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Oxidized humic acids from the soil of heat power plant

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Abstract: Humic acids isolated from the soil of heat power plant (HA-E) contaminated with oil were analyzed by the Fourier Transform Infrared spectra (FTIR). In comparison with humic acids standard (HA-S) lack of intense broad band of stretching vibrations of hydrogen-bonded hydroxyl groups (3600-3200 cm⁻¹) is evident. HA-E have a peak at 1649 cm⁻¹ which could belong to carbonyl groups. HA-E are heavily oxidized and among isolated microorganisms Achromobacter denitrificans may be responsible for such intensive oxidation of HA-E. To phylogenetically diverse nitrate-reducing microorganisms that have the capacity to utilize reduced HA as electron donors in soils, we can add A. denitrificans.

Keywords: humic acid; microorganism; FTIR; contaminated soil

INTRODUCTION

Structurally diverse, humic acids (HA) contain numerous functional moieties, including carboxylic acid, ketone, quinone, and phenolic/alcoholic hydroxyl groups. HA may be of particular relevance to inherent soil denitrification. HA are recalcitrant and degrade relatively slowly. However, reduced redox-active functional groups within HA can be readily oxidized as electron donors for bacterial respiration. The hydroquinone content of reduced HA are considered important humus-borne electron donors of this type, and microbial oxidation of hydroquinones to corresponding quinones has been demonstrated to support nitrate, perchlorate, arsenate, and selenate reduction. Although nitrate-dependent humic acid-oxidizing bacteria (NHOx) are common in the environment, their prevalence and metabolic activity in soils are still not well known.

We decided to isolate HA-E to determine number of presented microorganisms by serial dilutions method on agar plates, to identified microorganisms and to compare FTIR spectra of humic acid standard with FTIR spectra of humic acid isolated from samples of soil contaminated with oil from soil of heat power plant.

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EXPERIMENTAL

The number of microorganisms

We determined the number of presented microorganisms by serial dilutions method on agar plates at 28 °C. We used several types of media:

- nutrient agar for total chemoorganoheterotrophs (NA)
- malt agar for yeasts and molds (SA),
- mineral base medium for hydrocarbon degraders (UG) with D2 diesel fuel
- nutrient agar for anaerobes (HAG)

Identification of microorganisms using API tests

Analytical profile index (API) tests were realized by the „BioMerieux Industry“ manual. We used API 20 NE, Rapid 20E and API Coryne systems for detection of microorganisms.

Isolation of humic acids

Following standard procedure, the humic acids isolated from soil contaminated with oil from soil of heat power plant, was conducted with an alkaline sodium pyrophosphate solution (ISO 5073:1999). HA-E was precipitated with hydrochloric acid.

FTIR

FTIR spectra were obtained in solid state using attenuated total reflectance – ATR sampling technique on Thermo-Nicolet 6700 (Thermo Fisher Scientific, USA) spectrophotometer at wave numbers between 400 and 4000 cm⁻¹ and spectral resolution of 4.0 cm⁻¹ in transmission mode. Spectra were analysed using Omnic 7.3 software.

RESULTS AND DISCUSSION

The number of microorganisms

The obtained results (Table 1) show that number of UG microorganisms is high in comparison with other types of microorganisms, which can indicate high level of oil pollution in soil.

<table>
<thead>
<tr>
<th>Media</th>
<th>Number of microorganisms, CFU g⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA</td>
<td>4.3×10⁶</td>
</tr>
<tr>
<td>SA</td>
<td>2.6×10³</td>
</tr>
<tr>
<td>UG</td>
<td>3.5×10⁴</td>
</tr>
<tr>
<td>HAG</td>
<td>1.8×10⁷</td>
</tr>
</tbody>
</table>

Identification of microorganisms using API tests

We identified several microorganisms, such as Pseudomonas putida, Pseudomonas aeruginosa, Achromobacter denitrificans, Pseudomonas sp., Bacillus cereus, Rhodococcus sp., Aeromonas hydrophila, etc.

FTIR spectra

In FTIR spectra of HA-S, intense broad band of stretching vibrations of hydrogen bonded hydroxyl groups (3600-3200 cm⁻¹) can be observed (Fig. 1). Standard has a very strong band at 1737 cm⁻¹ that is assigned to C=O stretching
of COOH group and band at 1229 cm\(^{-1}\) that corresponds to in-plane bending vibrations of OH groups from COOH (Fig. 1).

![FTIR of HA-S](image)

Extracted humic acids (HA-E) luck in OH groups and in aliphatic chains than standard HA, because pick is absent in the 3000-3700 cm\(^{-1}\) and 2920-2850 cm\(^{-1}\) areas. Pick at 1649 cm\(^{-1}\) could belong to carbonil group (Fig. 2).

We have previous reported that HA promote zymogenous microbial consortium growth\(^{10}\). HA influences microbial consortium growth via protection from oxidative stress, and this mechanism is highly significant in oxidative conditions (i.e. the presence of free iron). We indentified A. denitrificans from samples of soil contaminated with oil from soil of heat power plant. It was shown that A. denitrificans can induce biodegradation of sulfamethoxazole in a bacterial consortium and Leucobacter sp. GP\(^{11}\). A. denitrificans efficiently utilizes 16 phthalate diesters and their downstream products through protocatechuate 3,4-cleavage pathway which makes A. denitrificans SP1 a very attractive candidate to be employed as an efficient biofactory in waste water treatment processes\(^{12}\). A. denitrificans strain SP1 efficiently remediates di(2-ethylhexyl) phthalate\(^{13}\).

Nitrate-dependent HA oxidizing organisms isolated from agricultural soils were phylogenetically diverse and included members of the Alphaproteobacteria, Betaproteobacteria and Gammaproteobacteria\(^{14}\).
CONCLUSION

Our present results indicate that HA from samples of soil contaminated with oil from soil of heat power plant are oxidatively degraded mainly by Achromobacter denitrificans. When we compared isolated HA with Aldrich humic acid sodium salt (H16752) as standard, there were differences which may offer a new promising improvement in cleaning technologies in all environments, especially for soil (bioremediation) and high levels of pollution in water and air.

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ИЗВОД

ОКСИДОВАНЕ ХУМИНСКЕ КИСЕЛИНЕ ИЗ ТОПЛАНЕ

Хуминске киселине, изоловане из земљишта контаминираног нафтом из топлане (HA-E) је анализирано уз помоћ инфрацрвеног спектрометра (FTIR). У поређењу са стандартом хуминских киселина (HA-S) уочљив је недостатак интензивног пика вибрација водоничке везе из хидоксилне групе (3600-3200 cm⁻¹). HA-E има пик на 1649 cm⁻¹ који вероватно припада карбонилној групи. HA-E су веома оксидоване, а претпоставља се да су микроорганизми Achromobacter denitrificans за ову интензивну оксидацију HA-E. Они су
филогенетски различити нитрат-редуктујући микроорганизми који имају капацитет да редукшу хуминске киселине где се понашају као електрон донори у земљишту.

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