Enhanced Applied Bioremediation

by Dr. J.R. Collins

The successful use of microbes in biodegradation is not a simple matter. Some of the key complicating factors are: not having the contaminants available to the organisms, toxicity of contaminants (or other materials) to the organisms, microbial preference for some contaminants or naturally occurring chemicals over other contaminants, partial degradation of contaminants producing hazardous or toxic byproducts inability to remove contaminants to very low concentrations, and aquifer clogging from excessive biomass growth.

For the more complex compounds such as oils and tars, it is unlikely that any single organism can degrade even a majority of the myriad of component compounds.

WHAT ABOUT THE MICROBES DEVOURING OIL WELLS?

Since oil was first discovered surface microbes have been concurrently injected into wells with drilling mud, water, etc with no ill effects. Apparently only very specialized microbes can survive in these very harsh conditions although microbes have been found almost two miles deep in an oil well.

ENZYMES AND BIOREMEDIATION

Living organisms are chemical machines. Microorganisms have evolved a variety of enzyme regulatory mechanisms to accommodate the changing needs of the microbial cell in a changing environment. Their knives, forks. and spoons are the various enzymes they produce. They involve numerous chemical reactions, each broken down by a specific enzyme. The breaking down of available nutrients and the building of cell constituents are not single step processes.

Enzymes are the microbe's catalysts, they speed that process without being used up in the reaction just as our utensils speed our partaking of our food but are not used up in the process. A living cell contains more than 1000 different enzymes, each an effective catalyst to some chemical reaction. These enzymes must act together and sequentially in a coordinated effort so that all chemical activities in the cell are smoothly integrated with one another. One consequence of this enzyme coordination is the making and distribution of materials as required by the microbe for normal growth and metabolism.

There are untold numbers of "enzyme" products on the market that the manufacturers claim will efficiently remediate hydrocarbons.

While specific enzymes are quite capable of degrading certain substrates or portions of substrates, the drawbacks to using enzyme mixtures include:

1. Changing types or kinds of pollutants that evolve as substrate breakdown proceeds.

2. Since enzymes are extremely specific, there must be sufficient quantities of each sequential enzyme present at the time it is needed.

3. If any one enzyme is missing, the process stops.

4. Proper enzymes must be added continuously and timely because they are being depleted, as well as being diluted, in the process.

5. Enzymes are produced by living cells. Unless the proper enzymes are constantly added or living microbes are present, bioremediation can cease.

6. By using microbes capable of producing each enzyme as it is needed, which it does because the organism wants to utilize the food, the lack of sequential enzymes is avoided.

PETROLEUM HYDROCARBONS OR CARBOHYDRATES?

Carbohydrates (carbon + [hydrate]water) the most abundant organic compounds in nature. Carbohydrates are usually short chains of carbon, hydrogen, oxygen and sometimes other elements. An example of a carbohydrate is sugar which is a combination of carbon. hydrogen and oxygen. Petroleum is a hydrocarbon. It is a combination primarily of carbon and hydrogen, but in different configurations (the way or sequence in which the various atoms are tied together). Since both carbohydrates and hydrocarbons are combinations of primarily carbon and hydrogen, it follows then that if some living cells or organisms can use sugar for a carbon source (food), others are able to use petroleum hydrocarbons for food.

HYDROCARBONS AS A FOOD (CARBON) SOURCE

Hydrocarbons are suitable sources of carbon (food) for microbial cell growth and energy. The relative complexity of the substrate determines the ease and speed of bioremediation. The more complex molecules require more microbial activity because of the many possible intermediate breakdown products produced. Even PCB's (polychlorinated biphenyls) can be bioremediated but special consideration must be given to such particulars as controlling the chlorine and the by-products released in the process since chlorine is toxic to microbes.

Crude oils are complex mixtures of hydrocarbons of varying molecular weight and structure comprising the three main chemical groups, paraffinic, naphthenic and aromatic. These hydrocarbons range from simple volatile substances to complex waxes and asphaltic compounds that cannot be distilled. Enhancing bioavailability through the use of potent efficient emulsifiers and surface active agents is a virtual necessity for efficient cleanup, especially of the higher boiling point and higher viscosity fractions and residues.

Gasoline, fuel oil, alcohols., ketones, and esters have been successfully bioremediated at contaminated sites via established bioremediation processes. The gasoline components benzene, toluene, ethyl benzene, and xylene (together known as BTEX) are relatively easy to bioremediate. Diesel is composed chiefly of unbranched paraffins and is also relatively easy to bioremediate.

FOOD (CARBON SOURCE) AVAILABILITY

Bioavailability of the substrate is directly related to the available substrate surface area. The more surface area that is exposed to the microbes the more efficiently they can subject the substrate to their secreted enzymes. It is much like comparing the efficiency of feeding 10,000 people out of one room filled with food or feeding that same 10,000 from individual servings already set at tables.

INCREASING BIOAVAILABILITY

When the substrate (pollutant-contaminant) is emulsified into colloidal sized droplets smaller than the microorganisms, it can be more efficiently used thus enhancing and greatly speeding

degradation. Whether referring to bioremediation performed by indigenous (on site) or added microorganisms, any acclimatized microbe will use the pollutant more efficiently as food when it is "served" to them individually.

BIOREMEDIATION TEMPERATURE REQUIREMENTS

A certain amount of heat is necessary for chemical reactions, even when broken down by enzymes. to proceed at a rate fast enough to sustain life. The rate of most enzyme-catalyzed reaction (breaking the substrate down) increases by a factor of 2 [doubles] for each 10 degree C. increase in temperature. up to the temperature at which the particular enzyme begins to deteriorate.

Temperature requirements are limited and not controllable in many bioremediation programs. Temperatures in the human comfort ranges support and enhance the speed of bioremediation. Temperatures near or below freezing will cause the microbes to go dormant and therefore bioremediation will cease until warming occurs. This does not destroy the bioremediation capability permanently. Enzymes simply cannot be exuded from the cell into solid water (ice). Bioremediation recommences a warmer tempatures build. Temperatures sufficienlty high to kill the microbes (starting at 140 degress F.) will effectivly stop the bioremediation process but this is not expected in most environment applications.

CAN BIOREMEDIATION BE ACCELERATED?

Bioavailability enhancing mixtures exponentially augment all microbes by making their food more available to the microbes and their multitudes of rapidly produced descendants. This is best accomplished by breaking the substrate into tiny droplets (colloids) which are suspended in water thereby increasing the surface area available to the microbes while at the same time providing the necessary oil-water interface. Bioremediation takes place only at the oil-water interface. This provides for an extremely rapid microbial population explosion and more rapid and efficient bioremediation. Microbes capable of producing enzymes that act on the hydrocarbons must be present with adequate moisture and minerals supplied if necessary. The major consideration then must be the bioavailability of the substrate.

The use of any chemicals detrimental to the microbes will diminish or destroy subsequent microbial activity and bioremediation. Bioavailability enhancing agents should not only more rapidly prepare the food (substrate) for the microbes but at the same time act as a food source for them. By adding the microbes to the enhancing agents prior to use, the microbes can begin to repair their tissues and enzyme systems and thus more rapidly initiate the bioremediation response.

SUBSTRATE EMULSIFICATION

When the substrate is acted upon by effective surface active agents, the result is a distribution of its particles in the other material , e.g. tiny droplets of milk fat (cream) in milk. In this size range, the relative surface area of the colloidal particle (droplet) is so much greater than its volume that unusual phenomena occur. For example the particles do not settle out of suspension by gravity, and are small enough to pass through filter membranes.

WHAT ARE COLLOIDS?

Molecules and atoms sometimes swarm together under the influence of intermolecular forces, and the large conglomerates behave like macromolecules: these are the colloids. Colloids can be distinguished from true solutions by the presence of particles that were too small to be observed with a normal (light) microscope yet were much larger than normal molecules. Colloids are now regarded as systems in which there are two or more phases with one (the dispersed phase) distributed in the other (the continuous phase). Colloids are thermodynamically unstable with respect to the continuous phase: surface tension favors small surface areas. Moreover, at least one of the phases has small dimensions (in the range of 10-9-10-6m.) Emulsions are colloidal systems in which the dispersed and continuous phases are both liquids, e.g. oil-in water or water-in oil. Such systems require an emulsifying agent to stabilize the dispersed particles.

DISPERSIONS AND EMULSIONS ARE SIMILAR

Dispersion. (1) A two-phase system of which one phase consists of finely divided particles (often in the colloidal size range) distributed throughout a bulk substance, the particles being the disperse or internal phase and the bulk substance the continuous or external phase. Under natural conditions the distribution is seldom uniform. but under controlled conditions the uniformity can be increased by addition of wetting or dispersing agents (surfactants) such as a fatty acid. The various possible systems are: gas/liquid (foam), solid/gas (aerosol), gas/solid (foamed plastic), liquid/gas (fog), liquid/liquid (emulsions), solid/liquid (paint), and solid/solid (carbon black in rubber). Some types, such as milk and rubber latex , are stabilized by a protective colloid which prevents agglomeration of the dispersed particles by an adherent coating.

Solid-in-liquid colloidal dispersions (loosely called solutions) can be precipitated by adding electrolytes which neutralize the electrical charges on the particles. Larger particles will either gradually coalesce and rise to the top or settle out, depending on their specific gravity.

Emulsion. A stable mixture of two or more immiscible liquids held in suspension by small percentages of substances called emulsifiers. These are of two types: (1) Proteins or carbohydrate polymers, which act by coating the surfaces of the dispersed fat or oil particles, thus preventing them from coalescing; these are sometimes called protective colloids. (2) Long-chain alcohols and fatty acids, which are able to reduce the surface tension at the interface of the suspended particles because of the solubility properties of their molecules. Soaps behave in this manner; they exert cleaning action by emulsifying the oily components of soils. All such substances, both natural and synthetic, are known collectively as detergents.

Note that dispersions is a more general term. Dispersions are seldom uniform and the dispersed material is not necessarily of colloidal size. Emulsions are colloidal systems in which the dispersed and continuous phases are both liquids. Such systems require an emulsifying agent to stabilize the dispersed particles. Without the emulsifying agent, the order will collapse and the dispersed phase will coalesce and reform into a liquid phase which will separate from the other liquid. This is the process that causes milk fat to separate from milk and oil to float on water.

By compromising the intermolecular forces within the substrate, the substrate surface molecules can be more easily separated from the substrate proper to form the dispersed phase of the emulsion. When this is done with organic chemicals, rather than high pH and high shear stress inorganics (soaps) which could damage the microbes, bioremediation can proceed rapidly. The microbes not only survive in the mixture, they, multiply exponentially thus increasing the efficiency of the mixture. The organic chemicals utilized in the emulsifying mixture must not only be non-toxic to "oil-eating" microbes, the chemicals actually must serve as food (carbon source) to the microbes. By the use of the organic chemicals, a stable emulsion of the substrate is formed which provides for continuous bioremediation of the substrate by microorganisms until the substrate and the organic chemicals themselves are consumed. The colloidal emulsion remains stable and bioremediation progresses rapidly and continuously until biodegradation is complete.

HOW MUCH CAN SURFACE AREA BE INCREASED?

A sugar cube contains about one and one-half square inches of available surface area for reaction with its environment (six half-inch square faces). If that cube were to be cut into 10 nanometer cubes, the available surface area of that same sugar cube increases 100,000 times (a cube 13 feet on each edge). Such is the effect of using the organic chemicals to make more substrate surface area available to any microbes which can utilize that substrate. No matter how small the microbes (bacteria and yeasts are large enough to be seen with an ordinary light microscope), limited numbers can crowd to a finite amount of surface. Were that relative amount of surface to become virtually infinite, almost limitless numbers of microbes would have food available without competition for them and their abundant offspring produced as a result of the feeding frenzy. There would be little competition for the food and extensive dilution of waste that might otherwise compromise them.

Colloids are not only readily available to the microbes but are also immediately available to the descendants of the microbes. In warm. moist environmental conditions with adequate food available (as presented by the tremendous available surface area), each microbe can divide into two cells as often as every 20 minutes. With this doubling many times per hour there is an exponential explosion of microbe population so they efficiently utilize the food source and thus obliterate the contamination.

WHY NOT USE HIGH pH SOAPS OR EFFICIENT CAUSTIC CLEANERS?

High pH is the basis for many bacteriocidal, virucidal and fungicidal cleaners and disinfectants commonly used in hospitals and food establishments. Prior to the use of organic emulsifiers, adding living microbes to the high pH high shear concentrated cleaners (or dispersants) would mean virtual instant death for the microbes.

OXYGEN IN BIOREMEDIATION

Oxygen is essential for most organisms yet can be poisonous to others. In aerobic bioremediation oxygen must be present for degradation to occur. The hydrocarbon is broken down yielding water (H20) and carbon dioxide (C02) as end products. Note that oxygen atoms are added to the hydrogen and carbon components of the hydrocarbons to make the end products. Adding atomically-bonded oxygen catalysts will speed the bioremediation processes when aerobic organisms are present.

INORGANIC NUTRIENTS

A typical bacterial cell is 50% carbon, 14% nitrogen; 3% phosphorus; 2% potassium; 1% sulfur; 0.2% iron, and 0.5% each of calcium, magnesium, and chloride. Therefore all organisms also require nutrients containing some form of nitrogen, hydrogen, oxygen, sulfur, phosphorus, and numerous other trace elements even though the primary building blocks are carbon. The addition of inorganic nutrients in a formulation that will make available the necessary elements to assure complete nutrition to the growing microbe population is essential.

ENHANCED APPLIED BIOREMEDIATION OF SOIL CONTAMINATION

Soil presents complex problems primarily because of the extreme variations in soil characteristics from location to location as well as specific areas within locations. Although limited permeability will slow contaminant penetration, desired infiltration by the remediation procedure will also be limited. Integra Environmental, Ltd. has developed an array of efficient microbe supporting emulsifiers and surface active agents that also act as liquid cleaners and carriers for the application of selected combinations of broad spectrum "oil eating" microbes to contaminated sites. Products are formulated for specific applications such as hard surfaces, soil remediation, parts cleaning, marine and fresh water oil spills, etc. Also supplied are microbe stimulating biocatalysts and inorganic nutrients.

Integra Environmental, Ltd. manufacturers specialized patented bioremediation enhancing agents. These products are designed for specific applications such as on hard surfaces (concrete, plastic, and metal), soil (injection and land farming), water (both marine and fresh as in cooling operations), on and off vehicle parts washing, emergency spill response, etc. Check with Integra Environmental for products Listed on the U. S. EPA National Contingency Plan, South Coast Air Quality Management District (California),and other listings, certifications and applications.

Specific information is available from INTEGRA ENVIRONMENTAL, LTD.
5825 Centralcrest, Houston, Texas 77092

PH (713) 680-1234, FAX (713) 680-1608