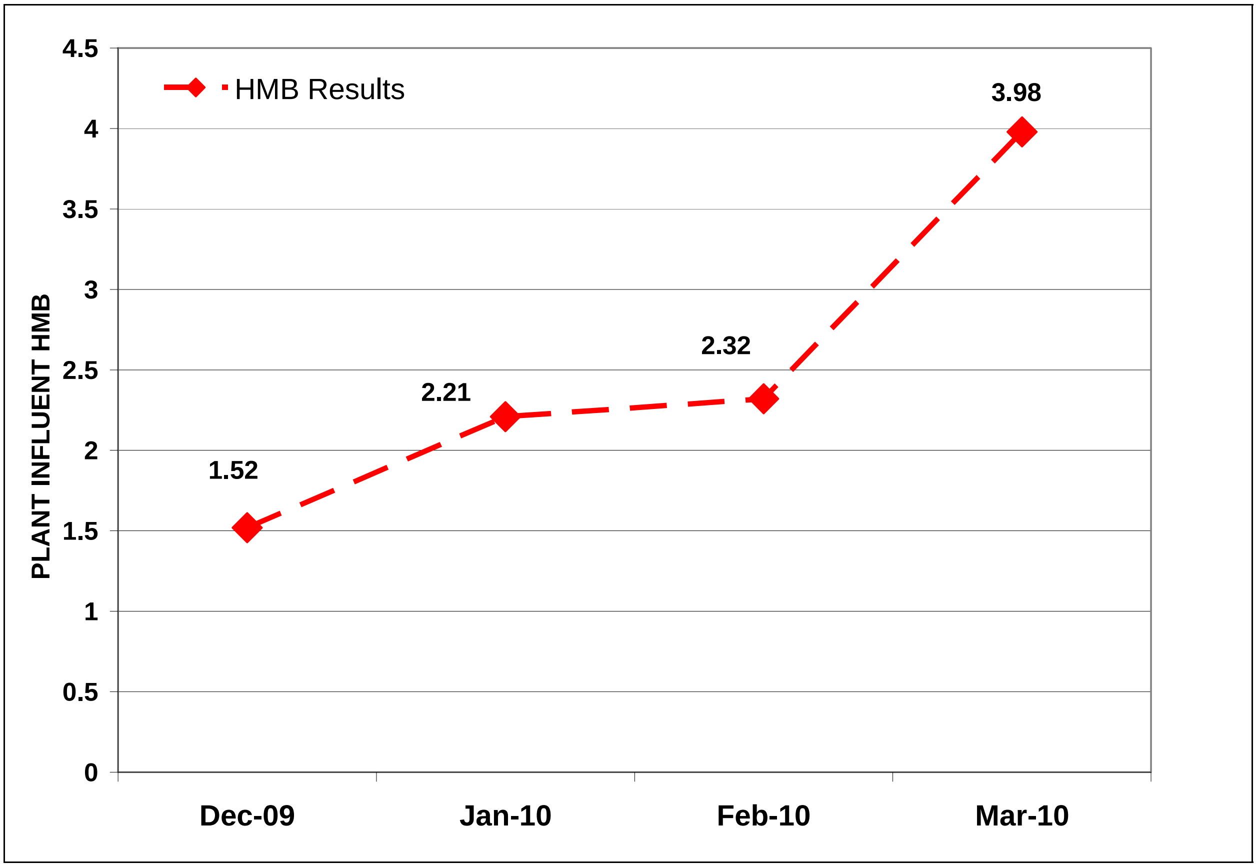
Bioaugmentation replaced previous chemical injections. During the 6 months prior to bioaugmentation, the average influent solution sulfide concentration was 7.9 mg/L. After 3 months of treatment, the average solution sulfide concentration dropped to 5.6 mg/L. Average daily influent sulfides decreased 29% from 7.9 mg/L to 5.6 mg/L as shown in Figure 8.

**Figure 8 – Influent Total Sulfides Entering the WWTP for 8 Consecutive Months**




The HMB results showed a gradual increase in metabolic activity. The influent baseline sample obtained prior to dosing in the collection system was 1.52. As of March 5, 2010, influent samples demonstrated a 161% increase over the baseline value at 3.98. Samples were also obtained at the Water Street pump station at the location of the old WWTP prior to entering the 4.5 mile force main. HMB results showed improvement at this location with a 25% increase in metabolic activity from 2.1 to 2.64; however, the baseline was not recorded prior to full collection system microbial treatment.

**Figure 8 – Influent HMB Results**




## CONCLUSION

The combination of BOD and TSS pounds converted in the sewer provided 78,500 lbs of additional capacity to the treatment plant in three (3) months. The cost of treating one pound of BOD or TSS at the plant was calculated using values of $0.155 and $0.064 per pound of BOD and TSS respectively. These values were provided by a contract operator for the actual cost of treatment. Converting BOD and TSS in the sewer is forecasted to save the WWTP $34,540 annually.

Using the collection system as an active part of the treatment process increases the efficiency of the WWTP and extends the life of existing infrastructure. Bioaugmentation in the collection system repopulates the wastewater and sewer biofilm with bacteria that excrete enzymes to rapidly degrade complex organic substrates to bio-available substrates, are more flexible in their oxygen requirements, and are better adapted to the wastewater environment than the intestinal bacteria that are introduced continually through normal wastewater collection activities.

The continuous addition of *Bacillus* bacteria allows a gradual repopulation of the sewer biofilm by bacteria that are more efficient degrading compounds in the wastewater than the bacteria that are present in untreated conditions. By optimizing the vast collection system, external bioaugmentation improves the economics of wastewater treatment.

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# Influence of Bioaugmentation in the Sewer to Improve the Economics of Wastewater Treatment in a Full-Scale System

Rich Schici, Senior Project Engineer
In-Pipe Technology® Company, Inc., 100 Bridge Street Wheaton, IL 60187

## SUMMARY

### BACKGROUND

- The high cost of upgrading a municipal wastewater treatment plant for increased organic capacity or stringent effluent requirements has urged many investigators to examine bioaugmentation as a means to improve the economics of wastewater treatment.
- Sewers are large biological reactors, whether we like it or not, that are often seen as a means to an end of the wastewater treatment plant, but actually provide similar treatment capabilities as a trickling filter if the microbiology is properly harnessed.
- Using a proprietary formulation of heterotrophic, facultative *Bacillus* bacteria added to the outer reaches of the wastewater collection system, the study tracks the progress of bioaugmentation in the sewer at a full-scale Sequencing Batch Reactor (SBR) wastewater treatment plant in Massachusetts.

### OBJECTIVES

- Analyze the impacts of *Bacillus* bacteria on wastewater characteristics and take advantage of the residence time of the wastewater within the sewer to degrade the waste.
- Investigate the effects of sewer bioaugmentation to reduce influent load, control Fats, Oil, and Grease (FOG) in the collection system, and improve effluent quality at the wastewater treatment plant with zero capital cost and no additional energy requirements.
- Measure biomass of the organisms containing the *catalase* enzyme using the HMB IV instrument to determine the increase in catalase positive *Bacillus* bacteria and the rate of metabolism.

### DESCRIPTION OF HMB IV ANALYSIS

- The HMB measures the activity of *catalase*, a peroxidase enzyme.
- Catalase is not present in strict anaerobes, but is present in facultative anaerobes or strict aerobes.
- The instrument measures only the biomass of organisms containing the catalase enzyme and not the total biomass of a sample.
- HMB test is unit less, not a count of bacteria present, and not a measurement of the water pollutant level.
- As metabolism increases, so too must the production of *catalase* enzyme. The HMB test measures the amount of *catalase* in the sample and thus measures the metabolic activity of the bacteria in the sample.

## METHODS


In an effort to utilize the miles of existing pipe and convert the passive sewer system into a meaningful treatment step, bioaugmentation in the sewer offers sustainable solutions through utilization of existing infrastructure with a natural, biological process.


Aerobic and facultative organisms use the *catalase* enzyme for all metabolic activity. When oxygen enters the bacterial cell, it is converted to the highly toxic compounds superoxide ($O_2$-) and hydrogen peroxide ($H_2O_2$). The enzyme *superoxide dismutase* converts superoxide to hydrogen peroxide and oxygen.

$$2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2$$

The enzyme *catalase* converts hydrogen peroxide to water and oxygen.

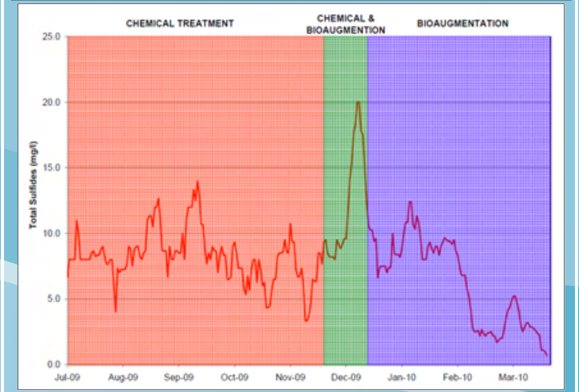
$$2H_2O_2 \rightarrow 2H_2O + O_2$$

The microbiological treatment for a system is carefully engineered and driven by:

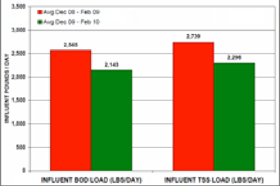
- Organic Loads
- Flow Distribution
- Collection System Layout
- Treatment Objectives

Battery-powered units hold a one (1) liter replaceable reservoir with a 30 day supply that provide time controlled treatment.
Dosing panels were installed at 25 locations in the sewer system and plant.

## RESULTS



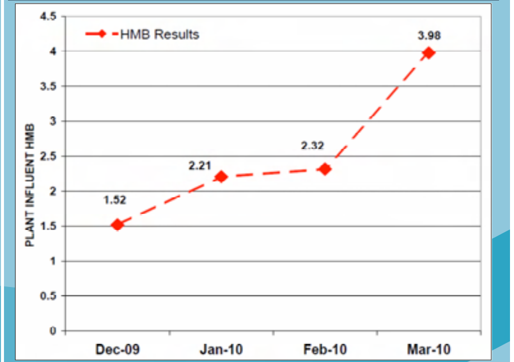

Influent Total Sulfides Entering the WWTP for 8 Consecutive Months
(Bioaugmentation replaced previous chemical injections)




Influent BOD & TSS Load Entering WWTP


Effluent BOD & TSS Load Discharged from WWTP

## RESULTS




## CONCLUSIONS

Sewer bioaugmenation with *Bacillus* bacteria had the following impacts on wastewater characteristics:

- Influent BOD load decreased 16% from 2,565 lbs/day to 2,143 lbs/day
- Influent TSS load decreased 16% from 2,739 lbs/day to 2,296 lbs/day
- Effluent BOD load decreased 21% from 126 lbs/day to 99 lbs/day
- Effluent TSS load decreased 6% from 153 lbs/day to 143 lbs/day
- Average daily influent sulfides decreased 29% from 7.9 mg/L to 5.6 mg/L
- HMB at the plant influent increased 161% from 1.52 to 3.98
- The combination of BOD and TSS pounds converted in the sewer provided 78,500 lbs of additional capacity to the treatment plant in three (3) months
- Converting BOD and TSS in the sewer is forcasted to save the WWTP $34,540 annually