

SOIL BIOCHEMISTRY

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Summary

Soil biochemistry is one of the branches of soil science dealing with the formation and decomposition of soil organic matter, biochemical reactions of carbon, nitrogen, phosphorus, sulfur, metals and xenobiotics in soils, and biochemistry of the plant-root rhizosphere. This chapter is divided into eleven sections, the first of which provides a definition of soil biochemistry and its scope of influence. In section 2, the overall concept of soil organic matter and the formation mechanisms of humic substances are presented. Sections 3 and 4 discuss the characteristics of biomolecules in soils including the genetic material DNA and enzymes which are the catalysis of all biochemical reactions. Sections 5 to 7 deal with the cycling of the major microbial and plant nutrients. The biochemistry of carbon, nitrogen, phosphorus and sulfur divide the material into logical units of study.

Section 8 focuses primarily on the complexation reactions of metals in soils. The degradation of xenobiotics treated in section 9 investigates the relationship between the structure of chemicals and their degradation, as well as some of the ecological aspects of applying xenobiotics to soil. Section 10 describes the plant root rhizosphere and its influence in augmenting many important biochemical reactions. The final chapter deals with future developments in soil biochemistry.

1. Introduction

Soil can be considered as a tissue, containing mineralogical compounds, organic matter, water and air in which biochemical processes occur. These reactions govern elemental cycles, decomposition and synthesis of humic substances, formation of soil structure and behavior of a variety of pollutants. Soil biochemistry is one of the branches of soil science dealing with the formation and decomposition of soil organic matter, reactions of the carbon, nitrogen, phosphorus, and sulfur cycles, metal biochemistry of soils, and the biochemistry of the plant-root rhizosphere.

Soil biochemical reactions concern primarily the organic fraction, though it is made up of only 5% of the total soil volume. This fraction serves as a storehouse of essential nutrients for plant and microbial growth, is a major source of energy for microorganisms, and plays an important role in determining soil structure. The biochemical reactions associated with the organic matter fraction are greatly affected by shifts in environmental factors such as oxidation-reduction (redox) potentials, pH, water and temperature.

The redox potential (E_h) is the measure of a substance to donate or accept electrons. It is directly affected by oxygen concentration and is also correlated with pH. In soil only a narrow range of redox potentials are possible because of the instability of water in strong oxidizing or reducing conditions. The presence of various reduced chemical species in soil is often a better indicator of the redox status of a soil than measured E_h values.

The soil pH controls many of the important variables in the soil environment such as the size and nature of the microbial community, the composition of the soil solution, the composition of the soil organic matter, and the activity of enzymes. The measurement of soil pH is one of the most common and important analytical techniques in soil biochemistry. It has often been observed that the optimum pH of an enzyme is 0.5-2.0 units higher when assayed in soil as compared to that in solution.

Temperature plays an important role in controlling the growth and activity of microorganisms, partial pressure of gases, redox potentials, and diffusion of solutes and gases. Biological activity can occur in soils at greater temperature extremes than would be expected in studying isolated systems due to the heterogeneity and complexity of soils.

The microbial biomass comprises approximately 1 to 4 percent of the total organic matter in soil. The microbial cell in soils can be viewed as being in a dynamic state, adapting to shifts in the environment. The majority of microorganisms cannot survive the transfer from the soil onto a culture medium making it difficult to assign a specific function to a specific organism in the soil. The soil biochemist, therefore, is not so much interested in an accurate description of each of the species of soil microorganisms but in their biochemical activity.

Soil chemical, physical and biological properties can vary considerably between soil samples. This happens not only between soils from different mapping units but also

between soils within a small unit area, or in individual soil horizons. What is generally measured in the soil is the sum of reactions at individual locations or micro-sites in the soil. However, to understand the soil biochemistry, one must have a good insight of the activities that can differ from one micro-site to another.

The cycling of nutrients within nature is necessary to avoid that large pools of compounds are formed containing one or more basic nutrients. The cycling of carbon is a good example of such a situation. The energy tied up in the carbon-hydrogen bond is constantly being released by microorganisms so that they can harness this energy for their own growth and reproduction. The cycling of nitrogen, phosphorus, sulfur, and several metals is often based on the transformations of carbon. Several factors affect the cycling of nutrients in soil. Parent material composition, climate, and activities of man (cultivation, fertilizer additions, pollution, etc) all affect the system, causing changes in the rate of cycling and in pool sizes of individual compounds of each nutrient.

The steady increase of population and human activities causes a continuous accumulation of organic and inorganic pollutants such as xenobiotics and heavy metals in the soil and, therefore, much more attention is nowadays paid to soil remediation. Many biochemical processes in soil can change the availability and toxicity of such contaminants, and many enzymes including hydrolases and oxidoreductases are responsible for the degradation of organic contaminants and the formation of humic polymers. Some organic ligands may bind heavy metals and reduce their availability to plants and microorganisms. A number of heavy metals could be transformed to gaseous forms via enzymatic reactions. It is certain that soil biochemistry plays more and more important roles in the bioremediation and restoration of soils.

2. Chemistry of Soil Organic Matter

2.1. Pools of Organic Matter in Soils

Soil organic matter includes litter, light organic fraction, microbial biomass, water-soluble organics and humus. Litter is defined as the macro-organic matter on the soil surface. This pool is particularly important to the cycling of nutrients in forest soils and natural grasslands. In cultivated soils, crop residues are incorporated into the soil well in advance of planting, and the litter becomes part of the light fraction.

The light fraction consists mainly of plant residues in varying stages of decomposition; it is used as an indicator of the changes in labile organic matter as affected by tillage, crop rotation and environmental conditions affecting microbial activity. The size of the light fraction is considerably less than that of stable humus, but can constitute as much as 30 percent of the organic matter in some soils.

The microbial biomass plays a dual role in the soil as an agent for the decomposition of plant residues and as a labile pool of nutrients. Water-soluble organics are important both from the view of plant nutrition and the environment (binding of metals and xenobiotics). In most agricultural soils, the bulk of the organic matter occurs as stable humus.

2.2. Formation of Humic Substances

Humic substances are a series of relatively high-molecular-weight, yellow to black colored substances formed by secondary synthesis reactions. The formation of humic substances is one of the least understood aspects of soil biochemistry. Figure 1 depicts several pathways for the formation of humic substances during the decay of plant and animal remains in soil. The classical theory is that humic substances represent modified lignin (pathway 4). However, the majority of present-day investigators favor a mechanism involving quinones (pathways 2 and 3). In practice, all four pathways must be considered as likely mechanisms for the synthesis of humic and fulvic acids in nature, including sugar-amine condensation (pathway 1). All pathways may be involved in humus formation, but not to the same extent in all soils or in the same order of importance. However, it is difficult to say which pathway is more important in a specific soil.

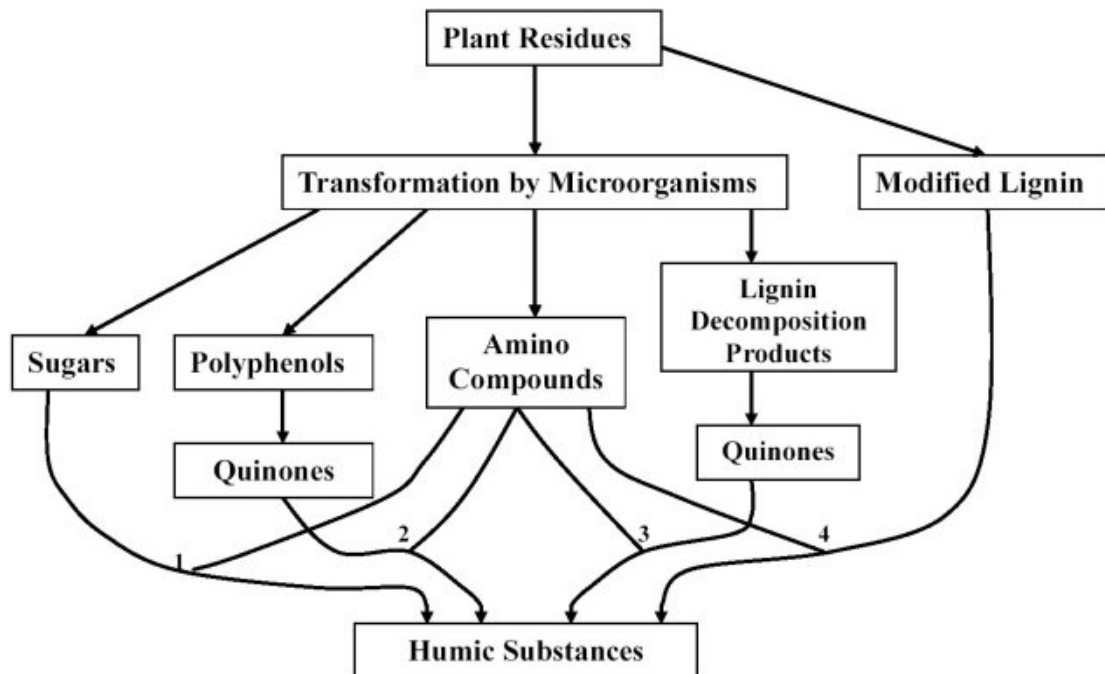
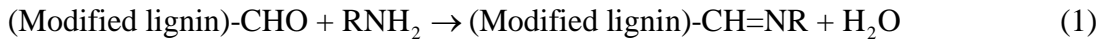


Figure 1. Pathways for the formation of humic substances (Stevenson and Cole, 1999)

2.2.1. The Lignin Theory

For many years it was thought that humic substances were derived from lignin, though there is still a lot of uncertainty about this issue. According to this theory, lignin is incompletely utilized by microorganisms and the residuum becomes part of the soil humus. Modification in lignin includes loss of methoxyl (OCH_3) groups with the generation of *o*-hydroxyphenols and oxidation of aliphatic side chains to form COOH groups. The nitrogen contained in humic acids results from the condensation of modified lignin with protein, the latter being a product of microbial synthesis. Stabilization of the protein is believed to occur through formation of a Schiff base.



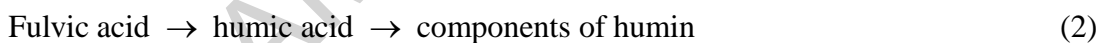
In aerobic soils, lignin may be broken down into low-molecular-weight products prior to humus synthesis. Oxygen is required for the microbial depolymerization of lignin, and fungi that degrade lignin are not normally found in excessively wet sediments. Therefore, modified lignins will make a major contribution to the humus of poorly drained soils.

Demethylation and oxidation of side chains lead to the formation of products enriched in acidic functional groups (COOH and phenolic OH) with lower C but higher oxygen contents than the original lignin. The COOH groups arising from the side chains of lignin are not sufficient to account for the high COOH content of humic and fulvic acids. Additional COOH groups may arise from ring cleavage of aromatic components of lignin. The *o*-dihydroxy-benzene units resulting from demethylation of lignin would be subject to oxidation to quinones capable of undergoing condensation reactions with NH₃ and amino acids.

2.2.2. The Polyphenol Theory

In pathway 3, lignin still plays an important role in humus synthesis, but in a different way. Phenolic aldehydes and acids released from lignin during microbiological attack undergo an enzymatic conversion to quinones, which polymerize in the presence or absence of amino compounds to form humic-like macromolecules. Pathway 2 is somewhat similar to pathway 3 except that the polyphenols are synthesized by microorganisms from non-lignin C sources. The polyphenols are then enzymatically oxidized to quinone and converted into humic substances.

Quinones of lignin origin, together with those synthesized by microorganisms, are the major building blocks from which humic substances are formed. In this model, the first step consists of the breakdown of all plant biopolymers into their monomeric structural units, some of which polymerize enzymatically to produce humic molecules of increasing complexity. The formation of humic substances would thereby follow the order:



Possible sources of phenols for humus synthesis include lignin, microorganisms, uncombined phenols in plants, glycosides and tannins. Polyphenols are not stable and subject to further decomposition by bacteria, actinomycetes, and fungi. They may also undergo recombination, either alone or with other organic molecules, after conversion of quinones. The conversion of phenols to quinones is likely to be carried out by polyphenoloxidase enzymes. For the products derived from lignin, oxidation to quinones and consequent polymerization to humic substances must be preceded by demethylation to account for the low OCH₃ but high phenolic OH contents of humic and fulvic acids.

Other changes include oxidation of aldehyde components, decarboxylation and hydroxylation, and coupling of intermediates. Self-condensation of quinones or free

radicals are greatly enhanced in the presence of amino compounds, such as amino acid, peptides, and proteins.

2.2.3. Sugar-Amine Condensation

For pathway 1, humus is supposed to be formed from sugars. Reducing sugars and amino acids, formed as by-products of microbial metabolisms, undergo a non-enzymatic polymerization to form brown nitrogenous polymers. Unlike the lignin theory, the starting material consists of low molecular weight organic compounds, from which large molecules are formed through condensation and polymerization. Sugars and amino acids are produced in abundance in the soil through microorganism activity. This theory could explain the formation of humic substances in environments where lignin and lignin degradation products are not prevalent.

Many different types of reactions can lead to the production of dark colored pigments in soil. The major pathway for the formation of humic substances in most soils appears to be through condensation reactions involving polyphenols and quinines. Polyphenols derived from lignin, or synthesized by microorganisms, are enzymatically converted to quinones, which undergo self-condensation or combine with amino compounds to form N-containing polymers. The number of precursor molecules is large and the number of ways in which they combine is astronomical, thereby accounting for the heterogeneous nature of the humic material in any given soil.

3. Soil Enzymes

The transformations of many chemical compounds in soil are catalyzed by proteins called enzymes. Like all catalysts, enzymes increase the rate of a chemical reaction without themselves undergoing permanent alteration. Soil enzymes are composed of four classes: hydrolases, lyases, oxidoreductases, and transferases. Hydrolases catalyze the hydrolysis of a variety of bonds, such as esters, glycosides and peptides. Lyase enzymes catalyze the non-hydrolytic removal of a group from a substrate with the resulting formation of a double bond. Enzymes involving the catalysis of oxidation-reduction reactions are called oxidoreductases. Enzymes that transfer a specific grouping from one molecule to another are transferases. Some soil enzymes catalyze reactions both inside and outside the organisms; some can only function outside the producing cell because of the large size of their target substrate; and others, being involved in the central aspects of metabolism, do not function extra-cellularly. Only a limited number of enzymes have been extracted from soil.

3.1. Classification by Location

The overall enzyme activity of a soil is a composite of various intra- and extra-cellular enzyme components produced by microorganisms or derived from animal and plant sources. Intra-cellular enzymes may exist in different cellular compartments of living cells, such as the cytoplasm or periplasm, or they may be associated with the cell membrane and wall. Extra-cellular enzymes are produced and secreted by living cells and operate at a distance from the parent cell, either as free enzymes in the liquid phase or as enzymes still associated with the external surface of root epidermal or microbial

cell wall.

3.2. Classification by Function

Two groups of enzyme activities appear to predominate in the soil: hydrolases and oxido-reductases. Hydrolases, such as polysaccharidases and proteinases, transform macromolecular substrates into smaller compounds. The depolymerization of carbohydrates and proteins and the subsequent mineralization of their monomeric products have special importance in the biological cycling of both carbon and nitrogen in soils. Polysaccharidases (e.g. amylases, cellulases, xylanases, dextranases) catalyze the first stage of the depolymerization of polysaccharides to oligo- and monosaccharides. These, in turn, can be assimilated by living organisms or undergo further decomposition in soil by the action of hydrolytic enzymes such as α - or β -glucosidases and α - or β -galactosidases. The final hydrolysis products of these enzymes, such as glucose or galactose, constitute important energy sources for soil microorganisms.

Hydrolysis of saccharose by invertase is another significant reaction of carbohydrate metabolism in soils. The presence of invertase has been correlated with microbial activity and is regarded as a useful "fertility index".

Proteins in soils are hydrolyzed to peptides and amino acids by proteinase and peptidase activities. Urease catalyzes the hydrolysis of urea into ammonia and carbon dioxide, which is an important reaction in the soil nitrogen cycle. In the P and S cycles the inorganic and organic forms are closely associated through mineralization and immobilization processes mediated by abiotic and biotic activities.

Herbicide- and insecticide-hydrolyzing activities, such as those of propanil-acylamidases or malathion- and parathion-esterases, have been studied in various soils. These enzymes which, in the presence of active co-factors, catalyze the oxidation of numerous organic compounds by removal of electrons and hydrogen, are believed to be localized only within intact viable cells. Treatment of soils with bacteriostatic or bactericidal compounds destroys their activity.

The transformation of atmospheric H_2 in moist soils is assumed to be catalyzed by hydrogenases. Reduced organic compounds, mostly phenolic, may be oxidized by the action of peroxidase. The presence of hydrogen peroxide, which is transformed to water and O_2 , is required during this reaction. Hydrogen peroxide, a potential toxic intermediate of respiration, may also be destroyed by catalase, which produces molecular oxygen and water. This decomposition of hydrogen peroxide makes catalase an important enzyme of soil biochemical activity.

Peroxidases like manganese and lignine peroxidases and polyphenol oxidases such as laccases and tyrosinases catalyze the oxidation of a variety of phenols, aromatic amines, and other compounds. The oxidative activities of these enzymes, such as the oxidative coupling reaction, play an important role in humus formation. Oxidoreductive enzymes also participate in the degradation or incorporation of xenobiotic substances into soil organic matter.

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Biographical Sketch

Qiaoyun Huang is a Professor of Soil Biochemistry and Head of Department of Soil Science and Plant Nutrition, Faculty of Resources and Environment, Huazhong Agricultural University, Wuhan, China. He holds a PhD in Soil Science (1992) from the same University.

He has been active for more than ten years in teaching, research in the fields of soil enzymology, soil mineral interactions with organic components and microorganisms. He has authored or co-authored more than 80 scientific papers in peer-reviewed journals and books. He was the chairman of the 4th International Symposium on Interactions of Soil Minerals with Organic Matter and Microorganisms (ISMOM2004), September 20-23, 2004 in Wuhan, China. His research has mainly focused on behavior and activity of biomolecules in soils, and on microbial transformation of heavy metals.

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