

# TRITIUM-LABELED HUMIC PREPARATIONS AS A PERSPECTIVE TOOL FOR MEMBRANOTROPIC STUDIES

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## I. INTRODUCTION

Humic substances (HS) are ubiquitous in the environment. HS are characterized by a surfactant-like structure, containing both hydrophilic domains, such as carboxylic and phenolic groups, and aliphatic and aromatic moieties. Because of this amphiphilic character, HS behave as natural surfactants and can adsorb on a large amount of natural surfaces, including biological membranes (Vigneault et al., 2000). Thus, HS may change the structure and fluidity of the membrane as well as its permeability (Visser, 1982). In spite of many studies demonstrating adsorption of HS on biological surfaces (Campbell et al., 1997; Fein et al., 1999; Vigneault et al., 2000), the direct measurements of HS partitioning to biological cells are still missing. The main reason is a lack of the radioactively labeled preparations identical in the structure to the natural HS. The goal of this work was to obtain and use for the membranotropic studies the tritium labeled HS. The most hydrophobic coal HA were used for this purpose as, supposedly, exhibiting the highest affinity for cell membranes.

## II. MATERIALS AND METHODS

### **Preparation and SEC-characterization of tritium-labeled HA**

Leonardite humic acids (HA) were isolated from the commercial preparation of potassium humate Powhumus (Humintech, Germany). The tritium-labeled preparations were obtained using thermal bombardment technique. For this purpose, the diluted HA solution was frozen onto the walls of a cylindrical glass reactor equipped with an axial tungsten wire. The reactor was evacuated, and then gaseous tritium was let into the system. When the temperature of filament reached 1500-2500 K, the tritium would atomize on its surface, and bombard the walls of the reactor. Low pressure of molecular tritium (0.5 Pa) was used to avoid the secondary reactions.

The <sup>3</sup>H-HA sample obtained was purified using dialysis. This allowed to eliminate exchangeable tritium from OH-, COOH-, and NH<sub>2</sub>-groups. To obtain preparations of different molecular weight (MW) the dialysis membranes of two cut-offs, namely 12 and 2 kD, were used. Thus, two samples of <sup>3</sup>H-HA differing in MW (>12 and >2 kD) were prepared.

The molecular weight distribution of the <sup>3</sup>H-HA samples was determined using size exclusion chromatography (SEC) according to (Perminova et al. 1998) using ABIMED-HPLC-system. Both UV (254 nm) and radioactivity detection were used to compare chromatograms. Radioactivity measurements were conducted using the liquid scintillation counter RackBeta 1215 (LKB Wallac) and scintillation cocktail Ultima-Flo M (Packard Instrument Co).

### **Bacteria cultivation and partitioning experiments**

Bacteria *Escherichia coli* XL1 strain was cultivated at 37°C in 2YT medium supplemented with 30 µg/ml of tetracycline overnight. For partitioning experiments, 1 mL of fresh overnight culture was transferred into 100 mL flask with 50 mL M9 media containing 30 µg/mL of tetracycline and added with both non-labeled HA (5-50 mg/L) and labeled <sup>3</sup>H-HA to obtain radioactivity of 0.5 mCurie/L. Cell growth was monitored by measuring the absorbance at 600 nm and subsequently converted into cell number per L.

Total radioactivity of media including bacteria was measured after 10-h cultivation at 37°C as described above. Then cells of *E. coli* were harvested by centrifuging (30 min, 5000 rpm) and

radioactivity of supernatant reflecting equilibrium concentration of HA was measured. Data obtained were used for the sorption isotherms plotting.

### III. RESULTS AND DISCUSSIONS

#### SEC-characterization of tritium-labeled HA

The SEC analysis revealed that the elution profile of the labeled HA sample dialyzed using membrane with cutoff 2 kD was very close to that of the initial HA sample indicating their identity. On the other hand, the SEC elution profile of  $^3\text{H}$ -HA sample of MW > 12 kD was shifted to higher MW values compared to the initial HA indicating a depletion of the labeled preparation with the low molecular weight fractions during the purification stage.

#### Studies on membranotropic properties of HA

To evaluate membranotropic properties of HA, the partitioning of the obtained samples was studied between the aqueous phase and bacterial biomass. The partitioning was quantified using sorption isotherm technique. Figure 1 shows isotherms characterizing sorption of the obtained  $^3\text{H}$ -HA samples of different MW onto *E. coli* cells.

As can be seen the samples under study differed drastically in their sorption affinity for *E. coli* cells. The sample depleted with low MW fractions had much higher sorption affinity compared to the sample identical in MW characteristics to the initial HA. So, the calculated values of distribution coefficient (Kd) were 0.066 and 0.002 L/million cells for HA with MW higher than 12 and 2 kD, respectively.

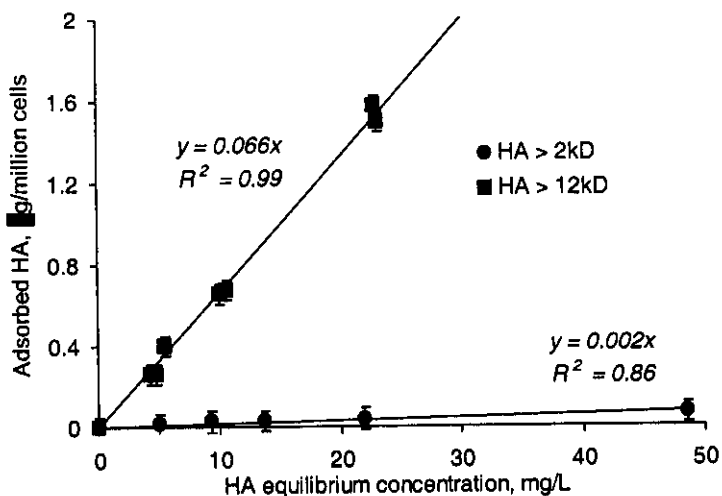


Figure 1 – Sorption isotherms of coal HA of different MW onto bacteria *Escherichia coli* cells.

The conducted experiments revealed a considerable difference in adsorptive properties of HA samples used towards *E. coli* cells. Maximum values of adsorbed HA at the highest initial concentration studied (50 mg/L) for preparations of MW higher than 12 and 2 kD were 0.15 and 0.01 µg/million of cells respectively. This finding demonstrates a leading role of molecular weight in membranotropic properties of HS: the higher MW, the higher sorption affinity for bacterial cells is.

### IV. CONCLUSIONS

Partitioning of two tritium-labeled coal HA preparations of different molecular weight to the *Escherichia coli* cells was studied. Maximum amount of sorbed HA accounted for 0.01 µg per million of bacteria cells at the initial concentration of HA 50 mg/L. Significant differences were observed

between the sorptive properties of HA of molecular weight higher than 2 and 12 kD. This observation indicated the leading role of molecular weight in membranotropic properties of HA in relation to bacterial cells. The higher molecular weight of HS is, the higher sorption affinity for bacterial cells they display.

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### **References**

- Campbell, P.G., Twiss, M.R., and Wilkinson, K.J. 1997. Accumulation of natural organic matter on the surfaces of living cells - implications for the interaction of toxic solutes with aquatic biota. *Can. J. Fish. Aquat. Sci.* 54, 2543-2554.
- Fein, J.B., Boily J.-F., Guclu, K., and Kaulbach, E. 1999. Experimental study of humic acid adsorption onto bacteria and Al-oxide mineral surfaces, *Chem. Geol.* 162, 33-45.
- Giles, C.H., Smith, D., and Hultson, A. 1974. General treatment and classification of the solute adsorption isotherm. I. Theoretical. *J. Colloid Interface Sci.*, 47, 755-765.
- Perminova, I.V., Frimmel, F.H., Kovalevskii, D.V., Abbt-Braun, G., Kudryavtsev, A.V., Hesse, S. 1998. Development of a predictive model for calculation of molecular weight of humic substances. *Wat. Res.* 32, 872-881.
- Vigneault, B., Percot, A., Lafleur, M., and Campbell, P.G.C. 2000. Permeability changes in model and phytoplankton membranes in the presence of aquatic humic substances, *Environ. Sci. Technol.* 34, 3907-3913.
- Visser, S.A. 1982. Surface active phenomena by humic substances of aquatic origin, *Rev. Fr. Sci. Eau.* 1, 285-296.