

***Summary***  
***Grease Trap Samples Experiment***  
***Total System Solution (TSS) Performance Analysis***  
***FOG Degradation***

***Sponsor: JSH international***

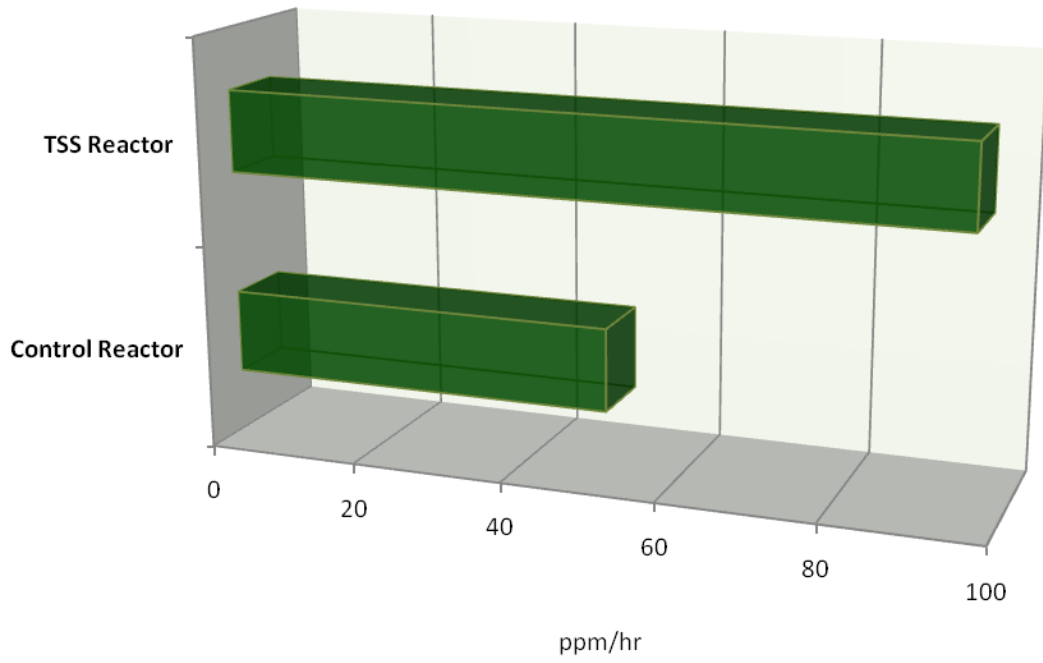
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***Results and Conclusions***

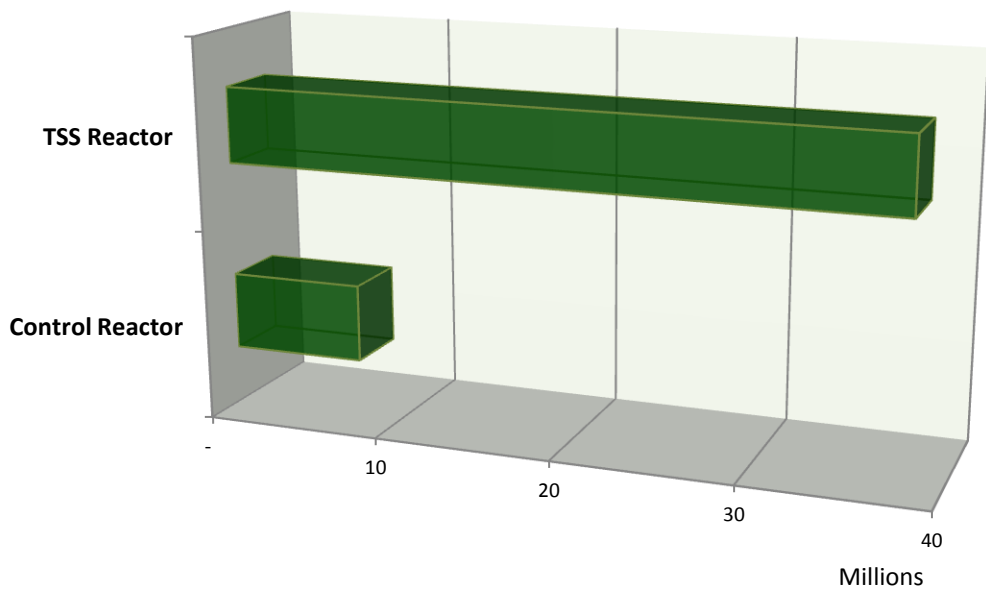
The effectiveness of the Total System Solution (TSS) product for the degradation of fats oil and greases (FOG) in grease trap samples was investigated in a controlled laboratory setting. The results reported here were obtained from March 18, 2010 to March 21, 2010. The experimental results indicate that TSS increases the rate of FOG degradation by a factor of two (2) compared to a control experiment where no TSS was used. The microbial concentration in the reactors was also measured at the end of the experiments. Cell counts indicate that the TSS product increases the cell count in the reactor by a factor of five (5). The TSS product enhances microbial metabolic rates causing up to a five-fold increase in microbial cell counts, and an increase in the rate in which microbes may use FOG as a nutrient. The effectiveness of the TSS product may be higher in grease traps where there is no mixing to provide contact between the microbes and the FOG material, and where the microbial population is more likely to include more microbes that can more effectively degrade FOG. After the experiments were concluded, the liquid material in the reactors maintained a fluid consistency even after 24 hours without stirring. There was no evidence of material that could clog grease traps or pipe network. The FOG degradation data presented here, along with cell count studies, indicate that microbes degrade FOG and use it as a nutrient. The byproducts of this degradation are smaller organic molecules that are unlikely to react or combine to form larger molecules in a grease trap or pipe network.

The results presented here are for experiments conducted at a temperature of 25°C (77° F) with a single application of the TSS product at a concentration of 500 ppm. The TSS concentration used is equivalent to a single 16 ounce treatment of TSS in a standard size interior grease trap.

### Rate of FOG Degradation



### Cell Counts



## ***Experiment Description and Data Analysis***

The experimental equipment consisted of two identical Wheaton MBF-250 Bioreactors equipped with 2-L glass vessels. The glass vessels were autoclaved prior to each use. One reactor was used as a control and no TSS was added. A second reactor was charged with the TSS product (TSS reactor). A NESLAB Circulator Water Bath was used to maintain the reactor temperature at 25° C (77° F). The reactors were covered to simulate the dark conditions in a grease trap. A 5% (by volume) grease concentration in tap water was used and the solution was stirred at 1100 rpm for 72 hours. A TSS product concentration of 500 ppm was introduced in the reactor as a single application. Samples were taken every 12 hours and the FOG concentration was measured using an Infracal TOG/TPH analyzer. Figure 1 shows the bioreactor system used. The system is equipped with an H<sub>2</sub>S gas analyzer as shown in the Figure.



Figure 1: Bioreactor System

## **Microbial Cell Count Results**

Figures 2a and b show the bioreactor system at the start and after the completion of the 72 hour experimental run. In Figure 2 a, the contents of the reactors are being stirred at 1100 rpm. Figure 2a qualitatively confirms the concentration measurements indicating homogeneity of reactor contents during the experiments. The reactor on the left of Figure 2b is the control reactor and the reactor on the right in the Figure is the TSS reactor. There are significant differences in the appearance of the two reactors. The milky appearance of the TSS reactor contents indicate a significant increase in microbial cell counts compared to the control reactor. This is confirmed by preliminary cell count results. The cell count in the control reactor was  $7.6 \times 10^6$  CFU compared to  $3.8 \times 10^7$  CFU in the TSS reactor. This is a five-fold increase in microbial cell concentration.



Figure 2a: Bioreactor system at start of experimentation



Figure 2b Bioreactor system at completion of experimentation

These results indicate that the TSS product has a significant effect in the metabolism of microbes and as a result, significantly enhance the microbial cell counts in grease traps.

## FOG Degradation Study

The FOG concentration in the TSS reactor is significantly lower than the FOG concentration in the control reactor (with 95% confidence). The ratio of FOG concentration between the control and TSS reactors was as large as two (2) for the duration of the experiment. Another important parameter is the rate of FOG degradation. The rate of FOG degradation in the TSS reactor was 96 ppm/hr compared to 51 ppm/hr in the control reactor. Thus, the rate of FOG degradation in the TSS reactor can be approximately twice that obtained in the control reactor.

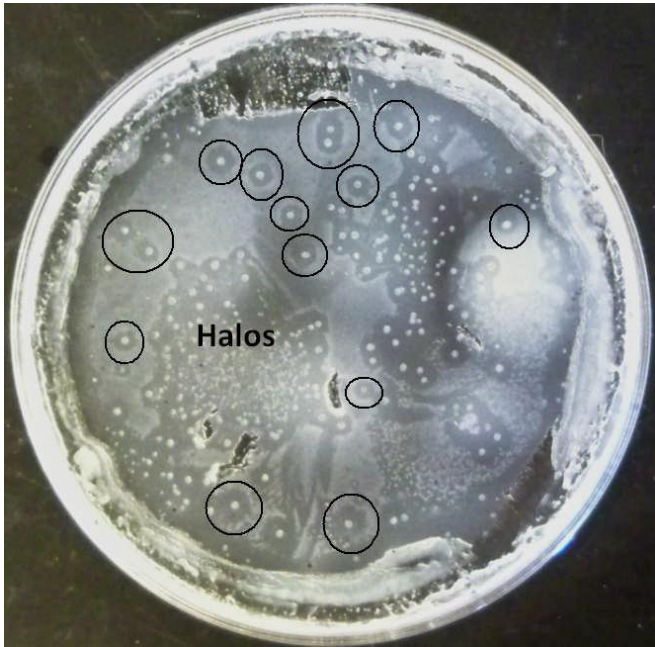


Figure 3: Microbial colonies showing vicinity FOG breakdown

The experimental results and available research indicate that microbes secrete enzymes that break down the FOG prior to utilizing it as a nutrient. This is confirmed by the plate results obtained and shown in Figure 3 below. The Figure shows colonies of microbes (milky white) surrounded by clear halos (highlighted by black circles). A black background is provided for easier viewing of these results. The enzymes secreted by the microbes make the FOG easier to use as a nutrient. This mechanism allows for all microbes to benefit from the FOG breakdown associated with the enzyme secretion by individual microbes. In other words, the microbes secrete enzymes that make FOG easier to use as a nutrient by all microbes. These breakdown products are more soluble in hexane, and FOG (materials soluble in hexane) concentration increases as confirmed by the FOG measurements in this study.

Microbes feed on these breakdown products and FOG concentration decreases. Once microbes have used most or all of the breakdown products, it will be necessary to breakdown additional larger molecules. FOG concentration begins to increase because additional breakdown products are formed which are more soluble in hexane, and the cycle begins again. This is confirmed by FOG measurements in this study after 48-60 hrs during some experimental runs. An increase in FOG concentration during an experiment is due to microbial activity and breakdown of larger molecules, which is a desirable result.

As typical of biological systems, responses are complex in nature and depend on a variety of factors. This should not be a deterrent in efforts to optimize the effectiveness of the TSS product in enhancing the degradation of FOG. It will be important to take all of the relevant system characteristics into account.