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Technical Discussion #4

Bioremediation of Odors

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This discussion is designed to give a brief background and review of both microbiology and odor perception and to present the scientific principles involved in Custom GT Tabs as they apply to odor control. Custom Biologicals, Inc. strongly believes that chemical and mechanical methods of odor control are both dangerous and expensive. Bioremediation of odors as practiced by Custom Biologicals, Inc. is rapidly replacing most of these methods.

The bioremediation of odors has a number of industrial applications such as: elevator shaft odor control, car wash recycle water odor control and oil water separators.

F-TREAT and Odor Control

Custom Biologicals' GT Tabs product is a live synergistic blend of selected microorganisms specifically chosen for their ability to rapidly metabolize organic material into carbon dioxide and water. The bacteria in Gt Tabs actually digests and eliminates the odor at its source rather than merely masking or attempting to chemically neutralize the odor.

GT Tabs controls odor in different ways depending upon the origin of the odor. If the odor is produced by indigenous bacterial decomposition of organic material such as sewage or urine, the bad odors are produced because bacteria incompletely oxidize and degrade organic compounds, such as proteins, carbohydrates, and starches instead of completely oxidizing them to carbon dioxide and water.

In this instance, GT Tabs works on a competitive principle called competitive inhibition. By applying GT Tabs, selected microorganisms are added at approximately one million

times the concentration of the indigenous bacteria. The microorganisms in GT Tabs utilize essentially all of the available organic material and oxidize it to carbon dioxide and water. The indigenous bacteria are so outnumbered that they cannot successfully compete for available organic material. This greatly restricts their growth and also greatly diminishes the emission of organic compounds that produce the characteristic odor. The small amount of incompletely oxidized organic material that they may still produce is quickly utilized by the microorganisms in GT Tabs further reducing the odor.

Examples of this type of activity would be wastewater or sewage treatment plants, elevator shafts, lift stations, oxidation ponds, trash cans, dumpsters, pet areas, kitchen areas, carpets, nursing homes, locker rooms, sewage treatment systems, and restrooms.

If the odor is caused simply by, the presence of an organic compound the microorganisms in GT Tabs will rapidly degrade the organic chemicals destroying the odor. Examples of this type would be non-hydrocarbon chemical spills, industrial wastewater, agricultural waste, fish and other food preparation.

Another major odor problem is the production of hydrogen sulfide (rotten egg smell) from sulfur containing amino acids. All of the microorganisms in GT Tabs degrade sulfur containing amino acids without producing hydrogen sulfide, and, in fact, utilize some hydrogen sulfide in growth.

All of the bacterial species utilized in GT Tabs are Class 1 bacteria, as defined by the American Type Culture Collection (ATCC). Class 1 bacteria are, by definition, non-pathogenic and non-opportunistic. These organisms are safe and will not cause infections or ill effects in humans, animals or plants. The United Sates Department of Agriculture (USDA) has approved this blend of organisms for use in USDA federally inspected facilities, including food production plants. Each organism has been approved by the microbiologists at the USDA laboratory and every production batch is certified to be Salmonella-free.

NATURE OF BACTERIA

Bacteria are considered prokaryotic organisms as their genetic material is not enclosed in a special nuclear membrane and they normally reproduce by the process of binary fission, one cell asexually splitting into two. Bacterial cells may be spherical or spiral but the majority are rod shaped (cylindrical) and are about one micron wide and 2 microns long. One micron equals 0.001 millimeter. For classification and identification purposes, bacteria can be divided into two large groups, Gram positive or Gram negative, depending on their reaction to a specialized staining procedure.

Microbial Growth Requirements

Bacteria as living organisms have certain chemical and physical growth requirements. The basic knowledge of these requirements is especially important in considering bioremediation and odor control.

1. **An Energy Source –** This is needed primarily for biosynthetic reactions to make polymers for the bacterial cell such as proteins from amino acids and RNA and DNA from nucleotides. Some bacteria can utilize light energy, however the ones that we are concerned with oxidize chemical compounds to obtain their energy. The bacteria in F-TREAT are chemoorganotrophs as they utilize organic compounds for their energy source. The bacteria in F-TREAT completely oxidize the organic compounds using them as an energy source by removing hydrogen from the compound and transferring it to oxygen to form water, as show below:

$$C6H12O6 + 6O2 = 6CO2 + 6H20 + energy$$

The organic material is completely oxidized and converted from a solid to a gas (CO2). If the organic material is incompletely oxidized, as with facilitative anaerobic and anaerobic bacteria, the organic compound that acts as the hydrogen acceptor usually has a bad odor.

2. **A Carbon Source –** Carbon is required for all of the polymeric units in the cell such as DNA, RNA, and proteins. Some bacteria can utilize carbon dioxide as a sole carbon source; however, the organisms concerned with odor control are heterotrophs, as they require an organic source of carbon.
3. **A Nitrogen Source –** Bacteria are very versatile as to their nitrogen source as they can use atmospheric nitrogen (gas), ammonia, nitrate, nitrite, and organic nitrogen. Nitrogen is a component in the amino acids of proteins and in the purines and pyrimidines of RNA and DNA.
4. **A Phosphorus Source** – Phosphate is a component of the nucleotides composing RNA and DNA and is required in energy transfer reactions.
5. **A Mineral Source** – Minerals such as magnesium, manganese, iron, and essentially every thing listed on your daily vitamin and mineral tablet bottle are required.

Bacterial Growth Process

Bacteria are prokaryotic organisms that reproduce primarily asexually by a process known as binary fission. One organism splits into two organisms and each one is capable of reproducing further. The principle of bioremediation is based on the tremendous growth potential of bacteria with the binary fission

process. For illustration purposes, lets consider the growth potential of one single bacterial cell, assuming a 20 minute generation time, for a few hours.

Hour 1	Hour 2	Hour 3	Hour 4	Hour 5	Hour 6	Hour 7
1 - 2 - 4 -	8 - 16 - 32 -	64 - 128 - 256 -	512 - 1024 - 2048 -	4096 - 8192 - 16384 -	32,768 - 65,536 - 131,472 -	262,944 - 525,888 - 1,051,776

In little more than 6 hours the population has increased over 1 million fold. This tremendous growth rate is the secret to successful remediation. In the case of odor control bioremediation, the odor causing compound is used both as a carbon source and an energy source to produce the million fold increase in cell mass.

Odor Detection in Humans

The major sense of odor detection is controlled by the olfactory nerves and is responsible for odor perception and recognition. Its performance is a function of molecular structure, configuration of odor reception sites, and signal generation at these sites as a result of a reaction between odorant and an enzyme, and relative concentrations of the reactants. A change in any one of these factors can change the perception of the odor.

The process that humans use to perceive odors follows these steps:

1. The odorant molecule travels along nasal air passages to the olfactory cleft where it fits into an odor receptor site.
2. A chemical reaction occurs between the resident enzyme (most probably ATP) and the odorant.
3. This reaction causes production of a specific coded electrical signal which is transmitted to the brain where it causes perception and recognition of the odorant characterized by that particular signal.

An odor can be perceived only if all of several criteria are available: there must be an available odor site, there must be sufficient enzyme to react with the odorant, there must be no interference with the pattern of signal generated by the reaction, and the concentration of the odorant must be high enough to create signal strong enough to be perceived.

Chemical and Mechanical Methods of Odor Control

1. Deodorizing by odor fatigue: If a very active consumer of the signal-producing enzyme is introduced into the environment, it can prevent the chemical reaction that produces the code signal characteristic of the malodor. It will consume the enzyme, leaving none available for

reaction with the malodor. It may also cause a secondary signal to be generated thereby altering the overall odor perception and recognition. These compounds are generally non-specific. They block all perception. Ionone, ketones, and aldehyde have all been used for this purpose.

2. Deodorizing by blocking.
    a. Mechanical: This can be accomplished either by closing the receptor sites via mechanical means such as masks, filters, or nostril plugs, or by chemically blocking or damaging the sites. While this technique is cumbersome, transitory and uncomfortable it is preferable to the latter, which could be dangerous.
    b. Chemical: The blocking effect of chemicals such as formaldehyde can be harsh and dangerous and more than transitory. These substances are powerful enough to cause a radical physiological change and can have severe and harmful long-range effects upon the body.

3. Masking by reodorizing: perfumes and fragrances function in this manner. Products of this type do little to alter either the basic perception of the odor character or the intensity of the malodor. The intent of their use is to cause so many signals to be sent to the brain, most of which are pleasant, that the impact of the malodor is relatively weak in relation to the overall impact. This approach can be effective with low levels of malodor. Effectiveness is debatable with high levels of malodors. Most often both malodor and fragrance are perceived, and the potential for exacerbating a problem exists.

4. Deodorizing by chemical reaction: If a malodor can be made to react chemically with an introduced substance, it will become something else and smell differently. This type of deodorizing reaction is primarily an oxidation-reduction or metal salts. Oxidizing agents include chlorine, (chlorine/caustic) sodium and calcium hypochlorite, chlorine dioxide, potassium permanganate and hydrogen peroxide. All are effective, but non-specific. Thus they react with non-malodorous organics and nitrogen based compounds, which increases the cost of their use. AS a group, these products generally pose safety problems such as toxicity, the production of toxic by-products, inherent corrosive and explosive characteristics. Metal salts are also used for deodorizing. They bind and precipitate. There effectiveness is restricted to addressing sulfides in solution. They do not react with malodorous organics such as amines and mercaptans. Those most commonly in use are ferrous and ferric chloride, ferrous and ferric sulfate.

5. Deodorizing by counteraction/neutralization. Defined as the mutual diminution of two odors, counteraction or neutralization is achieved by the application of a second odorous substance to the original malodor so that the combination of odors becomes inoffensive. The simultaneous reaction at the receptor sites in the olfactory cleft cause the generation of a signal other than that characteristic of the malodor. The combined signal may either overpower the malodor signal or cause the brain to recognize a different pattern, which results in perception and recognition of a pleasant odor or no odor at all.