

Assessment of the Possibility of Humic Acid Extraction from Vermicompost with Urea

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Abstract The aim of this study was to introduce new possible extractants for humic acid and determination of qualitative and quantitative characteristics of humic acid that were extracted with the new extractants. In the present study, vermicompost, a plant growth promoting biofertilizer containing many humic substances, was used as initial substrate for humic acid extraction. In order to determine the optimal concentration, urea was used in three concentration levels (0.1, 0.5 and 1 mol L⁻¹). In this regard, urea 0.1 M was incapable of extracting humic acid within 24 h, and the maximum amount of humic acid was extracted with 0.5 M urea. Thereafter, to ensure the nature of humic acid extracted with urea and its quantitative characteristics, indices such as elemental analysis, acidic functional groups content and total hydroxyls content were determined and additional spectrophotometric measurements carried out as well. Further research could be conducted with these extractants to determine the nature of extracted humic acid. Finally, more research on the use of urea as a new extractant is recommended.

Keywords Functional groups • Humic acid • Vermicompost • Urea • Ratios of E_4/E_6 and E_3/E_5

Introduction

During the process of vermicomposting, a large part of the organic matter is mineralized to carbon dioxide, ammonia and water, and the remaining portion is transformed into stabilized, mature organic matter that chemically resembles indigenous soil humic substances (HS) (Benitez et al. 1999). The highest humic acid

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(HA) yields are often obtained by the classical isolation method including aqueous alkaline solution extraction after a pretreatment with dilute aqueous acid. However, basic extractant solutions may produce HS and thus HA oxidation, hydrolysis and cleavage reactions; these kinds of transformations may be different according to the source of HA, the isolation conditions and the variation in the extraction time. The extraction with alkaline solution has to be carried out under nitrogen atmosphere; thus, the damage produced by the oxidation can be suppressed or minimized, but the extraction method remains too harsh. At the same extraction time using mild extractants such as sodium pyrophosphate and cation exchange resins, the HS yields are significantly lower than with alkaline solutions. The diverse solubility of HS in the different extractants can result from the differences in the chemical structure of the macromolecules or the physical protection of the organic macromolecules which are complexed with the mineral fraction through the cation bridges (Stevenson 1994). The combinations of different extraction methods and various fractionations processes provide more accurate information about humic substance and their fractions which can have different properties (Nifant'eva et al. 1994).

Urea is fully water soluble and makes alkaline solutions with pH above 8.3. Therefore, in this study, the possibility of HA extraction using urea to create alkaline conditions was evaluated.

Materials and Methods

The organic material which used for humic acid isolation was vermicompost that obtained from cow manure and crop residues in a ratio of 50–50%. The samples of vermicompost were mixed with 0.1, 0.5 and 1 mol L⁻¹ urea under N₂ at a ratio of 1/10 (w/v) (vermicompost/extractants). The treated slurry was left to stand for 24 h in the dark at room temperature; then the supernatant was separated by centrifugation at 10,000 rpm. The suspension obtained after filtration was diluted with distilled water and precipitated slowly at a pH of 1.17–1.50 with HCl. The precipitate was purified with HCl/HF 0.1/0.3 mol L⁻¹ and washed with distilled water until negative AgCl test was obtained, then finally dried at temperatures below 50°C. Extraction of humic acid was conducted in five replications.

The elemental composition was determined by an Elementar Analysen System GmbH Vario EL. Acidic functional groups were determined according to conventional methods described by Page (1982). E_3/E_5 and E_4/E_6 ratios were determined according to conventional methods described by Campitelli et al. (2006) and Abbt-Braun and Frimmel (1999). All of these analyses were carried out in three replications.

Results and Discussion

Urea had not been used as an extractant in previous techniques, so three different concentrations (0.1, 0.5 and 1 mol L⁻¹) were used to make alkaline conditions. Among the concentrations of urea used, 0.5 mol L⁻¹ urea had better results (Table 1).

The humic acid isolated with urea (HA-urea) has higher N content probably due to the nitrogen in the extractant solution (Table 2). In general, there was a decrease in C/N ratio with increasing saturation with N (urea). This decrease in C/N was attributed to an increase of N in the humic structure, rather than C removed from the humic structure, because there was no decrease in dissolved organic carbon (DOC) values for the samples with increased exposure to N (urea). The H/C ratio represents the degree of the HA unsaturation (Campitelli et al. 2006; Stevenson 1994). This ratio for HA-urea 0.5 M is higher than that obtained for HA-urea 1 M, indicating that HA-urea 0.5 M has a great degree of saturation, probably due to the different acid–base characteristics of the urea solution or the different kind of reactions that could take place with the macromolecule.

The acidic functional groups' content (total acidity, carboxylic and phenolic-OH groups) of the HA-urea, specifically the phenolic-OH, were high which is in agreement with the higher H content for HA-urea and the probable disaggregation produced by the urea solution (Table 3).

The E_4/E_6 ratio is related to the degree of condensation of the aromatic C network (Campitelli et al. 2006), and the E_3/E_5 ratio changes inversely with the

Table 1 The yield of humic acid extracted from vermicompost

Extraction [with]	HA yield (%)
Urea 0.1 M	0.22
Urea 0.5 M	1
Urea 1 M	0.7

Table 2 Elemental composition (ash and moisture-free basis), H/C and C/N ratios of the HA studied

HA sample	C (%)	H (%)	N (%)	S (%)	O (%)	Ash (%)	H/C	C/N
HA-urea 0.5 M	53.1	4.9	5.1	0.4	36.5	1.7	1.12	6.41
HA-urea 1 M	53.3	4.3	5.9	0.5	36	1.8	1.00	6.12

Table 3 Acidic functional groups contents (mmol g⁻¹), E_3/E_5 and E_4/E_6 ratios of the studied HA

HA sample	Total acidity	Carboxylic groups	Phenolic-OH group	Total hydroxyls	E_3/E_5	E_4/E_6
HA-urea 0.5 M	7.63	3.89	3.74	2.9	5.5	5.2
HA-urea 1 M	7.75	4.14	3.61	2.74	5.7	5.2

mean molecular size (Lguiratia et al. 2005). According to the results of this study, HA-urea had higher E_3/E_5 and E_4/E_6 ratios (Table 3). These results are probably due to the effect of extractant type on the characteristics of extracted humic acid from vermicompost. Interactions between urea and HA produce the disaggregation of the macromolecules and generate the small size and more aliphatic structures, while interactions between NaOH and HA can produce aggregation of the macromolecules and thus the greater size and more aromatic structures.

Conclusions

Urea solutions have the ability to extract humic acid. A solution of 0.5 M urea had the maximum yield of extraction. Among the measured characteristics, extracted humic acid using urea had more functional groups, aliphatic structures and greater degree of saturation. On the other hand, humic acid extracted with urea had smaller size, but in general, there were no large differences between the characteristics of the extracted humic acid using various concentrations of urea. Finally, more research on urea as a new extractant is recommended.

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