

Carbon Changes in Environment, from Total Organic Carbon to Soil Organic Matter

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Abstract

Composition of wastewater is complicated. It is rich in micro- and macroelements, plus many carbon species. During wastewater treatment carbon changes chemical form and creates sewage sludge as a by-product. Sewage sludge is rich in nutrients and organic compounds, and possesses a potential soil-forming value. Hence it can improve soil properties, especially when applied to poor sandy soils or sludge. The last part of carbon changes occur in soil when organic matter is produced across specialized microorganisms. The aim of our current work is to present the main carbon fractions in three environmental matrices: wastewater, sewage sludge, and soil.

Keywords: carbon, total organic carbon, soil organic matter, ecoanalytics

Introduction

Carbon is an essential part of life on Earth. About half the dry weight of most living organisms is carbon. It plays an important role in the structure, biochemistry, and nutrition of all living cells. Living biomass holds about 600 gigatons of carbon, most of which is wood. Soils hold upwards of 3,000 gigatons, mostly in the form of organic carbon, with perhaps a third of that inorganic forms of carbon.

Carbon can be divided into a few different forms, depending on methods applied to separation from matrix:

- Carbon extracted by sodium pyrophosphate – the extractable carbon fraction included all the easily mineralizable organic fractions and other more humified and hence more biodegradation-resistant fractions. These fractions decreased during biochemical reactions due to biodegradation.
- Soluble carbon at pH 2 – this carbon fraction is composed of the most biodegradable carbon compounds: fulvic acids, sugars, and organic acids. Urban residues such as wastewater and city refuse had a high proportion of this carbon fraction.

- Carbon precipitated at pH 2 – this carbon fraction is defined as “humic acids” and decreased during the biochemical processes. The decreases were smaller and more gradual than those of soluble carbon at pH 2.
- Water-soluble carbon – this is the most easily biodegradable carbon fraction because it consists of sugars, phenols, and organic acids apart from the soluble fraction of fulvic acids,
- Soluble carbon-precipitated carbon at pH 2 – this carbon fraction indicates the mineralization of the fulvic acids and more biodegradable carbon fractions and, to a smaller extent, of humic acids.

Organic matter has been defined as: simple substances such as sugars, amino acids, and other small molecules, identifiable high-molecular weight compounds such as polysaccharides and proteins, and humic substances. Organic matter is heterogeneous, consisting of basic chemical and mineral building blocks. Essentially, seven major elements constitute organic matter:

- carbohydrates and sugars
- protein
- fats
- hemicelluloses
- cellulose

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- lignin
- mineral matter

The first three constituents are decomposed relatively rapidly, whereas hemicelluloses, cellulose, and lignin are much more difficult to degrade. Finally, mineral matter does not biodegrade. The composition of organic matter varies with its source.

The aim of this work is the presentation of the main different carbon fractions in several environmental matrixes (wastewater, sewage sludge, soil).

Wastewater

The efficiency of a wastewater treatment plant depends on correct performance of individual processes during the treatment. The removal of carbon compounds is reached in the oxygenation process, nitrogen compounds are removed during nitrification, and denitrification processes and phosphorus in dephosphatation process. Each of these processes involve specialized microorganisms. The above-mentioned processes occur during biological part of wastewater treatment and in practice is realized in activated sludge method or other systems (eg. rotating beds with microorganisms biofilm) [1].

Activated sludge is a process for treating sewage and industrial wastewaters using air and a biological floc composed of bacteria and protozoans. The process involves air or oxygen being introduced into a mixture of primary treated or screened sewage or industrial wastewater combined with organisms to develop a biological floc that reduces the organic content of the sewage. This material, which in healthy sludge is a brown floc, is largely composed of saprotrophic bacteria but also has an important protozoan flora composed mainly of *Amoebae*, *Spirotrichs*, *Peritrichs* (including *Vorticellids*) and a range of other filter-feeding species. Other important constituents include motile and sedentary *Rotifers*. In poorly managed activated sludge, a

range of mucilaginous filamentous bacteria can develop, including *Sphaerotilus natans*, which produces a sludge that is difficult to settle and can result in the sludge blanket decanting over the weirs in the settlement tank to severely contaminate the final effluent quality. This material is often described as sewage fungus but true fungal communities are relatively uncommon. The combination of wastewater and biological mass is commonly known as mixed liquor. In all activated sludge plants, once the wastewater has received sufficient treatment, excess mixed liquor is discharged into settling tanks and the treated supernatant is run off to undergo further treatment before discharge [2]. Fig. 1 shows the most often observed carbon species present in wastewater. Dissolved organic matter (DOM) is defined as the organic matter that is able to pass through a filter 0.45 μm . Conversely, particulate organic matter (POM) is that carbon that is too large and is filtered out of a sample.

Dissolved and particulate organic matter are important components in the carbon cycle and serve as primary food sources for microorganisms in WWTP. In addition, DOM alters aquatic ecosystem chemistries by contributing to acidification in low-alkalinity, weakly buffered, freshwater systems. Furthermore, DOM forms complexes with trace metals, creating water-soluble complexes that can be transported and taken up by organisms. Finally, organic carbon, as well as other dissolved and particulate matter, can affect light penetration in aquatic ecosystems, which is important for the ecosystem's phototrophs that need light to subsist. The biodegradable dissolved organic carbon fraction consists of organic molecules that heterotrophic bacteria can use as a source of energy and carbon [3].

The characterization of DOM is useful for the understanding of the underlying mechanisms responsible for the complexation, reduction, and mobilization or immobilization of heavy metals, radio nuclides, pesticides, and other toxic chemicals [4-6]. Natural organic matter of the aquatic environment (including wastewaters) is a complex of sub-

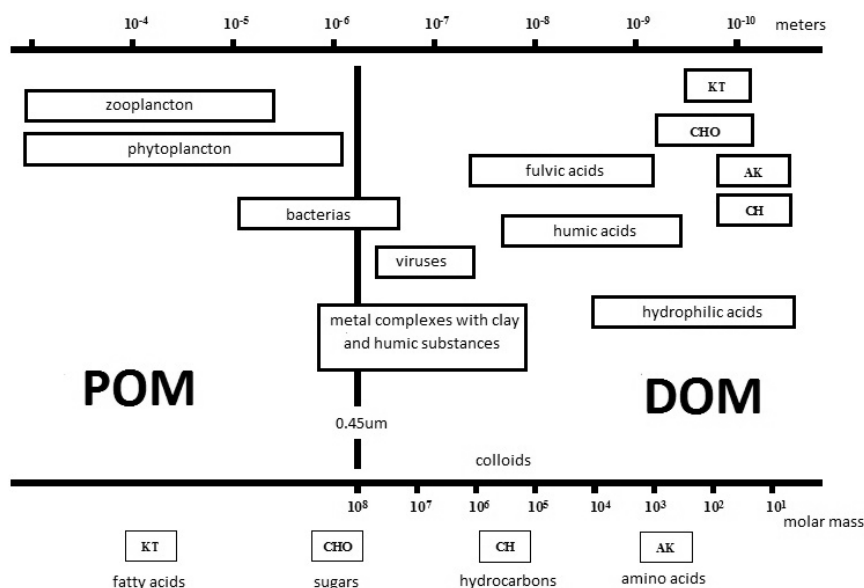


Fig. 1. Divide of carbon species present in wastewaters.

stances which may be classified into the groups of humic and non-humic substances, whereas about 25% of the dissolved organic carbon (DOC) in oceans, rivers, and lakes are non-humic substances [7]. DOM in natural waters is a highly complex mixture of organic compounds [8-9], so its overall concentration is usually reported as total organic carbon (TOC) and humic substances are an important component of the TOC, dominated by DOC [6]. The TOC or DOC concentration is a measure of carbon content in water that gives no information on the nature of organic substances themselves.

Aquatic humic substances are one of the main characteristics to be measured for water studies, but humic matter has no homogeneous, exactly specified chemical composition which makes it difficult to measure the content of humic matter in water [3]. The actual concentration of humic matter in water can only be determined after isolation [8, 10] and gravimetric determination [11] of the dried isolate, but the isolation is not a rapid and easy way to measure the aquatic humus content.

Sewage Sludge

Part of the settled material, the sludge, is returned to the head of the aeration system to re-seed the new wastewater entering the tank. This fraction of the floc is called return activated sludge. Excess sludge is called surplus activated sludge or waste activated sludge. Surplus activated sludge is removed from the treatment process to keep the ratio of biomass to food supplied in the wastewater in balance. Surplus activated sludge is stored in sludge tanks and is further treated by digestion, either under anaerobic or aerobic conditions prior to disposal [12].

Sewage sludge obtained as a by-product reflects the chemical composition of the treated sewage, but the composition of sewage itself is determined by the industrial wastewater inflow to the treatment catchment. Quantitative and qualitative composition of the sewage sludge is very complicated. It is rich in organic matter, nitrogen, phosphorus, calcium, magnesium, sulphur and other microelements necessary for plants and soil fauna to live. So it is characterized by the large manurial and soil-forming value. Except for the indispensable elements to live, sludge can contain toxic compounds (heavy metals, pesticides) and pathogenic organisms (bacteria, eggs of parasites) [13-16].

The most preferred method of neutralization of sewage sludge is composting. It is a complicated process aimed at:

- destruction of pathogenic organisms
- stabilization of organic matter-ripening
- drying of the sludge
- production of material that can be environmentally used or sold

To reach the above aims, the sludge has to be mixed with structural materials in suitable proportion to obtain a C:N ratio of about 30:1 in the compost. In practice the most often used structural materials contain cellulose (i.e. wood shavings, sawdust, bark, straw, leaf litter). The transformation occurring during the composting of materials containing cellulose with the sludge has an enzymatic character.

Municipal waste materials are also used in composting. Many parameters influence the composting process (temperature, moisture, aeration, time) [13].

The composting process transforms clammy consistency of the sludge to a sallow form of cloddy structure. Thanks to its structure, it is easy to use it in field applications. The potential use of the obtained product of composting is agricultural use – fertilization of soils, planting of trees and shrubs.

Humic Substances

The process of “humification” can occur naturally in soil, or in the production of compost. The importance of chemically stable humus is thought by some to be the fertility it provides to soils in both a physical and chemical sense, though some agricultural experts put a greater focus on other features, such as disease suppressiveness. Physically, it helps the soil retain moisture by increasing microporosity, and encourages the formation of good soil structure. Chemically, the incorporation of oxygen into large organic molecular assemblages generates many active, negatively charged sites that bind to positively charged ions (cations) of plant nutrients, making them more available by ion exchange. Biologically, it allows soil organisms (microbes and animals) to feed and reproduce [17]. Humus is often described as the “life-force” of the soil. Yet, it is difficult to define humus in precise terms; it is a highly complex substance, the full nature of which is still not fully understood. Physically, humus can be differentiated from organic matter in that the latter is rough looking material, with coarse plant remains still visible, while once fully humified organic matter becomes more uniform in appearance (a dark, spongy, jelly-like substance) and amorphous in structure, and may remain such for millennia or more [18]. That is, it has no determinate shape, structure or character. However, humified organic matter, when examined under the microscope without any chemical treatment, may reveal tiny but clearly identifiable plant, animal or microbial remains that have been mechanically but not chemically degraded. This points to a fuzzy limit between humus and organic matter. In most recent literature, humus is clearly considered as an integral part of soil organic matter (SOM) [17].

Plant remains (including those that passed through an animal gut and were excreted as faeces) contain organic compounds: sugars, starches, proteins, carbohydrates, lignins, waxes, resins, and organic acids. The process of organic matter decay in the soil begins with the decomposition of sugars and starches from carbohydrates, which break down easily as detritivores initially invade the dead plant organs, while the remaining cellulose and lignin break down more slowly. Simple proteins, organic acids, starches, and sugars break down rapidly, while crude proteins, fats, waxes, and resins remain relatively unchanged for longer periods of time. Lignin, which is slowly transformed by white-rot fungi, is one of the main precursors of humus, together with by-products of microbial and animal activity. Humus, the end product of this manifold process, is thus a

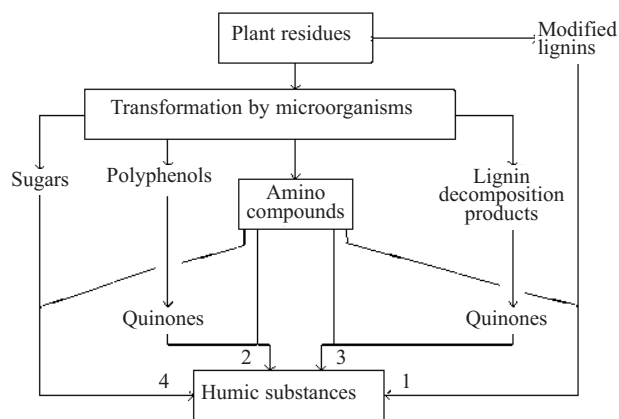


Fig. 2. Mechanisms for the formation of humic substances [19].

mixture of compounds and complex life chemicals of plant, animal, or microbial origin, which has many functions and benefits in the soil (Fig. 2) [19]. Most humus in the soil is included in animal faeces of more or less dark color according to their content in organic matter. Earthworm humus (vermicompost) is considered by some to be the best organic manure there is.

Humic substances can be divided into three main groups (Fig. 3).

Humic acids is the fraction of humic substances that is not soluble in water under acidic conditions ($\text{pH} < 2$) but is soluble at higher pH values. They can be extracted from soil by various reagents insoluble in dilute acid. Humic acids are the major extractable component of soil humic substances. They are dark brown to black in color.

Fulvic acids is the fraction of humic substances that is soluble in water under all pH conditions. They remain in solution after removal of humic acid by acidification. Fulvic acids are light yellow to yellow-brown in color.

Humins is the fraction of humic substances that is not soluble in water at any pH value and in alkali. Humins are black in color [20].

A decline in water-soluble organic carbon is often used as an indicator of compost maturity. Organic matter of fresh compost consists of many easily degradable and water soluble substances, whereas mature compost is rich in stable,

decomposition-resistant and high molecular mass humic-like substances that have limited ability to dissolve in water [21]. Humic substances formed during composting show colloidal properties and may cause aggregation of organic and inorganic matter. This is a very desirable feature of organic matter because it influences many physicochemical properties of compost and soils. To investigate the colloidal properties of compost organic matter, a 0.01 M CaCl_2 solution was often used instead of water in the same way as in the study on water-extractable fractions. The aggregation of humus origin substances takes place in the presence of polyvalent cations like Ca^{2+} [22].

The UV-Vis absorption spectrum of alkali-soluble humic substances (humic or fulvic acids) is generally non-specific. The absorbance monotonically decreases with an increasing wavelength at a low absorption of above $\lambda = 500$ nm. Most of the solar energy absorbed by humic materials is between $\lambda = 300$ and $\lambda = 500$ nm. Despite no direct information can be extracted from the humic UV-Vis spectrum, the technique is widely used for determining the molecular properties of humic material. There are three important regions in the spectrum where exact absorbance is measured: about $\lambda = 280$, $\lambda = 400$ -500 nm and above $\lambda = 600$ nm. UV-Vis analysis of alkali extracts is generally based on the assumption that the absorbance at $\lambda = 260$ -280 nm is due to lignin and quinone moieties, i.e. the material at the very beginning of the transformation. The absorbance at $\lambda = 460$ -480 nm reflects the organic material at the beginning of humification and the absorbance at $\lambda = 600$ -670 nm is said to be indicative of strongly humified material with a high degree of aromatic, condensed groups. The following absorbance ratios may then be calculated: $Q2/6 = A_{280}/A_{664}$, $Q4/6 = A_{472}/A_{664}$, and $Q2/4 = A_{280}/A_{472}$. On the basis of these absorbance ratios the degree of maturation (humification) of organic matter may be determined. The absorbance ratios ($Q2/6$, $Q4/6$, $Q2/4$) of 0.5 M NaOH extracts reflect the degree of organic matter maturity in bulk compost [23].

Humic substances constitute the most important fraction of compost organic matter. Mature and well humified compost applied to a poor sandy soil may greatly improve its properties, increasing fertility and crop productivity. Moreover, humic substances strongly interact with pesticides to speed up their biodegradation, and with metal ions influencing their transport and plant accessibility. They also have the ability to buffer pH and to act as a potential source of nutrients for plants. The levels of humic fractions (HA, FA, HM) in compost represent the degree of maturity [23].

Application of Analytical Chemistry in Study of Carbon Changes

The most common methods used to characterize the humic substances in the 1960s were the so-called destructive testing methods (oxidation, reduction, pyrolysis). A number of non-destructive methods have been developed since then. These include elementary analysis, the assay of oxygen-containing functional groups, and nitrogen-containing components. The properties of humic substances can also be described with the help of instrumental analytical methods.

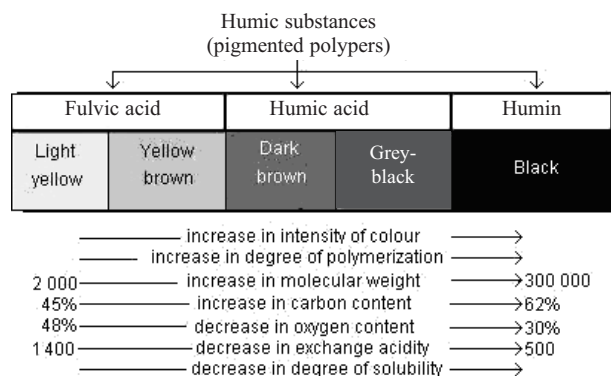


Fig. 3. Chemical properties of humic substances [20].

These include potentiometric and conductometric titration, and within the category of spectral methods, UV and VIS, fluorescent, infrared, NMR, and ESR spectroscopy. But other methods may also be used, including x-ray diffraction, surface tension measuring, determination of molecular weight, steam pressure and membrane osmometry, size-excluded chromatography (SEC), field flow fractionation (FFF), electrophoresis, (ITP), ultracentrifuge, viscometry, and mass spectrometry. Other methods worth mentioning include light dispersion, x-ray dispersion, and electron microscope and ultrafiltration [14, 24-28]. Sometimes the simplest methods, e.g. elemental analysis (CHN), give fundamental information about the structure of analyzed compounds (Table 1). Between humic and fulvic acids, peptides, cellulose, sugars, and lignins, huge differences in content of carbon, hydrogen, oxygen, and nitrogen are observed [28].

Table 1. Elemental composition of basic soil organic matter constituents [28].

	C	H	O	N
Humic acids	52.0-62.0	3.0-5.5	30.0-33.0	3.5-5.0
Fulvic acids	44.0-49.0	3.5-5.0	44.0-49.0	2.0-4.0
Peptides	50.0-55.0	6.5-7.3	19.0-24.0	15.0-19.0
Cellulose	44.4	6.2	49.4	-
Sugars	45.4	6.1	48.5	-
Lignin	62.0-69.0	5.0-6.5	26.0-33.0	-

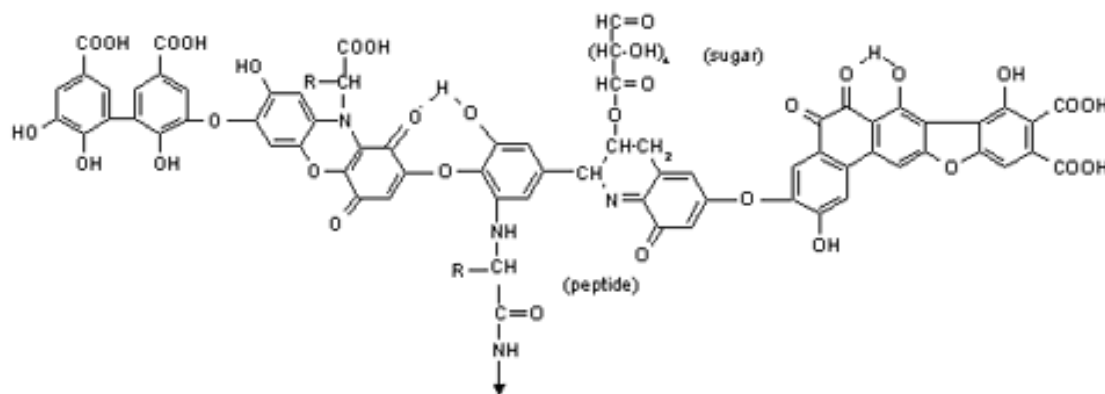
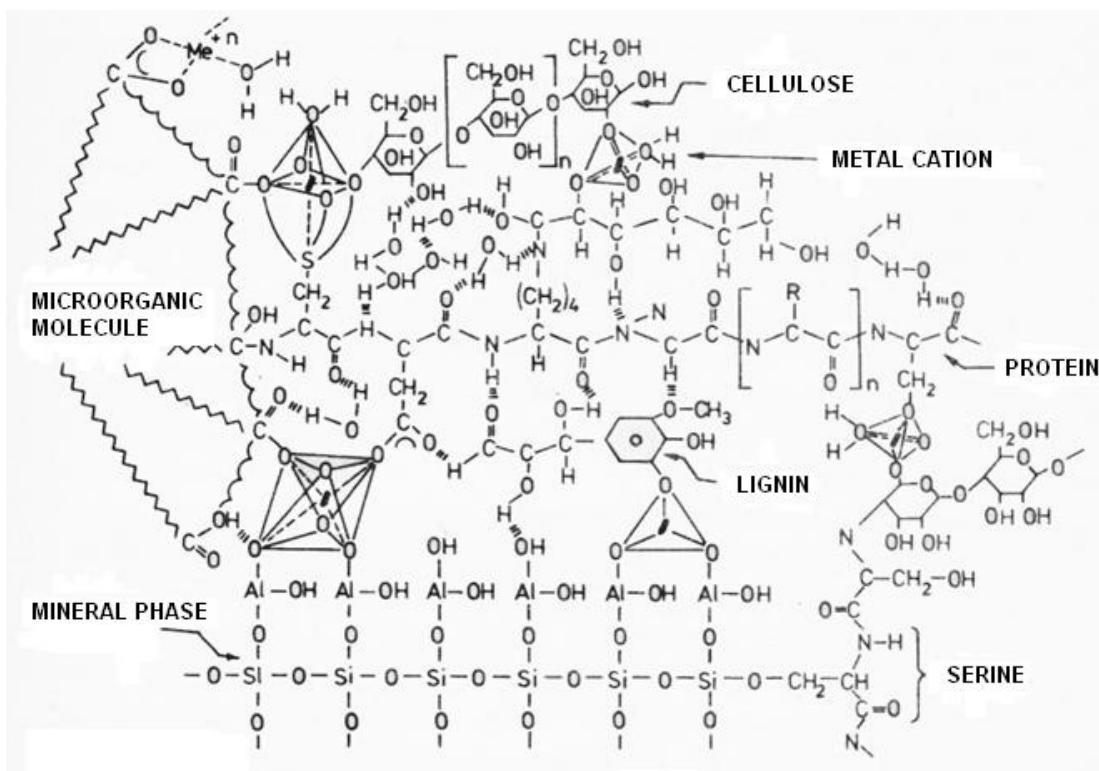


Fig. 4. Hypothetical structures of humic acid [18].

One could assume that due to the ubiquitous nature and structural and chemical properties of humic substances, the environment is dependent on them. It is known that the specific functional groups of humic acids are responsible for chelating various compounds in the environment, thereby improving nutrient utilization and preventing metal toxicity in waters, soils, and thus possibly in plants, animals and humans as well.

Humic acids from peats show significant levels of phenolic carbons (C₆) and methoxyl carbons (-OCH₃) associated with the presence of lignin-like materials. Lignin, being the starting material of humic and fulvic acid, and various phenolic compounds such as vanillin, vanillin acid, resorcinol, ferulic acid, protochatechuic acid, and benzoic acid, are the degradation products of these lignins. It is apparent that humic substances consist of a heterogeneous mixture of compounds for which no single structural formula will suffice. Nevertheless, humic acids are thought to be complex aromatic macromolecules with amino acids, amino sugars, peptides, and aliphatic compounds involved in linkages between the aromatic groups. The hypothetical structure for humic acid is shown in Fig. 4. It contains free and bound phenolic OH groups, quinone structures, nitrogen and oxygen as bridge units and carboxylic acid groups variously placed on aromatic rings [18].

Conclusions

Humic substances exist in all environments including soils, groundwater, streams, estuaries, and oceans. They are very reactive and are important participants in many geochemical reactions and processes. The functions they perform are multiple and varied and include, but are not limited to, the mobilization and transport of metal ions, contribution to the cation-exchange capacity of peat, soil, and water, and binding of various organic molecules such as carbohydrates, lipids, and proteins.

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