HUMIC ACID NANOTUBE MEMBRANES
AS REVEALED BY SCANNING ELECTRON MICROSCOPY

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SUMMARY

The issues and controversies, associated with humic acids as natural compounds or artifacts produced during extractions, have been examined in earlier papers in sufficient details. The common belief is now that they are products of the environment, though transient in nature. An alleged absence of a well-defined structure characteristic for humic substances appears to be a remaining stumbling block for acceptance of the compounds as discrete chemical entities. At first widely considered as large polymers, the idea became so popular that it was happily embraced by most scientists for almost a century. However, the biopolymer concept seems now to become of age and many people appear anxious to apply the new micelle and supramolecular theories introduced by the end of the 20th century. The latter more modern theories advance the ideas that small soil organic compounds are capable of self assembling into humic acid molecules in the form of micelles or as poorly defined random molecular units. These two theories were closely followed by the discovery with electron microscopy of the occurrence of humic acid nanotubes, which, in contrast, suggested the presence of some orderly humic assemblies, such as in micelles. In an effort to study it further, humic acids were extracted from lignite by the present author using the usual standard NaOH method. Samples were then prepared for scanning electron microscopy (SEM) by the rapid liquid-N method developed by the author. The humic samples were scanned by the scientific staff of the University of Georgia Electron Microscopy Center using a Zeiss SEM at 20 kV and a spot size smaller than usual aimed at getting better results with organic substances. The results showed the presence of nanotube membranes as manifested clearly in the characteristic honeycomb arrangements. At one instance a nanobud was observed, indicating involvement of fullerenes in the structure of the humic substances. The observation above does not only agree with the author's nanotube concept, but is also in support of the supramolecular and especially the micelle concept. The humic acid nanotube membranes are presumably the most stable thermodynamic entities that can be formed by self assembly. Such a more orderly molecular arrangement relates better than random bundles to explaining issues of consistent carbon and nitrogen contents and reproducible spectroscopic features of humic substances.

INTRODUCTION
From Biopolymers to Supramolecular Nanotubes

Humic substances have traditionally been considered as either natural or artificial compounds produced during extraction (Aiken et al., 1985; Stevenson, 1994; Frimmel et al., 2002; Tan, 2003). With the advancement of humic acid chemistry and consequent availability of considerable amounts of new data, they are now believed to be important components of natural organic matter (NOM) in soil and water (Jackson, 1975; Schnitzer, 2000; Ghabbour and Davies, 2003; 2006; Hayes and Clapp,

2001; Steinberg, 2003). They are also major constituents of seve ral geologic deposits, such as lignites or leonardites, which are valuable sources of humic ac ids for agricultural and industrial applications (Burdick, 1965; Stevenson, 1994; Tan, 2003). As is the case with many of his articles, the author uses here the term *Humic Acids* also for quick reference only. After humic acids were isolated by Berzelius in 1839, mos t people believe them to be poly mers, as a dvanced in the biopolymer theory, which became so popular that the concept was embraced by most scientists for almost a century. By definition a pol ymer is composed of repeating units, normally connected by strong covalent bonds. Applied to humic acid chemistry, polymerization---emphasizing aggregation of small molecules into large biopolymers or humic acids—seems to provide a workable hypothesis. It is among other things not running afoul with the carbon and nitrogen composition of humic acids, always reported within relatively consistent ranges. As stated above, it was the hypothesis for almost one century, until it was contested in 1986-1999 by the micelle or micellar concept (Wershaw, 1986; 1999; von Wandruska, 1998), followed thereafter in the new millennium by the supramolecular concept (Piccolo, 2001; 2002). The two concepts—received with only subdued critiques due in part to perhaps a *polymer concept fatigue*---have been examined and the analyses reported in a previous book and as a web site a rticle by Tan (2011a, and b). Micelles are defined in the literatur e as aggregates of amphiphilics or surfactants dispersed in a liquid medium, but in clay mineralogy the silica tetrahedron and aluminum octahedron layers are also called micelles or clay micelles. Both Wershaw and von Wandruska believe humic acids to be aggregates of amphiphilics, surfactants or detergent-like compounds in the form of micelles. In contrast to polymers, the components in micelles are held together by noncovalent bonds, for example by the weaker van der Waals and hydrogen bonds. Though, micelles are ordinarily visualized as spherical in shape, cy linders and bilayers are also found. The bilay ers are often called membranes. The supramolecular concept emphasizes self assembly of molecules or substances into a multi-component unit held together also by van der Waals, hydrogen and hydrophobic bonds, pi-pi interactions and other electrostatic forces. As stated before, these forces are considerably weaker than covalent bonding, hence resulting in formation of a less stable assembly. Therefore, the compound is easily to break apart. The product is formed initially because it is the most stable thermodynamic arrangement that can be attained by self assembling of the components. A "wrong" product is then ea sily disassembled and the components rapidly reassembled into the "proper" product with the most stable thermodynamic assembly, all by themselves. Such a molec ular self assembling process also applies to the development of micelles and membranes. Here--- in the mechanisms process--- lies then the relation between Wershaw's and Piccolo's ideas. The difference is that Piccolo and coworkers, in trying to apply this brilliant concept of molecular self-assembly to formation of humic acids, believe that the end products—humic acids—are poorly defined, randomly assembled substances. However, the theory of supramolecular chemistry suggests instead that the process of self assembling tends to yield products with somewhat orderly or re cognizable structures (Lehn, 1995; Steed, 2009). With a randomly assembled product, it will be very difficult, if not an impossible task, relating such an ill-defined compound to humic acid, e xhibiting, as stated above, consistent ranges of carbon and nitrogen contents, and reproducible infrared absorption and nuclear magnetic resonance (NMR) features (Orlov, 1985; Tan, 2003; 2011a). As reported earlier, the present author then started to reexamine all his previous data for the *impossible* task finding a humic molecule with some kind of orderly molecular arrangement. As if by accident, he stumbled in his published SEM papers on the

occurrence of a "fishnet" structure, characteristic for nanotubes, formerly identified "erroneously" as *perforated sheets* (Tan, 1985; Lobartini and Tan, 1988). As discussed in his paper, posted in the web site (Tan, 2011b; http://drkhtan.weebly.com/), the small fishnet networks exhibited by the humic assemblies are called nanotubes, because of their sizes, ranging from nanometers (1 nm = 1 x 10^{-9} m) to micrometers (1 μm = 1 x 10^{-6} m). As the name nanotube implies the particle should be tubular, but for reasons unknown to the author, the name is an "umbrella" name that often applies to nanotubes of different forms or shapes, e.g., spheres or balls, tubes or cylindrical, ellipsoidal, sheets and ribbons, etc., with all of them characterized by the peculiar structure, the author calls a "***fishnet***" structure. A well-known spherical nanotube particle is *Fullerene,* which has the shape of a tiny soccer ball. The discovery by the present author of nanotubes in his humic acid SEM images may well prove to be the "*missing link*" that can pump new lifein Piccolo's concept, making it now more viable and exciting. However, for arriving at definite conclusions, it is deemed necessarily by the author to investigate the issue more thoroughly. The search is on for obtainingmore information in support of his important discovery. This is then his story for the hunt and tracking down more of the elusive "fishnet" networks that can strengthen his proposed nanotube concept of humic acids.

MATERIALS AND METHODS

Humic acid was extracted from lignite with a 0.1 *M* NaOH solution at a lignite to solution ratio of 1:10, due to the lignite' s extremely high c ontent of humic substance s. This ratio was a lso the recommended ratio of the International Humic Substances Society(IHSS). The procedures followed are standard procedures of the I HSS and Soil Science Society of America. After dialysis of the isolated humic acid fraction against distilled water, it was freeze-dried and stored for SEM analyses. Preparation of humic acid samples for SEM were conducted bythe rapid liquid-N method developed by the author (Tan, 1985). The frozen specimens were dried under a high vacuum (6.5 x 10^{-10} MPa) overnight at room temperatures in a vacuum evaporator, before they were sputter-coated with an Au-Pd alloy for subsequent analyses by SEM. Scanning electron microscopy was conducted by the staff of the University of Georgia Electron Microscopy Center employing a ZEISS 1450EP variable SEM instrument at 20 kV and a probe size of 250pA. A small spot size was used aimed at getting better images of organic substances. Some gun alignments were also performed prior to imaging to avoid drifting of the gun over time. For more details of the extraction procedures of humic acid and sample preparation for SEM reference is made to Tan (2005)

RESULTS AND DISCUSSIONS

Occurrence of Nanotube Membranes. The results show that the humic acids under investigation are assembled bynumerous particles of nanotubes, as recognized by the extensive networks of fine "fishnets" present in allthe SEM micrographs. The fishnets areafter all not that elusive as expected. At the bottom and at the right of Figure 1A, they seem to be arranged like the cells of honeycombs. A close-up of a spot at the bottom of the above image not only shows clearly the honeycomb structure (Figure 1B), but also demonstrates the presence of a tubular or cylindrical assembly, torn at the top. A subsequent close-up taken at higher magnification (Figure 2A), confirms definitely the honeycomb characteristics as seen in beehives, validating the idea for the occurrence of nanotube membranes. The drawings in Figures 2B, C, and D, provided for comparisons, suggest the several types of nanotube networks close similarities with a humic acid fishnet structure as demonstrated by the present SEM images. However, the honeycomb structure as shown in Figure 2A is more neat-
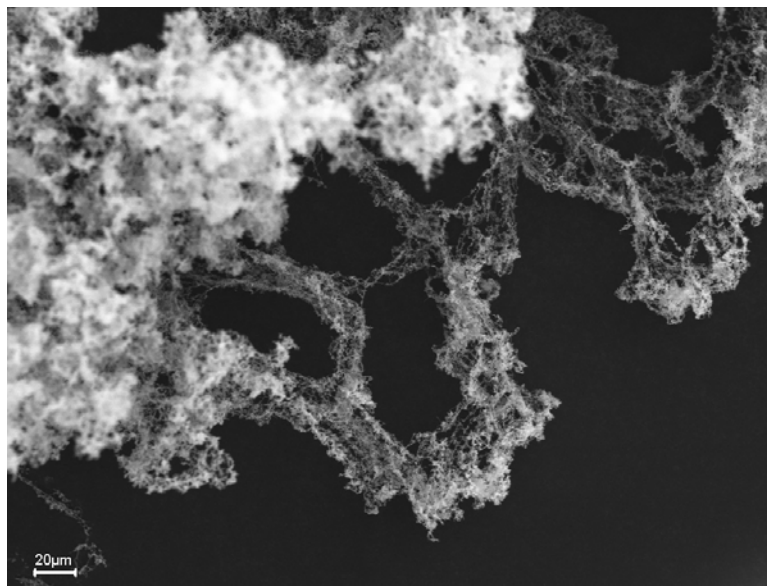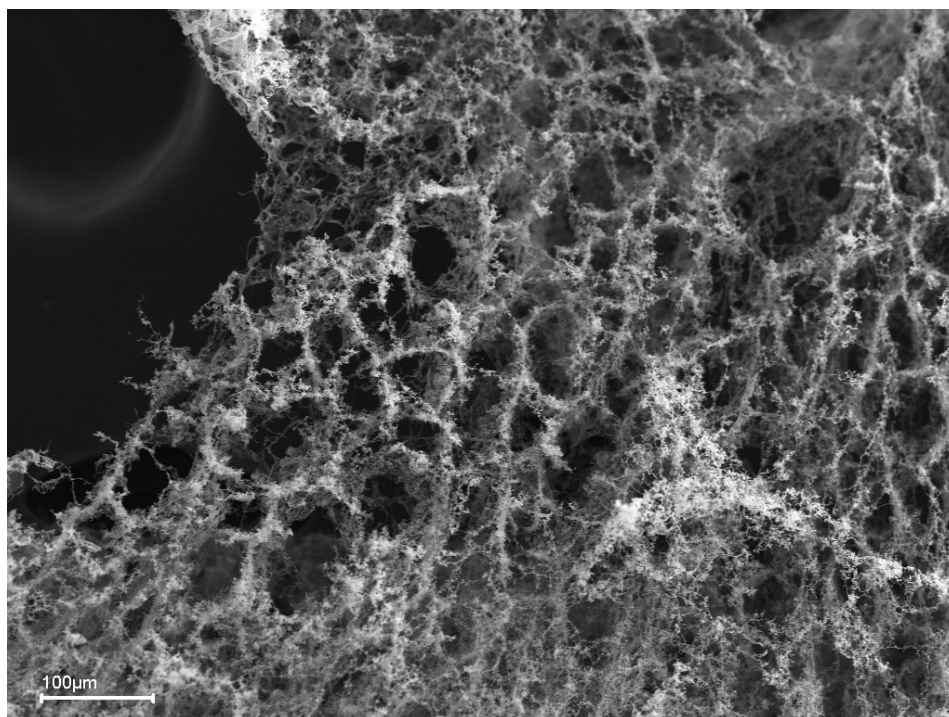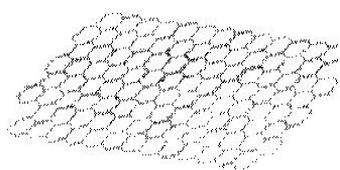
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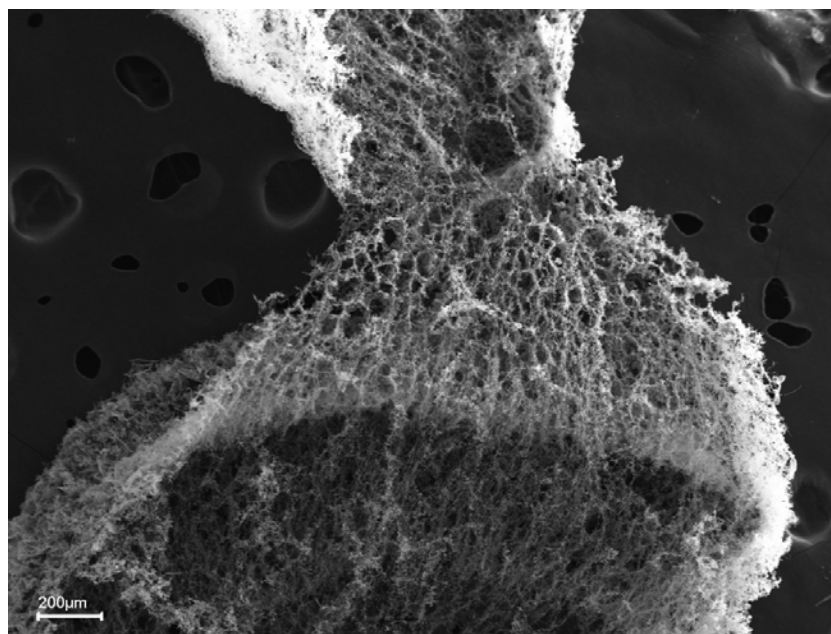
B

Figure 1.   Scanning Electron Microscope images of humic acids isolated from lignite exhibiting the "fishnet" structures. **A**. Nanotubes, on bottom and right, seen assembled into membranes; **B**. A close-up of a nanotube membrane with a torn cylindrical assembly, exposing the characteristic honeycomb structural arrangement.
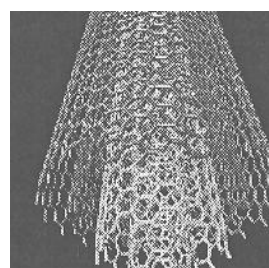
A

B   C   D

Figure 2. **A**. A nanotube membrane section of figure 1B showing the honeycomb assembly at higher magnification; **B**. Drawing of a single nanotube sheet; **C**. Roll-up of a single-walled nan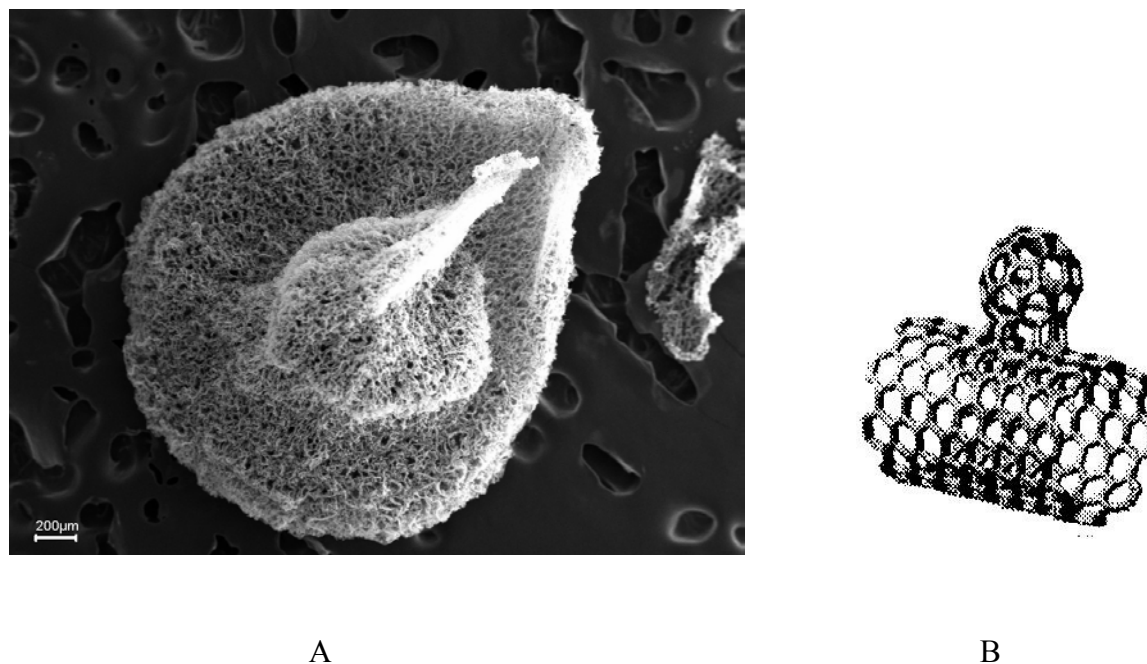otube; **D**. A multi-walled nanotube. Source of drawings: http://www.nanotech-now.com/nanotube-buckyball-sites.htm/ , accessed 7/2/2011.

A	B

Figure 3. A. Scanning electron micrograph of humic acid e xhibiting a "***nanobud***," with the characteristic fishnet structures in a honeycomb arrangement. On the right is visible a small torn cylindrical nanotube membrane. **B.** A drawing of a single-walled nanobud shown for comparison. Source of drawing: http://en.wikipedia.org/wiki/carbon_nanotube/ Accessed 7/2/2011.

ly arranged, resembling a beehive more closely than a randomly constructed multi-walled nanotube assembly in Figure 2D. The discovery of nanotube membranes is an extension of Wershaw's idea of humic molecules aggregating into micelles. It also provides to Piccolo's concept a positive direction for further developments than the c oncept of random assemblies can do. The latter appears to be deadlocked on relating them to the many established properties reported for humic substances, an issue which will be discussed in some more detail below.

Occurrence of Nanobuds. In addition to the discovery of nanotube membranes, it appears that perhaps nanobuds are also formed during the self a ssembly of humic ac id molecules due to the presence of *look alike nanobuds* in some of the micrographs. Figure 3A shows for example a humic acid nanotube membrane with on top a spherical or ball-like structural arrangement suspiciously

resembling a nanobud. In supramolecular chemistry and nanotube science, nanobuds are fullerenes assembled and bonded by covalent bonds on the outer walls of nanotubes as shown in Figure 3B. The buds are supposed to function as anchoring the nanotubes in the molecules. Because of the nanotube membrane appearance as shown in Figure 3A above, the author suggests calling it a humic acid *nanobud membrane*.

Conclusion. In conclusion the author wishes to re iterate that the re sults discussed above neither contradict nor invalidate Wershaw's and Piccolo's new ideas. The presence of a more orderly molecularly assembly provides a better advantage in relating them to the several consistent and/or reproducible characteristics exhibited by humic substances, which would otherwise be a very difficult task when considering them as random, ill-defined, bundles of small organic substances. For example, it is common knowledge that the carbon content is consistent within the range of 40 to 50% for humic acids, with somewhat higher values for fulvic acids. The nitrogen contents, on the other hand, are in the range of 2 to 4 %for humic acids, with the lower values usually reported for fulvic acids. Infrared absorption and nuclear magnetic resonance features are also reported to be reasonably reproducible. The infrared absorption spectrum is often used in the identification of fulvic from humic acids, and/or hymatomelanic acids (Orlov, 1985; Schnitzer, 2000; Stevenson, 1994; Tan, 2003). The author realizes that the above is to some unfortunately a controversial issue, though the pictures shown are very straightforward and speak clearly for themselves. However, hardcore critics may disagree as usual, but the author wonders how one can explain rationally random assemblies of humic substances to exhibit consistent elemental compositions and/or reproducible spectral characteristics. Wouldn't it be more reasonable to expect them to be at random too?

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