15 May 1981, Volume 212, Number 4496

SCIENCE

Sludge Decomposition and Stabilization

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Summary. Sludges are labile putrescible materials which must undergo a heterotrophic decomposition process and be subject to humification before becoming stabilized substances. An understanding of the principles underlying these processes may give rise to better management plans for the application of sludge to land. Procedures are suggested for the evaluation of sludge stabilization rates.

Sludge stabilization has been defined in a number of ways over the last half century. For many civil and environmental engineers, sewage is believed to have been stabilized when its labile organic component is properly digested, either anaerobically or aerobically. Modern practitioners of sludge composting would probably agree that stabilization is achieved through processes that were reviewed by a prominent scientist nearly 50 years ago (*1*), processes in which readily decomposable material is converted into colloidal, humified lignoproteinaceous material and human and animal pathogens disappear or are rendered ineffective. Today students in engineering are still being exposed to definitions which, like the foregoing and the following, bear labels or are descriptive but fail to provide insight into the basic nature of the sludge stabilization process. Thus, sludge stabilization has been defined as a process through which sludge becomes able to resist putrefaction (*2*), as a process in which sludge is made less odorous and putrescible and contains a lower content of pathogenic organisms (*3*), and as a property which permits sludge to be disposed of without damage to the environment and without creating nuisance conditions (see below) (*4*).

On the basis of technical information provided below, it can be stated categorically that sludges are not stable organic substances. If they were, complaints would not commonly be made with regard to the placement of sludge upon land. Controversies surrounding the presence of pathogens (*5*), excessive concentrations of heavy metals (*6*), and the production of malodor speak to the contrary.

The basic scientific information needed to provide a definition and to establish procedures for measuring the rate and extent of sludge stabilization is available. What is needed, however, is testing and application of this information. The objective of this article is to present the information, to propose a workable definition for sludge stabilization (a definition that ties in with both the basic information and the three definitions given above), to describe several factors relating to management whereby the rate of sludge decomposition and stabilization can be enhanced, to discuss the highly probable consequences of sludge stabilization in light of the basic information, and to suggest procedures for evaluating the sludge stabilization process. As a starting point, it is necessary to describe the fossilization pathway of the carbon cycle.

The Fossilization Pathway of the Carbon Cycle

Kerogens, coals, and petroleum oils are the earth's major fossil fuels; they may be considered as relatively stable substances in view of their ages. In becoming fossilized, organic matter passes through a two-step process. The first, humification, is mainly aerobic and relatively rapid, whereas the second, carbonification, occurs more slowly and anaerobically. The entire process is continuous and gradual, but differentiable products arise in sequence. In the coalification process, decaying organic material is first converted to humus and then to peat, brown coal, lignite, subbituminous, bituminous, and anthracite coal. Evidence for this sequence is based on the observation that humus deposits are not found below coal, lignite deposits have not been found above peat, bituminous coals above lignites, and so on.

Table 1 provides elemental data for some of the broadly differentiable members of this sequence. It also presents elemental data for a typical American diet and municipal sludges. Beginning with the human dietary intake and continuing toward digested sludge, the carbon concentration in the organic matter decreases. Thereafter, with the onset of humification and continuing through coalification, the carbon concentration increases (*7*). An arrangement of the members of the entire sequence, from dietary food to coal, on a plot (*8*) of the atomic ratio of hydrogen to carbon versus the atomic ratio of oxygen to carbon shows that sludge must undergo extensive decarboxylation, dehydration, and demethanation on becoming humified (Fig. 1).

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0036-8075/81/0515-0743$01.75/0

Humification as an Index to Stabilization

An arbitrary and artificial but reasonably rational distinction must be made between humification and carbonification, if a definition is to be obtained for sludge stabilization. In humification an equilibrium is established between the production and oxidation of humus, and the reaction is driven toward the production of carbon dioxide and water as newly decaying organic material is added to humified material in the presence of oxygen. Carbonification, in contrast, occurs anaerobically and, upon exposure of the organic matter to sufficiently high temperature and pressure, irreversibly. Carbonification thus occurs increasingly in deeper layers of organic matter, where correspondingly fewer microorganisms are present and large amounts of methane are produced instead of carbon dioxide.

Support for the arbitrary distinction between humification and carbonification is derived from observations that newer humus is less stable than older humus (*9*); that organic materials do not accumulate significantly in well-aerated soils (*1, 10*), despite repeated doses or different loading rates (*1, 11*); and that the application of fresh organic wastes stimulates the decomposition of humus (*12*). In view of this and because humified material appears to be nonamenable to putrescence, it appears proper to say that sludge is stabilized when it becomes humified. At what point in the decay process, however, is organic matter humified, and are there varying degrees of humification?

Humus

Humus is the dark-colored colloidal organic material found in soils. It cannot be characterized in specific physicochemical terms; it does not have a specific molecular weight, melting point, boiling point, refractive index, ultraviolet, infrared, and nuclear magnetic resonance spectra, and the like (*13*). Humus, in fact, cannot be separated physically from nondecaying materials, such as living microbes or recalcitrant molecules such as lignins, which reduce the rate of organic decomposition; nor can it be separated chemically from nonhumic materials, such as carbohydrates and amino acids.

Procedures for isolating and fractionating humic matter are well established (*14, 15*). Briefly, undecomposed biological material such as leaf fragments are removed from a sample, which is then finely ground (about 40 mesh). The sample is then usually shaken in acid to remove exchangeable calcium and centrifuged. Humic and fulvic acids are extracted into alkali from the centrifuged sediment, and the organic residue is known as humin. Acidification of the extract causes humic acids to precipitate, thus separating them from fulvic acids.

Fulvic and humic acids consist mainly of complex hydrophilic polyelectrolytic polymers of benzene rings that are variously substituted, partially to completely, with either or both phenolic and carboxyl groups, together with large amounts of aliphatic carboxylic acids; up to about 70 percent of humic and fulvic acids may be made up of these subunits, which form water-soluble and water-insoluble complexes with metal ions and hydrous oxides and interact with clay minerals and hydrophobic organic compounds such as alkanes (*13*). About 10^{18} stable free radicals are found per gram of humic or fulvic acids (*16*). Fragmentation patterns derived through mass spectrometry reveal remarkable similarities between soil humic acids, model humic acids, composted straw, peat, and melanins (humic substances secreted by fungi) (*17*). Many of the chemical and spectroscopic properties of model humic acids are also indistinguishable from those of soil humic acids (*18*) and melanins (*19*). Reactions that produce model humic acids are virtually complete in minutes to a week, depending on the starting material (*18*), although the reaction period required for the production of humus in nature is unknown. Humic acids differ from fulvic acids in having larger molecular weights and from humins (the alkali-insoluble component of humus) in having smaller molecular weights. On the basis of the analyses in Table 1, fulvic acids may be younger forms of humic acids, although contrary views may be expressed in the literature (*14*). Fulvic acids also contain greater amounts of carboxyl, alcoholic hydroxyl, and phenolic groups than humic acids (*13*). These findings, together with a trend toward a higher concentration of free radicals (*20*) and lower concentration of purines and pyrimidines (*21*) in humic over fulvic acids, a longer mean residence time of humic over fulvic acids (*22*), and more carbohydrate in fulvic than in humic acids (*23*), lend support to the data in Table 1 in suggesting that fulvic acids are antecedents of humic acids.

Fulvic acids undergo a considerable amount of demethanation (possibly preferential losses of methylene) in being derived from foodstuffs (Fig. 1). Moreover, in the event that they are precursors of humic acids, they lose an appreciable amount of carboxyl en route; this is in line with the analytical data (*13*). Fulvic and humic polymers are linked with amino acids, carbohydrates, peptides, and amino sugars (*24*), probably through bonding of nitrogen to carbon on a phenol or by quinonimine formation (*25*), as well as possibly through peptide linkages (*24*), and by way of the thioether linkage of sulfur to ring carbon (*15*). On the basis of electron spin resonance studies, humic acids are believed to be semiquinone type radicals with high cation exchange and oxidation-reduction capacity (*26*). A schematic of some of the chemical findings is given in Fig. 2, which should not be considered a model but rather a device to convey current conceptions. An outstanding feature of these molecules, one which is especially germane to this article, is their long residence time in nature, 25 to 1400 years (*22*).

Consideration of the extent to which

Table 1. Ultimate analyses of organic materials in the fossilization pathway (in percentage of dry, ash-free weight).

Element	Carbohydrate*	Protein†	Fat‡	Mixed diet§	Sludge, digested‖	Sludge, activated¶	Fulvic acids (*13*)	Peats, recent (*62*)	Humins (*13*)	Humic acids (*13*)	Peats, old (*62, 63*)	Lignites (*62, 63*)	Coals, midrank (*63*)	Coals, anthracite (*63*)
Carbon	44	58	75	53	27	32	47	53	56	59	59	70	85	92
Hydrogen	6	7	12	7	4	4	4.4	6	5.7	5	6	5	5	4
Nitrogen		11		2			2	1	5	3	2		1.5	
Oxygen	49	23	12	36	42	37	46	38	33	34	31	25	8	2

*$C_6H_{10}O_5$. †$C_{72}H_{112}N_{10}O_{22}S$. ‡$C_{16}H_{32}O_2$. §60 percent carbohydrate, 20 percent protein, 20 percent fat. ‖Directly from digester, dried at 105°C (*58*). ¶Directly from aeration tank, dried at 105°C (*58*).

the degree of formation of humic substances ought to be considered as an index of sludge stability requires insight into (i) the natural processes that occur as organic matter decomposes and (ii) the factors that govern decomposition rates. Insight into these factors is needed also for the prudent selection of procedures for the management of sludge placement on land.

The Heterotrophic Decomposition Process

Humification in nature is achieved through the heterotrophic decomposition process, in which myriads of biological organisms interact with each other and with decomposing organic material. Three indexes have been used to evaluate the process: (i) the percentage of carbon released, (ii) the fractional life or mean residence time, and (iii) accumulation.

In general, about 50 to 85 percent of the carbon is released under temperature conditions from labile organic materials such as decaying food or composts during the first 6 months of decay. The losses follow an exponential curve and, if no additional material is added during 5 years, low percentages of the original carbon remains as a residue (*1, 9*). Half-lives or mean residence times of individual components in decaying material range from days to years, and the products into which they are derivatized, such as humic material, may linger for centuries (*22*). In the absence of conditions that lead to the production of peat, kerogens, and coal, organic matter does not accumulate appreciably in soil. Thus from data of Jenkinson and Rayner (*27*), if we assume a weight of 2500 tons of soil 23 centimeters deep per hectare, farmland with an initial 0.01 ton of carbon per ton of soil received a total of 0.14 ton of carbon per ton of soil (35 tons of farmyard manure per hectare per year) but showed only 0.032 ton of carbon per ton of soil after 119 years of annual application; organic carbon as sludge applied at rates up to 77.5 tons per hectare per year resulted in soil residues of less than 7.5 tons per hectare per year (*11*); and the application of 369 tons of sludge per hectare during 6 years resulted in only a twofold increase in the organic matter of the soil (*28*). Accumulation data with respect to the application of sludge to land indicate that heavy metals become more concentrated as the organic matter is oxidized, unless they diffuse into adjoining untreated landscapes or into deeper regions of the soil. If all conditions are equal except the dose rate of organic material, the rate of heavy metal accumulation can be expected to be proportional to the quantity (not concentration) of heavy metal applied, since the steady-state accumulation of organic material in soil is inversely related to the dose (*29*).

Factors That Govern the Rate of Heterotrophic Decomposition

The availability of nutrients, the presence of recalcitrant molecules, the accumulation of phenols, perturbations of the environment, temperature, and moisture are the main factors that limit the rate or the extent, or both, of decomposition of organic matter. Nitrogen, sulfur, potassium, phosphorus, calcium, and magnesium are the principal macronutrients required in the heterotrophic decomposition process. Except for nitrogen and sulfur, however, none of these elements, nor micronutrients such as molybdenum or cobalt, need be considered as important regulators of the process. Nitrogen and sulfur are exceptional because, under anaerobic conditions, they may become gaseous and escape from the decomposing material. A number of studies have shown that nitrogen in excess of about 2 percent (dry weight) may be

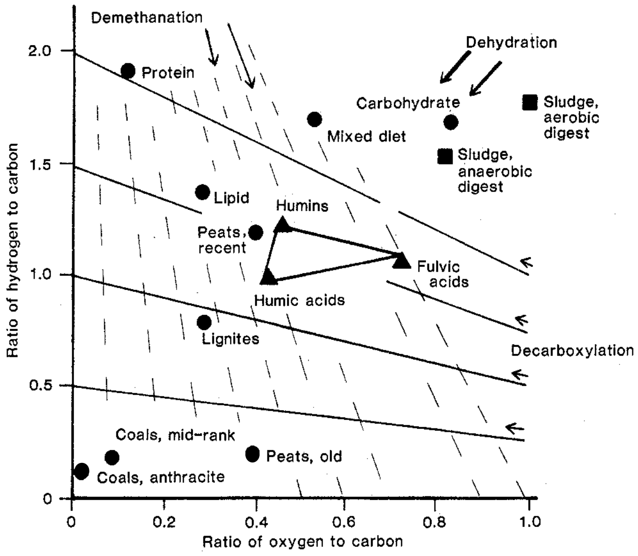

Fig. 1. The fossilization pathway of the carbon cycle.

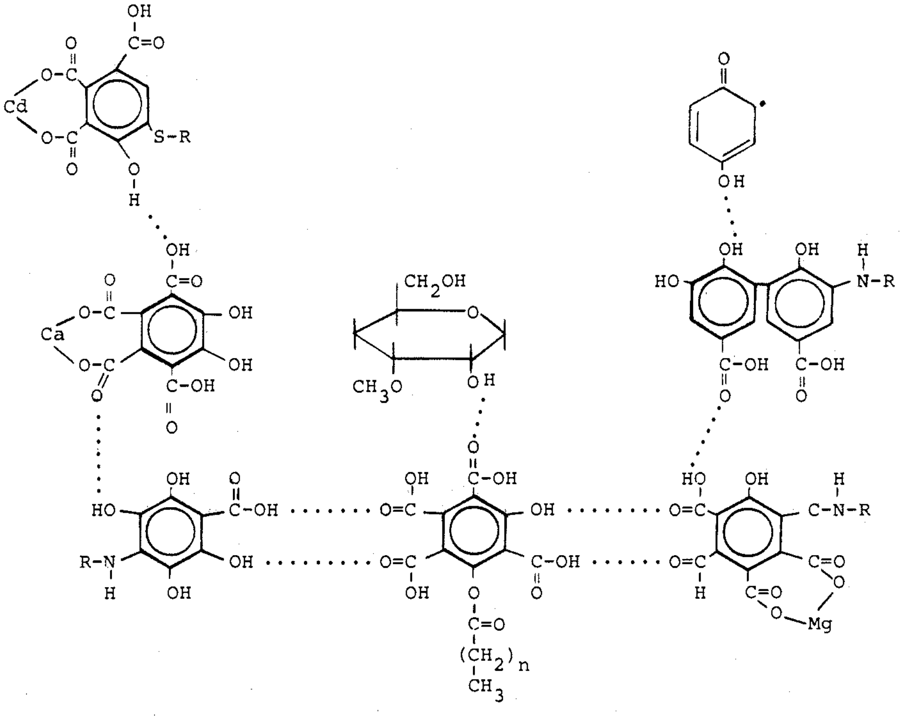

Fig. 2. A schematic of humic material; R may be an alkane, amino acid, fatty acid, purine, or some other molecular residue.

converted to ammonia or molecular nitrogen and released into the atmosphere, that concentrations around 1.7 percent suffice to support microbial needs, and that concentrations below about 1.5 percent fail to support rapid decay. Sulfur and phosphorus are needed at concentrations of about 0.15 and 0.2 percent, respectively, if decomposition is to proceed rapidly (*1*, *30*). Sludges generally contain sufficient or more than sufficient amounts of all the necessary minerals to permit rapid losses of carbon. In studies of sludge decomposition, it has generally been reported that the rate of release of carbon during early weeks to months is linearly proportional to the load, and a constant percentage of carbon is released, independent of the load (*1*, *11*, *12*).

Recalcitrant molecules, such as tannins, lignins, and melanins, tend to reduce the rate at which organic matter decomposes. Each of these classes of compounds is to some extent resistant to decomposition because they contain phenolic groups. Lignins, melanins, and humic substances are resistant, in addition, because of the numerous bond types, the highly random assembly of monomeric constituents in these polymers, and possibly their high concentration of free radicals. Because of the large variety of bond types, a wide variety of microbes may be needed to effect decomposition. The randomness characteristic is associated with high entropy. The high free radical concentration implies a high degree of reactivity, including possibly bonding on to, and destruction of, microbes, and may facilitate the repolymerization of components (*31*) that arise from the polymers and cannot be assimilated by the microbes.

Phenols accumulate increasingly during the heterotrophic decomposition process as humification proceeds. This in turn decelerates decomposition and thus protects labile components, such as carbohydrates and amino acids, from destruction. Less carbon dioxide is released per unit time from organic matter in the presence of lignins, tannins, and model humic acids than in their absence (*1*, *32*). The greater decomposition of sludge that is placed upon the soil as opposed to sludge that is mixed into the soil (*33*) may be related to the reduced rate of decay that occurs with mixing.

Perturbation of the environment constitutes a fourth factor governing the rate of heterotrophic decomposition. One type of perturbation, referred to as the priming effect, is the result of adding fresh decaying material (primer) to older decaying organic matter. The primer accelerates the exponentially declining rate of decomposition of the older material (*34*). This results in a greater net release of carbon than can be obtained as the sum of carbon dioxide and methane released from the primer and older material in isolation from each other. In some cases priming may even result in the release of a greater percentage of carbon from the older organic matter than from the primer itself (*12*). The greater net release of carbon in either event is due to the introduction of numerous and novel microbes and enzymes into the older material by way of the primer. Larger amounts of organic matter can be oxidized per unit time by the application of small amounts of primer on land often than by the application of large quantities infrequently. Excessive amounts applied to land may result in severe decimation of earthworm populations (*35*) and possibly other soil fauna.

Table 2. Some principal genera of bacteria commonly found in sewage sludges (*39*, *40*).

Sludge	Gram stain
Aerobic	
Pseudomonas	–
Flavobacterium	–
Alcaligenes	–
Micrococcus	+
Bacillus	±
Anaerobic	
Lactobacillus	±
Clostridium	+
Bacteroides	–
Peptococcus	+
(Methanogens)	±

Repeated wetting and drying of decaying material, in contrast to keeping the moisture constant, is another example of an acceleratory perturbation (*36*). Subsurface layers of sludges in lagoons, which may remain wet continuously, do not decompose as rapidly as sludges placed thinly and repeatedly upon a landscape. Cultivation also provides an acceleratory effect, whether achieved by plowing or through the activities of earthworms (*37*). Mere physical disturbance of decaying material constitutes still another form of acceleratory perturbation.

The last important factors are temperature and moisture. During the first few days to 2 weeks or so, heterotrophic decomposition of fresh material generally proceeds at a higher initial rate with increasing temperatures, to the thermal death point of microbes, although in field studies where decay rates are determined after 6 months or more the overall rate appears to be independent of temperature (*11*, *12*). Excessive moisture may lead to anaerobiosis with consequent reductions in the rate of decomposition and in the concentration of nitrogen, whereas in soils with a moderate amount of water where microbial activity is sufficient significant differences in rate are not seen after 4 months or less (*11*, *12*).

Sludges differ widely from one to another, partly because of differences in their source material and partly because of the dynamic nature of the heterotrophic decomposition process. Despite the variance found (*38*), it should be possible to monitor both the rate of decomposition and stabilization. This compels us to consider the chemical composition of the organic matter in sludges.

Chemical Composition of Sludge

The average dry weight of a bacterium is about 0.3×10^{-12} gram. Since rich anaerobic (*39*) and activated or aerobically digested sludges (*40*) may contain about 10^{10} to 10^{12} viable and dead bacteria per gram, a large amount of the material in fresh sludge is bacterial biomass or derivatives thereof. Some of the major genera found in these sludges are given in Table 2, and their main chemical components are listed in Table 3. An appreciable proportion of the biomass in aerobically digested sludge may be protozoal, and a smaller proportion includes rotifers, nematodes, and fungi. In addition, both types of sludge include microbial excretory and secretory products, tannins, lignins, humus, recalcitrant organic material, and minerals. Despite the fact that the chemical constituents of sludge are predominantly those of its biomass, it is perhaps useless, in view of the continuous and rapid changes that characterize the heterotrophic decomposition process, to obtain proximate analyses of sludges in terms of class compounds, such as carbohydrates, fats, and proteins. Ultimate (elemental) analyses should prove to be more helpful, since they are related to the humification process and to the value of sludge as a fertilizer or soil amendment, although other types of analyses, as described later, may provide a more practical route.

Sequence of Events in Sludge Decomposition

When anaerobic sludges are placed on soil and exposed to air, many obligate anaerobic bacteria are killed and become subject to decay, since they are deficient in certain enzymes which protect living organisms against the toxic action of oxygen and oxygen derivatives, such as the hydroxyl and superoxide radicals (*41*); obligate anaerobes distant from oxygen survive until they too are exposed to air. When aerobic sludges are placed upon land, obligate aerobic microbes die and decay when ambient conditions become anaerobic, although surface-dwelling aerobes and facultative aerobes and anaerobes may survive. With time, for either type of sludge, the dead cells are subject to lysis and decomposition, potentially giving rise to putrescence. In the presence of sufficient oxygen, as may happen if the sludge is spread thinly enough on land, the potential for putrescence diminishes, increasingly so as the carbon/nitrogen and carbon/sulfur ratios increase. During this transition, a considerable amount of interorganismal transformation occurs, wherein various kinds of microbes feed upon one another (*42*). Extracellular enzymatic decomposition processes also occur, both within the sludge and at the sludge-soil interface as a result of soil enzymes (*43*). All of these activities lead to an increase in mineralization and humification.

Table 3. Principal chemical constituents (*64*) in bacterial cell walls. Remarks in column 2 pertain to the corresponding contents in column 1.

Gram-negative bacteria	Gram-positive bacteria
Amino acids	
Alanine, glutamic acid, and either diaminopimelic acid or lysine	The same, but much less
Glycine or aspartic acid	The same, but much less
Serine or threonine	The same, but much less
N-Acetylglucosamine	The same, but more
Glucosamine	
Dominant sugars	
Glucose	
Galactose	
Mannose	
Arabinose	
Rhamnose	
Other components	
Various lipids	The same, but less
Muramic acid (*O*-lactyl sugar moieties)	Teichoic acid (ribitol and glycerol moieties)

Mechanism of Humification

It is generally believed that the process of humification is an oxidative one, since it is accompanied by a relative increase in the number of carboxyl, phenolic hydroxyl, and carbonyl groups and a reduction in the methoxyl content (*13*). At the heart of the process is the production of phenolic and carboxylic benzene rings. Some of these rings are synthesized by microbes from common metabolites (*44*). Others are secreted by fungi after being derived from lignins, and still others are obtained from soil organic matter through extracellular biological activity (*45, 46*).

Polymerization of the phenocarboxylic acids is believed to proceed mainly through short-lived free radicals whose production may be catalyzed enzymatically (*47*), by clay minerals (*48*), or autocatalytically in alkali (*15*). The enzymatic reactions are catalyzed by any of various oxidases, such as laccase or polyphenoloxidase with oxygen as an electron acceptor or by peroxidase with hydrogen peroxide. Hydrogen peroxide may be furnished by fungi (*49*) and perhaps other microorganisms which secrete this oxidant into their environment; or it may arise as a product of any of several metabolic reactions, such as those that are catalyzed by amino acid oxidases or glucose oxidase. Once formed, the free radicals rapidly condense as suggested in Fig. 2. Bonds are also formed between nitrogen- and sulfur-containing compounds and the polymers or their reactive phenolic precursors, and between functional groups on carbohydrates, fatty acids, and other classes of compounds. All types of bonds are possible, including pi, covalent, Van der Waals', and ionic bonds (*13–15*).

Consequences of Humification

Protection of labile organic material, an increase in the cation-exchange capacity, the prevention of malodor, and the destruction of most types of pathogens are the main consequences of the humification process. Evidence in support of the idea that labile organic materials are protected by humification includes the fact that proteins and certain other forms of easily decomposable organic material, when first complexed with phenols and converted into products with properties indistinguishable from those of humic acids, are more resistant to degradation than when freely exposed (*50*). Protection may be afforded, in part, by the toxicity of hydroxyl and superoxide radicals generated through certain phenols (*41*), and the long-lived phenolic radicals themselves, to microbes. Protection is also due in part to chemical bonds between sulfur- and nitrogen-containing groups of labile organic metabolites and the polyphenolcarboxylate units of humic and fulvic acids, and possibly to sequestration of such metabolites through hydrogen bonds and electrostatic bonding, thus precluding their accessibility to destructive enzymes or microbes. A third possibility relates to the movement of the humus material into the latticework of clay minerals within spaces too small for the ingress of microbes.

The increase in cation-exchange capacity is attributable mainly to the increase in the number of carboxyl groups per unit weight of organic material. Up to about 500 milliequivalents of cations can be held per 100 grams of fulvic and humic acids (*15, 51*), which is about three times the capacity of the best soil minerals, vermiculite and montmorillonite (about 150 milliequivalents per 100 grams) (*52*).

Severe reductions in the numbers of both animal and plant pathogens are suggested to result from humification for three reasons. First, the biological and ecological conditions in sludge are alien and therefore less favorable for the survival of pathogens. Second, the increasingly higher concentrations of phenols which appear during humification are conceivably less tolerated by pathogens than by the native soil biota. Finally, the process of humification itself results in the conversion of protoplasmic material into humic and fulvic acids. Although it is generally believed that high temperatures in compost piles cause the destruction of pathogens, humification may be equally important, though experiments are needed to determine whether this suggestion is valid.

With all of the preceding as a framework, I suggest that sludge reaches an acceptable level of stabilization, that is, is free of potential putrescence and malodor, is maximally capable of forming relatively stable complexes with heavy metals, and has acceptably low concentrations of pathogens, when it has been through the process of humification. The only facet of the definitions provided in the introduction that is not included in this definition is the nuisance factor on application to land with respect to certain compounds such as polychlorinated biphenyls (PCB's) and insecticides. The extent to which these types of compounds are destroyed is not known. Because a sufficient amount of work has been accomplished on xenobiotics generally (*53*), it is perhaps safe to say that scarcely any type of covalent bond is invulnerable to enzymatic alteration (*54*). In certain molecules, however, the presence of structural features that are never encountered in natural products is a cause for concern. Thus, although hundreds of natural products contain carbon-chloride bonds (*55*), none occur as PCB's. Accordingly, it is best to prevent the synthesis of potentially dangerous compounds. Moreover, for existing synthetics in the biosphere, it is worth determining whether sludges may be useful agents for their destruction. This possibility exists since thousands of strains of microbes are possible within gram quantities of sludge, and, whereas single strains or species may be unable to fully degrade a certain compound, such as cyclohexane, a mixture of microorganisms may (*56*).

Evaluation of Sludge Decomposition and Stabilization

Because of the sequential changes that occur during the heterotrophic decomposition of sludge and in view of certain characteristics of humified mineral soils—highly stable entities from a practical viewpoint—several properties of sludge may be measured as a way of evaluating sludge decomposition and stabilization on land. Possibly the most suitable ones are ash and nucleic acid or ATP (adenosine triphosphate) concentrations, followed by or along with humification values.

I suggest ash concentration as an indicator because mineral soils supportive of plant growth generally have less than 6 percent organic matter and do not become malodorous when wet. It is tempting to believe that the ratios of carbon to nitrogen or carbon to sulfur may be good indicators also, since these ratios tend to decrease when fermentative materials such as plant vegetation decompose and to increase with the decomposition of potentially putrescible materials such as protein and sludge. Sludges, however, often have carbon/nitrogen ratios that already approximate the values that are characteristic of humus. In view of this and because the carbon, nitrogen, phosphorus, and sulfur ratios (*57*) found in stable soils (220:20:5:2) are similar to those of microorganisms per se and sludges, it is clear that the sparsity of organic matter and the richness of mineral material and not the ratio of carbon to nitrogen or carbon to sulfur are essential characteristics of a stabilized sludge. Hence, the value or need of ash determinations.

Sludges typically contain 40 to 70 percent organic material when freshly released from waste-water treatment tanks. Much of this material is composed of microbial biomass, rich in nucleic acids. The smaller microbes, 10^{-6} meter in diameter, may be composed of up to 28 percent nucleic acids, whereas the larger ones, 10^{-5} to 10^{-4} meter long, have a concentration of about 5 percent or more. Measurements have revealed concentrations in fresh sludge that exceed values obtained on freeze-dried preparations of *Escherichia coli* and *Saccharomyces cerevisiae*, and substantially lower concentrations in aged sludges and soils (*58*). A measure of decreasing nucleic acid or ATP (*59*) concentration with time may prove to be a sensitive and specific indicator of the rate at which sludge decomposes, since bacteria and fungi are the ultimate biological agents of the heterotrophic decomposition process and their reduction in mass should coincide with a reduction in the amount of labile organic matter.

Of the various analyses that have been used to assess the degree of humification, the concentration of phenols per unit weight of organic matter may be most appropriate from the viewpoint of interpretation, ease, time required, and instrumental expense. Phenols, including fulvic and humic acids, tannins, and lignins, when evaluated in context with the pyrophosphate index (PP_{550}) of humification at 550 nanometers, show a high degree of correlation (*60*). In turn, values of the PP_{550} index, based on the optical density of extractable humic and fulvic acids into sodium pyrophosphate, increase as humification increases and are highly correlated with values of the extinction coefficient ($E_{4/6}$) (*61*). The latter, based on the light absorption of extracts at 465 and 665 nanometers, decreases as humification increases. Lower $E_{4/6}$ ratios are significantly correlated with higher molecular weights of humic and fulvic acids, higher concentrations of carbon, lower concentrations of oxygen and carboxyl, and lower cation-exchange capacity (*61*). Lower $E_{4/6}$ ratios also correspond to longer mean residence times (*22*) and higher concentrations of stable free radicals and phenols (*60*). A measurement of phenol concentration expressed as the percentage of organic matter may be more meaningful than values of PP_{550} and $E_{4/6}$ since the latter two measures do not provide chemical information and cannot be directly related to any specific chemical moiety in sludge. All of this is in need of testing, and, until attempts are made, environmental and civil engineers will continue to need a better definition of sludge stability than they have at present.

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65. Research during the past 4 years which led to the formulation of the concepts expressed in this article was supported by the National Science Foundation. All statements and opinions are the sole responsibility of the author.

Chitin: New Facets of Research

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Summary. Research on chitin as a marine resource is pointing to novel applications for this cellulose-like biopolymer. Discovery of nondegrading solvent systems has permitted the spinning of filaments, for example, for use as surgical sutures. New methods for preparing the bioactive alkyl glycosides of *N*-acetyl-D-glucosamine (the monomer unit of chitin) and a microcrystalline chitin have encouraged their use as promoters for growth of bifidobacteria and as an aid in digestion of high-lactose cheese whey by domestic animals. Chitin-protein complexes of several crustacean species show great variability in ratios of chitin to covalently bound protein and in residual protein in the "purified" chitins.

Chitin, poly-β-(1→4)-*N*-acetyl-D-glucosamine, is a cellulose-like biopolymer distributed widely in nature, especially in marine invertebrates, insects, fungi, and yeasts. Both chitin and chitosan (deacetylated chitin) are being processed industrially in million-kilogram quantities in Japan, but in much lesser amounts in this country (*1*). Chitin is a source of glucosamine, which serves as a potentiator for antibiotics, and chitosan is used in wastewater treatment. The broad field of chitin chemistry, structure, and applications has been thoroughly reviewed by Muzzarelli (*2*) and updated at a recent international conference on chitin and chitosan (*3*).

Chitin has an unusual combination of properties, including toughness, bioactivity, and biodegradability, which makes it an attractive specialty material. With these factors in mind, studies were instituted to promote chitin from crab and shrimp shells as a marine resource and to alleviate a growing waste-disposal problem in the shellfish food industry. Previous investigations of chitin have included basic biological and physiological studies, in vitro syntheses promoted by enzyme preparations (*4*, *5*), and an examination of its digestibility (as crab meal) by calves (*6*), accelerating effects on wound healing (*7*), and sequestering of plutonium from aqueous systems (*8*).

The applications research (*9*) has largely been focused on chitosan because the free amino groups in this modified product contribute polycationic, chelating, and film-forming properties, along with ready solubility in dilute acetic acid. However, chitin itself is substantially lower in cost and, although intractable, appears amenable to modern manipulative technology. In this article, we outline approaches to chitin utilization via nondegradative solvent systems, microcrystalline chitin, and the monomeric alkyl *N*-acetyl-D-glucosamine (NAG) glycosides. We have also studied species variations in chitin among marine Arthropoda. The bioactivities of the chitin products open new avenues for future research, particularly in the biochemical, pharmaceutical, and nutritive supplement fields.

Solvents and Filaments

Chitin, like cellulose, is insoluble in ordinary solvents; it is crystalline, and sometimes oriented in its natural state, suggesting fiber-forming potential. Strong acids, fluoroalcohols, and certain hydrotropic salt solutions are chitin solvents but degrade the chitin or are incon-

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0036-8075/81/0515-0749$01.25/0