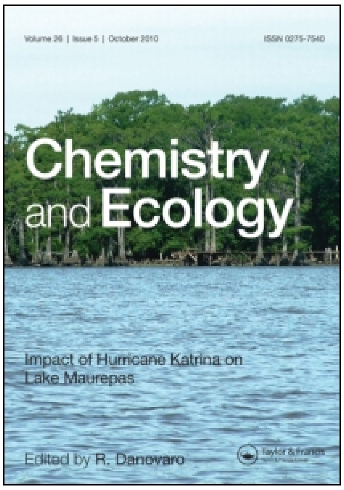

Chemistry and Ecology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455114

Historical and current progress in understanding the origin and structure of humic substances

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To cite this Article Hänninen, Kari(2010) 'Historical and current progress in understanding the origin and structure of humic substances', Chemistry and Ecology, 26: 4, 1 — 11

To link to this Article: DOI: 10.1080/02757540.2010.494158

URL: http://dx.doi.org/10.1080/02757540.2010.494158

Chemistry and Ecology
Vol. 26, Supplement, October 2010, 1–11

Taylor & Francis
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Historical and current progress in understanding the origin and structure of humic substances

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(*Received 28 September 2009; final version received 14 May 2010*)

Research into humic substances (HS) has focused selectively on the search for aromatic structures. All aromatic compounds are regarded as eligible HS constituents, whereas aliphatic, polysaccharide structural constituents are considered non-HS. Wet chemical methods for analysing HS produce 2–5% aromatic compounds from the original starting material. Only those compounds in the hydrophobic extraction phases are studied carefully. Compounds in the hydrophilic extraction phases, containing mainly aliphatics, are discarded. Solid-state NMR spectra from HS show 30–40% of carbon–carbon unsaturation, which is selectively interpreted as 'aromatic'. No recognition is given to the fact that NMR spectroscopy of HS cannot distinguish olefinic unsaturation from aromatic unsaturation in molecules. By considering this unsaturation to be more or less olefinic, solid-state NMR shows the structure of HS to be mainly aliphatic. Polysaccharides are recycled in humification via microbial metabolism. Lignin is not formed again in humification. During evolution, polysaccharides appeared first and lignin much later, and it is reasonable to suppose that the existing humification process accommodated lignin as an additional material to consume. Humification has evolved to become a key process in the production of fertile soils, and dearomatisation may be an important component of this role.

Keywords: humic substances; humification; dearomatisation; functional recalcitrance; NMR; olefinic unsaturation; non-humic aromatics

1. Introduction

Humification, defined as the transformation of dead organic matter into humic substances (HS), is an important phenomenon in nature. HS formation represents a complex physico-chemical process that produces a new class of organic compounds, absent in living organisms but supporting the existence and continuous development of life. Without humification, two opposing processes could take place: either (1) mineralisation of organic residues to oxides or (2) conservation of organic residues. In both cases, the continuation of life on Earth would be extremely difficult, if not impossible.

In humification, dead organic matter is mineralised microbiologically and chemically (abiotically) to solids and gases. The gaseous products are carbon dioxide and water under aerobic conditions, and methane and carbon dioxide under anaerobic conditions. The residual

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ISSN 0275-7540 print/ISSN 1029-0370 online

DOI: 10.1080/02757540.2010.494158
http://www.informaworld.com

solid products of humification are HS, or humus. HS are ubiquitous in soils, sediments and natural waters.

HS are fractionated into more homogeneous groups of compounds according to their solubility in alkaline and/or acidic solutions. Humic acids (HA) are a brown–black, polymeric, alkali-soluble, acid-insoluble group of HS compounds. Fulvic acids (FA) are yellow to brown polymeric, alkali- and acid-soluble compounds. Humins are insoluble in both alkaline and acidic solutions. All are heteropolymers with thousands of potential monomeric units. HA and FA are the most studied groups of HS.

The origins of HS are mainly major plant constituents, lignin and polysaccharides, although important animal constituents, proteins, fats and chitins, are also among them. Degradation of dead organic matter is connected to the formation of fresh HS, and to the degradation of old HS. These interlinked processes are more or less simultaneous and continuous. There is a transformation period during which both HS and non-HS exist. Humification can be regarded as a slow equilibrium reaction.

Depending on local climatic conditions, humified matter may accumulate to a certain extent. The significance of humification in evolution is to produce fertile soil for new plant (and animal) generations to grow. Humification is one of the pedogenic factors. Wind, water and solar UV radiation physically erode humified organic matter.

The need to improve soil fertility, and to better understand chemical reactions in soil, the ecotoxicological effects of persistent organic compounds, and composting and anaerobic digestion as technological processes, are among the driving forces behind efforts to understand the chemistry of HS.

The chemical structures and origin of HS were thought to have been solved several times during the last 200 years, but in fact they are still not clear. In the history of chemistry, such a long time span from the discovery of a group of natural chemicals without final elucidation of its major structural features, and at least those groups of compounds from which they originate, is unique. A definite structure for HS may be impossible to elucidate, but it is possible to describe HS according to the statistical distribution of various classes or groups of key structural types, and so to distinguish a certain HS sample from others. To achieve this, a sound understanding of the important structural types of HS is needed, and adequate technology for this is now available.

This article underlines the need for objective discussion to direct future HS research. There is a need for a sound understanding of which basic structural features of HS are most important.

2. Historical background of HS research

The German chemist Karl Franz Achard (1753–1821) was the first to extract HA with alkali from peat bogs in 1786 [1]. Since then, alkali extraction has been the preferred method for extracting HA. During that time, a Phlogistic concept of humus theory prevailed that was based on the importance of HS as nutrients to plants. In 1826, Carl Sprengel (1787–1859) published a comprehensive article on previous HS studies [2], in which he also refuted humus theory. Thomson [3] started to use the name ulmin for alkaline bark extracts of *Ulmus nigra*. Berzelius criticised this definition because several names would be needed for alkaline extracts according to their host plants [4]. Döbereiner [5] was the first to use the name humic acid. The name of ulmic acid was, however, used for a long time together with the name humic acid [6].

Efforts to prepare artificial HA-like substances (artificial ulmin) started in 1819 when Braconnot [7] added acids to starch and sucrose. The dark precipitate that formed looked like extracted natural HA. Glucose was soon found to give the same type of HS-like substances. Nearly 100 years later, Berthelot and André produced HA from sugar using hydrochloric acid [8]. Throughout the nineteenth century it was accepted as proven that HA are generated from polysaccharides.

Berzelius initiated the study of aquatic (freshwater) HS by isolating organic acids from a spring at Porla, Sweden. Later he noted that these acids were similar to those from soil, and named them crenic and apocrenic acids (organic acids from springs) [9]. Oden, in his summary of humus research [6], named these fulvic acids (yellow acids).

From the late nineteenth century onwards, three important findings greatly influenced the forthcoming studies of HS: (1) the aromaticity of organic compounds was discovered; (2) lignin was recognised as an aromatic substance and its importance to plants was better understood; and (3) microbes were detected by Pasteur. When researchers additionally found small amounts of nitrogen in HS (HA), a protein–lignin origin theory evolved. In the first two decades of the twentieth century these ideas were not yet generally recognised: Oden [6] did not discuss the aromaticity of HS.

In 1921, Fischer and Schrader [10] demonstrated that micro-organisms rapidly consume cellulose. Fischer and Schrader were adamant that polysaccharides could not be the precursors of HA. Among other acids, Fischer discovered mellitic acid as a component of black coal HA. He proposed that black coal HA had an aromatic core, a honeycomb-like structure. By analogy, not by experimental results at normal pressure and temperature, Fischer also deduced that HA from peat and brown coal must have the same type of core structure as black coal HA. The aromaticity was suggested to be derived from lignin. The lignin origin theory gave birth to the concept that the structure of HS is aromatic rather than aliphatic.

At the same time in the 1920s and 1930s, researchers like Gortner [11], Marcusson [12], and Hilpert and Littman [13] were strongly promoting the polysaccharide theory. Marcusson [14] was suggesting that the building blocks of HA were three condensed nuclei of furan. Heathcoat and Wheeler [15] studied the degradation products of HA, and came to a similar conclusion that the structural units of HA were heterorings, probably derivatives of pyrrol and furan.

At that time, it was thought that HS research would be more advanced because of its longer history compared with lignin research. Fuchs suggested that the aromatic structural hypothesis developed for HA of black coal should be applied by analogy to lignin as well. However, a young scientist, Freudenberg, had a different view and suggested that lignin was made of a network of interconnected phenyl propenoid units. Freudenberg and Fuchs had strong debates on this issue at various forums. Now it is generally accepted that Freudenberg's idea about the structure of lignin was correct, which means that Fuchs was wrong on that issue. However, Fuchs' view of the aromaticity of all HS has remained an accepted fact in humus chemistry. It is notable that since then, lignin chemistry has advanced considerably, whereas humus chemistry has advanced very little.

Waksman emphasised the importance of the bacterial and fungal alteration of plant matter in humus formation. He preferred proximate analysis, non-isolative methods of investigation. In his book *Humus* [16], Waksman strongly supported microbial alteration of lignins as the way in which HA are formed. Later, Kononova [17] in 1963, Schnitzer and Khan in 1972 [18], Stevenson [19] in 1982 and Orlov in 1985 [20] also supported the lignin origin theory of HA in their books. The authors of those books emphasised the importance of the aromaticity of HS and their polyphenolic origin, and did not address much of the evidence suggesting a polysaccharide origin for HA.

In the latter half of the twentieth century, scientists like Tyurin, Alexandrova, Flaig, Dushofur and Hayashi contributed much to HS research. Their main interest was to elucidate the aromaticity of HS. In 1981, the International Humic Substances Society (IHSS) was founded. Its objective was better coordination of HA research by collecting a bank of standard HA samples, so that researchers could work on documented samples and produce some coherence in their data. The IHSS has supported the publication of books in which the aromatic structure of HS was favoured [21,22]. In this way, it is likely that many researchers were encouraged to develop their views and begin looking just for aromatic compounds in HS. As a result, the polyphenol theory and a lignin origin of HS remain prevalent.

Researchers like Ikan, Susic, Farmer, Pisaniello, Anderson and Hepburn et al., studying the aliphatic structure of HS, mainly work within their research teams and publish their results as journal articles. In the context of a polysaccharide origin of HS, the discovery of HA of marine origin by Nissenbaum and Kaplan [23] proved important. These were recognised as purely aliphatic. Also important was the polymerisation of maleic anhydride to produce synthetic, aliphatic FA-like polymers in 1976 by Anderson and Russell [24]. Improvements in analytical techniques (GC, GC-MS, IR, NMR) allowed for an increasingly accurate analysis of HS. Discussion about the meaning of the results was rather frenzied in the 1980s. In 1985, Farmer and Pisaniello [25] wanted to abandon the lignin-origin theory and suggested an aliphatic structure for HS. According to them, NMR studies proved that FA were aliphatics, not aromatics. Criticism of Farmer and Pisaniello did not sway Schnitzer [26], who argued that the problem was an incomplete understanding of the NMR data. However, with increasing evidence during the 1980s and 1990s of an aliphatic nature of HS, most researchers came to accept that HS have at least some aliphatic structure.

3. Wet chemical methods in HA structural determinations

Research into HA structure uses reducing or oxidative wet chemical methods. However, there are relatively few studies of reduced HA. The basic feature of reduction is the formation of low molecular mass aliphatic compounds. The most prominent structures are diols or aliphatic ether polymers [27].

Most wet chemical studies have concentrated on oxidations. Cupric oxide oxidation, an important procedure in lignin chemistry, was adopted in HS research specifically to reveal the presence of lignin-derived aromaticity in HA. The compounds identified have mainly been aromatics, like phenypropenyl acids (vanillic, ferulic and syringic acids) and benzene polycarboxylic acids. Among the aliphatic compounds identified are oxalic, succinic and maleic acids, and various keto acids. Benzene polycarboxylic acids are similar to the mellitic acid that had been obtained by Fischer and Schrader in 1921. The results are taken as confirmation that HA are aromatic polymers and therefore have a lignin origin. However, yields in the oxidation studies have been rather low: only 2–5% of the original starting matter; loss of material is consequently 95–98%.

Wet chemical methods extract compounds which are solubilised in hydrophobic phases, like ethyl acetate. The yield could also be increased by analysing compounds which are solubilised in hydrophilic phases, like water, even though these compounds are mainly aliphatic [28]. Discarding compounds in hydrophilic phases is biasing the structural view of HA in favour of aromaticity.

4. Solid-state NMR spectroscopy of HA

The current main research interest is on solid-state NMR spectroscopy, which seems to give the expected result of HA fractions with plenty of aromaticity. In single compounds, NMR can differentiate carbons having olefinic and aromatic unsaturation. The number of magnetically non-equivalent carbons is low, and the NMR spectrum shows only a few sharp signals, the chemical shift values of which can be accurately determined.

HA, and all other HS polymers, have a very heterogeneous structure. In NMR spectroscopy this means that the number of magnetically non-equivalent carbons is very large. In terms of magnetic environments, HA may be even more heterogeneous than in terms of chemical structures. Each magnetically non-equivalent carbon gives a sharp peak with low intensity, but with a slightly different chemical shift value. A single, broad spectral band results, in which signals due to aromatic and (conjugated) olefinic carbons and protons are overlapping in normal one-dimensional ^{13}C NMR and ^{1}H NMR spectroscopy, respectively. Interpretation of this broad signal solely as aromatic on the basis of chemical shift values alone is not correct, because signals of olefinic carbons or protons cannot be distinguished from those of aromatic ones.

The more advanced two-dimensional ^{13}C NMR technique can distinguish conjugated olefinic and aromatic carbons in more and more complex structures. Synthetic and/or plant- and animal-derived compounds always have certain logically repeating structural entities. HA polymers are similar but it may be that none are identical, for example, in terms of repeating structural entities. In terms of the magnetic environments of their carbons and protons, individual HA polymers may be even more different. Because of their conjugated olefinic and aromatic carbons and protons, HA may have such similar chemical shift values that even with two-dimensional NMR spectra their signals are not resolved. Then, for example, in two-dimensional COSY spectra the cross-peaks cannot be accurately associated with a certain proton in the second dimension axis [29]. Assignment of a certain NMR signal of HA directly as aromatic in terms of chemical shift and coupling values would be possible only if it were proved that HA do not contain olefinic unsaturation. To date, however, nobody has shown this.

The automatic assignation of certain chemical shift values as ‘aromatic’ is biasing the concept of the structural view of HA in favour of aromaticity. Assuming that the ‘aromatic’ portion of the spectra would be more or less olefinic, we arrive at the conclusion that the structure of HA may be highly aliphatic with a substantial olefinic contribution.

Because of the conjugated olefinic double bonds, HA may be able to undergo reactions like keto-enoltautomerism. An acidic enolic hydroxyl may produce the same type of colouring reaction with the Folin–Ciocalteu reagent as phenolic hydroxyls. To date, this colouring reaction has been interpreted as being due to phenolic hydroxyls alone.

5. Microbial humification

There is evidence that the *in situ* formation of HS may have already started in plants when cells have died [30,31]. Plant enzymes cause this phenomenon. This alteration of organics to form HS enhances the degradation of plant material in soils.

Microbes are actually very potent utilisers of organic carbon, and they also utilise carbon even in its inorganic form. Bernalier et al. [32] studied H_2/CO_2-utilising acetogenic populations of methanogenic archaea. They isolated 20 H_2-consuming Gram-positive, strictly anaerobic acetogenic strains from four faecal samples for *in vitro* semi-continuous culture enrichment, with H_2/CO_2 as the sole energy source. Different morphological types were identified, providing evidence of generic diversity. All the acetogenic strains characterised used H_2/CO_2 to form acetate as the sole metabolite. Carbon dioxide is rather inert, so to use it as their sole source of carbon microbes need to have an excess of available hydrogen [33].

According to Waksman, degradation of lignin in nature was suggested in the nineteenth century [34]. Since then, plant biochemists have accepted that phenolic plant constituents are not just metabolically inactive storage products that simply accumulate during the life of the plant, but are subjected to relatively rapid turnover and degradation [35]. Numerous studies have shown that lignin is degraded by microbes or by fungi under aerobic conditions [36,37]. At the early stage of delignification of a tree, lignin is broken down more easily than hemicellulose and cellulose [38]. Actually lignin seems to be a preferred target of white-rot fungus at the early stages of degradation [39]. Cellulose crystals possibly prevent fungal access.

Microbial metabolism in the absence of oxygen is dominated by the nature of the available electron acceptor or hydrogen sink. Nitrate, ferric ion, sulphate and carbon dioxide serve as the preferred electron acceptor for denitrifying, iron-reducing, sulphate-reducing and methanogenic bacteria, respectively. In the reactions, the benzene nucleus is first reduced and then cleaved by hydrolysis to yield aliphatic acids for cell growth. Some micro-organisms found in soils and sediments are able to use HS as an electron acceptor for the anaerobic oxidation of organic compounds and hydrogen. This electron transport yields energy to support growth [40].

Anaerobic degradation of benzoic acid [41] proceeds via two pathways: (1) pimelic acid and (2) adipic acid. Both involve reduction (saturation) of the aromatic ring and incorporation of oxygen from water, leading to an enol prior to ring cleavage. Reduction of the aromatic ring destabilises the resonant electron distribution. The phototrophic nonsulphur bacterium *Rhodopseudomonas* attacks benzoic acid via the pimelic pathway, using coenzyme A as a cofactor. The pathway resembles the β-oxidation of fatty acids. Oxygen inhibits this degradation as it does other ring-cleavage pathways.

Cleavage of the aromatic ring takes place even in plants. Mithaisvili et al. [42] applied aromatic pollutants [1-6-^{14}C]-aniline and [1-^{14}C]- and [7-^{14}C]-benzoic acids to maize (*Zea mais* L.), kidney bean (*Phaseous vulgaris* L.), pea (*Pisum sativum* L.) and pumpkin (*Cucurbita pepo* L.). In the plants, xenobiotics were transformed by oxidative or reductive reactions, conjugation with cell endogenous compounds and binding to biopolymers. Ring cleavage was proved by the evolution of $^{14}CO_2$.

Micro-organisms carry out humification in succession; some microbial populations start the process and others continue it. New populations of microbes utilise not only the original biomass, but also remnants of the previous microbial populations. Bracewell et al. [43] concluded that the initial stages of humification in peats involved the loss of some of the primary polysaccharide and the accumulation of secondary polysaccharide features. This means that carbohydrates are recycled through microbial metabolism in humification. This provides a kind of functional recalcitrance to the carbohydrate structures. Lignin is not reformed once it has entered the humification process.

Because microbes utilise dead organic matter, its nutritive value in terms of usable carbon and nitrogen decreases. The role of microbes then decreases, but abiotic chemical reactions may continue degradation of HS. The rates of these reactions may be slower than those of microbiological actions. HS seem to ‘stick’ to almost any substance, including large proteins, minerals and soil particles. They can chelate ions, enhancing the dissolution of primary mineral particles, and envelope them so that HS may contain many times their own weight in mineral particles. This creates the possibility for catalytic chemical reactions to take place. Microbial action may make physical and chemical reactions easier, and vice versa.

Observations made by Schulten et al. [44] may be taken as proof of abiotic dearomatisation. Schulten et al. investigated the composition of organic matter in clay–organic complexes isolated from a New Zealand spodosol using the novel analytical combination of (1) labscale (500 mg) off-line pyrolysis, (2) direct inlet (5 mg) off-line methylation with tetramethylammonium hydroxide (TMAH) and pyrolysis-field ionisation mass spectrometry (Py-FIMS), and (3) Curie-point pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) with off-line and conventional on-line derivatisation with TMAH. In complex I, the organic matter was associated with both external and interlayer surfaces of a swelling clay. Complex II was obtained by removing the external portion with H_2O_2, leaving the interlayer organic matter essentially intact. For both complexes, a high proportion of the carbon (64–86%) and nitrogen (76–91%) was volatilised during pyrolysis.

Analysis of the pyrolysates showed mono- and dicarboxylic acids, alkanes, alkenes, n-alkylmonoesters and N-containing compounds to be major constituents. Thermal methylation of the material prior to (off-line) and during (on-line) pyrolysis gave rise to a wide range (between C_6 and C_{35}) of methyl esters of aliphatic and aromatic acids, methoxy derivatives of phenols and phenolic acids. Consistent with previous ^{13}C NMR spectroscopic measurements, the organic matter in both complexes was highly aliphatic in composition. Although there was little difference between complex I and complex II in the composition of the aliphatic constituents, complex II was greatly depleted in lignin-derived aromatics.

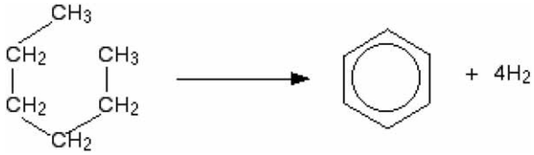
6. Microbial degradation of polycyclic aromatic hydrocarbons and the dogma of the honeycomb core of HA

Anaerobic degradation of naphthol, naphthalene and acenaphtalene under denitrifying, but not sulphate-reducing, conditions has been observed in freshwater–soil slurries using radiolabelled polyaromatic hydrocarbons (PAHs) and recovery of $^{14}CO_2$ [45]. Coates et al. oxidised PAHs under sulphate-reducing conditions in enrichments from marine sediments [46]. Rokne and Strand [47] demonstrated the anaerobic biodegradation of the PAHs, naphthalene, phenanthrene and biphenyl, in a fluidised bed reactor (FBR) enrichment.

In a later article, Rokne and Strand [48] continued re-feeding and mineralisation experiments with sub-cultures of the nitrate-reducing enrichment. The subcultures continued to remove the PAHs after three feedings. PAH biodegradation ceased when nitrate was depleted and resumed when the enrichment was fed nitrate, demonstrating that PAH biodegradation was dependent upon nitrate reduction. Tests with radiolabelled PAH confirmed that PAH was mineralised, although the extent of mineralisation differed greatly with different PAHs. Only partial mineralisation (17% of initial carbon) was observed when the culture was fed naphthalene, whereas almost complete mineralisation (96%) was observed with phenanthrene. PAH carbon was incorporated into cell mass and mineralised after complete biodegradation of the PAHs, with 78–102% recoveries of radiolabel for naphthalene and phenanthrene, respectively.

Chang et al. [49] added known concentrations of phenanthrene, pyrene, anthracene, fluorene and acenapthene to soil samples to investigate the anaerobic degradation potential of PAH. Consortia-treated river sediment taken from sites of known long-term pollution was added as an inoculum. Mixtures of soil, consortia and PAH (individually or combined) were amended with nutrients and batch incubated. Highest-to-lowest degradation rates for both soil types were phenanthrene > pyrene > anthracene > fluorene > acenaphthene. Faster individual PAH degradation rates were observed in those cultures containing a mixture of PAH substrates than in those with a single substrate. Degradation was enhanced for PAH by the addition of acetate, lactate or pyruvate. The addition of municipal sewage or oil refinery sludge to the soil samples stimulated PAH degradation. Results show the highest-to-lowest order of biodegradation rates to be sulphate-reducing conditions > methanogenic conditions > nitrate-reducing conditions. The results show that sulphate-reducing bacteria, methanogens and eubacteria are all involved in the PAH degradation and that sulphate-reducing bacteria constitute a major component of the PAH-adapted consortia.

The oil refining industry generates benzene from aliphatic hydrocarbons at high pressure and high temperature. This is called catalytic reforming and the temperature is ~500 °C and the pressure varies either side of 20 atmospheres. For example, hexane, C_6H_{14}, loses hydrogen and turns into benzene (http://www.chemguide.co.uk/organicprops/arenes/manufacture.html).



Natural formation of black coal may be induced by tectonic processes if a large layer of organic matter is subjected to high pressure and temperature due to tectonic movements, and the whole organic layer is then aromatised. Black coal formation would then be a completely geological process, compared with mainly microbiological humification, so the aromaticity of black coal does not necessarily imply that HS, or its HA fraction, must be aromatic as well. Because the experiments also provide strong evidence that polycyclic aromatic ring systems are degraded during humification, there seems to be little room for the dogma of a polycyclic aromatic core, the honeycomb structure of HA.

7. Evolutionary considerations

The development of human understanding of the chemical characteristics and the origin of HS has followed the general development and understanding of chemistry, plant biology and microbiology. Nature, however, has 'known' these issues from the beginning, so it is relevant to consider them from an evolutionary perspective.

Humification has been running in the marine environment over eons with the same original process. Because aquatic plant forms still do not contain lignin, it may be presumed that humification has remained similar since it first started. This also suggests that polysaccharides have recalcitrance in humification.

On an evolutionary time scale, lignification is a relatively recent process in photosynthetic organisms. Vascular, lignin-containing plants were absent until the Early Devonian period, whereas humification and polysaccharides were present long before then. It is reasonable to suppose that for humification lignin was only an additional matrix for degradation rather than vice versa. Actually, there are other studies that confirm this conclusion: as dominance shifted from lycopsids and pteridophytes in the Paleozoic, to gymnosperms in the Mesozoic, to angiosperms in the Tertiary, plant architecture became more sparing in its use of lignin. Lignin-degrading organisms were rare or absent in the Paleozoic, but diverse and abundant in the Tertiary [50]. Otherwise we should now see two different humification processes: (1) an original 'polysaccharidephilic' process which would still be running in lignin-free environments; and (2) an 'aromaticphilic' process which would have been altered by the appearance of lignin, and which would be running especially in terrestrial environments.

The evolutionary importance of aromatic compounds is essentially to protect living organisms because they are rather resistant to biological actions. The evolutionary significance of humus is the improvement of the soil for future generations. Unlike humus, aromatic compounds do not support the growth of other plants. This would imply that humus is highly aliphatic rather than highly aromatic, which would further suggest that humification is a dearomatisation reaction.

Cows have a high-lignin diet (15–17%). If lignin did not degrade anaerobically, it would accumulate in cow dung and anaerobic treatment of cow dung would further enhance lignin accumulation. In terms of lignin content, hydrolysis of the residue from cow dung digestion would then be comparable, for example, with the black liquor of the pulp and paper industry. Both cow dung and its digestion residue can be used as a soil amendment with rather good results, but nobody has even tried to use neutralised black liquor as a soil amendment. From an evolutionary perspective, the most important advantage of ruminants is that they can also utilise the energy content of lignin because microbes in the cow's digestive tract degrade lignin. This further implies that lignin is dearomatised in humification, and that lignin does not have such a high recalcitrance as is commonly supposed.

8. Non-humic aromatics – a missing definition

Organic compounds of living organisms are classified as non-humic substances (non-HS). Many of them have solubilisation similar to HS. The method for extraction of HS is not selective. A standard procedure for the purification of HS has been suggested by the IHSS, the basic idea of which follows the concept of the polyphenol theory and is intended to remove polysaccharide structures using strong acids.

I tested the IHSS purification procedure with my 2000-year-old peat HA by several successive extractions. The final outcome was total loss of the original HA material. All material was solubilised – it was like peeling the apple to the core! Thus this peat HA seems not to have the presumed aromatic core structure! Rather it appears that peat HA contains carbohydrate structures as their essential structural parts. Consequently, purification is destroying chemically relevant information within the starting material, which actually makes the chemical analysis of HS more obscure.

It seems that all aromatics must be automatically eligible as constituents of HS without any purification procedure, otherwise there would be such a concept as non-HS aromaticity. We really should be as interested in non-HS aromaticity as in non-HS aliphaticity. For example, cupric oxide oxidation of HA produces trace amounts of lignin degradation products, such as vanillic, ferulic and syringic acids, which are considered to be structural constituents of HA. However, they are ubiquitous in terrestrial evironments and could have been derived from degrading lignin, being non-HS compounds. The thinking behind the purification procedure may reflect more our human expectations than the reality in nature.

The dilemma of the definition of HS is a kind of vicious circle. Aliphatic, carbohydrate-like structures are considered unwanted structural constituents of HS, whereas aromatic, lignin-like structures are considered wanted. But new aliphatic structural units cannot be accepted, and new aromatic ones cannot be found, because they may exist only in minor amounts. The scope for finding potentially new structural units of HS is reducing dramatically, sound structural conclusions cannot be made, and chemical research is not advancing. The dogmatic search for aromatics has biased the research. We need adequate methods to separate non-HS and HS, but first we need to know which chemical groups are important in the structure of HS.

9. Conclusions

Automatic destruction of polysaccharides in the name of purification may include automatic destruction of vital structural evidence of HS. Instead we need to take an iterative approach: (1) select those matrices from which we know that all organic material is at a certain level of humification, for example, samples of peat from a certain depth, composts or anaerobic digestates of a certain age; (2) try to study as much of the organic matter in the humic extracts as possible, not discarding hydrophilic portions just because they yield ‘too few’ aromatics; (3) produce a realistic view of the structure of the matrices; and then (4) start to find out how much of the structure is due to the non-HS.

Wet chemical studies current show that HS (HA and FA) fractions contain only minor amounts of aromatic constituents, but this evidence is neglected. The research focus has now turned to NMR because it seems to give more aromatic structure. NMR, however, rather reliably tells only the degree of carbon–carbon unsaturation, the sum of olefinic and aromatic double bonds, but no more. Depending on the contribution of olefinic unsaturation, the structure of HS (HA and FA) may even be highly aliphatic.

No microbial mechanism produces new lignin during humification which reduces the importance of its recalcictrance. Polysaccharides, however, are recycled via microbial metabolism which provides them a kind of functional recalcitrance. These facts suggest that polysaccharides are of major importance as source materials in humification. Dearomatisation may be an important feature of humification.

Acknowledgement

Professor Roger Jones is thanked for revision of the English of the text. Kari Hänninen is presently working as associate professor at the University of Jyväskylä. He has spent 30 years researching humus chemistry. In 1987 he finalised his dissertation in that field. Presently he is teaching and researching environmental chemistry (stability of the climate) and environmental biotechnology (composting, anaerobic digestion).

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