ACREC
Solutions

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Success in Improving Method to Count Bacteria in High Fat Materials

Researchers at Clemson University's Animal Co-Products Research and Education Center (ACREC) have discovered a way to accurately count bacteria in high fat materials. Previous attempts to enumerate bacteria in rendered animal co-products have proven a challenge due to the high fat nature of the materials. In earlier experiments conducted at Clemson to determine thermal death time calculations, extreme errors in bacterial counts left researchers unable to conclusively determine the amount of heat required to kill particular bacterial species in rendered materials. ACREC researchers realized that unless improved methodology could be created to ensure true bacterial counts, their microbiological studies on rendered animal co-products could be in error. Therefore, Clemson University ACREC researchers initiated a study to find a better way to enumerate bacteria in high fat materials.

Traditional bacterial enumeration methods involve plating samples on microbial media in petri dishes. After incubation in a warm growth chamber, bacterial colonies are counted on the petri dish. However, because samples often can contain thousands to millions of bacteria per gram, it is not possible to count such high numbers of bacterial colonies on a single petri dish. Therefore, the microbiologist's primary tool for all microbial enumeration is a technique known as serial dilution. In this method, samples of product are aseptically measured and diluted into a water-based, sterile dilution buffer. The technique relies on the ability to evenly distribute sample, and thus inherent bacteria, in the aqueous buffer. Subsequent dilutions are made so that the final sample plated on a petri dish is countable. With a little simple math, the microbiologist can then determine how many bacteria per gram were in the original sample.

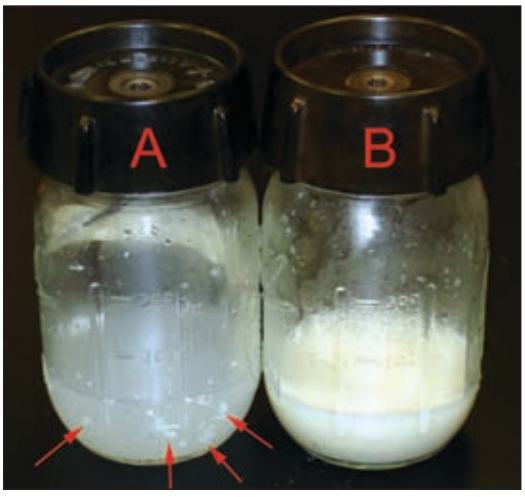

Figure 1: High fat sample diluted in standard buffer (A) and in the improved ACREC buffer (B).

However, because fat and water do not mix, high fat samples tend to clump in the dilution buffer. As shown by the arrows in Figure 1A, clumps of fat did not evenly mix into the aqueous dilution buffer. As a result, subsequent dilutions are confounded by these clumps. If sample is not evenly diluted throughout the dilution buffer, extreme errors in final enumeration occur. Unless accurate methodology can be created to ensure accuracy in bacterial counts, all microbiological studies on rendered animal co-products may be questioned.

Microbiologists Annel Greene and Thomas Hughes initiated a study on determining a better way to enumerate bacteria in high fat samples. Master of science graduate student Robert "Rob" Valerio has conducted the initial phases of the study and has achieved very promising results. Valerio tested 30 different emulsification systems until he discovered the combination that allowed greatest dispersal of high fat samples in the aqueous buffer. He observed fat globule size and dispersion using light microscopy and rated each combination for effectiveness. As shown in sample

ACREC graduate student Rob Valerio.

B in Figure 1, Valerio discovered a method that allowed even distribution of the fat into the water-based dilution buffer. In subsequent experiments using lard and added bacterial cultures, Valerio has achieved encouraging results that indicate the new method allows accurate enumeration of bacteria in the high fat samples. A manuscript of results is being prepared for submission to a refereed journal.

Valerio will continue his work by testing his new system on high-fat food products such as hamburger and peanut butter. Upon completion of these tests to ensure that the method is accurate in food systems, the next phase of the study will be to re-examine rendered animal co-products for bacterial content. The success achieved in this project will allow ACREC researchers to understand bacteria in the rendered product and to validate the success of rendering cooking systems in killing bacterial contaminants. In addition, the success of this project could open many other opportunities for improving detection via rapid methodologies as

well as improved microbial media systems for *Salmonella* and other potential pathogens.

Valerio is a native of Syracuse, NY, but now calls Greenville, SC, home. He earned his bachelor of science degree in biological sciences from Clemson University in May 2008. He soon thereafter entered graduate school at Clemson studying for a master of science in food science. His hobbies include soccer, tennis, basketball, and fishing, and he is an accomplished musician who writes music for piano, guitar, and strings. As an undergraduate, Valerio planned to pursue a career in medicine but after a few short weeks working on a senior research project, he realized that laboratory research was his true passion. He has worked as a volunteer for medical-based charities and helped to build homes for Habitat for Humanity.

The Clemson University ACREC is very proud to have Rob Valerio working to create an accurate method of enumerating bacteria in rendered animal co-products. The results of this project will provide not only renderers but also food processors with a powerful tool in their bacterial control efforts. **R**