Fractionation of humic acids on bacterial surfaces

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Humic substances (HSs) actively adsorb toxicants from solutions (Perminova et al., 2006). HSs can be adsorbed on the cell bacterium surface and minerals for this reason they are decrease impact and bioavailability of different substances in the solutions. HSs are adsorbed on the cell surfaces of microorganisms (Fein et al.,1999, Tikhonov et al., 2013) and minerals (Zhou et al., 2001), reducing the effects and bioavailability of various substances in solutions. It has been shown that under adsorption of HSs on goethite fractionation occurs. HSs of low molecular weight were adsorbed as the first components, which were quickly replaced by high molecular weight fractions (Zhou et al., 2001). HSs with the high molecular weight were sorbed onto *Bacillus subtilis* predominantly (Maurice et al., 2004). There is little information about adsorption onto bacterial cells, collected from the different habitat. The aim of the study was to research the fractionation of HSs on bacterial cells isolated from natural waters.

Materials and Methods: We used humic acids (HAs) Aldrich (cas 1415-93-6), which were dissolved in 0,1 M phosphate-buffered saline (pH 7). Water samples were collected from the natural water of bog, stream and Mezha river in the Central Forest State Natural Biospheric Reserve (Tver region, Russia). The site of sampling is pristine in terms of local pollution. Bacteria were cultured on nutrient agar (Himedia, M001) and were identified as *Pseudomonas* sp., *Bacillus amyloliquefaciens*, *Pseudomonas* sp. and *Paenicbacillus* sp. according 16S rRNA gene sequence. Sorption of HSs onto bacteria were provided according methods (Tikhonov et al., 2013). The molecular mass of HSs was determined by size-exclusion chromatography (Ilina et al., 2014).

Result and discussion: Bacteria sorbed 25 – 150 mg HAs per gram of dry biomass bacteria (figure 1). The most sorbed culture was *Paenibacillus* sp., forming a bacterial capsule. Earlier it was shown the role of bacterial capsules in increasing of adsorption heavy metals (Drozdova et al., 2015) and other substances. HSs may be considered as biofilms, so presence of capsules can inductive changes of initial biofilms, creating the new properties, for example resistance to UV-radiation or bacteriophages (Tikhonov, 2017). During sorption of HAs onto bacterial surface there were their fractionation (figure 2). All the strain sorbed HAs with high molecular weight 6000 – 10000 predominantly. The similar results were obtained in study with fulvic acids (Maurice, 2004). In our opinion predominant sorbed HAs with high molecular weight onto bacterial cells are the universal phenomena in an environment, which create the additional properties from outer disruptors.

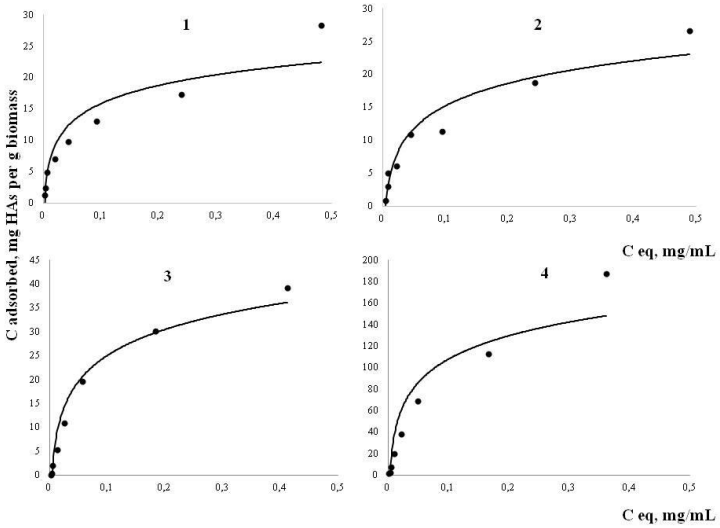

Figure 1: Adsorption isotherms of HAs for *Pseudomonas* sp. (1), *Bacillus* sp. (2), *Pseudomonas* sp. (3) and *Paenibacillus* sp.

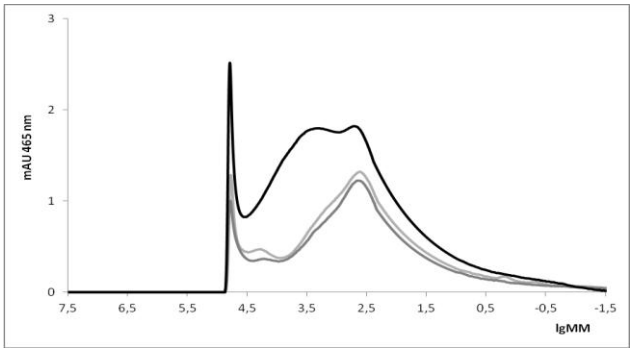

Figure 2: Chromatogram of the initial HAs (black) and after sorption with *Paenibacillus* sp. (dark grey) and *Pseudomonas* sp. (light grey)

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