Studies of a Microbial Mixture with Useful Properties for the Treatment of Organic Sewage

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Background:
The importance of bacteria in the treatment of raw sewage and water purification is well known. Here, we report on Sewper Rx, a unique mix of bacteria with powerful properties for treating sewage and other water cleaning applications, and we describe properties of the bacterial components of the treatment mixture which likely account for its enhanced organic solids digestion and odor reduction.

Description of the Bacterial Mix:
Studies of the bacterial mix have shown that when administered in accordance with dosing recommendations into a sewage plant, or other waste transport or collection system, the mixture is capable of reducing organic solids by up to 80% within as little as one month as shown in this 250,000 gallon per day (GPD) waste water treatment (WWT) plant (Figure1) and, additionally, of significantly reducing odors. Total solids are reduced significantly, when measured by truckloads of solid waste removed in the second example, a 1 million GPD WWT plant, Figure 2.

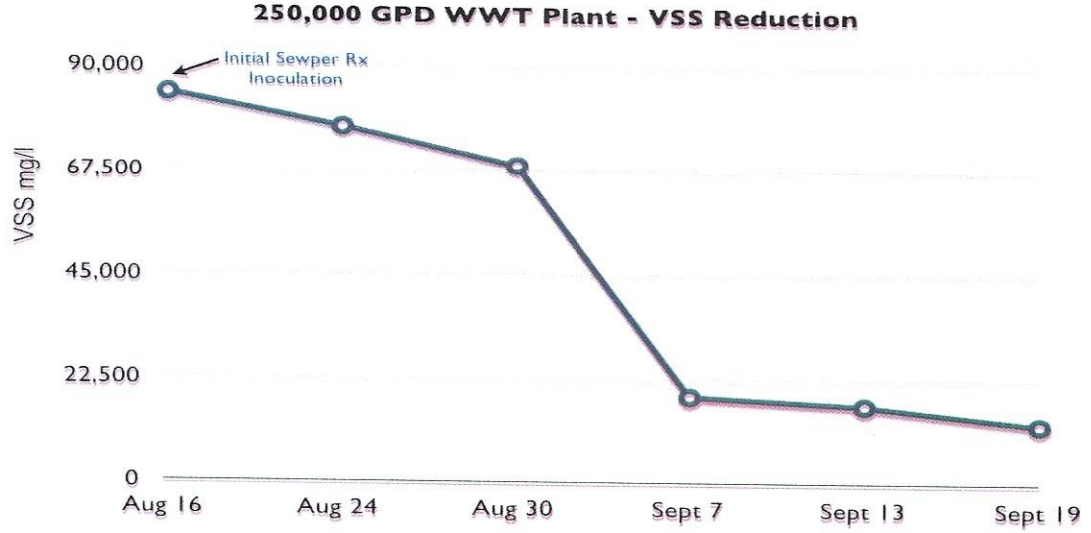

Figure 1 - Wastewater treatment plant VSS solids reduction within 30 days of initial inoculation of Sewper Rx.

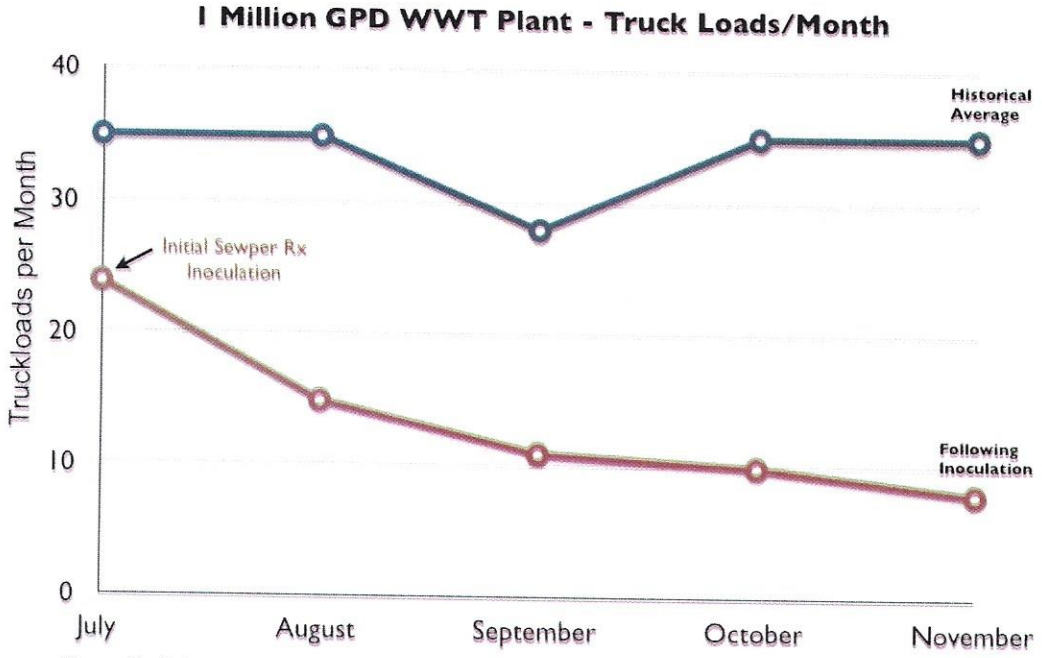

Figure 2: Wastewater treatment plant with normal monthly sludge truckloads averaging 35 loads per month exhibited a reduction within 30 days of initial inoculation of Sewper Rx patented bacterial mix. Reduction in loads averaged 77% below the norm within 5 months.

Sewper Rx is a patented product developed through a unique manufacturing process that results in a polymicrobial mixture composed of three distinct Gram-negative bacteria (a scientific designation reflecting the inability of the bacteria to retain a dark blue stain, known as Gram-stain). The three bacteria belong to the Genera *Pseudomonas*, *Citrobacter*, and *Erwinia* and are commonly found in nature. Together, in Sewper Rx, they express special properties that make them superior in reducing sewage solids and odors.

We have performed a number of tests on these three bacteria to identify properties that allow us to begin to understand why they have superior organic solids and odor reducing properties. The bacteria are motile (i.e. all three have appendages that give them the capability to move independently) which allows them to attack organic solids (VSS) and migrate into the plant when introduced upstream. Some can become adherent, enabling them to form biofilms. The bacteria are capable of reproducing (doubling) in very short times (as little as 24 minutes) and all can grow in the presence of oxygen and appear to be able to survive well and multiply in reduced oxygen conditions. All of the bacteria have a waxy coat composed of lipopolysaccharide. One of the bacterial species is capable of the fermentation of sugars in the absence of oxygen and another species can grow under anaerobic conditions

Utility in Sewage Treatment:
These properties of the bacterial mix provide a strong indication of why they are superior in reducing organic solids and odors.

Why is the mixture so efficient?:
The bacterial biofilm is sticky and likely permits the organisms to attach to the linings of the process chambers rather than immediately flow through the system, or to remain at the site of introduction. It is likely that the ability to form a biofilm explains why the mix is so efficient and doesn't require frequent repeated dosing. While bacterial biofilms allow the organisms to stick to the structure of the plant, once it has grown to a significant colony size, some of the organisms break free and travel to other parts of the system. We have evidence from studies in plants where dosing was performed in lift stations, sometimes miles ahead of the plant, that the bacteria do get into the plant and exhibit a reduction of solids there, as well as in the sewers leading to the plants. While we do not currently have firm data to support this point, there is much anecdotal evidence that it occurs.

Why is the mixture so superior to standard treatment procedures?:
The finding that all of the bacteria in the mix are motile is a further indication why the mix has superior properties. Stirring, with oxygenation, is considered a vital step in sewage processing and motile bacteria have the ability to move to gradients with oxygen, nutrients (i.e. solid organic material) and ideal temperature climes, giving them an ecologic advantage and hence provide improved organic and odor reducing efficiency. Also, it is quite likely that there is a synergistic action of the three bacteria. Some documented tests have shown that the consistent use of the Sewper Rx mix has reduced energy costs in the aeration process over 20%.

Why is the mixture so effective at eliminating organic solids?:
We used the BIOLOG phenotype array technology to determine how the bacterial mixture utilized organics (carbon sources) and other substrates compared to the bacterium *Pseudomonas* alone. Our data clearly demonstrate enhanced carbon (i.e. organic) utilization by the mix, as shown in Table 1.

Table 1. Carbon utilization by Sewper Rx is enhanced compared to *Pseudomonas* species alone. Some of the additional carbon sources that are efficiently metabolized by the mixture are listed below.

D-Sorbitol	Galactopyranosyl-D-Arabinose
Gentiobiose	Uridine
D-Glucuronic Acid	Dulcitol
D-Galactonic Acid-g-Lactone	b-Methyl-D-Glucoside
p-Hydroxyphenyl Acetic Acid	D-Glucose-6-Phosphate
N-Acetyl-Neuraminic Acid	D-Fructose-6-Phosphate
Formic Acid	D,L-a-Glycerol Phosphate
m-Hydroxyphenyl Acetic Acid	D-Glucose-1-Phosphate

D-Glucosaminic Acid	Inosine
Methylpyruvate	D-Galactose
m-Inositol	Adenosine
Sucrose	D-Glucosamine
D-Mannose	Thymidine
Adonitol	2`-Deoxyadenosine
Maltotriose	Glycerol
D-Arabitol	b-Methyl-D-Galactoside
L-Rhamnose	D-Trehalose
N-Acetyl-D-Glucosamine	D-Cellobiose
Pyruvic Acid	a-D-Lactose
N-Acetyl-D-Mannosamine	D-Mannitol
D-Ribose	L-Arabinose
Maltose	D-Melibiose
Palatinose	D-Xylose
Arbutin	D-Raffinose
Quinic Acid	g-Amino-N-Butyric Acid
D-Ribono-1,4-Lactone	d-Amino Valeric Acid
a-Methyl-D-Galactoside	Salicin
4-Hydroxybenzoic Acid	a-D-Glucose

This wide spectrum of carbon utilization of the mix is a clear indication of the superior properties of the Sewper Rx mix and accounts for the enhanced ability and speed with which the mixture is able to reduce solids and clarify waste solutions. The mix is capable of efficiently utilizing a large proportion of the components of organic solid waste with resulting clarification of the sludge solution.

Why is the mixture so effective at reducing Ammonia?
Bacteria that are capable of aerobic respiration (like those in Sewper Rx) are important in the nitrification process - breakdown of ammonia to nitrate. However, anaerobic processes are typically essential for denitrification (further breakdown of nitrate to nitrogen gas). Denitrification completes the nitrogen cycle by returning nitrogen gas to the atmosphere. This is usually accomplished by anaerobic bacteria and requires separate reactors for denitrification and nitrification processes. At least two of the bacteria in Sewper Rx can perform aerobic denitrification (Reference 1. Kim et al, 2008; Reference 2. Huang and Tseng, 2001). Thus, the complete breakdown of ammonia to nitrogen gas can potentially be accomplished in a single reactor under aerobic conditions.

Why does the treatment significantly reduce or eliminate hydrogen sulfide odors so efficiently?
Our phenotype array data show that the *Pseudomonas* species present in Sewper Rx is mostly responsible for the strong and speedy sulfur reduction observed with the mixture. This property of *Pseudomonas* is well documented in the scientific literature (Reference 2: Chung, Huang and Tseng, 1995). *Citrobacter* has also been shown to reduce hydrogen sulfide odors (Reference 3: Huang and Tseng, 2001). It is also entirely

possible that there is a symbiotic relationship among the bacteria in the mix which speeds up and enhances the process, but this needs to be further evaluated.

Why does the treatment work on fats, oils, grease?
Sewper Rx has also been shown to significantly and quickly reduce fats, oils and grease (Figure 3), common by-products of commercial restaurant and food process operations. There is scientific evidence that *Erwinia* produces lipases (enzymes that break down oils and fats) in significant amounts (Reference 4: Hankin and Sands, 1974) and this may account for Sewper Rx's ability to degrade these substances.

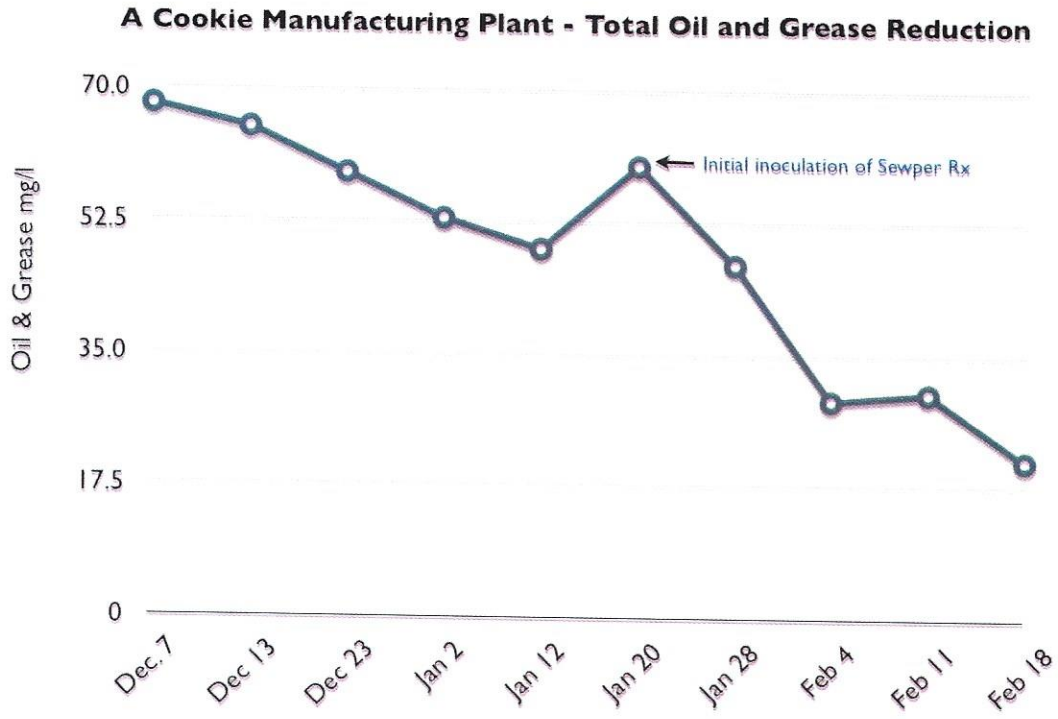

Figure 3 - The wastewater treatment plant (SBR) of a cookie manufacturing facility reduced their oil and grease content by over 60% in under 60 days with Sewper Rx. **In this graph it should be noted that the plant was not operational for produced goods from mid December through mid January, at which time production resumed. Sewper Rx inoculation took place on January 20.**

Discussion:

We have demonstrated that a proprietary product with enhanced utility in the processing of sewage and other water purification consists specifically of a mix of three different Gram-negative bacterial isolates. Because of the unique manufacturing process, the microorganisms in the mixture have novel properties which enable enhanced processing of solid waste and reduction of odors. We postulate that there may be a synergistic action of the three bacteria (along with several less prominent bacterial strains present in the mixture), with innate properties of the constituents conferring these activities. Using phenotype array technology, which identifies metabolic and substrate utilization of the bacteria, it appears that Sewper Rx has special enhanced activity for utilization of carbon containing compounds (i.e. organic, solid waste). Other

properties of the mixture that are also important are the ability to grow in microaerophilic (reduced oxygen) conditions as well as the possession of flagella, which confer motility, allowing the microorganisms to migrate to the microenvironment(s) ideal for their growth. Our studies show that the bacteria in the mix have an extremely quick doubling time with high growth yield - a further indication of why they are so efficient.

At least two of the bacteria can form biofilms, which enables them to stick to the walls of wastewater transport channels and the physical plant. These biofilm properties indicate that the bacterial mix can be introduced at specified points in the system and will remain there, while further colonizing sites downstream. As it grows, colonies of bacteria may be released into other locations of the plant where they can either attach to the walls of the plant or remain in the flow of the system, further enhancing the efficient digestion of organics.

We have demonstrated by phenotype array analysis that certain bacteria in the mix are capable of high nitrification (breakdown of ammonia to nitrates), but can also accomplish the denitrification pathway resulting in nitrogen production from the nitrates. This could account for the speed and efficiency with which solid organic material in sewage operations is processed and the rapid clearing of the fluid component by the bacterial mix.

The properties we describe are consistent with the recommended treatment process that it is only necessary to apply the mix at monthly intervals in modest quantities. As such, this leads to considerable cost savings to the plant operations with the added bonus of reduced load on machinery and further maintenance savings. Documented tests have also identified that the addition of the bacterial mix have reduced electricity cost up to 20% in the aeration basin alone.

The mix has also been shown to significantly reduce odors in hog farm lagoons and primary clarifiers in wastewater plants. Our phenotype array data indicate that at least one of the bacteria has an affinity for sulfur and this can lead to reduction of hydrogen sulfide and other odiferous vapors which likely accounts for the observed odor reduction.

A final indication from use of the bacterial mix is that it is capable of reducing fats, oils and grease. Our studies of the bacterial mix in a cookie plant showed more than 60% reduction in a 60-day period post inoculation. While our studies on this capability are less comprehensive, we do know that at least one of the bacteria present in the mix is known to produce lipase enzymes. Lipases are an important enzyme catalyzing the ability of the microorganism to utilize fatty compounds. (Reference 4)

In conclusion, our findings support the many experiences where the Sewper Rx bacterial mix has proven to be more effective in reducing carbon solids than the normal populations of transient wastewater plant bacteria, while requiring less oxygen for support. The resulting financial savings in aeration electricity, post treatment of sludge

hauling/tipping fees, and odor reduction, provide confirmation that this bacterial mix can be more than beneficial in modern wastewater treatment practices.

References:

Ref 1. Mia Kim, S. Jeong, S. Cho, Y. Kim, M. Kim, E. Ryu, and S. Lee. (2008). Aerobic denitrification of *Pseudomonas putida* AD-21 at different C/N Ratios. Journal of Bioscience and Bioengineering. **106**, No. 5, 498-502.

Ref 2: Ying-Chien Chung, Chihpin Huang, and Ching-Ping Tseng (1996): Biodegradation of Hydrogen Sulfide by a Laboratory-Scale Immobilized *Pseudomonas putida* CH11 Biofilter. Biotechnol. Prog. **12:**773-778

Ref 3: Chihpin Huang and Ching-PingTseng (2001): Nitrate Reduction by *Citrobacter* diversus under aerobic environment. Appl. Microbiol. Biotechnol. **55:**90-94

Ref 4: **L.** Hankin and D. Sands (1974): Bacterial production of enzymes in activated sludge systems. Water Pollution Control Federation, **46**, No. 8, 2015-2025.