Carl Sandburg, in his “The People, Yes,” writes of the meeting of an Indian and a white man. The latter, impressed with his own importance, draws a small circle in the sand and says, “This is what the white man knows.” The Indian, much wiser of the two, draws an enormous circle and then correctly points out, “This is where the white man and the red man know nothing.”

Sandburg’s story is an appropriate way of introducing a few summary remarks about this symposium. Just a few years ago, shortly before most of the research presented here was initiated, our knowledge could be described by a small circle. As a result of the work presented at this symposium as well as the work of other scientists and engineers in the last few years, we can view our accomplishments as the somewhat larger circle. However, in reality, the present status is best described by the enormous circle drawn in the sand by the wise Indian. Our ignorance of the topic far outstrips our knowledge. Although much has been accomplished, we still face a vast circle of ignorance. I shall illustrate some of the areas of ignorance, but clearly the choices represent only a small sampling, and those issues are derived from my experience.

The conference is concerned with biodegradation to reduce toxicity and exposure. The various presentations have clearly demonstrated that biodegradation does, in fact, often accomplish these ends; however, any objective evaluation of a technology must consider not only its benefits but also its risks. The benefits of the technological uses of biodegradation, specifically in the area of bioremediation, are great. In some instances there can and likely will be an increase in toxicity, an increase in exposure, and therefore an increase in risk. The risk may be large, it may be small, or it may be infinitesimal. Nevertheless, it would be irresponsible of scientists who are interested in the benefits of a technology not to state clearly the nature and the possible magnitude of the risk. On one hand, it is possible that biodegradation will reduce the concentration of the pollutant to a level that is undetectable and will generate no low-molecular-weight products or only products that are known to be of no hazard. On the other hand, it is known that, at least in certain instances, the concentration is markedly reduced, but a low level remains undetected: Although the concentration represents less of a hazard than the high concentration, a technology other than bioremediation might have led to environmentally acceptable residual levels. Considering our large circle of ignorance, little information exists on the concentrations remaining following extensive microbial degradation, and society will be loathe to accept a technology until that type of information is available.

Risk elimination and risk reduction are beneficial aspects of microbial transformation, but ample evidence exists that sometimes the risk is increased as a result of the metabolic processes of microorganisms in natural environments or in engineered systems. Enhancement may be associated with the formation of products that have greater toxicity than the precursor molecules, products that may have equal or even lesser toxicity but persist for longer periods, and products with enhanced mobility. Although the actual concentrations of the persistent or mobile compounds may be lower, they do, because of their longer periods of persistence or greater transport, represent greater exposures. Enhanced mobility as a result of microbial action could be a consequence of the formation of less hydrophobic, nonionic, or anionic products generated from the parent substrate. In addition, microorganisms produce surfactants, and a surfactant may increase the risk by increasing the exposure to the parent compound because of its greater mobility. Again, giving heed to the large circle of ignorance, the persistence and mobility of many, and possibly most, of the products of microbial transformation are unknown. Such information is essential if bioremediation is to be considered as an appropriate way of reducing or eliminating risks.

In contrast, some of the toxic products of microbial metabolism have been characterized. A few of these are illustrated in Table 1. The process of converting an innocuous compound to a toxic product or the conversion of a molecule of low toxicity to one of high toxicity is known as activation. The activation may result in the formation of carcinogens or teratogens that affect humans or animals, acute toxicants of importance to public health or natural populations of animals, or phytotoxins. Although some of the products of activation are rapidly destroyed, others are slowly tranformed and hence represent issues of real concern.

A circle of modest size has been generated from a small one in the area of inoculation of environmental samples or, as it is sometimes called, bioaugmentation. In this approach to bioremediation, it is hoped that toxicants will be eliminated by the addition of microorganisms to natural environments. Indeed, reports in the peer-reviewed literature suggest that this approach is really quite simple and that many chemicals have been destroyed by the addition of one or another bacterium.
or fungus to environmental samples. Typical instances are given in the first two columns of Table 2. However, careful reading of the methods in these publications suggests a problem, which is intimated by information in the third column. An investigation that involves inoculation of sterile samples from natural environments or several grams of soil in a petri dish, flask, or bottle does not represent an environmental study but is simply a test conducted with a heterogeneous culture medium. Nature is not two-dimensional, and applying conclusions based upon two-dimensional laboratory models to a three-dimensional environment is not appropriate for meaningful assessments of the utility of inoculation procedures. It is quite likely that bioaugmentation or deliberate addition of microorganisms to natural environments will work in certain circumstances, and several examples exist of success. However, individuals who have grown exotic species of plants in solution culture under ideal conditions in a growth chamber know that frequently the same plants fail in pots of soil, and agronomists and horticulturists who have grown plants in pots in the greenhouse know that most of the plant species will not be successful under field conditions. Thousands of years ago, farmers learned what many who work with microorganisms still do not know: environmental constraints must be overcome in order for most introduced species to become successful. Those environmental constraints are overcome by the farmer when he modifies the soil structure to permit root development, provides adequate nutrients to permit good plant growth, and adds selected chemicals to inhibit competing species, parasites, and predators. The mere addition of seeds to a field almost never leads to successful establishment. Unfortunately, little is known about the environmental constraints on inoculation with microorganisms. Beyond the obvious need for adequate pH, temperature, and moisture, nutrients in addition to the target organic compound must be available, predation and parasitism have to be overcome, the mobility of the organisms through environmental media has to be adequate for them to reach the compound, and the molecule of concern must be available for utilization. Microbes do not live by substrate alone.

Consider the circle of ignorance on microenvironmental distribution of organic pollutants. Soils and subsoil materials have pores, micropores, and nanopores. These vary in size from millimeters in diameter to nanometers. In contrast, bacteria are typically in the micrometer-size range, and the hyphae of fungi may have a diameter ten times larger. Should chemicals become deposited and sorbed in these small pores, it is doubtful that a microorganism added to the soil surface would be able to move through pores with smaller diameters than the cell or the hypha to locate a substrate at some distance from the site of inoculation. Indeed, distances in terms of a microorganism should be visualized in the realm of centimeters or less. Moreover, if the compound is sorbed to a somewhat inaccessible surface associated with a micropore, the rate of desorption and the tortuous path of the diffusing chemical to reach the organism might be so slow as to make bioremediation impractical. Many of the major pollutants are sorbed in soils and subsoil materials, witness that they are still present when nonsorbed compounds have long since been washed out. Thus, how relevant is the addition of a microorganism in a two-dimensional environment to problems in which the compound is at some distance away and the small pores cannot be colonized by the somewhat larger celled organism?

Knowledge of the biodegradation of many organic compounds in aqueous solution is extensive; however, in nature many compounds are not readily available to microorganisms. The compound may be sorbed, it may be present in a nonaqueous phase liquid (NAPL), or it may be deposited in an inaccessible microenvironment. Desorption may bring the compound back into solution, but the rates of desorption of some compounds are very slow, or much less than 100% of the added compound that is desorbed. A compound present in a NAPL may partition to the aqueous phase, but the rate of partitioning may often be extremely slow. Many pollutants are thus rendered less available because of their sorption, presence in a nonaqueous liquid, or location in a physically inaccessible microenvironment; yet a large circle of ignorance characterizes our knowledge of bioavailability. Indeed, in some cases, knowledge simply does not exist. This is especially true for compounds within the micropore or nanopore matrix of soils. On the other hand, evidence exists that some sorbed compounds are readily degraded, at least compounds that have been recently sorbed and do not undergo "aging." This is illustrated in Figure 1. As indicated in the top panel, phenanthrene is extensively sorbed by a series of soils; however, as illustrated in the bottom panel, there is extensive degradation nevertheless. The rapid degradation may be associated with a rapid rate of desorption, and it
remains to be shown whether there is significant degradation of such compounds when desorption is very slow or undetectable. Similarly, preliminary studies of test compounds in individual organic solvents, as well as in oils, suggest that microorganisms may be able to degrade some chemicals that are initially present in NAPLs. Such rapid degradation is evident for phenanthrene initially in cyclohexane in the presence of microorganisms capable of degrading that aromatic hydrocarbon (Figure 2). However, the same compound, when initially present in several other NAPLs, is not actively degraded by the same organisms.

It is not surprising that scientists tend to emphasize their accomplishments, and without question, research in biodegradation and bioremediation has progressed remarkably in recent years. Public interest, the attention of scientists, and the provision of research funding have resulted in an impressive literature and the widespread application of that scientific information to practical engineering solutions; however, the circle of ignorance still remains enormous. We should proudly proclaim our accomplishments, but we should also point to the information gaps that prevent a more rapid or more frequent accomplishment of practical bioremediation.

It is worth bearing in mind an Arab proverb which, to suit the purposes of the present discussion, has been modified slightly:

He who knows not and knows not that he knows not, he is a fool. Shun him.

He who knows not and knows that he knows not, he is on the road to wisdom. Follow him.

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