

## THE GENESIS AND STABILITY OF NITROGEN IN PEAT AND COAL

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Coal consists of the fossilized remains of bog and swamp-shore vegetation, which, following diagenesis, was preserved by water and modified by heat and pressure. Geochemists generally accept as valid the concept of a coalification series: vegetation-peat-brown coal-lignite-bituminous coal-anthracite.

Coal commonly contains 1-2% N in fixed forms which are highly resistant to biodegradation. The woody tissues of the originating vegetation--predominately tree-ferns--probably averaged about 1% N, which was of a biodegradable nature. Assuming them to be similar in structure to present-day woody tissues, about 20% of tree-fern cells contained proteinaceous material. Humic substances derived from woody and more succulent tissues as a result of microbial activities and oxidative polymerization reactions probably contained 2-5% N, although the average N content of the peat resulting from plant tissue deposition and decay remained at about 1%. The N content of bituminous coal generally is slightly higher than that of peat and lignite--about 1.5 to 1.75%--but decreases to less than 1% in anthracite.

The C/N ratio typically increases from about 50/1 in wood to 65/1 in peat and lignite. However, the C/N ratio decreases in low-rank bituminous coals, then increases with increase in rank (55/1 in high-rank bituminous coals to 100/1 or greater in anthracite). It is evident that N was gained relative to C (or C was lost) during the latter stages of diagenesis.

The accretion of N by peat may continue after its formation and subsequent submergence in waters through deposition of microbial (microflora and microfauna) protein. The total amino acid content of peat is considerably higher than in the original plant material; at least 5-10% of peat organic matter is considered to be derived from microbial tissue (1). For example, the weight of bacterial tissue per acre foot of peat might range between 35-700 pounds at any given time, corresponding to about 5-115 pounds of N. This range is calculated by assuming 35-700 million bacteria per gram of moist peat (2, 3), a volume of 1 cubic micron per organism, and a cell density of 1.04. Soil bacteria usually outnumber actinomycetes by 10:1, and fungi by 100:1, but the weight of bacteria is about equal to and half that of the actinomycetes and fungi, respectively. As can be seen, the total living and newly deceased biomass in peat could be substantial. Obviously, N accretion through deposition of microbial tissue implies the presence of sufficient fixed N to sustain microbial growth. Such N could be obtained from *in situ* biological fixation of atmospheric N<sub>2</sub> or from the transport of fixed N in sediments and aquatic life to peat beds.

Determining how and in what forms the N was preserved may be more instructive in understanding the genesis of N in coal than speculating on the primary sources of N in a coalification series. The time, manner, and circumstances of nitrogen accretion during or shortly after diagenesis is of lesser importance if one accepts the concept of a coalification series progressing from peat to anthracite rather than the concept advanced by Fuchs (4), which holds that lignites are formed under aerobic conditions and bituminous and anthracite coals are formed under anaerobiosis. More importantly, a question central to understanding the presence of fixed N in coal is how nitrogenous products of microbial activity acquire stability against further microbial and chemical decomposition over geological times.

#### SOIL HUMUS FORMATION

Humus, as used here, refers to the debris of higher plants, microflora, and microfauna in various stages of biochemical and chemical alteration. Early work on soil humus formation was much influenced by chemical studies of humus in peats and coals. Granting that the factors influencing humus formation in soils, marshes, peat bogs, etc., may be very different, one nevertheless may be justified in seeking common mechanisms for humus formation under widely different conditions because of the known gross similarities of certain humic substances (alkali-extractable, acid-precipitable substances) in soils and in lake and marine sediments.

Recent N tracer ( $^{15}\text{N}$ ) studies support the postulate of Jansson (5) that there exists in soil a large pool of relatively passive organic N, all or part of which is in equilibrium with a much smaller pool of labile organic N. Usually, when N is added to soil, some becomes immobilized through microbial activity, entering first a pool of labile organic matter, then being redistributed among progressively more refractory, bioresistant substances in the passive pool (6-10). Current knowledge of the organic forms of N in these pools is based largely on the chemical analysis of organic N compounds released by hydrolysis of soils with hot acids [for reviews, see Bremner (11, 12)]. Such hydrolysis studies indicate that the labile organic N pool consists largely of bound amino acids and amino sugars (mainly glucosamine and galactosamine), existing perhaps as mucopeptides (amino acid--amino sugar complexes), teichoic acids (ester-linked alanine-organophosphate polymers), and chitinous substances. Amino acids and hexosamines normally comprise 20-50% and 5-10%, respectively, of the total organic N of soils. Purines, pyrimidines, ethanolamine, and other N compounds also have been identified in soils, but the chemical nature of about half of soil organic N is not well understood. It is this unidentified, relatively stable fraction that is of geochemical interest.

In soils, peat bogs, and like environments, labile forms of N are assimilated by microorganisms. The microorganisms die and decay, the tissues of one generation being food for succeeding ones. With each cycle of generation and decay, waste products of metabolism are released to the microenvironment. Ammonium N and amino acids can be considered

such wastes. As long as these waste products re-enter vital processes, and then recycle as part of new living tissue, there is no mechanism for acquiring stability. However, the recycling processes may be interrupted by chemically binding labile nitrogenous substances to other intracellular or extracellular constituents to form biochemically resistant and chemically inert materials, or by decreasing their physical accessibility to enzymatic and nonenzymatic attack.

The "physical inaccessibility" concept is supported by the finding that destruction of physically stable microaggregates in soil consisting of clay mineral-organic matter complexes renders the organic matter more susceptible to chemical and microbial attack (13). Also, the entrapment of ammonia within clay lattices is another protective mechanism for soil N. Clay-protected N could survive the coalification processes and appear in coal as part of clay mineral contaminants in low-rank coals.

Much work has been reported in support of the view that stable N complexes in soil are produced by reactions of lignin- and cellulose-derived phenols or quinones with amino acids [e.g., see (11-20)] and the reactions of oxidized lignins with ammonia (21). The dark-colored substances produced by such reactions have chemical properties similar to the humic substances found in soils, peats, and sediments. A detailed analysis of the extensive work on the role of lignin in humification processes is beyond the scope of this paper.

Chemical fractions of humic substances (e.g., humic, fulvic, and hylatomelonic acids) are probably not simple chemical entities, but more likely are mixtures of components of heterogeneous structure. They are noncrystalline, which suggests that they are not formed through enzymatic activity. Although the dark humic substances exhibit the properties of oxidized lignins, it is reasonable to assume that lignins must be drastically altered before entering oxidative polymerization or polycondensation reactions with ammonia and amino acids (22, 23). Natural and unaltered lignins are too chemically inert to form complex, biologically stable polymers with amino acids. Also, formation of such polymers extracellularly in soil from lignin residues may not be extensive because only small amounts of lignin degradation products are found in soils and peats. For example, alkaline-nitrobenzene oxidation of soil organic matter yielded syringyl, guaiacyl, and p-hydroxyphenyl residues amounting to less than 1% of the total soil C; for peat, the yield was 1 to 4% (24).

The proximity of reactants to each other, their concentrations, the reaction site microenvironment, and the time that each reactant is released to the microenvironment are factors which may determine the extent of formation of dark humic substances and their nature. These factors themselves are influenced by the type of vegetation undergoing diagenesis (e.g., woody tissues are characterized by relatively high protein and lignin contents as compared to sphagnum moss). As plant tissue decays, loosely bound proteins and carbohydrates are attacked first, followed by peptides and methylated polysaccharides. Lignins are relatively resistant to biodegradation. It appears, therefore, that

ammonia and amino acids would be released from plant residues to the soil at a particular microsite, and then transformed or assimilated before lignin- or cellulose-derived phenols and quinones are produced at the microsite. Even though amino acids may persist unmodified in soils for many years, there is evidence that a considerable portion of soil amino acids is not bound to lignin (11) and may form stable complexes with transition series metals (25).

The intact cell is a potential reaction chamber in which chemically active substances may be intimately associated at high concentrations in a microenvironment favorable for reaction. Recently, considerable attention has been given to the possibility that humic substances are formed by autolysis of microorganisms (23). The presumption that stable organic N polymers can be formed intracellularly is supported by evidence that dark humic substances are formed by chemical reactions of biochemically produced reactants, that the sequence of reactant production in soil may not be conducive to extensive extracellular formation of humic substances, and that the dark humic substances formed in different media and at different geological times are similar.

#### POSSIBLE INTRACELLULAR FORMATION OF HUMIC SUBSTANCES

Living cells contain the enzymes needed for degrading their autogenous substances (e.g., proteins, peptides, and cellulose) and for the further reaction of the degradation products (e.g., the oxidation of phenols to quinones and polyphenols by phenolases). However, ligninase has not been isolated from higher plant or microbial tissue, notwithstanding the specific evidence of ligninase activity in fungal cells. In woody cells, lignin is deposited in the interstices between the micellar strands and microfibrils of cellulose and other microcrystalline components of the cell wall. After the cell or tissue dies, removal of the lignin leaves the cell wall morphologically intact. Within the protection of a relatively bioresistant cell wall, high concentrations of monomeric substances can accumulate as autolysis proceeds.

Quinones can condense with  $\alpha$ -amino acids through the amino group, leaving the acidic carboxyl groups free. Cross-linking with adjacent quinones could occur through diamino-amino acids (e.g., lysine) or thiol-containing amino acids (e.g., cysteine). Polycondensation would result from intermolecular interactions between compounds possessing at least two functional groups capable of esterification. The order and pattern of polycondensation would be irregular, resulting in a 3-dimensional amorphous substance (a heteropolycondensate co-polymer with side chains) composed of many phenolic-quinolic-amino acid units. It has been postulated that such polymeric substances, by virtue of their isolated resonance groups, would absorb light of all wavelengths and appear dark in color, be soluble in alkalis because of their free carboxyls, be slightly soluble in organic solvents because of their predominantly condensed aromatic nature, and be precipitated from alkaline solution by acids and heavy metals (26). Such co-polymers would be resistant to chemical and microbial attack because of their high molecular weight, their cross-linked, covalent bonding, and their heterogeneous, predominately nonlinear structure of nonrepeating units.

The humic substances formed within the cell would be released by extracellular microbial and chemical attack on the cell wall. Since their overall mode of formation is similar, dark humic substances found in different environments (e.g., soils, peats, and sediments) would be expected to have similar gross properties. In time, and depending on environmental factors, the linear side chains of the large, irregularly spherical co-polymer would be degraded, leaving a substance that would require the fortuitous, unlikely association of several enzymes to effect its further degradation.

#### NITROGEN FORMS IN COAL

Little is known with certainty about the N forms in coal and it appears premature to speculate about the metamorphic conversion of humic substances whose structures are only grossly identified. There is evidence that N occurs largely as heterocyclic structures (27, 28), such as nicotinic acid (29). A presumptive analysis of coal extracts after physical and chemical treatment yielded the following hypothetical distribution of nitrogen structures: Water phase--purine bases and structures with urea, amino acid, and peptide units (35%), carbazole structures yielding ammonia (10%), low-molecular weight cyclic bases and phenylamines (3%); Chloroform phase--hydrophilic bases, nonbasic N compounds, and fatty amines (23%); Residual coal--high-molecular weight N compounds (3%). Upon pyrolysis, 26% of the N compounds yielded N<sub>2</sub> (30).

Among the amino acids found in peat were glycine, aspartic and glutamic acids, alanine, leucine, threonine, and valine (31). Monoamino and diamino acids have been identified in peat, lignite, and subbituminous coal, but no diamino acids have been found in bituminous and anthracite coals (28). Glycine and aspartic and glutamic acids were found in hydrolysates of anthracite estimated to be over 200 million years old (32).

Free porphyrins have been identified in polar solvent (e.g., ether or pyridine) extracts of bituminous coal (33). Metal-porphyrin complexes may be present in concentrations as high as 7000 ppm, about 100 times those of free porphyrins. Nickel and vanadium complexes are especially stable. Porphyrins in coal originate in the chlorophyll and respiratory pigments of green plant tissue and have been preserved in water-logged peat because of their greater stability in anaerobic biological environments. Their presence lends support to the hypothesis that pressure and moderate heat are the agents of peat metamorphosis because porphyrin stability increases with pressure and decreases with heat. Also, studies of porphyrins in bitumens give support to the hypothesis that petroleum is formed in brackish environments from asphaltic constituents resulting from the alteration of marine and terrestrial plant and animal organic matter (34).

### TRACER STUDIES

Use of the N tracer,  $^{15}\text{N}$ , has contributed greatly to an understanding of the turnover of N in biological mineralization-immobilization reactions, processes which proceed simultaneously and in opposite directions [for an extensive bibliography, see Hauck and Bystrom, (35)]. However, N tracer techniques have not been used effectively to clarify the chemical nature of humic materials, largely because experiments have not been devised to make maximum use of  $^{15}\text{N}$  for this purpose. It would be instructive to follow the detailed, sequential decay of higher plant and microbial tissue labeled with  $^{13}\text{C}$  and  $^{15}\text{N}$ , and to attempt to isolate labeled polycondensation products from cell populations in various stages of autolysis. Comparisons should be made of the distribution of tracers in different humic fractions, as affected by the precursor tissue. For example, woody tissue may produce more highly aromatic, humic substances, such as those found in coals, while the corresponding humic fraction from proteinaceous tissue may be more aliphatic in character. However, such comparisons might be valid only if care is taken to separate extracellular from intracellular formation of humic substances, should such a distinction exist.

Carbon isotope studies show no correlation between isotopic composition, degree of coalification, and geological age of coals (36). From this one may infer that the C in coal was derived largely from land plants which absorbed carbon dioxide of uniform C isotopic composition and that no measurable C isotope fractionation occurred during diagenesis.

Slight but significant variations have been observed in the N isotopic composition of different peats and coals (37-39). The N in coal tends to have a slightly higher  $^{15}\text{N}$  concentration than vegetation in the vicinity of the coal seam (38), but definite conclusions are not justified from the limited data available. In another study (39) C isotope analyses indicate that some Dutch natural gas deposits originated from recoalification of coal seams 3000 and 5000 meters below sea level. Nitrogen in gases obtained from these deposits was enriched in  $^{15}\text{N}$ , while ammonia obtained from the coal (coke-oven gas) was depleted in  $^{15}\text{N}$  (relative to atmospheric  $\text{N}_2$ ), leading to the speculation that the N in natural gases was not derived from the coal. Measurements of N isotope ratios in natural gases have also been used to explain the parallel increase in He and  $\text{N}_2$  in gases with increase in age of reservoir rock (40) and to elucidate the origin of N in gases associated with crude oils and sedimentary materials (41).

The limited number and scope of the studies referred to above make it difficult to assess whether further studies of this kind will prove useful in clarifying the genesis of coal, kerogen, petroleum, and natural gases. Obviously, if the N isotope ratio of N occluded in methane, for example, is similar to but slightly lower than that of a nearby deposit of organic material, then one has reason to assume that the methane was derived from that deposit. Also, a slightly lower N isotope ratio for N in methane suggests the occurrence of isotope fractionation as C-N bonds are ruptured during gas formation.

There is a time gap of about 200 million years separating current studies of humification processes and the events which converted vegetation to coal. Because the isotope ratio reflects the cumulative effects of nitrogen cycle processes, in-depth studies of the N isotopic composition of peats, coals, and their associated materials may prove helpful in separating events that occurred during different geological times.

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