are concentrating their efforts on the possibility of developing new systems for nitrogen fixation using recombinant DNA technology.

One area of research is directed toward introducing the "package" of nitrogen-fixing genes from bacteria into plant cells. If this were achieved, plants might be capable of directly fixing nitrogen from the atmosphere. This would be a tremendous advance not only for agriculture but for the world at large in terms of producing food more economically and abundantly. Obviously, considerably more research is necessary before this kind of genetic engineering can be attempted at a practical level. For instance, nitrogenase is easily destroyed by oxygen, and some means of protection of this enzyme complex from oxygen would have to be provided in order for a plant cell to be able to fix nitrogen.

Alternatively, it may be possible to modify certain bacteria in a manner so that they would develop a relationship with the root system of other plants, as the *Rhizobium* species grow with legumes. For example, a symbiotic bacterial nitrogen-fixing system with cereal grains would have a tremendous effect on grain production both in yield and cost.

BIOCHEMICAL TRANSFORMATIONS OF CARBON AND CARBON COMPOUNDS: THE CARBON CYCLE

Carbon Dioxide Fixation

The ultimate source of organic carbon compounds in nature is the carbon dioxide present in the atmosphere (or dissolved in water). The process, carbon dioxide fixation, was discussed in Chap. 11. Although green plants and algae are the most important agents of carbon dioxide fixation, bacteria are also capable of synthesizing organic matter from inorganic carbon. The occurrence of photosynthesis among microorganisms has already been described. Other examples of carbon dioxide transformation or incorporation into organic compounds by bacteria are:

1 Utilization of carbon dioxide by autotrophic bacteria; the carbon dioxide represents the sole source of carbon for these organisms and is transformed by a reduction reaction to carbohydrates. The general reaction is

$$CO_2 + 4H \rightarrow (CH_2O)_x + H_2O$$

2 Carbon dioxide fixation by heterotrophic microorganisms is common among bacteria. A specific example of this type of reaction is

$$\underset{\text{Pyruvic acid}}{CH_3COCOOH} + CO_2 \rightarrow \underset{\text{Oxalacetic acid}}{HOOCCH_2COCOOH}$$

Organic Carbon Compound Degradation

The organic carbon compounds that eventually are deposited in the soil are degraded by microbial activity. The end product, carbon dioxide, is released into the air and soil. Fresh air contains approximately 0.03 percent carbon dioxide by volume. Bacteria and fungi are the principal microorganisms that degrade organic carbon compounds.

Under most natural systems of vegetation, e.g., forests, the amount of organic material in the soil remains approximately the same from year to year. This results from a balance established between the annual litter fall and death of the plants and the capacity of microorganisms to degrade these tissues.

The most abundant organic material in plants is cellulose. It is readily attacked by many species of bacteria and fungi. The initial enzymatic attack is by cellulase which splits this long-chain polymer of glucose to cellobiose, which contains two glucose units. In turn, the cellobiose is split to glucose by the enzyme β-glucosidase; glucose is metabolized readily by many microorganisms. Complete oxidation yields CO_2 and H_2O. The process can be summarized as follows:

1 $\text{Cellulose} \xrightarrow[\text{cellulase}]{\text{enzyme}} \text{cellobiose}$

2 $\text{Cellobiose} \xrightarrow[\beta\text{-glucosidase}]{\text{enzyme}} \text{glucose}$

3 $\text{Glucose} \xrightarrow[\text{of many microorganisms}]{\text{enzyme systems}} \text{carbon dioxide, water, and/or other end products}$

An example of the breakdown rate of glucose (and microbial growth) by soil microorganisms is shown in Fig. 25-13. Similar degradation pathways occur for the other major plant tissue substances such as hemicellulose, lignin, and pectin. Carbon dioxide may also originate from the decarboxylation of amino acids, as well as from the dissimilation of fatty acids. All of these transformations may occur in the soil.

A general summary of the carbon cycle is shown in Fig. 25-14.

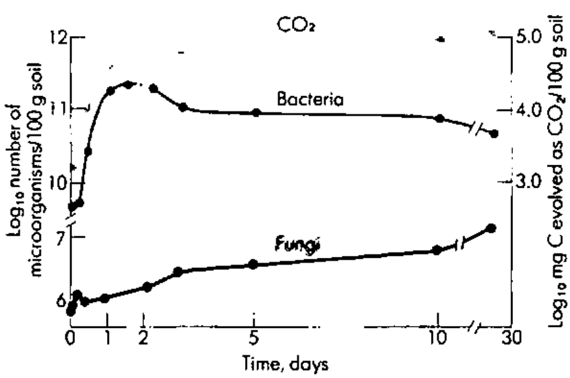


Figure 25-13. Plate counts of bacteria and fungi and cumulative CO_2 evolution during the incubation of soil treated with glucose. (*Courtesy of B. Behera and G. H. Wagner, Soil Sci Soc Am Proc,* 38:591, 1974.)

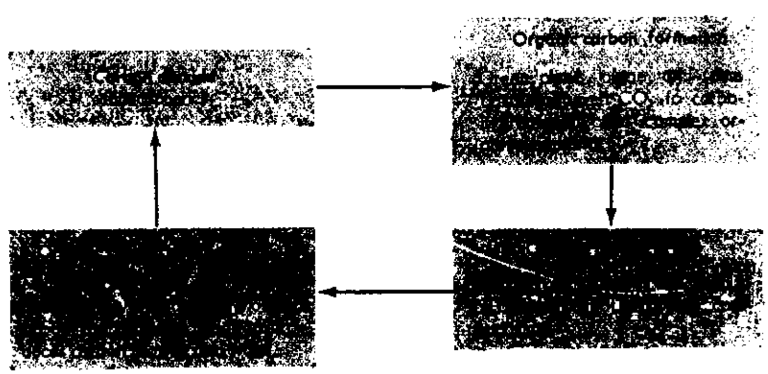


Figure 25-14. Carbon cycle (schematic), showing the role of microorganisms.

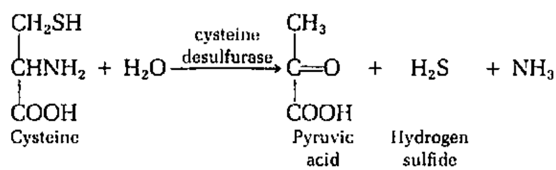
BIOCHEMICAL TRANSFORMATIONS OF SULFUR AND SULFUR COMPOUNDS: THE SULFUR CYCLE

Sulfur, like nitrogen and carbon, passes through a cycle of transformations mediated by microorganisms (see Fig. 25-15). Some species oxidize and others reduce various sulfur compounds. The microbial transformations of sulfur have counterparts in the microbial transformation of nitrogen. For example, sulfide and ammonia are reduction products of the dissimilation of some organic compounds; both may be oxidized by various bacterial species. Some of the biochemical changes by microorganisms involved in this cycle may be summarized as follows:

1 Sulfur in its elemental form cannot be utilized by plants or animals. Certain bacteria, however, are capable of oxidizing sulfur to sulfates. The classical example is *Thiobacillus thiooxidans*, an autotroph; the reaction involved is

$$2S + 2H_2O + 3O_2 \rightarrow 2H_2SO_4$$

2 Sulfate is assimilated by plants and is incorporated into sulfur-containing amino acids and then into proteins. Degradation of proteins (proteolysis) liberates amino acids, some of which contain sulfur. This sulfur is released from the amino acids by enzymatic activity of many heterotrophic bacteria. The following reaction is an example:



3 Sulfates may also be reduced to hydrogen sulfide by soil microorganisms. An example of bacteria involved in this process is the genus *Desulfotomaculum*, and the reaction suggested is

$$4H_2 + CaSO_4 \rightarrow H_2S + Ca(OH)_2 + 2H_2O$$

4 Hydrogen sulfide resulting from sulfate reduction and amino acid decomposition is oxidized to elemental sulfur. This reaction is characteristic of certain pigmented (photosynthetic) sulfur bacteria and is expressed as

$$CO_2 + 2H_2S \xrightarrow{\text{light}} \underset{\text{Carbohydrate}}{(CH_2O)_x} + H_2O + 2S$$

A laboratory technique which facilitates isolation of various sulfur-metabolizing bacteria is the Winogradsky column shown in Fig. 25-16. The column contains mud, $CaSO_4$, plant tissue (a source of carbohydrate-cellulose), and water. It is exposed to daylight and incubated at room temperature. The microbiological events can be summarized as follows:

1 A variety of heterotrophic microorganisms oxidizes various substrates, depleting the oxygen supply and creating anaerobic conditions:

$$\text{Organic matter} + O_2 \rightarrow \text{organic acids} + CO_2$$

2 Organic acids serve as the electron donors for the reduction of sulfates and sulfites to hydrogen sulfide by anaerobic sulfate-reducing bacteria, e.g., *Desulfotomaculum*:

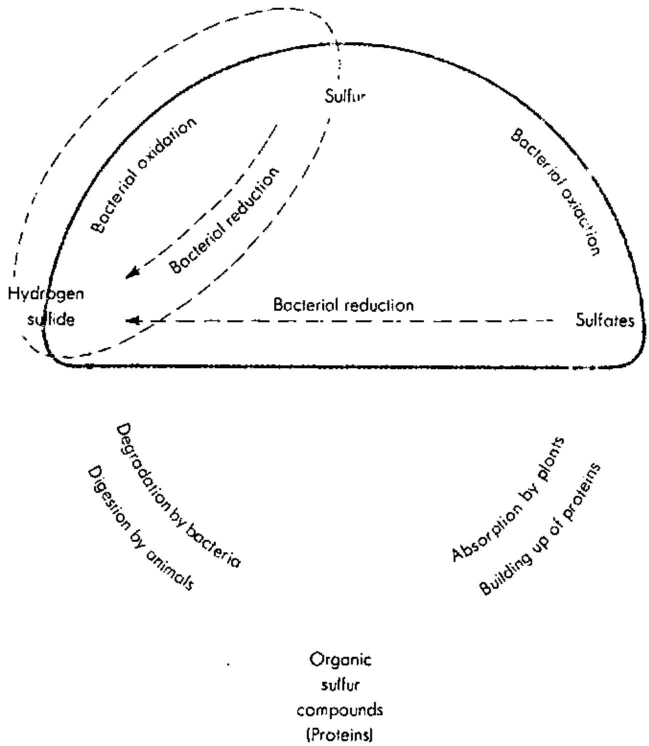


Figure 25-15. Sulfur cycle (schematic), showing the role of microorganisms. (*After Bunker.*)

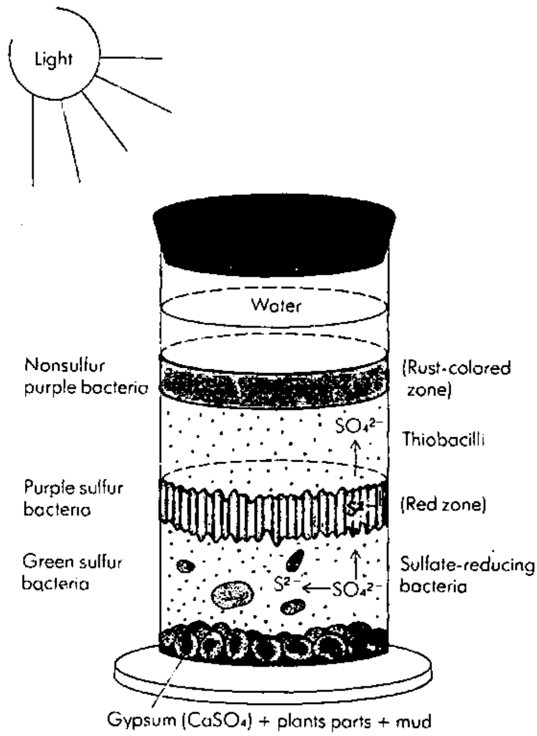


Figure 25-16. A Winogradsky column showing areas of localization of sulfur-metabolizing bacteria. See text for explanation of sequential developments. (*Courtesy of T. Hattori, Microbial Life in the Soil, Marcel Dekker, Inc., New York, 1973.*)

$$\text{Organic acids} + SO_4^{2-} \rightarrow H_2S + CO_2$$

3 Photosynthetic microorganisms such as the purple and green sulfur bacteria (*Chromatium* and *Chlorobium*) use hydrogen sulfide as the electron donor to reduce CO_2:

$$CO_2 + H_2S \xrightarrow{\text{sunlight}} (CH_2O)_x + S$$

4 The aerobic sulfur-metabolizing bacteria, *Thiobacillus* spp., develop in the upper portion of the column and oxidize reduced sulfur compounds (sulfides, elemental sulfur, sulfite). Final oxidation product is sulfate; sulfur accumulates:

$$\text{Reduced sulfur compounds} \rightarrow SO_4^{2-} + \text{accumulation of S}$$

5 The nonsulfur purple bacteria (*Rhodospirillum, Rhodopseudomonas,* and *Rhodomicrobium*) are facultative phototrophs; they grow aerobically in the dark and anaerobically in the light and can utilize sulfide at low levels. They are capable of utilizing hydrogen gas as an electron donor in photosynthesis:

$$CO_2 + H_2S \xrightarrow{\text{light}} (CH_2O)_x + S$$

$$CO_2 + 2H_2 \xrightarrow{\text{light}} (CH_2O)_x + H_2O$$

BIOCHEMICAL TRANSFORMATIONS OF OTHER ELEMENTS AND THEIR COMPOUNDS

The preceding discussion was concerned with transformations of nitrogen, carbon, and sulfur and their compounds. But this represents only a part of the elements and their compounds that are subject to assimilation and dissimilation by microorganisms. The metabolic activity of microorganisms (production of acids) solubilizes phosphate from insoluble calcium, iron, and aluminum phosphates. Phosphates are released from organic compounds such as nucleic acids by microbial degradation. Bacteria change insoluble oxides of iron and manganese to soluble manganous and ferrous salts. The reverse is also possible.

From these examples of biogeochemical changes that take place in the soil, it should be apparent that microorganisms do, indeed, perform numerous and essential functions that contribute to the productivity of soil.

BIODEGRADATION OF HERBICIDES AND PESTICIDES

Herbicides are chemical substances that kill plants, especially weeds; pesticides, as the term denotes, are chemical substances that destroy pests. In the context of soil, we think of those pests which adversely affect economic crops—weeds, insects, and pathogenic microorganisms. Thus a more specific nomenclature for substances classified as pesticides would be herbicides, insecticides, fungicides, and nematocides.

The wide-scale application of herbicides and pesticides, while improving the crop yield, raises questions as to the short- and long-range effects as they are deposited in the soil. Are they degraded by soil microorganisms, and if so how rapidly? Do they have a temporary (or permanent) effect upon the soil microbiota? Do they constitute a form of runoff pollution to streams and rivers and as such affect aquatic plant life? These are some of the questions that concern the soil microbiologist as well as other soil scientists, biologists, and environmentalists. Naturally, a major research effort is directed toward answering the questions asked above, as well as others. An ideal pesticide compound would be one that destroys the pest quickly, and, in turn, the pesticide compound would be degraded to more elementary nontoxic substances. The soil is the

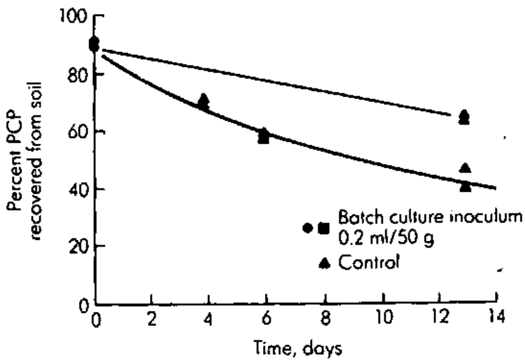
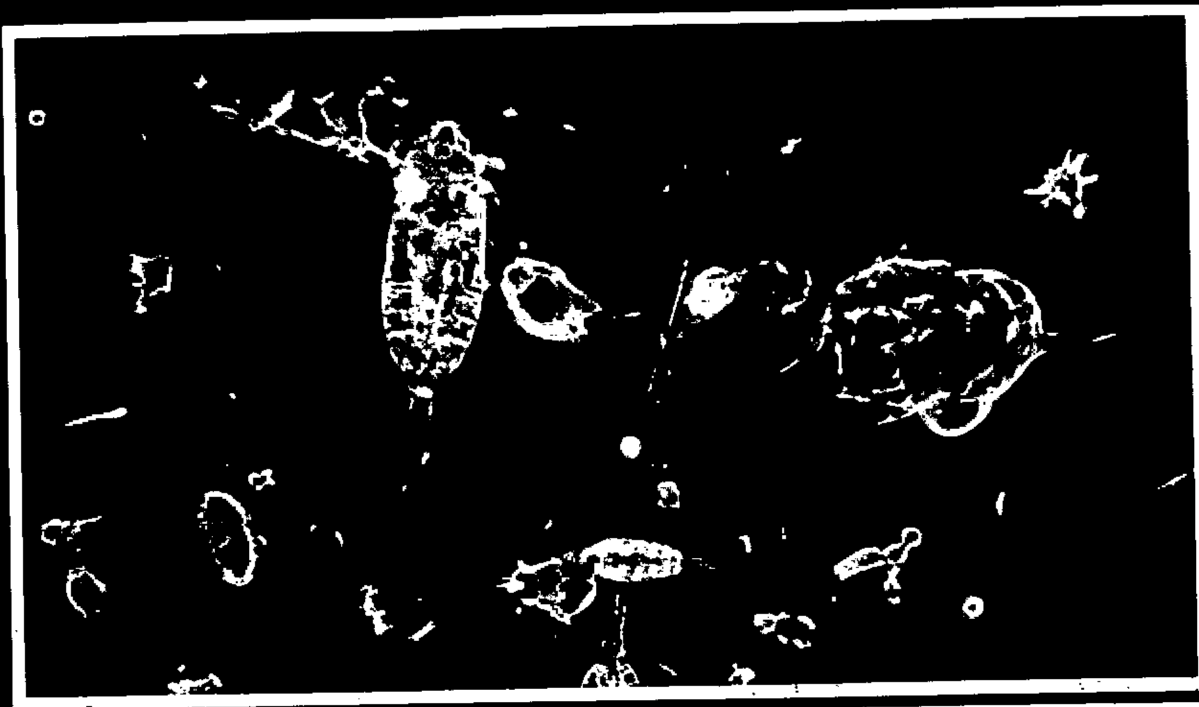


Figure 25-17. Degradation of PCP (pentachlorophenol) in soil by indigenous and inoculated bacteria under laboratory conditions at 30°C. (Courtesy of *R. U. Edgehill and R. K. Finn, Appl Environ Microbiol,* **45:**1122, 1983.)

a



b

Marine plankton:

a Diatoms, copepods, crustacean larvae, protozoa, animal eggs, and other organisms.
b Diatoms.

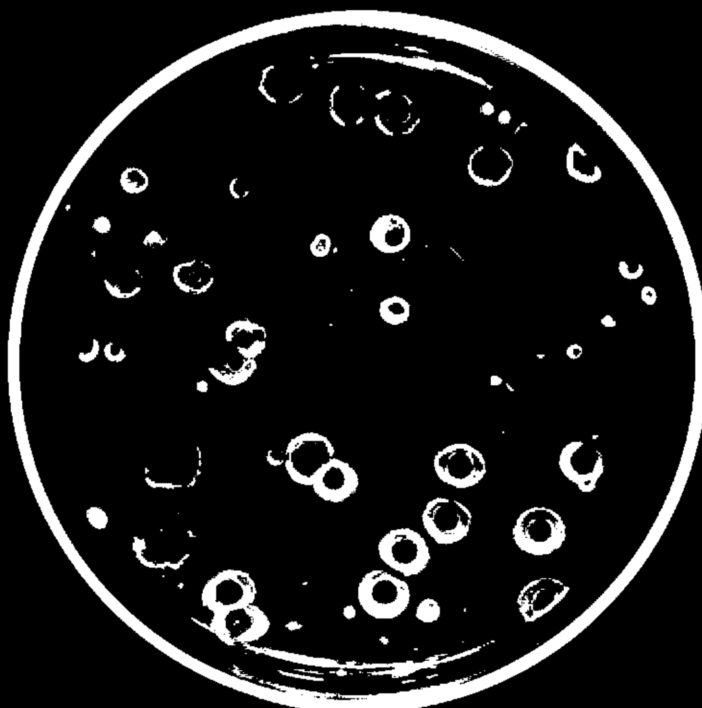
(Figure a courtesy of D. P. Wilson; Fig. b courtesy of Dr. Boris Gueft.)

Room air samples:

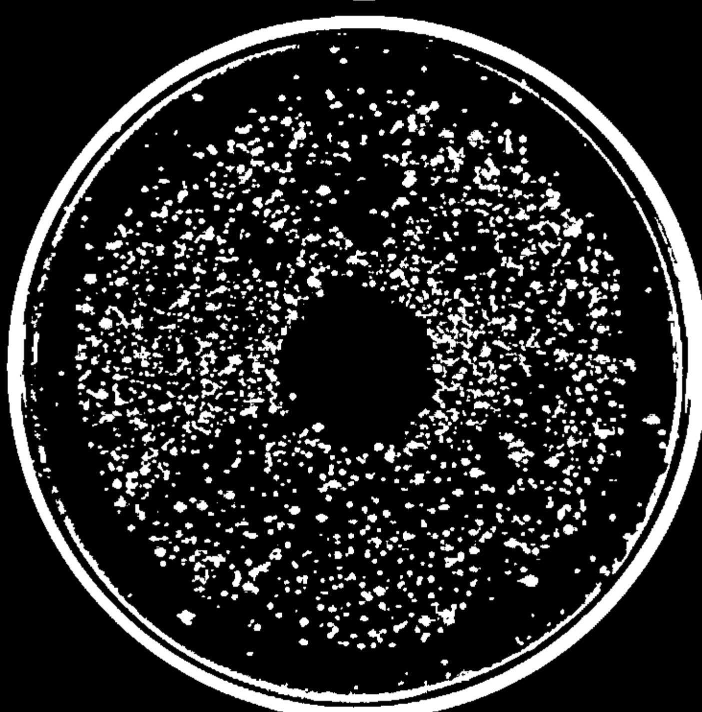
a Exposure of agar medium in petri dish to room air
b Collection of microorganisms by special air sampling device.

(Figures a and b courtesy of Environmental Services Branch, National Institutes of Health, Public Health Service.)

a



b



"sink" which receives the pesticide, and it is the soil microbiota that we depend upon to degrade the compound. As an example to illustrate the results of research on this topic, Fig. 25-17 shows the rate of disappearance (degradation) of a pesticide deposited in the soil. This aspect of soil microbiology, namely, the impact of, and the fate of, pesticides deposited in the soil, is a subject of growing concern.

QUESTIONS

1 Describe how the physical composition of soil influences the magnitude and diversity of the microbial flora.
2 Describe one contribution made by Winogradsky and one by Beijerinck to our knowledge of soil microbiology.
3 Assume that you made a microscopic count on a soil sample and a standard nutrient agar plate count from the same sample. What generalizations are likely with respect to the comparability of the counts?
4 How could one proceed to enumerate, by cultural techniques, the various physiological groups of microorganisms present in soil?
5 Compare the microbial flora of soil in the region of the rhizosphere to that in an area at a distance from the rhizosphere.
6 What is meant by the term *mineralization*? Give an example.
7 Assume that some protein material is buried in the soil. Trace the changes it may undergo as a result of microbial attack. Identify bacteria capable of bringing about each of the changes.
8 Distinguish between symbiotic and nonsymbiotic nitrogen fixation. Name several genera of bacteria that are nonsymbiotic nitrogen fixers.
9 What are the components of the bacterial nitrogen-fixing system?
10 How may the process of nitrogen fixation be determined experimentally?
11 Describe the process by which *Rhizobium* spp. invade the root system of a leguminous plant.
12 How is recombinant DNA technology being explored to develop new means of nitrogen fixation?
13 Tremendous amounts of plant material, largely cellulose, are deposited annually on the earth's surface. Insofar as microbiological events are concerned, what happens to this cellulose?
14 Illustrate, with reactions, the manner in which organically bound sulfur is released by microbial dissimilation.
15 Describe the mineralization process as related to the dissimilation of organic phosphorous compounds.

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