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SPORE CONTENT VS. SPORE COUNTS

Quantifying Microbial Content

There is a lot of discussion today about quantifying the microbial content of granular materials, such as a product formulation, or even soil. Several states require that microbial ingredients be quantified by indicating on the label the number of colony-forming units in a given quantity of the product. To enforce this, it is necessary to be able to count the spores present in the mixture. Researchers had already devised various methods for extracting and counting spores of a given kind present in soil. Many of these techniques are being utilized to determine the number of mycorrhizal fungal spores present in a product.

Counting Bacteria (cfu's)

Techniques abound for performing counts of this type, with each technique designed to extract or detect the particular type of propagule in question. For counting bacterial content, a sample of the product is mixed in a given quantity of water and stirred. This is then reduced to 1/10, 1/100, 1/1000, etc. dilution, and a small measured volume (1 ml) is placed on an agar plate to grow the bacteria. By counting the colonies that form, you can use the dilution factor and the volume of the solution used as inoculum to calculate the total colony forming units (cfu's) in the starting material. This sounds good in theory, but there are several problems inherent in the technique:

1. Some of the bacterial cells stick together, so a clump of several cells will form one colony, and will be counted as one cell.
2. Different species and strains of bacteria have different requirements for nutrients, for temperature, for pH, and other factors. You can only count the ones that grow under the conditions you provide.
3. Different strains and species of bacteria take different amounts of time to grow. At room temperatures, molds can grow rather quickly. *Bacillus* will grow in about 24 hours. Actinomycetes take a few days. Of course, all this depends on the temperatures used.

Typically, total aerobic counts are performed in 48-hours on a general nutrient agar grown at room temperature. This standard method is used so that everyone's counts are comparable. It is (or should be) understood that no single technique will count all the propagules.

Counting Fungal Spores

Similarly, various techniques are used to count the number of fungal spores present in soil. This involves drying, sieving, washing, eluting, and density separation techniques. These techniques have been employed to extract and count the number of mycorrhizal fungi spores present in products. As expected, there are several problems inherent in these techniques:

1. In mixed granular materials like soil or product formulations, spores are among the tiniest particles present, and can easily lodge inside cracks and crevasses of larger mineral particles.
2. Electrostatic forces can also compound the difficulty of extracting spores, causing them to adhere to surfaces of charged soil or mineral particles.
3. Spores can collect charged dust particles that adhere to their surfaces, changing their density and the way they elute in density gradient solutions.

In short, just as various limitations hamper bacterial counting techniques, the same is true for fungal spore counts. It is (or should be) understood that no single technique will count all the spores.

Efficiency of Spore Counting Techniques

Plant Health Care, Inc. has performed various tests to determine how many spores can actually be counted by current extraction techniques. This was done by making a standard spore mixture. Independent researchers who do spore counting using the latest techniques were given some PHC products that were made without spores. These researchers were also given some of our concentrated VAM fungal spore cocktail. They counted the spores in the cocktail, and mixed them into a given quantity of the dry product formulations. Then the spores were extracted and counted again by the same researchers, using their latest counting techniques. Since they already knew the number of spores they had added, they were able to compare the results of their extraction methods to the known spore count. In all cases, spore counts determined by extraction techniques were significantly lower than the known number of spores originally added to the mix. Typically, the researchers were able to detect only about 25 to 50% of the spores present, depending on the technique used. Currently, there is no technique that can effectively detect the majority of VAM fungi spores in a mixed granular product. The same is likely true of results obtained with soil.

Actual Counts vs. Representative Counts

Even though you could never devise a workable method to provide a true count of the spore population, you could devise a technique that could provide a consistent numerical result. Even though this result had a built-in error, it could provide you with some indication of the spore numbers. As long as the degree of error inherent in your technique is consistent, then the counts that it provides are still useful, as long as you understand their limitations and the fact that the counts do not represent the actual population size, but only provide a proportional “shadow” of the true value. In other words, if an actual count is not possible, then a technique is useful if it can provide a consistent representative count. A representative count has a built in error, but the count rises and falls proportionately with the actual population.

Interpreting Representative Counts

In the case of the spore counts, the accuracy of the representative count method was about 25 to 50%. However, the actual accuracy of a representative count is not always known. Often, it must be estimated using statistical analysis.

The proper way to interpret a representative count is to understand that it is only a shadow of the real population. If the population grows, the shadow grows. But the representative count is not equivalent to the actual population size. So one must never use a representative counting technique to verify a spore count unless you know the accuracy of the technique, or can estimate it statistically.

Practical Considerations

State regulations require that we indicate on the label the numerical number of microbes in our products. This is easily done, since we know how many we add. The problem occurs when someone tries to count the number of spores by using a representative counting method (the only kind available), without understanding that these methods will never result in a number that is equal to the actual spore count. All the representative counting techniques currently available significantly underestimate the actual spore count. They only give a numerical value that is proportional to the actual spore number. They never produce a number equal to (or even close to) the actual spore number. These techniques are not supposed to provide an actual count. However, if the investigator (state agent, lab investigator, customer, etc.) doesn't understand the difference between representative counts vs. actual counts, then he or she will always conclude that the product does not live up to the label.

Applying the Statistical Accuracy Conversion

When someone confronts you with a spore count obtained by testing a product, be sure to ask them for the statistical accuracy of their technique. Point out that all current counting techniques provide representative counts, and not actual counts. You will need the statistical accuracy value to convert their figure to an approximate actual count. If they cannot provide the figure, then point out that in our experience, the statistical accuracy of the current extraction techniques has been about 30 to 35% on average. Using this value, the experimental figure should be tripled.