SPECTROSCOPIC METHODS TO ASSESS BIODEGRADATION OF HUMIC ACIDS BY MICROSCOPIC FUNGI

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The interactions of humic substances (HS) and microscopic fungi (micromycetes) are very important for understanding of sustainable soil functioning and transformations of aquatic dissolved organic matter (DOM). Microscopic fungi are actively involved in the processes of HS synthesis, transformation and mineralization due to production of extracellular nonspecific oxidative enzymes (1,2). The degradation of lignin and related compounds by fungi from the "white-rot" group occurs only in presence of an easily metabolisable carbon source, e.g. glucose (1). Some species of micromycetes can be grown on media with humic acids (HA) or coal-originated materials as the sole carbon source (2). The main direct effects of HS on microorganisms include stimulation of biomass growth and biosynthetic activity (3). The aim of our study was using spectroscopic methods to assess the possibilities of micromycetes to transform HAs as primary organic material.

The research object was a strain of *Alternaria alternata* (Fr.) Keissl. This strain was kindly provided by Marfenina O. E. and Ivanova A. E. from the collection of the Soil Biology Department at Soil Science Faculty (Lomonosov Moscow State University). The fungi culture was grown on the liquid Czapek medium (CzLM) with addition of 0.3 or 3 g/L of sucrose. The effect of two HAs, isolated from peat and coal humic products, has been studied. The sampling of supernatant liquid was performed on 14th days. The samples were filtered through a filter with a pore size of 0.2 microns before spectral measurements. Absorption measurements were carried out with a double-beam spectrophotometer Solar PB2201 (Belarus) within the spectral range 200-1000 nm and wavelength step 1 nm placing liquid samples into a quartz cuvette with the 1 cm optical path length and 4 ml volume. The fluorescence spectra were measured using a luminescence spectrometer Solar CM2203 (Belarus) at several wavelengths of the exciting radiation (270, 310 and 355 nm) for aqueous solutions and suspensions in quartz cuvettes at 90 degree geometry.

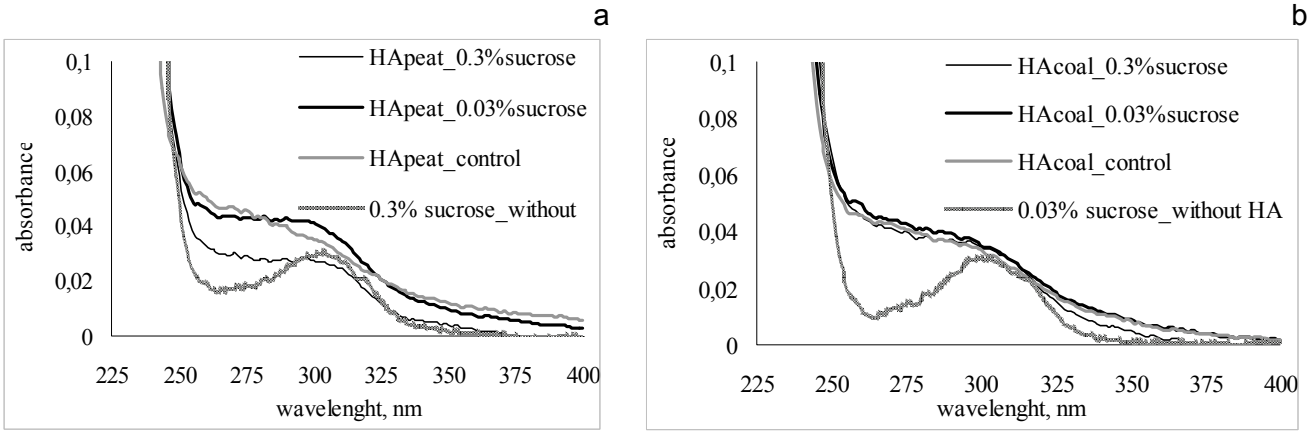

Figure 1: Absorbances of culture liquids of A. alternata after growth on the mediums with peat HA (a) and with coal HA (b) measured against distilled water

Typical UV-VIS absorption spectra for HA solutions in water do not feature extremes showing absorbance values decreasing monotonically along with the wavelength increase. The absorption spectra of HA in CzLM changed during the process of the micromycete growth: the absorbance values, the peak positions and the shape of absorption bands were modified. We can assume the degradation of the peat HA by micromycetes at 0.3% sucrose using the reduction of absorbance values (fig.1a). We have not observed analogous degradation of the coal HA by micromycetes (fig.1b). The fungi supernatant liquid had the specific absorption spectra with maximum at about 300 nm and relatively large optical densities. The appearance of these peaks was associated with the production of fungi metabolites in the process of their growth.

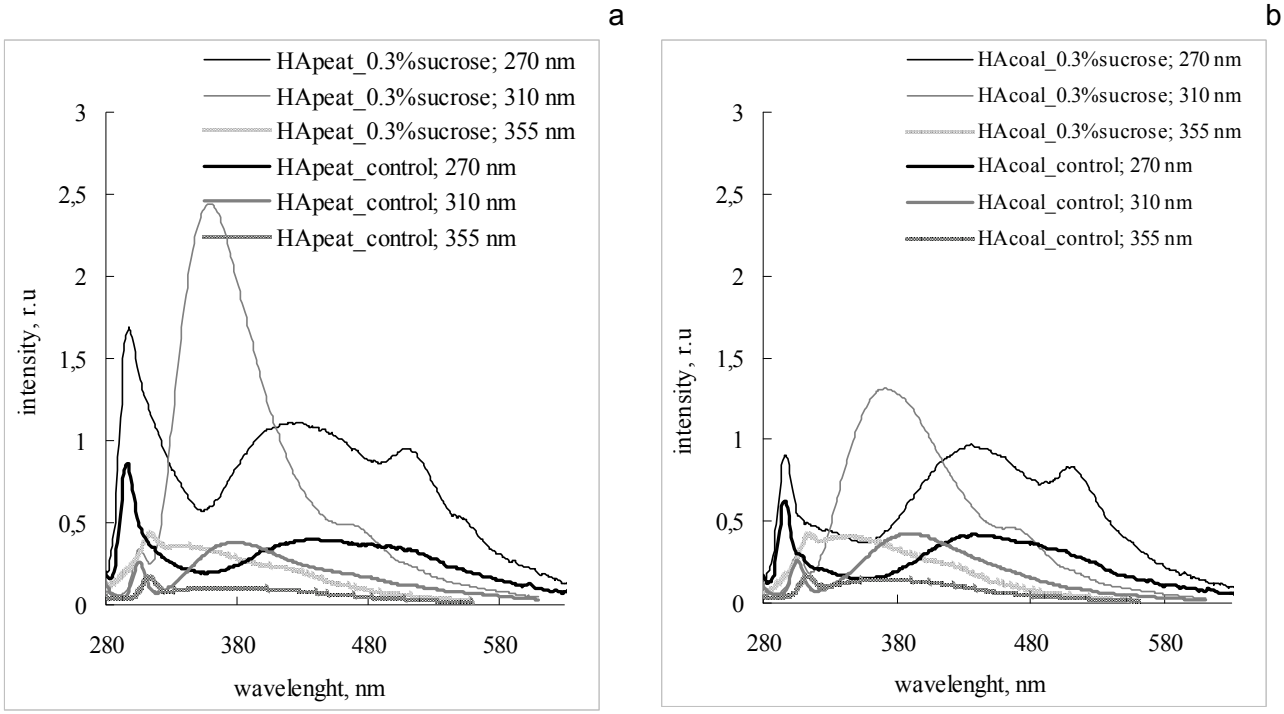

Figure 2:. Fluorescence spectra of CzLM with addition of HA and filtered fungi culture for A. alternata grown with addition of HA

The analysis of fluorescence spectra of supernatant liquid recorded during fungal growth supported the idea of HA transformation by microscopic fungi. To characterize spectral properties of HA and their changes due to fungi growth we used a number of indices: the fluorescence emission maximum position and its variation upon changes of the excitation wavelength, fluorescence intensity of protein-type emission band taken at 350 nm with excitation at 270 nm $F_{350.}$ The data presented on fig. 2 do not demonstrate principal differences in the fluorescence emission maximum position between coal and peat HAs. However we can observe the greater intensity of protein-type emission band taken at 350 nm in the medium with peat-originating HA compared to coal-originating HA.

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