AQUAINTECH INC.

AQUAculture INformation TECHnology

Bacillus Spore Counts What do they really mean?

Products that contain Bacillus spores are ideal for many different types of applications. The spores are shelf stable and if the moisture content is kept low, they can remain viable for very long periods of time (centuries). Bacillus species are exploited for their wide range of enzyme production capabilities and this makes then a powerful tool for degrading organic matter in aquatic ecosystems.

Many people fail to grasp what the number of spores in a product means. Firstly, spores cannot be counted using traditional techniques. They have a charge and they stick to each other making traditional approaches of enumeration highly likely to under-estimate the counts by a factor of ten or more. Secondly, not all the spores in the product germinate (the process of becoming actively growing bacterial-vegetative-cells) and those that do, do not at the same time. Different species and different strains of the same species can have different germination rates.

There are many factors that impact growth of the vegetative cells. Species and strain differences, types and quantity of nutrients available, presence of growth limiting factors, temperature, salinity, etc. are among them. They grow until something limits their growth and then they die off and return to background levels.

Those spores that germinate the earliest will produce vegetative cells that have access to all the nutrients that they require to grow (assuming that the environment provides them). There is no practical way for all the spores to germinate and access nutrients. Bacterial cells divide by splitting so that one cell becomes two and two becomes four, etc. The time that it takes for this to occur is also dependent on many variables. Conservatively assuming that it takes an hour for this to occur, one cell can divide to become more than 8 million cells in 24 hours. By 31 hours there would be more than a billion cells. If the bacteria could grow for another 17 hours (48 total) and divided at the same rate there would be more than 100 trillion cells! This is from a single cell.

Clearly this does not happen as this level of cells would fill the pond up with a bacterial sludge and we know that the viable cell counts never reach much higher than a small fraction of this. Not all the spores germinate and of those that do their growth is impacted by the environment. Higher spore counts do not relate to higher bacterial growth and thus activity. <u>Higher spore counts are a sales gimmick</u> (clients think that they are getting more value for their money) and since we cannot design a product that works the same in every environment we include high enough spore counts to ensure that even in the worst of environments there are enough spores present to get the job done. Products with a fraction of what we put into our tablets work just as well in many environments.

The bottom line is that the <u>number of spores</u> is not related ultimately to how well the product works, although enough spores need to be present to account for environmental variability.

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