Triglyceride Degradation Capability and Cell Density of an Engineered Bacterial Consortium at Varied pH and Temperature

by
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Introduction

The broad aim of this study was to understand the amount of triglyceride degradation that occurs across a range of pH values and temperatures as a result of BESTechnologies' bacterial consortium. This experiment determined the effectiveness of BESTechnologies' Biofeed FS® bacterial consortium in reducing grease accumulation in a laboratory model grease trap. The experiment was designed to measure the effectiveness of the consortium over 28 days at low, medium and high pH, and at low, medium and high temperature conditions.

Materials and methods

Physical Design

To simulate field conditions a 1:5500 volumetric scale model grease trap was developed. This model was based on existing information about common restaurant grease traps. We assumed that the average grease trap measured 1.22 m wide, 1.83 m long and 1.83 m deep, with a total volume of 4080 l. We further assumed that an average grease cap increases 5 cm per month or a total of 112 liters. No flow rate was estimated. Our model grease traps consisted of hollow-walled beakers with a volume of 750 ml and a grease accumulation of 22.4 ml per month.

Each hollow-walled beaker was attached in series to a PolyScience model 210 flow-thru cooler and heating recirculation system for temperature control. A double junction Kynar pH/temperature probe, connected to a digital pH controller was used to monitor temperature and pH. Each simulated trap was assigned its own pH/temperature probe and controller. For all pH trials we attempted to maintain a temperature of 18° C. Target pH values were 4.5, 7 and 9 for low pH, medium pH, and high pH trials respectively. For all temperature trials we attempted to maintain a pH of 7. Target temperatures were 8° C, 18° C and 28° C for low, medium and high temperatures respectively.

For all trials with the exception of that for high temperature, traps 1 and 2 received 22.4 ml grease at the beginning of the month. Traps 3, 4, and 5 received 1 ml grease per day for 22.4 days. Each trap initially contained about 725 ml tap water. The grease consisted of beef, pork, and chicken fat and corn oil. The three fats were obtained from a local butcher and sterilized in the autoclave. Mazola corn oil was purchased from a local grocery and was also sterilized. 5.6 ml of grease was aseptically measured into 20 small beakers (yielding a total of 22.4 ml per trap), covered and stored for introduction to the traps. The estimated sum of triglyceride mass was 20.6g based on densities from Pearson and Cox[1].

Because the high temperature test was the first performed, only three traps were used. The control trap and two experimental traps received the entire 22.4 ml of grease initially as explained above.

A proprietary blend of bacteria marketed under the tradename BESTech Biofeed[2] was hydrated in a Mylar bladder bag within a dispensing unit. Each bladder bag was filled with five gallons of tap water and allowed to grow at room temperature for 24 h. Every week, one liter of bacterial suspension from the bladder bag was transferred into four polyethylene bottles that fed the automatic bacterial dosing system. An EFD aliquot dispenser inoculated traps 1 through 4 with 20 µL of the bacterial consortium six times per day (every two hours from 0800 through 1800 hours), seven days per week. Personnel observed the automated inoculation system at least one time per day to confirm that it was functioning properly. Prior to the testing, the inoculation doses from the automated inoculation system valves were calibrated against the syringe used prior to the automated inoculation system's operation.

To maintain desired acidity, the pH in the traps was increased to the target pH using 1N hydrochloric acid. Through the remainder of the month, if an increase in pH was necessary, it was effected with 1N NaOH. If a decrease in pH was necessary, it was effected with 1N hydrochloric acid (HCl). Natural buffering of the system began on about the third day. Microbial respiration decreased pH, making it easier to maintain the lower pH experiments.

Data Collection

The pH/temperature meters were calibrated and then stored in water for use the following day. Readings of temperature and pH were taken daily Monday through Friday.

The Partition-Gravimetric Method for measuring oil and grease[3] was used to describe grease reduction. The samples were acidified (to < pH 2 with HCl) in the traps and poured into separatory funnels for separation and extraction. The traps and the Kynar probes were rinsed with very hot water, and the rinseate added to the funnel.

Technicians recorded all parameters in the traps (temperature in the closed loop system, pH, inoculation times and grease reduction) in a logbook dedicated to this experiment. Chemical preparations, observations and results of samples taken during the month were also recorded in the book.

Data Analysis

The decision was made to dispense grease into grease traps 1 and 2 at startup and add grease to traps 3, 4, and the control trap continuously. In order to achieve results that can be compared to a control it is necessary to pool data from traps 1, 2, 3, and 4 or discard data from traps 1 and 2. To increase the degrees of freedom it is preferable to pool the data. However, this could only be done once it was proven that that the two treatments were not statistically different. In order to make this determination, an ANOVA was calculated using the General Linear Model (GLM) nested with respect to each test, e.g. high temp or low pH (SPSS SYSTAT 1998). Because results of this treatment indicated no statistical difference, the data was analyzed under the assumption that all of the treated traps were replicates.

Several descriptive statistics were calculated in order to compare the reduction of grease. A comparison between mean grease reduction and the control was made for each of the six parameters. Standard error was reported for the means. Standard error was selected to indicate variance while taking into account differences in sample size. To confirm that the two treatments (bacteria / no bacteria) were statically different, an ANOVA nested with respect to test status was calculated to determine if the control was statically different from the variable.

Several plots were constructed to indicate the change in cell counts over time in the bladder bag and the grease traps. Regression and confidence intervals were calculated for the population change in the bladder bag. Because of sample size only a histogram of mean cell density was plotted for each grease trap.

Results

Controls

Within each experimental treatment except for high temperature, two methods were used for supplying grease and oil. Traps 1 and 2 were inoculated at the beginning of the trial with 22.4 ml of mixed lipids. Traps 3 and 4 and the control were inoculated daily with 0.8 ml of mixed lipids. Because of this experimental design, nested GLM analyses of variance for lipids and cell counts were run to prove that there was no statistical difference between the two treatments. All data was plotted in frequency distributions and skewed values were considered to confirm that parametric assumptions were valid. Results of the nested GLM on lipid degradation indicate that there is no statistical difference between lipid measurements from traps supplied with grease initially and those supplied daily. $P = 0.331$, F-ratio = 1.254, df = 5.

Because there was no statistical difference between the two treatments the data was pooled to provide an adequate sample size for meaningful analysis. The results of this pooled data are reported in the following pages.

The model grease trap system controlled pH and temperature well. Data on pH and temperature control are reported in figures 1 through 4. There was very little fluctuation in pH and acceptable fluctuation in temperature.

Triglyceride degradation

The results of this study were quite positive. The BESTechnologies Biofeed bacteria degraded between 50% and 73% percent of grease at a wide range of temperatures and pH values. Figures 5. and 6. show the mean reduction in grams of grease. In general, grease degradation was higher at lower pH and at higher temperatures. The median temperature bar in figure 5. is anomalous; testing of the dispensing volume that month indicated that the volume was not as consistent as it should have been. Because fresh batteries were not utilized, the dispensing volumes had dropped to one-third of their intended volume by day 28. This may account for the decreased grease degradation.

The differences between adding bacteria and not adding bacteria are statically significant. A GLM ANOVA was calculated to determine the effect of bacterial addition on grease degradation. When the ANOVA was nested with regard to

the particular test (e.g. high temp or low pH), the results were: F ratio, 35.600, P = 0.000. For the non-nested ANOVA the results were: F ratio, 121.749, P = 0.000. This indicates that there is a less than 0.1% chance that adding bacteria had no effect.

The most striking information from this portion of the study is the marked degradation of triglycerides in the traps receiving repeated bacterial inoculation. The traps were not sealed or sterile before inoculation. The control trap was in an environment where lipase-secreting bacteria were present and almost certainly contaminated the trap. Despite their likely presence, there was little lipid degradation. One might expect that a triglyceride rich environment would quickly be colonized by lipid-degrading organisms because of a competitive advantage, but there is no indirect evidence of that. The reasons for lipid-degrading bacteria apparently not achieving dominance are not clear. It is possible mycotoxins from fungal growth could have influenced community composition. Ecological systems tend to be self equilibrating so it seems bio-augmentation is necessary to keep the balance tipped in favor of lipase secreting organisms. In this system the clearest conclusion that can be drawn is that bio-augmentation is necessary for significant triglyceride degradation even when small quantities of lipid degrading bacteria are already present.

Conclusions

This study is the first comprehensive laboratory modeling of a grease trap since the 1994 studies by the Center for Crops Utilization Research at Iowa State University[4]. In these trials as well as those in 1994, the product performed well across a variety of conditions. In general, grease degradation was significant across a range of the temperatures and pH encountered in typical grease traps. Most importantly, there was a strong correlation between adding BESTechnologies Biofeed bacteria and grease degradation.

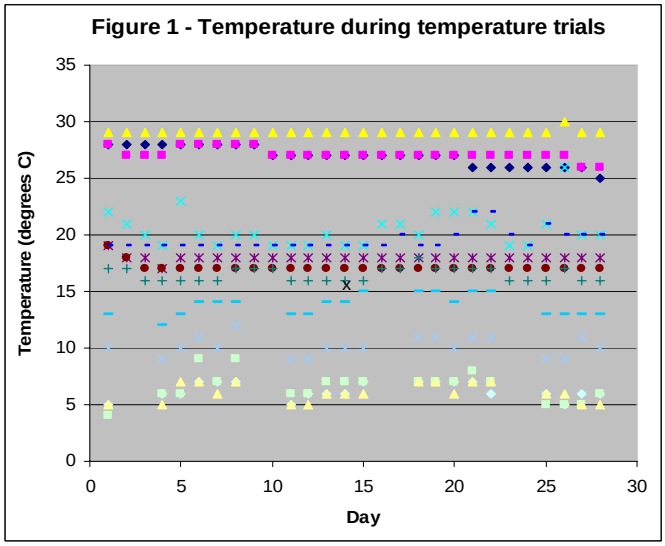
References

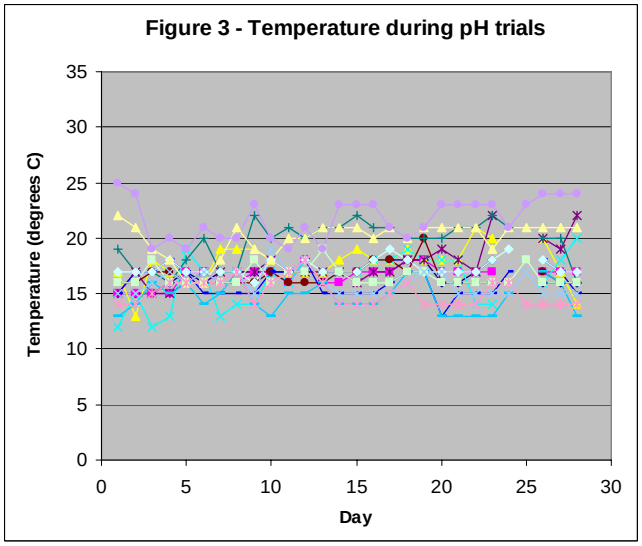
[1] "The Chemical Analysis of Foods," D. Pearson and H. E. Cox, Chemical Press, New York, 1971

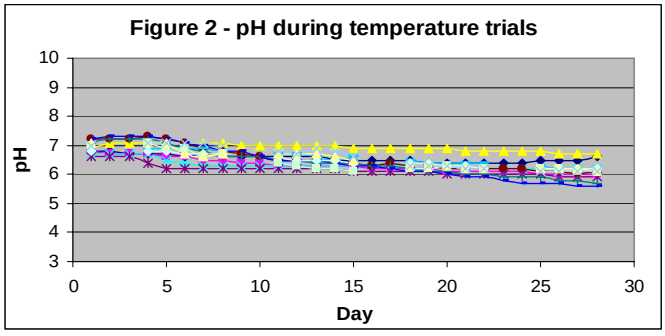
[2] Available from BESTechnologies, Inc., 7329 International Place, Sarasota, FL 34240 USA, www.bestechcorp.com

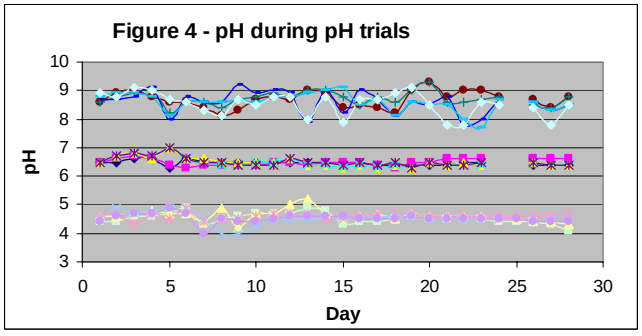
[3] "Standard Methods For the Examination of Water and Wastewater," American Water Works Association., et al., American Public Health Assoc., Washington, DC, 1995

[4] "Bioremediation of Food Industrial Wastes," A. L. Pometto III and C. S. Oulman, Center for Crops Utilization Research, Iowa State University, Ames, Iowa, USA, 1994









Figures 1.-4. Control of temperature and pH
These figures illustrate the tight control of pH and temperature for each simulated trap and trial. pH and temperature were successfully controlled with a PolyScience cooling / heating recirculator and manual addItions of HCl or NaOH.

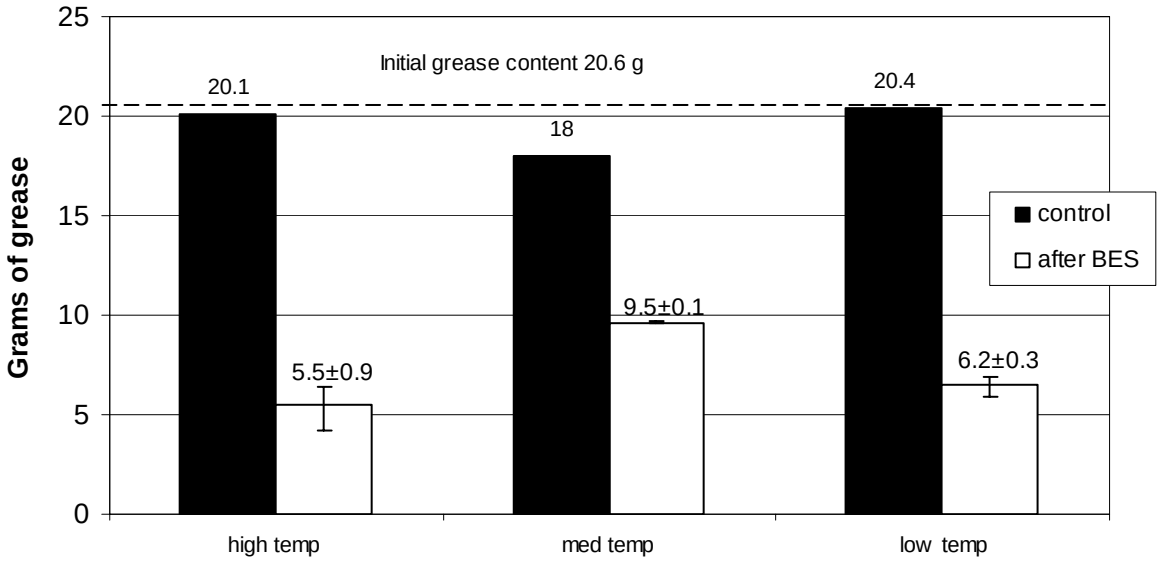


Figure 5. Mean reduction in grease at different temperatures.
High temperature was defined as 28º C and all units were kept between 26º C and 30º C and a pH of 6.7-7.2. Medium temperature was defined as 18º C and samples were kept between 17º C and 21º C, (pH 6.0-7.3). Low temperature was defined as 8º C and temperature ranged between 5º C and briefly 14º C, (pH 6.1-7.1). Grease reduction was measured using the FOG test from *Standard Methods for the Analysis of Water and Wastewater 18th ed*. All values are in grams ± standard error. Overall reduction in grease was 60% to 80%.

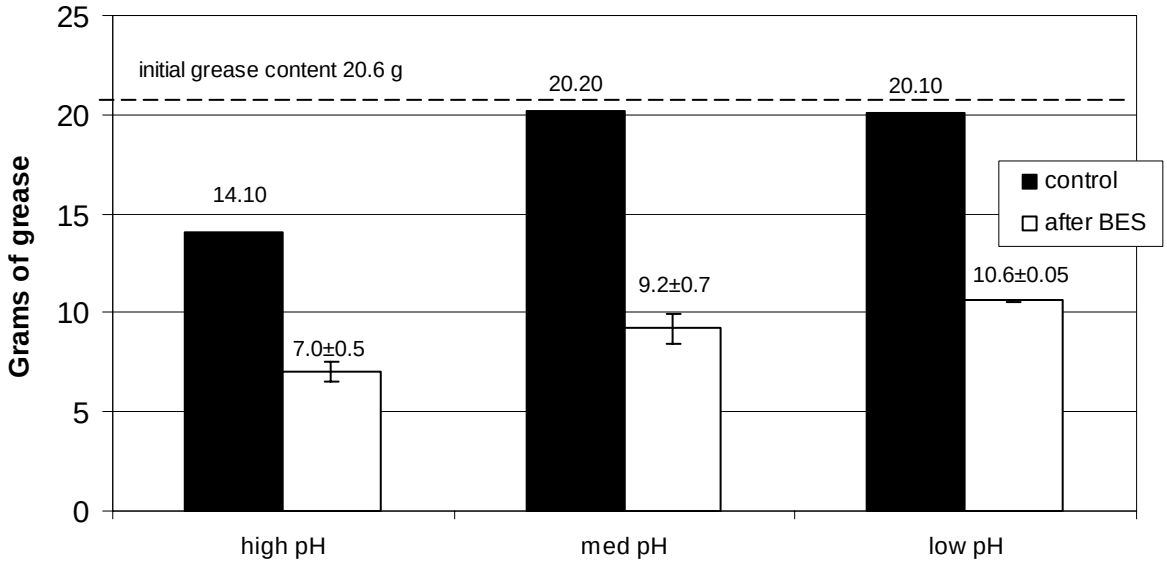


Figure 6. Mean reduction in grease at varied pH
High pH was defined as 9.0 at a temperature of 18º C. Actual pH ranged from 7.8-9.1, temperatue from 13º C to 21º C. Median pH was defined as 7.0 at a temperature of 18º C. Actual pH ranged from 6.4-7.0, temperature from 15º C to 22º C. Low pH was defiend as 4.5 at a temperature of 18º C. Actual pH ranged from 4.0-4.9, temperature from 14º C to 21º C. These values represent a 55-70 percent reduction in grease at a wide range of pH values.