A white paper on Effect and Efficacy of BiOWiSH™ for Reducing Solids in Dairy Wastewater

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Abstract: BiOWiSH™ wastewater treatment formulations were tested for their ability to reduce the solids content of Dairy wastewater. Significant reductions in Total Suspended Solids (TSS) were observed with a single dose of these products. Particle size analysis shows that the BiOWiSH™ treatments reduce the size of suspended particles across the size range from 0.7 to 5.0 µm.

Background: Our laboratory started working with BiOWiSH™ products in 2008-2009. Since 2010, we have been continuously working directly with BiOWiSH Technologies, Inc. on product development, product formulation, and product testing for wastewater bioremediation. During our tests, we have seen the additive effect of BiOWiSH's™ technology on degradation and clarification of wastewater. For testing purposes, we selected treated waste from the California Polytechnic State University (Cal Poly) Dairy lagoon. Dairy waste is generally recognized as one of the most difficult wastewaters to treat due to the presence of high amounts of ligno-cellulosic material, un-digested straw, organic solids, and other waste end-products. Literature shows very slow or negligible reduction of Total Suspended Solids (TSS) over long periods of time for treated dairy waste.

We examined the effect of BiOWiSH's technology on reduction of Biochemical Oxygen Demand (BOD), TSS and denitrification and compared the results to a control, which contained only indigenous microbiology from the waste water facilities (in this case from Cal Poly Dairy lagoon waste). A good number of grey (commercial and unpublished) research studies indicate that BiOWiSH™ is effective in reducing BOD and TSS in septic systems and untreated municipal waste.

RESULTS FOR BIOWISH™ WASTE-WATER TREATMENT FORMULATION COMPARED TO A CONTROL

A typical result is shown in Figure 1 below. In this experiment, a single dose of the BiOWiSH™ product was added to dairy wastewater and TSS was measured over a 75 day period.

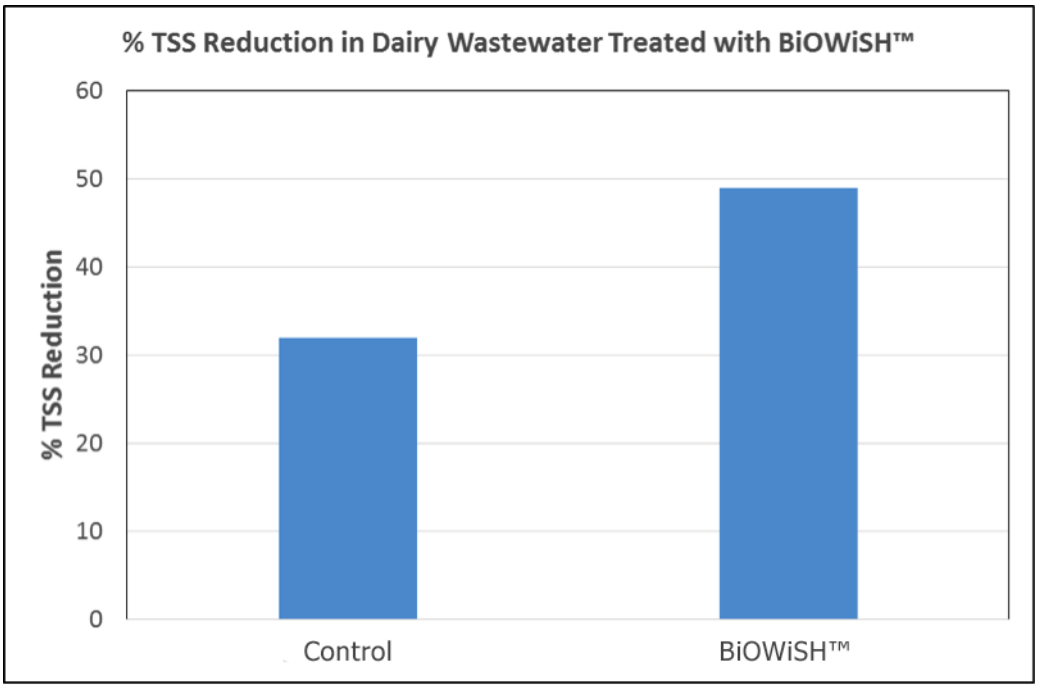

Figure 1: Effect of BiOWiSH™ on Reduction of Total Suspended Solid in Dairy Waste

In Figure 1, we see that the BiOWiSH™ product contributes about 16% more reduction in total suspended solids compared to the control (without BiOWiSH™).

In our subsequent studies, we have also seen that TSS reduction can be further enhanced when BiOWiSH™ is repetitively dosed. Our recent studies show that a single second dose 2 weeks after the initial dose reduces TSS by an additional 10% compared to a single dose. Currently, detailed studies are being conducted to optimize the re-dose interval, as we've determined that the addition of more biology over an extended period has increased the TSS reduction.

Possible Mode of Action of BiOWiSH™: To determine the possible mechanism of this enhanced degradation by BiOWiSH™, we conducted a series of particle size distribution analyses. In these experiments we monitored how the particle size distribution changes as a function of time post BiOWiSH™ dosage. It is well known that decreasing particle size increases solubility in water facilitating transfer into the bacterial cell and subsequent conversion to CO_2, H_2O and biomass. Also, conversion to CO_2 is important, as it is an odorless gas and as such, does not elicit offensive malodor like H_2S or other VOC's associated with anaerobic biodegradation.

Previous studies with particle size distribution show that degradation of particle sizes in the range 2.5 to 1.6 microns is the rate limiting step in the biodegradation process. A simple mechanism for the microbial degradation of waste solid particles is shown in Figure 2 (below) with breakdown of the 2.5 to 1.6 micron size as the rate limiting step for the dairy waste system:

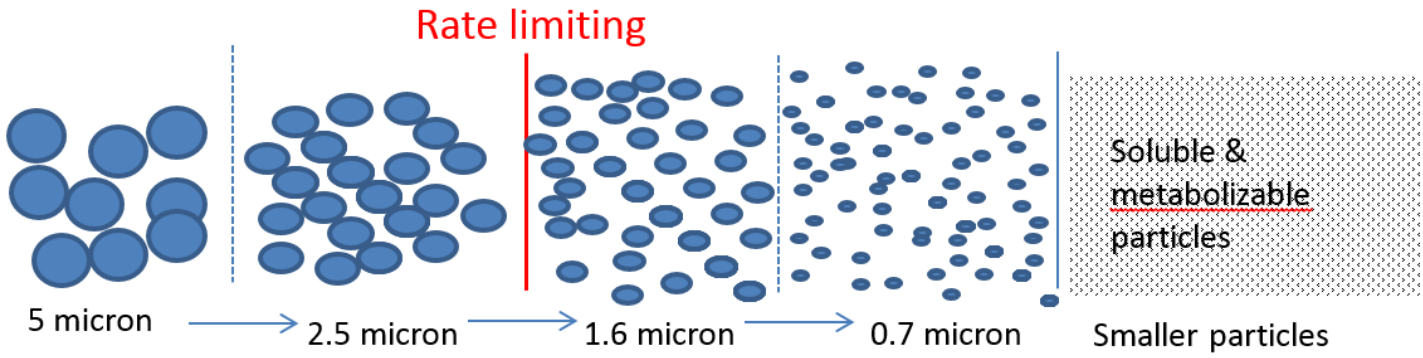

Figure 2: Schematics of Step-wise Degradation of Particles by Bacterium

In wastewater, a large fraction of 5.0 micron particles are inert cellulosic or lignin containing materials. It is known that biodegradation of these particles is extremely slow for most naturally occurring wastewater microbes. For the biodegradable fraction of the particles, extracellular enzymes, produced by microbial growth, stepwise degrade the carbon source to smaller particle sizes. As the microbial carbon source is exhausted, or the bacteria cannot further metabolize the recalcitrant particles, overall solids reduction slows or comes to a complete halt. Our laboratory data show that in the case of indigenous dairy waste bacteria the particle size fraction between 1.6 to 2.5 microns becomes recalcitrant and native microorganisms cannot further degrade this particular range of particles. This appears to be the rate limiting step for dairy waste. This situation may be different for other types of waste. For dairy waste, we noticed that the BiOWiSH™ product enhanced the rate of degradation of particles in the 2.5 to 5.0 micron range as shown in Figure 3 below.

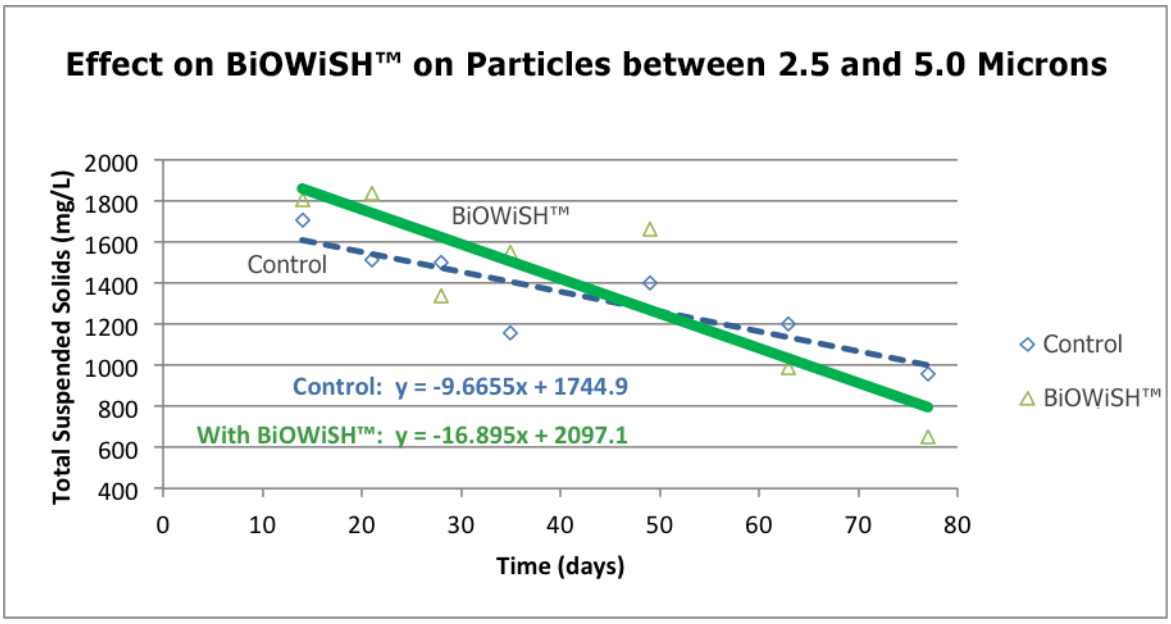

Figure 3: Effect of a Single Dose of BiOWiSH™ on Particles Between 2.5 and 5.0 micron.

If we assume zero order kinetics, we notice that the BiOWiSH™ product has a significantly higher rate of particle size reduction (-16.9 mg/L-day) than the control (-9.7 mg/L-day) in this size range.

The particles between 2.5 and 5.0 microns are apparently being degraded into smaller particles. This is evident when we examine the particle size range between 0.7 and 1.6 microns with and without BiOWiSH™ (Figure 4) 2 weeks after the initial dose:

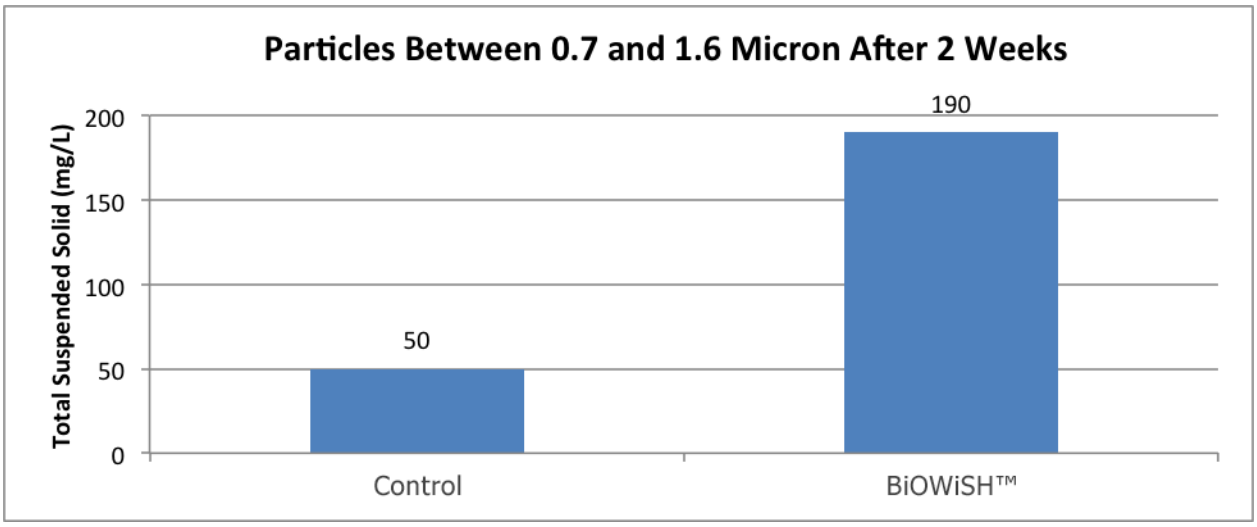

Figure 4: Total Suspended Solid Between 0.7 and 1.6 Micron Size After Initial 2 Weeks Period.

Addition of the BiOWiSH™ product increases the number of particles in the size range from 0.7 to 1.6 micron more than 3.5 fold. The increase in the number of particles in the 0.7 to 1.6 micron range and the concomitant decrease in particles in the size range 2.5 to 5 microns suggests degradation of larger particles to smaller particles by the BiOWiSH™ product.

When the BiOWiSH™ formulation is added to dairy waste we also notice a substantial difference in the particle size range between 1.6 and 2.5 micron versus the control:

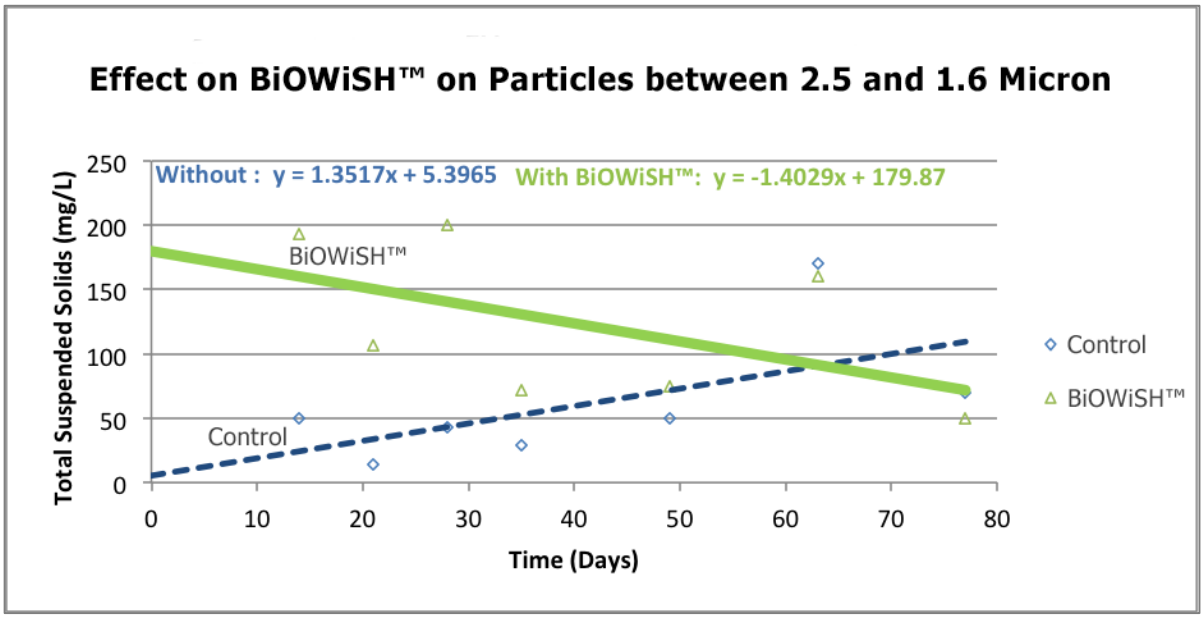

Figure 5: Effect of BiOWiSH™ on Breaking Particles Between 1.6 and 2.5 Micron.

Figure 5, shows the slow accumulation of particles in the size range of 1.6 to 2.5 microns when BiOWiSH™ is not added, consistent with previous observations that the degradation of particles in this size range is the rate controlling step in TSS reduction of dairy wastewater (see Figure 2 above). However, when BiOWiSH™ is added, we see continued decrease of TSS in this size range. As we know, specific enzymes or enzyme systems are needed to breakdown specific materials. Dairy waste contains substantial amounts of cellulosic materials and we hypothesize that the indigenous wastewater bacteria alone cannot break these down further, or the rate of breakdown is very slow. In the presence of BiOWiSH's technology, different enymes are produced and expressed that help to degrade the particle size fraction

between 1.6 to 2.5 microns to finer particles. This is accompanied by an increase in the particle size fraction from 0.7 to 1.6 micron when BiOWiSH™ is added as shown in Figure 4.

Figure 6 below, shows the zero order rate constants for the breakdown of the 1.6 to 2.5 micron fraction for the BiOWiSH™ treatment and the control. Here we observe that without BiOWiSH™ , the rate constant is +1.135 mg/L-day. This denotes accumulation of particulates between 1.6 and 2.5 micron. However, for the BiOWiSH™ product we see a rate constant of -1.40 mg/L/day. This is a remarkable improvement for the solid reduction process, especially when we consider that millions of solids are produced in the waste water industry.

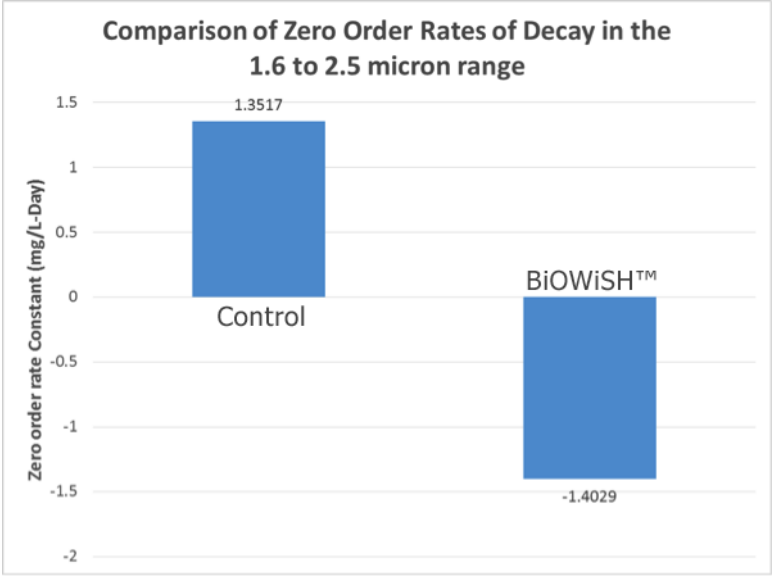

Figure 6: Zero Order Rate Constant Comparison For Particulates Between 1.6 and 2.5 Micron.

When we consider finer particles, which are bigger than 0.7 micron but less than 1.6 micron, we also see very similar behavior. The finer particles are not degrading any further in the control sample but do degrade when BiOWiSH™ is added. The result is shown in Figure 7 below:

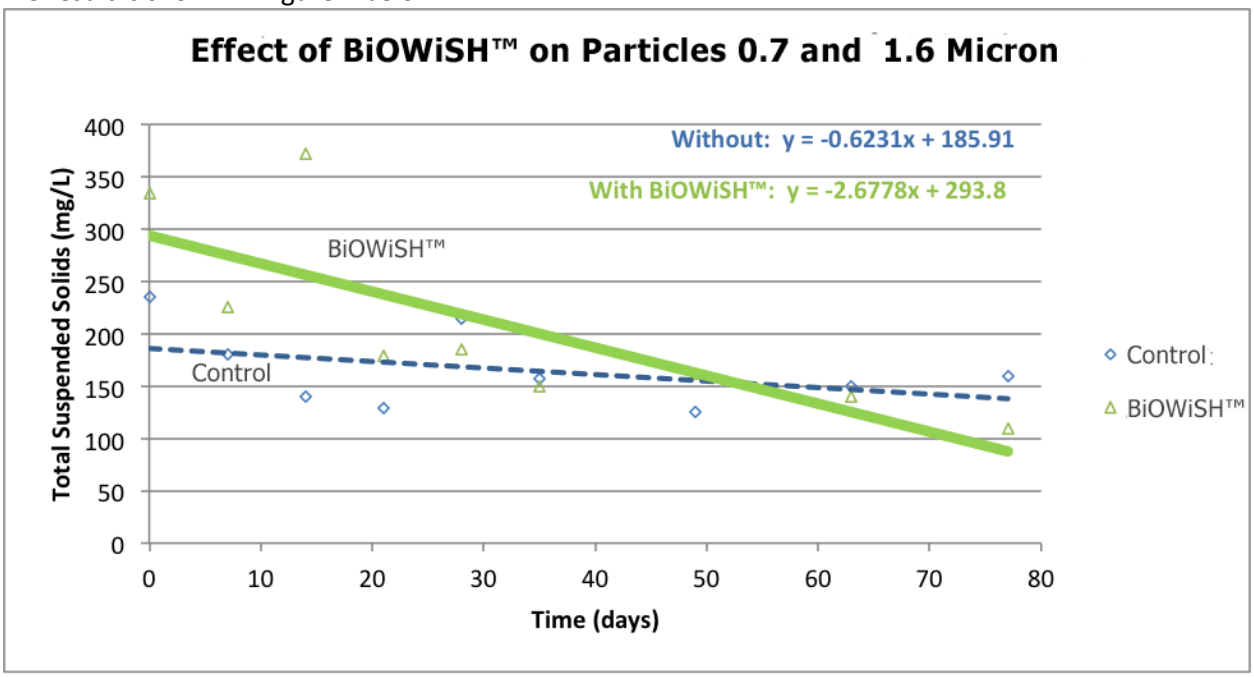

Figure 7: Effect of BiOWiSH™ on Particles Between 0.7 and 1.6 Micron Particles.

Another important observation is that regardless of the type of microbiology , with or without BiOWiSH™ , in general , dairy waste becomes increasingly difficult to degrade as the solid waste particles become smaller. This is a well known fact and bacteria have evolved various options to further degrade and metabolize these small particles. Figure 8 below shows the effect of particle size on the rate constant for breakdown of dairy waste particles:

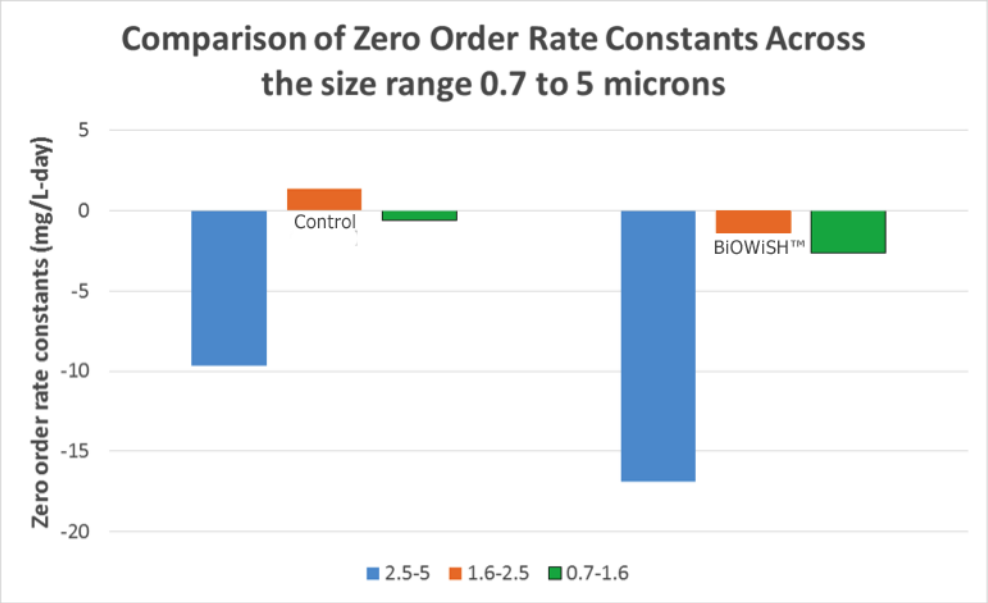

Figure 8: Effect of Particle Size on Zero Order Rate Constant.

From Figure 8, we can conclude that the BiOWiSH™ product is well suited as a biological additive for the treatment of dairy waste. In other investigations in our lab, we have also measured substantial decrease in BOD5 when BiOWiSH™ formulations are added to different wastewater systems.

Denitrification using BiOWiSH™: Another novel and interesting finding with BiOWiSH™ technology occurred during denitrification tests. The scientific literature indicates that denitrification happens strictly under an anaerobic environment. However, with BiOWiSH™ we observe significant denitrification (i.e., formation of nitrite and subsequent disappearance of nitrite/nitrate) even at a dissolved oxygen concentration of 3-4 mg/L of oxygen. In dairy wastewater we did not observe any denitrification in the absence of BiOWiSH™. However, with BiOWiSH™, we observed denitrification even at these low oxygen levels which increased further in the absence of any oxygen. In many tests, BiOWiSH™ technology has shown rates of denitrification that are significantly higher than published literature values. A typical denitrification result using one of the key micro-organisms in BiOWiSH™ is given in Figure 9 below. This data is taken from earlier work on BiOWiSH™ from 2012.

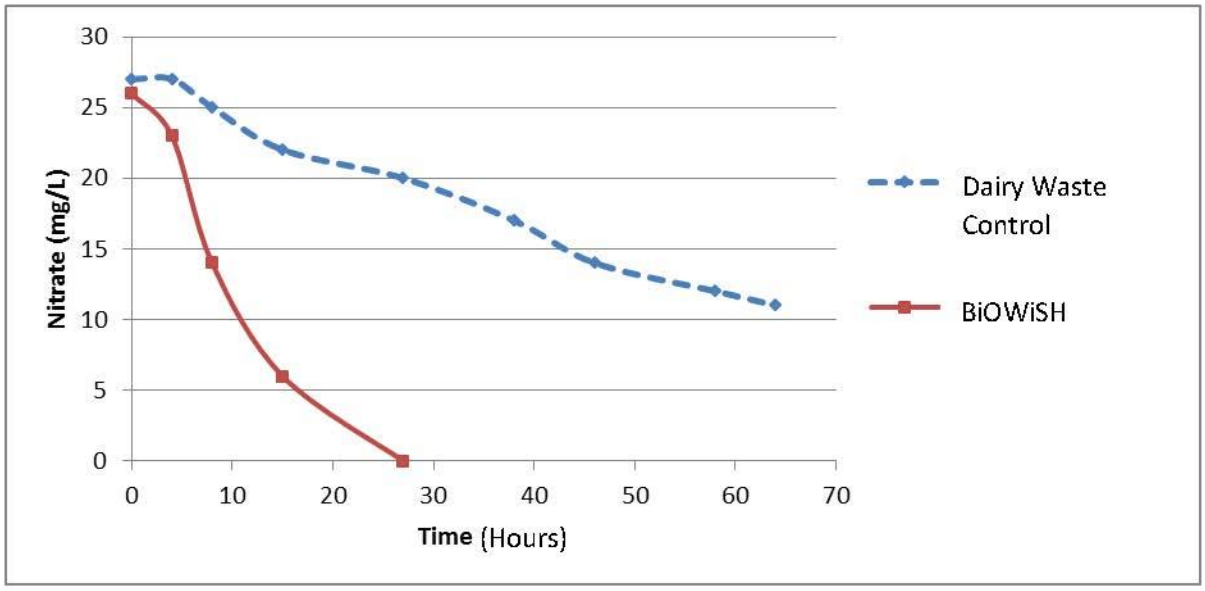

Figure 9: Denitrification Under Initial Aerobic Conditions – No Aeration

These observations suggest that BiOWiSH™ should be effective in denitrification under both aerobic and anaerobic conditions.

In conclusion, we can say that addition of BiOWiSH™ increases the efficiency of solids reduction in wastewater. The efficiency of solid reduction is a function of particle size, (i.e., the smaller the particles, the slower the process). The BiOWiSH™ product performed very well on dairy waste remediation by facilitating the breakdown of particles across the size range 0.7 to 5 microns. BiOWiSH™ overcomes the rate limiting step of breaking down particles in the size range between 2.5 and 1.6 microns, and enhances the efficiency of the overall solids reduction process. Addition of BiOWiSH™ also enhances the rate of degradation in each step of the particle breakdown of wastewater. In addition to solids reduction, we also observed significant denitrification rate enhancement (even at relatively high dissolved oxygen levels) and higher BOD removal when BiOWiSH™ formulations are added to augment wastewater remediation. This combination of BOD and TSS reduction with efficient denitrification is expected to result in significant odor reduction of treated wastes, which may be the subject of future investigations in this and other laboratories. At Cal Poly, laboratory trials are underway to expand the scope of testing of BiOWiSH™ wastewater formulations to other types of wastewater.