



*J. Serb. Chem. Soc.* 85 (3) 421–426 (2020)  
JSCS–5311

SHORT COMMUNICATION

**Oxidized humic acids from the soil of heat power plant**

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(Received 27 July, revised and accepted 9 September 2019)

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

**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.<sup>1</sup> 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.<sup>1,2</sup> The hydroquinone content of reduced HA are considered important humus-borne electron donors of this type, and microbial oxidation of hydroquinones to the corresponding quinones has been demonstrated to support nitrate, perchlorate, arsenate, and selenate reduction.<sup>1–7</sup> Although nitrate-dependent humic acid-oxidizing bacteria (NHOx) are common in the environment,<sup>2</sup> their prevalence and metabolic activity in soils are still not well known.

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<https://doi.org/10.2298/JSC190726099M>

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

## EXPERIMENTAL

### *The number of microorganisms*

The number of microorganisms present was determined by the serial dilutions method on agar plates at 28 °C. Several types of media were used:

- nutrient agar for total chemoorganoheterotrophs (NA),
- malt agar for yeasts and molds (SA),
- mineral base medium for hydrocarbon degraders (UG)<sup>8</sup> with D2 diesel fuel<sup>9</sup> and
- nutrient agar for anaerobes (HAG).

### *Identification of microorganisms using API tests*

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

### *Isolation of humic acids*

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

### *FTIR*

FTIR spectra were obtained in solid state using the attenuated total reflectance (ATR) sampling technique on Thermo-Nicolet 6700 spectrophotometer (Thermo Fisher Scientific, USA) at wave numbers between 400 and 4000 cm<sup>-1</sup> with a spectral resolution of 4.0 cm<sup>-1</sup> in the transmission mode. The spectra were analyzed using Omnic 7.3 software.

## RESULTS AND DISCUSSION

### *The number of microorganisms*

The obtained results (Table I) show that the number of UG microorganisms was high in comparison with other types of microorganisms, which could indicate a high level of oil pollution of the soil.

TABLE I. Number of microorganisms

Media	Number of microorganisms, CFU g <sup>-1</sup>
NA	4.3×10 <sup>6</sup>
SA	2.6×10 <sup>3</sup>
UG	3.5×10 <sup>4</sup>
HAG	1.8×10 <sup>2</sup>

### Identification of microorganisms using API tests

Several microorganisms, such as *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Achromobacter denitrificans*, *Pseudomonas* sp., *Bacillus cereus*, *Rhodococcus* sp., *Aeromonas hydrophila*, etc., were identified.

### FTIR spectra

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

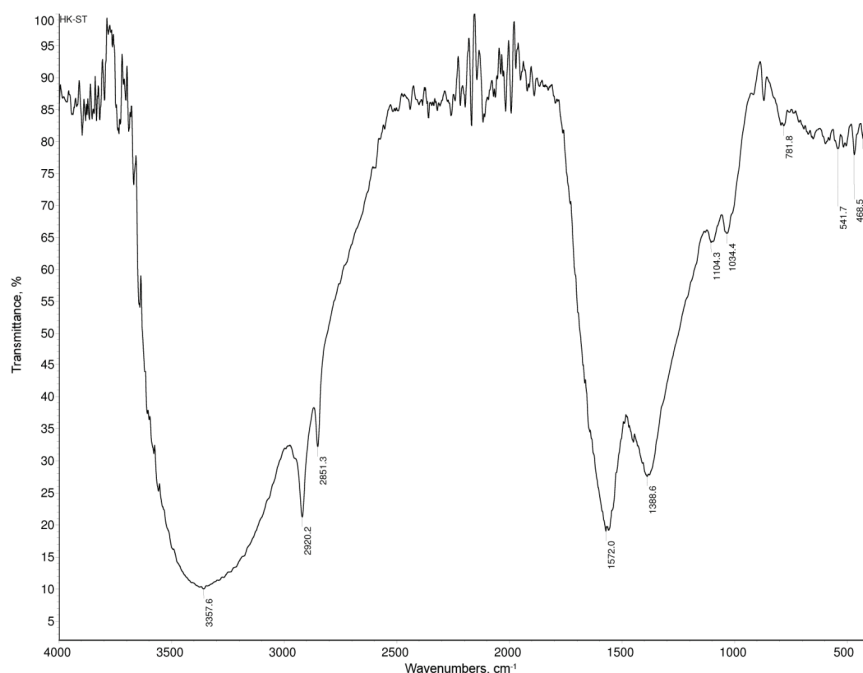


Fig. 1. FTIR of HA-S.

Extracted humic acids (HA-E) lack OH groups and aliphatic chains compared to the standard HA, because peaks is absent in the  $3000\text{--}3700\text{ cm}^{-1}$  and  $2920\text{--}2850\text{ cm}^{-1}$  regions are absent. The peak at  $1649\text{ cm}^{-1}$  could belong to carbonyl group (Fig. 2).

It was previously reported that HA promote zymogenous microbial consortium growth.<sup>10</sup> HA influences microbial consortium growth *via* protection from oxidative stress, and this mechanism is highly significant in oxidative conditions (*i.e.*, in the presence of free iron). *A. denitrificans* was identified from samples of

soil contaminated with oil from the heat power plant. It was shown that *A. denitrificans* can induce biodegradation of sulfamethoxazole in a bacterial consortium and *Leucobacter sp.* GP.<sup>11</sup> *A. denitrificans* efficiently utilizes 16 phthalate diesters and their downstream products through the 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.<sup>12</sup> *A. denitrificans* strain SP1 efficiently remediates di(2-ethylhexyl) phthalate.<sup>13</sup>

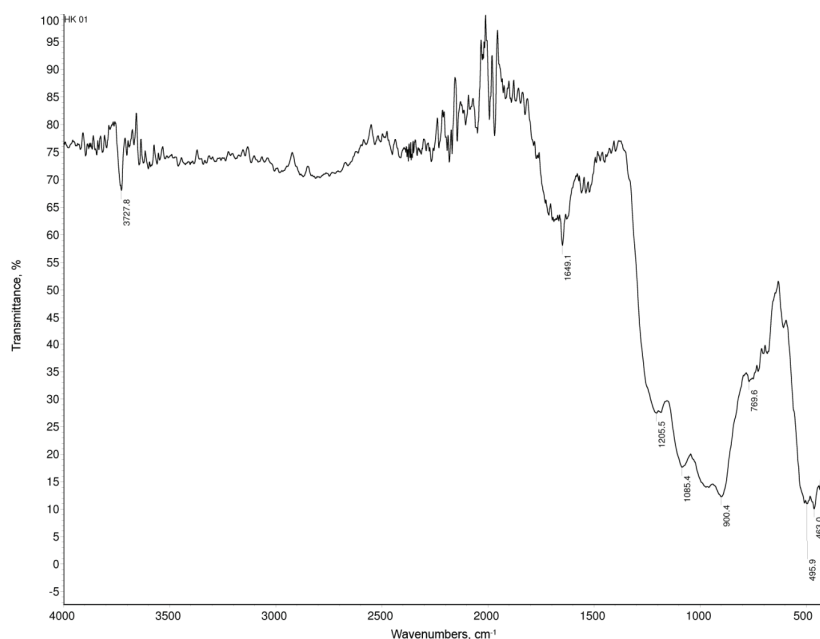


Fig. 2. FTIR spectrum of HA-E.

Nitrate-dependent HA oxidizing organisms isolated from agricultural soils were phylogenetically diverse and included members of the *Alphaproteobacteria*, *Betaproteobacteria* and *Gammaproteobacteria*.<sup>14</sup>

#### CONCLUSIONS

The present results indicate that HA from samples of soil contaminated with oil from a heat power plant are oxidatively degraded mainly by *Achromobacter denitrificans*. When the isolated HA was compared with Aldrich humic acid sodium salt (H16752) as standard, there were differences that 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.

*Acknowledgment.* This research is a part of Project III 43004 funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia. The authors have declared no conflict of interest.

## ИЗВОД

## ОКСИДОВАНЕ ХУМИНСКЕ КИСЕЛИНЕ ИЗ ТОПЛАНЕ

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Хуминске киселине, изоловане из земљишта контаминираног нафтом из топлане (НА-Е) је анализирано уз помоћ инфрацрвеног спектрометра (FTIR). У поређењу са стандардом хуминских киселина (НА-S) уочљив је недостатак интензивног пика вибрација водоничне везе из хидоксилне групе (3600–3200 cm<sup>-1</sup>). НА-Е има пик на 1649 cm<sup>-1</sup> који вероватно припада карбонилној групи. НА-Е су веома оксидоване, а претпоставља се да су микроорганизми *Achromobacter denitrificans* за ову интензивну оксидацију НА-Е. Они су филогенетски различити нитрат-редукујући микроорганизми који имају капацитет да редукују хуминске киселине где се понашају као електрон донори у земљишту.

(Примљено 27. јула, ревидирано и прихваћено 9. септембра 2019)

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