White Paper

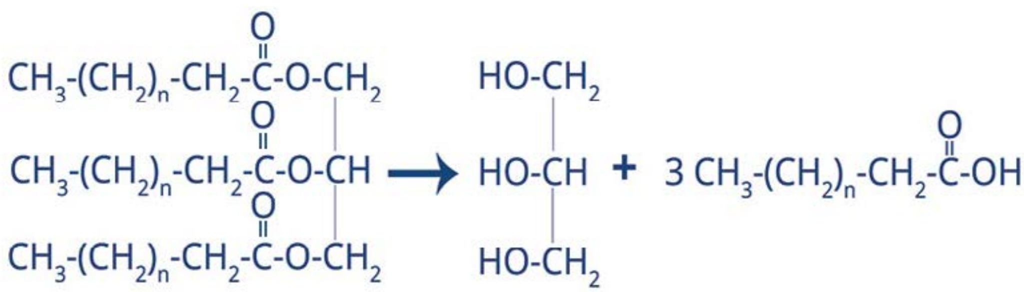
Removal of Fats, Oils, and Grease from Wastewater with BiOWiSH™

Background

Fats, Oils, and Grease (FOG) are common contaminants in wastewater systems. They discharge from a number of sources and pose a problem for sewer and septic systems because they are largely insoluble, accumulating over time in drainage lines and holding tanks. A layer then forms over the surface of water which leads to a decrease in dissolved oxygen content. This in turn reduces the natural microbial activity responsible for FOG breakdown.

The potential damage caused by the accumulation of FOG, as well as the additional processing required to ensure regulatory compliance for wastewater heavily polluted with FOG and BOD. This potential damage has led some municipalities to shift the costs associated with clean-up to consumers and businesses through contaminant surcharges or through mandatory pumping intervals for decentralized grease interceptor traps. For businesses such as food processing facilities that expel high FOG and BOD wastewater, these surcharges can total tens of thousands of dollars a month.

Naturally occurring microbial consortia comprising: aerobic, facultative, and obligate anaerobic organisms, are largely responsible for the removal of organic waste in advanced wastewater and sewage treatment systems. Microbial metabolism results in the breakdown of organic material. For FOG waste, this process initially involves cleavage of the ester bond between the hydrophobic (water repelling) fatty acid moieties of the FOG and the more hydrophilic (water loving) glycerol head group:



The fatty acids are then further broken down via β-oxidation to smaller, volatile fatty acids and ultimately, given the right conditions and microbes, to CO_2 and water. The glycerol is converted to metabolically useful intermediates such as Glycerol-3-phosphate.

This natural process often leads to undesirable consequences such as malodor from the production of short chain, volatile fatty acids, corrosion, and, if not highly efficient, blockage of sewer pipes with undissolved FOG material.

Bioaugmentation with BiOWiSH™

Bioaugmentation is the practice of adding actively growing, microbial strains into a native microbial community. This enhances the ability of the microbial community to respond to fluctuations in the environment or to degrade certain compounds. Bioaugmentation can be used to reduce FOG materials in wastewater and mitigate some of the negatives associated with partial break down. BiOWiSH™ Aqua FOG is comprised of a proprietary mix of natural surfactants, metabolically cooperative microorganisms, and small molecule metabolic co-factors that stimulate natural microbial systems in the breakdown of a broad spectrum of organic matter including FOG.

In vitro assays conducted at California Polytechnic State University show that BiOWiSH™ organisms express high levels of extracellular enzymes called esterase. These are the enzymes responsible for the initial breakdown of FOG through cleavage of the ester bond.

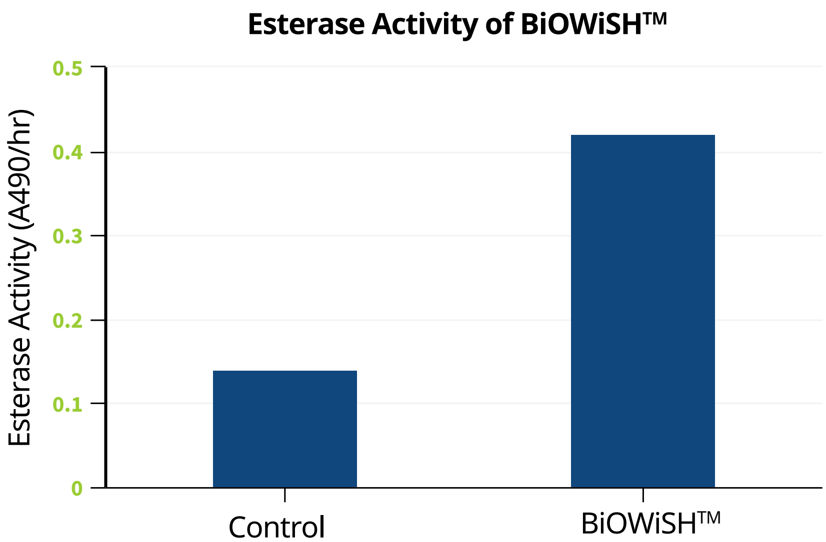


Figure 1: In vitro esterase activity of BiOWiSH™ organisms

When dosed into wastewater containing high levels of FOG, BiOWiSH™ Aqua FOG provides the following benefits:

1. **Odor control.** BiOWiSH™ operates as an effective facultative anaerobe allowing it to reduce the emission of volatile organic compounds.
2. **FOG reduction.** FOG levels are rapidly reduced, even in systems with relatively high flow/low retention times, in the presence of typical cleaning agents and in otherwise normally challenging conditions such as low temperature.
3. **Improved Effluent Quality.** Dependent on the available retention time in the system, significant improvements in BOD, COD, TSS, and TKN can be achieved.

Figure 2 shows the effect of BiOWiSH™ Aqua FOG dosage in a typical fast food restaurant grease trap:

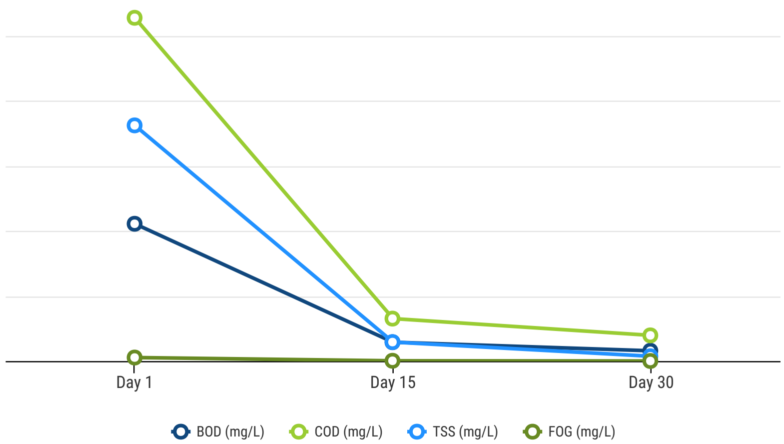


Figure 2: Effect of BiOWiSH™ Aqua FOG addition to Fast Food Restaurant Grease Trap

After 30 days BOD and COD were reduced by more than 90%, FOG and TSS were reduced by more than 95%. In addition, there was a noticeable reduction in the headspace odor above the trap.

A large industrial catering company in Southeast Asia dosed BiOWiSH™ Aqua FOG into a 9.6 m^3 grease trap with a waste flow from the kitchen of 25 m^3/day. After 24 hours the FOG dropped from 5,470 ppm to 800 ppm, a decrease of 85%.

Similar benefits are observed in septic systems:

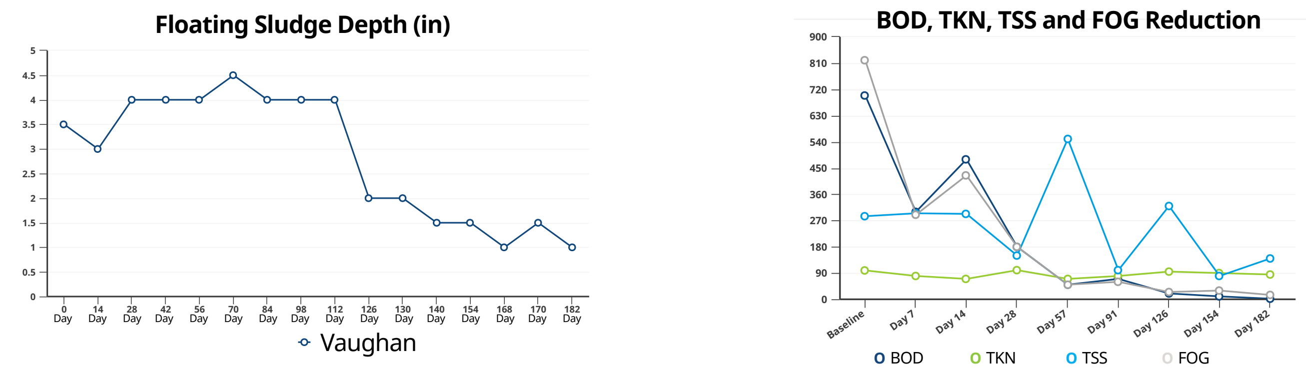


Figure 3: Effect of BiOWiSH™ Aqua FOG on septic systems after 90 days

And in industrial wastewater treatment systems where an estimated 4,444 tons of primary floating FOG were removed in 90 days:

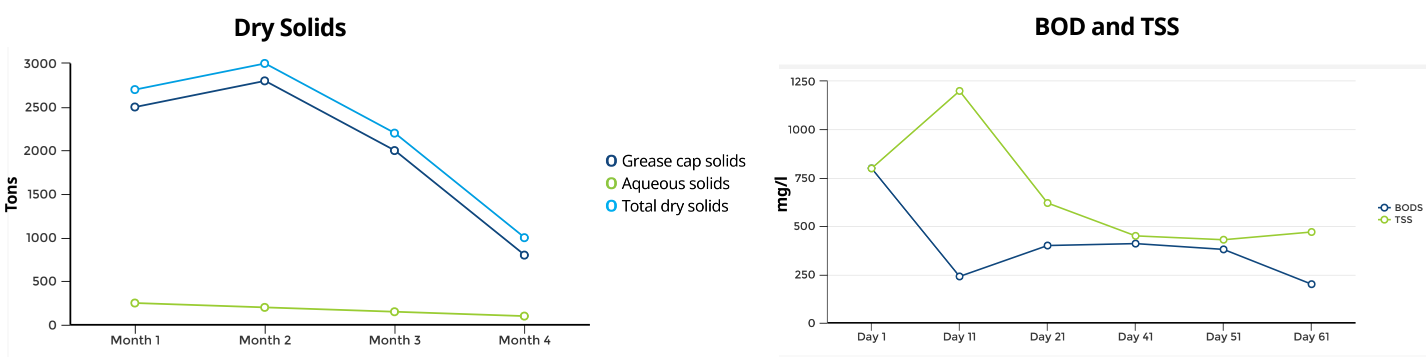


Figure 4: Dry Solids, BOD, and TSS reduction in JBS WWT site (Greely, Colorado USA) after BiOWiSH™ addition

Laboratory Demonstration of FOG Degradation

To better understand how BiOWiSH™ degrades FOG a series of in vitro assays were developed using vegetable shortening and olive oil as the substrate.

One of the main objectives behind these assays is to demonstrate that BiOWiSH™ Aqua FOG organisms can not only express the required biochemistry to cleave FOG, but will further use the resulting fatty acids as a suitable carbon source to support biomass growth. This action ensures that fats do not re-solidify at cooler ambient temperatures in downstream systems, a common problem with many emulsifying agents.

Materials and Methods

Solid Pour-Plate Assays

Preperation of vegetable shortening – Vegetable shortening (Crisco™) was dispensed into 250mL Pyrex beakers and melted using a SciLogex MS-H280 Pro Stirrer Hotplate. Once melted, shortening was dispensed in 75mL aliquots into 150mL beakers, which were capped with aluminum foil and autoclaved at 121°C, 15 psi, for 15 minutes. Shortening flasks were allowed to cool on the laboratory benchtop until warm to the touch before use in pour plates.

Preparation of water-based additives – Aqueous components of pour plates for treatments A, B, C and D consisted of 25mL DI H_2O prepared with the following additives: no additives (treatment A), 0.25g heat-killed BiOWiSH™ Aqua (treatment B), 0.25g live BiOWiSH™ Aqua (treatment C), and 0.25g live BiOWiSH™ Aqua FOG (treatment D). For treatment E, the aqueous component consisted of a bacterial metabolite suspension. Two 15mL aliquots of turbid mineral medium were aseptically removed from a 96-hour old Bushnell-Haas reactor flask (described below) and spun in a centrifuge at 6,000 x g for 10 minutes. 25 mL of the supernatant was decanted into a sterilized 100mL foil-capped 100mL beaker using a sterile serological pipette.

Preparation of solid pour plates – Beakers of 75mL sterile, melted vegetable shortening were combined with beakers of 25mL aqueous additive (both described above) and mixed vigorously by hand with a flame-sterilized metal spatula until a relatively homogeneous mixture was achieved. This mixture was then poured in ~30mL aliquots into triplicate sterile, plastic Petri dishes. Petri dishes were then allowed to incubate for 24 hours at 37°C before being allowed to cool benchtop at 25°C for a further 24 hours. Plates were then inverted and examined for evidence of emulsification. Plates containing clearly delineated, heterogeneous regions of water and shortening were considered poorly emulsified, whereas plates containing relatively homogeneous media were considered well emulsified.

Bushnell-Haas Reactor Flask Assays

Preparation of Bushnell-Haas Medium – Bushnell-Haas broth (Bushnell and Haas, 1941) was prepared according to the specifications found in the Difco Manual of Microbiological Culture Media. Broth was decanted in 150mL aliquots into 500mL Erlenmeyer flasks. Olive Oil (100% pure, Kroger Company brand) was added to reactor flasks in 50mL aliquots as the sole carbon source for the medium. One pair of Bushnell-Haas broth flasks was prepared without olive oil for use as controls. Reactor flasks were then capped with foil and autoclaved at 121°C, 15 psi for 15 minutes.

Preparation of Bacterial Inoculums – 1.0g of BiOWiSH™ Aqua Fog was aseptically added to a 500mL foil-capped Erlenmeyer flask containing 100mL sterile Trypticase Soy Broth (TSB, Difco) and incubated for 24 hours at 37°C, 150RPM. After incubation, four 15mL aliquots of turbid TSB were aseptically withdrawn and spun in a centrifuge at 6,000 x g for 10 minutes. Bacterial cell pellets were washed with sterile phosphate buffered saline (PBS, Cold Spring Harbor) to and then re-suspended in sterile PBS until an absorbance of 1.00 at OD600 was achieved.

Preparation of Reactor Flasks – Reactor flasks were prepared according to three separate treatments. Treatment A, a control for growth of bacterial inoculums in plain Bushnell-Haas broth, was prepared by adding a 1.0mL bacterial inoculum to 150mL flasks of Bushnell-Haas broth. Treatment B, a control for abiotic interaction between Bushnell-Haas broth and olive oil, consisted of full Bushnell-Haas medium with no bacterial inoculum. Treatment C consisted of Bushnell-Haas medium and a 1.0mL aliquot of bacterial inoculum. All reactor flasks were prepared in duplicate.

Sampling of Reactor Flasks – 5.0mL aliquots of broth were aseptically collected from below the olive oil layer of each reactor flask using sterile serological pipettes at T = 0h, T = 24h and T = 96h. OD600 readings were taken for each aliquot using an Agilent Cary UV/Vis spectrophotometer as a measure of bacterial growth.

Results

Solid Pour-Plate Assays

Qualitative results from solid pour-plate assays are displayed in Figure 5. While Treatments A and B resulted in heterogeneous plates with separate regions of shortening and water clearly delineated, Treatments C, D, and E showed varying degrees of emulsification:

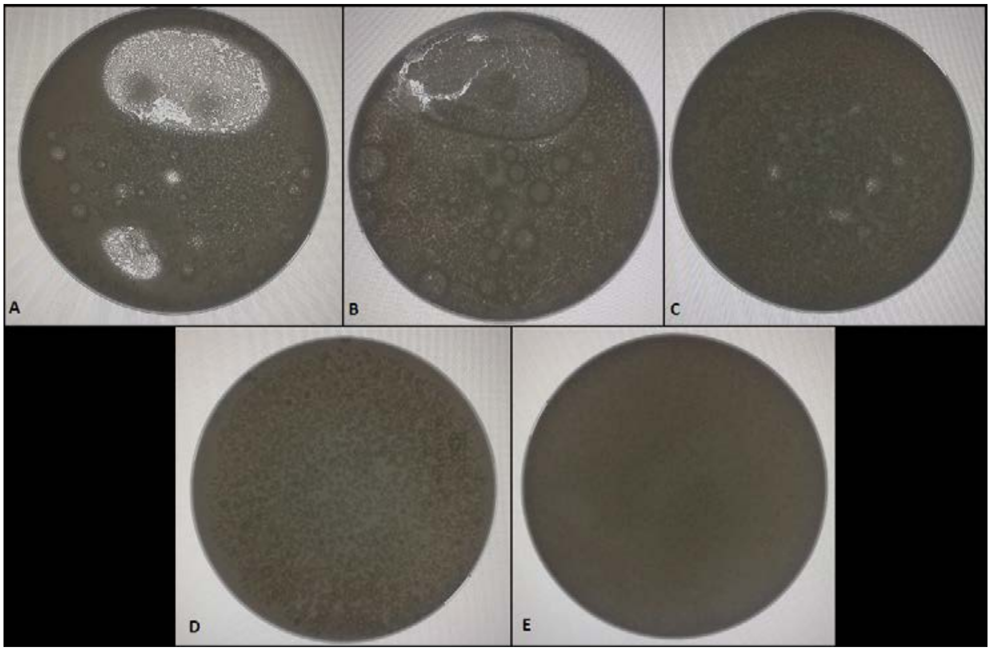


Figure 5: Emulsification of immiscible shortening and water in control (A), heat-killed BiOWiSH™ Aqua (B), live BiOWiSH™ Aqua (C), live BiOWiSH™ Aqua FOG (D), and BiOWiSH™ Aqua FOG metabolites (E).

Emulsification results from both the breakdown of the shortening into more soluble components and the generation of emulsifying agents such as mono- and di-glycerides through the effect of BiOWiSH™-generated esterase activity on the triglycerides.

Bushnell-Haas Medium Assays

BiOWiSH™ treatments showed a significant increase in turbidity of Bushnell-Haas broth in reactor flasks within 96 hours relative to control treatments (Figure 6), indicating logarithmic growth of bacteria in the broth using olive oil as the **sole** carbon source. Qualitative differences were also observed in the physical properties of the olive oil overlays between control and BiOWiSH™ treatments (Figure 7) as the oil is emulsified by the BiOWiSH™.

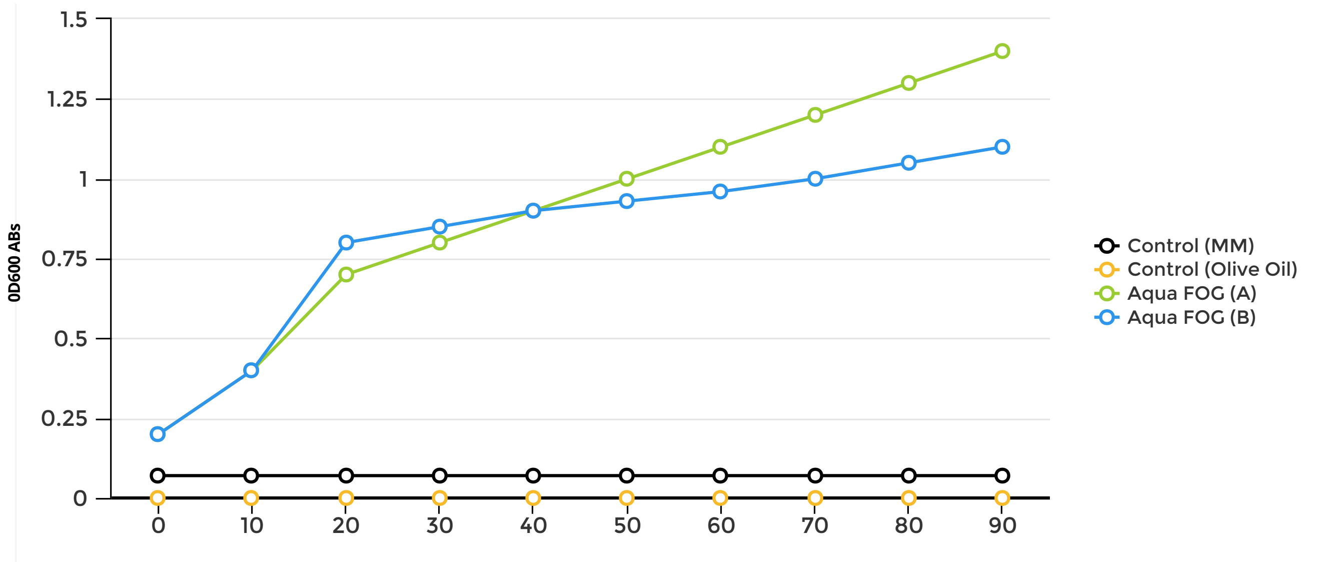


Figure 6: Changes in absorbance at OD600 in Bushnell-Haas medium reactor flasks. The increase in turbidity of the Bushnell-Haas broth in BiOWiSH™ treatments indicates that the bacteria are able to enter into logarithmic growth using olive oil as the sole carbon source.

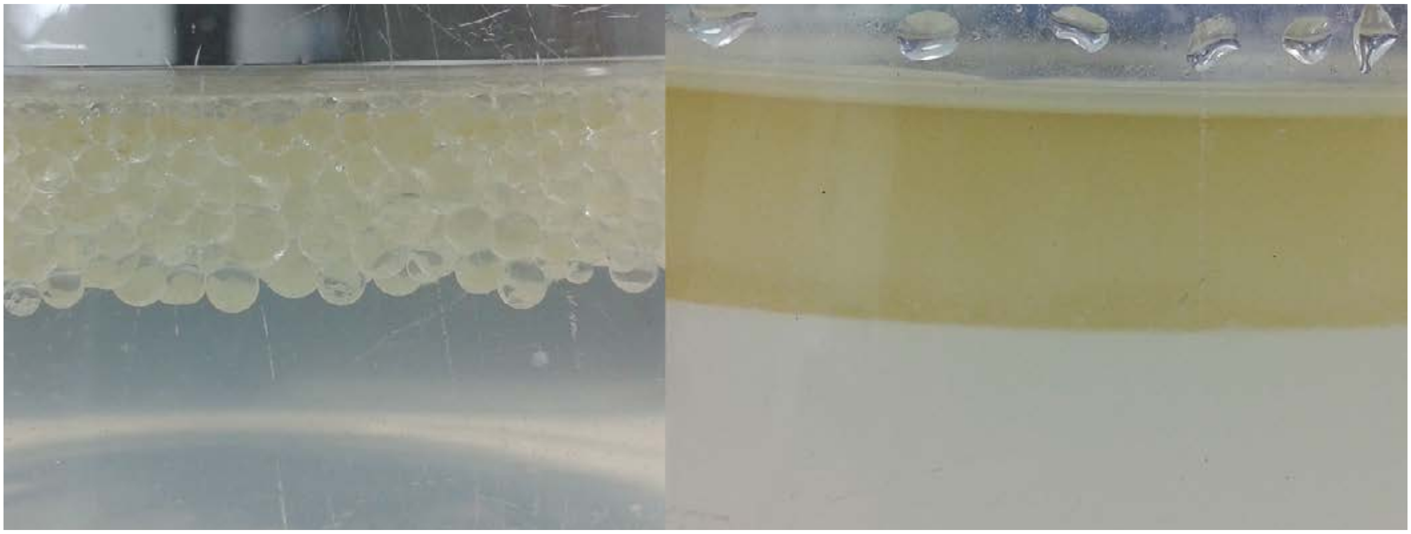


Figure 7: Changes in the physical characteristics of olive oil overlays in control (left) and BiOWiSH™ (right) Bushnell-Haas reactor flasks.

Testimonials

BiOWiSH™ Aqua FOG has been used across a range of wastewater systems and consistent benefits reported for both organic waste and odor reduction. Following are comments from users:

"Since the implementation of a BiOWiSH™ treatment regime no settled sludge has accumulated within the treatment system, pipe work blockages have been eliminated, and the working environment is no longer adversely affected by odour." – **Wickwar Brewing Company, Gloucestershire UK**

"The plant operators have confirmed complete odor elimination on the site which has increased the plant safety as well as air pollution." – **Alturas WWTP, California USA**

"BiOWiSH™ Odor has been crucial to the success of our client's composting program and we continue to use it to this day. It's easy to use and apply, and more importantly, it has effectively removed problematic odors from our site from day one while saving our client money on pull out costs."
– **McDonalds Global Corporate Headquarters, IL USA**

"After only 34 days, Maintenance Supervisor Hugh Keith reported a significant breakup of the fatty sludge in the E Section sludge tank. He also reported that the odor at the D Section lift station was nearly eliminated."
– **Thousand Trails, Chesapeake Bay, VA USA**

Conclusions

In Vitro studies and numerous field trials across multiple geographies show that BiOWiSH™ Aqua FOG:

1. Effectively reduces FOG accumulation in wastewater.
2. BiOWiSH™ Aqua FOG microorganisms are not limited to producing the necessary enzymes to cleave FOG, but can further degrade resulting compounds to support biomass development, and therefore prevent downstream solidification.
3. Reduces odors associated with FOG and FOG breakdown.
4. Prevents fat blockages in pipework.
5. Provides a simple, rapid, low cost way to manage FOG removal that is safe and environmentally sustainable.