THE OPPORTUNITIES OF SPECTRAL METHODS TO ASSESS THE BIODEGRADATION OF HUMIC SUBSTANCES BY MICROMYCETES

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Fungi are largely responsible for the biodegradation which can be defined as the breakdown or mineralisation of organic material. The organic carbon of the parental material is partly converted to new biomass of living organisms and some carbon remains in the form of metabolites released into environment (1). The aim of our study was to assess the opportunities of micromycetes (microscopic fungi) to transform humic substances as primary organic material using spectral methods.

The strains of three soil microscopic fungi species were used in this study: *Alternaria alternata*, *Cladosporium cladosporioides* and *Trichoderma harzianum.* The strains were kindly provided by O. E. Marfenina and A. E. Ivanova from the collection of the Soil Biology Department at Soil Science Faculty, Lomonosov Moscow State University. The fungi strains were grown in Czapek liqiud medium (CzLM) (pH 7.0 or 7.2) with and without addition of lingohumate (HS - humic substances) in concentration 0.2 g/L. The fungi strains were incubated at 25°C in the dark with constant shaking for 14 d. Fungi culture fluid was filtered through a filter with pore size of 0.2 μm before the measurement of the spectra. Absorption spectra were measured with a Unico spectrophotometer in the UV and visible spectral ranges. Fluorescence emission spectra were measured with a luminescence spectrometer Solar CM 2203 under excitation at 270, 310 and 355 nm for the investigated samples diluted in 10 times.

The absorption spectra of HS 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 approximately assess the degree of degradation of HS by micromycetes using the reduction of absorbances at a wavelength of 260 nm. The data presented in Table 1 demonstrate that the strain *A. alternata* was a more active destructor in comparison with the strains *T. harzianum* and *C. cladosporioides.* The fungi culture fluid showed specific absorption spectra with a maximum at about 289-290 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 (aromatic amino acids, a wide spectrum of other cyclic compounds, etc. (2,3), as well as the transformation products of HS. This way we can approximately assess a physiological response of the micromycetes to the presence of an additional source of organic carbon in their nutrient medium.

We can additionally assess the transformation of HS during the process of micromycete growth by fluorescence spectra observing the spectral band shape, wavelength of the emission maximum and the fluorescence intensity. The data presented on fig. 1 allow us to speak about the occurring transformation of HS in the process of growth of the strain *A. alternata.* It is important to say that correct conclusions can be formulated only based on combined data on the absorption and the fluorescence of the samples, as well as taking into account the conditions of micromycete growth.

Table 1: Absorbances measured against distilled water.

Wavelength, nm	260 nm	289 nm
distilled water	0.04896	0.04493
without dilution		
CzLM	0.20337	0.59281
CzLM+0.2g/L HS	3.11447	3.06349
A.alternata on CzLM	0.74401	0.50936
A.alternata on CzLM+0.2g/L HS	0.74377	0.60691
with dilution		
CzLM+0.2g/L HS	0.20337	0.19135
T.harzianum on CzLM	0.15018	0.15931
T.harzianum on CzLM+0.2g/L HS	0.16417	0.15337
C.cladosporioides on CzLM	0.11681	0.09645
C.cladosporioides on CzLM+0.2g/L HS	0.15900	0.12486

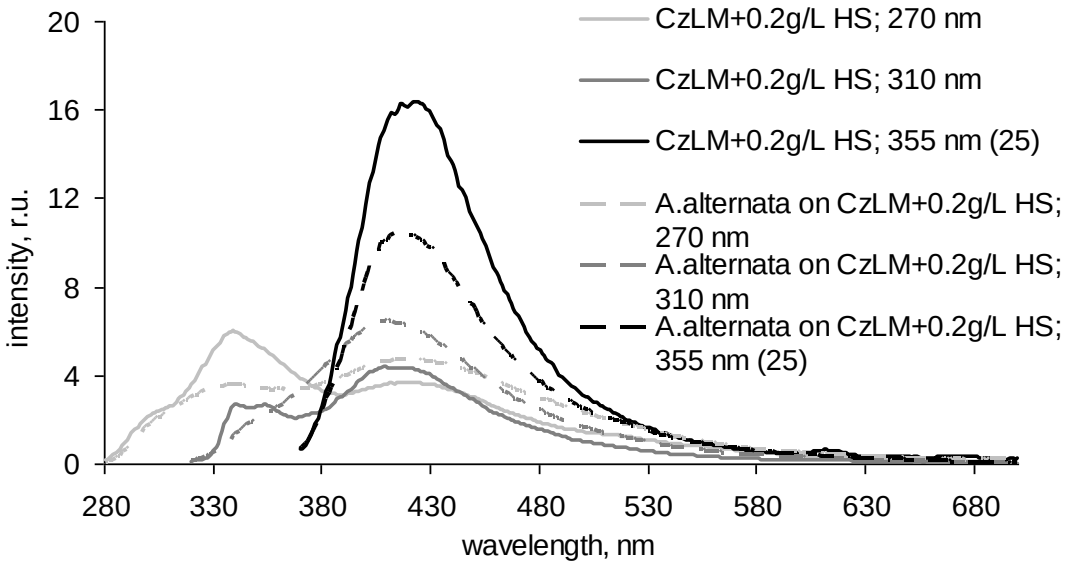

Figure 1: Fluorescence spectra of CzLM with addition of HS and filtered fungi culture fluid for A. alternata grown with addition of HS (sucrose content in CzLM – 3 g/L)

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