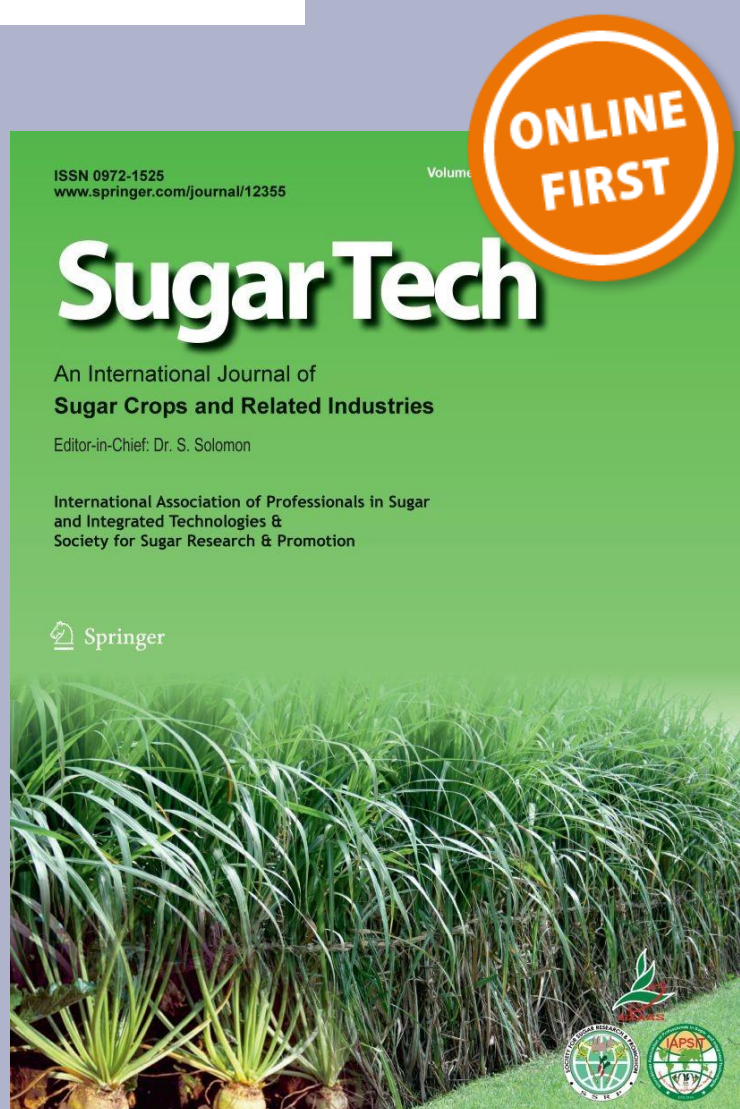*Chemical and Spectroscopic Characterization of Humic Acids Extracted from Filter Cake using Different Basic Solutions*

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RESEARCH ARTICLE

Chemical and Spectroscopic Characterization of Humic Acids Extracted from Filter Cake using Different Basic Solutions

Hamid Reza Behravan[1] · Paul Voroney[2] · Reza Khorassani[1] · Amir Fotovat[1] · Abdol Amir Moezei[3] · Mehdi Taghavi[4]

Abstract Filter cake is a valuable by-product generated during the process of making sugar from sugarcane in factories. The purpose of this experiment was to study the effect of different basic solutions on humic acid extraction rate. Chemical and spectroscopic characterization of humic acids extracted from filter cake has also been investigated. In this research, extraction and chemistry of humic acid have been studied in two separate experiments. In the first experiment, humic acid was taken from filter cake using the IHSS reference method in three concentrations (0.5 M, 1 M and 2 M) of KOH and two preparation liquids (water and vinasses) in the laboratory of the School of Environ- mental Sciences at the University of Guelph. Changes in the extraction rate of humic acid with type and concen- tration of base solution also were studied. In the second experiment, Fourier transform infrared (FTIR) and ^{13}C NMR were used to examine the chemistry of humic acid extracted from filter cake by 0.5 M KOH solutions (pre- pared using distilled water and vinasse). The results indi- cate that the highest extraction rate of humic acid was obtained when the 2 M KOH made from vinasse solution was used. Elemental analysis results, FTIR and NMR analyses as well as the E4/E6 ratio showed that all samples of extracted humic acid had similar chemical characteris- tics compared to the reference humic acid.

Keywords Filter cake · Humic acid · Sugarcane · Vinasse

Introduction

Humic acids (HAs) are organic compounds that can improve the physical, chemical (Khaled and Fawy 2011) and biological (Tikhonov et al. 2010) properties of soil. In addition, it is thought that they play a role in improving plant growth and crop yield. They also increase plant ele- mental uptake and increase the length and volume of roots, especially the root hairs of several plants (Canellas and Olivares 2014). HA increases plant uptake of nutrients such as phosphorus because of its effect on the growth and development of the root (Zandonadi et al. 2010; Canellas et al. 2002; Marques et al. 2008). Therefore, it can be considered as an effective element in improving the growth and yield of plants (Govindasmy and Chandrasekaran 1992). One of the most important effects of HA is improvement in elemental uptake through the spread of roots as root hairs (Canellas and Olivares 2014). Among other significant effects of HA on improving the elemental mobility and uptake of nutrients are an increase in the microbial population of soil (Puglisi et al. 2008, 2009) and the release of organic acids in the plant's rhizosphere (Piccolo 2002; Šmejkalová and Piccolo 2008). In addition to extracting HA, various fractionations have also been taken into consideration. In this connection, Pramanik and Kim (2014) suggested the ethanol method. This method relies on the polarity changes and the dielectric point of solution using different amounts of ethanol and HA.

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Humic acid can be extracted from various sources which are rich in organic matter such as composts, coal and soils (Enev et al. 2014). The extraction of HA from sugarcane by-products such as filter cake has also been considered in recent years (Busato et al. 2010). Filter cake is generated during the process of making sugar from sugarcane after purifying the syrup and collecting the remaining impurities through filters. Because of its high content of organic matter and nutrients, filter cake can be used as soil amendment in sugarcane fields and other cropping systems (Busato et al. 2010). In recent years, the use of compost, filter cake and other organic compounds has been consid- ered worldwide in an attempt to improve the organic matter conditions (Demattê et al. 2005).

The extraction of HA can be carried out using various extraction methods and extractants. Although several different basic solutions prepared from NaOH, KOH and Na pyrophosphate have been suggested by various researchers for the extraction of HA, the standard method is to use 0, 5 M NaOH and 6 M HCl, which is widely recommended (Swift 1996).

According to Hidalgo (2009), vinasse is a by-product of the sugar and ethanol industry. Once the main products (alcohol, ascorbic acid, etc.) are produced, the remaining stuff is called vinasse. Due to its high levels of nutrients (Prado et al. 2013), especially potassium, vinasse can be added to irrigation water as a soil amendment (Anonymous 2013). Vinasses can be used as a source for the extraction of HA, and in this study, it was used as a way to improve the HA extraction rate. There is no reference related to this, but because of the valuable characteristics of vinasse, there has been a lot of research regarding its elemental analysis and application as soil organic amendment (Perdue 2013). Researchers have considered increasing the extraction rate of HAs from different sources by creating changes in alkaline solutions, centrifuge time and temperature (Asing et al. 2009).

Stevenson (1994) has emphasized the importance of soil organic matters fractionation as a basic step to characterize them. Lamar and Talbot (2009) studied three HA extraction methods including the California Department of Food and Agriculture method (CDFA), the colorimetric method and the standard method (Swift 1996). In the current study, extraction of HA from filter cake and using vinasse as a substance to increase the extraction rate of HA have been investigated. The chemistry of HAs extracted from filter cake (with and without using vinasse) was also studied, and their characteristics were compared with the standard HA. Reference HA or standard HA samples are extracted from some organic materials such as leonardite, according to the standard method (Swift 1996).

Materials and Methods

The efficiency of extraction and the chemistry of the HAs extracted from filter cake were studied in two separate experiments as follows.

First Experiment

The first study was carried out on samples of filter cake and vinasse which had been collected from Hakim Farabi Sugar Factory and the alcohol production factory located in Khuzestan Province, Iran. Their chemical characteristics are shown in Table 1. The characteristics of filter cake and vinasse were determined by conducting the routine meth- ods. pH of filter cake and vinasse was measured with pH meter (WTW inoLab, Germany) in a filter cake: water ratio of 1:2 and in vinasse liquid sample. Organic carbon in both of them was determined using the method described by Nelson and Sommers (1996). Nitrogen was measured using the Kjeldahl method (Bremner, 1996). Phosphorus in filter cake was taken by Olsen et al.'s method (Olsen et al. 1954), and phosphorus in vinasse was measured using Murphy and Riley's method (1962). Exchangeable potas- sium in filter cake was determined by ammonium acetate method (Helmke and Sparks 1996). Potassium in the liquid sample of vinasse was measured by flame photometry (BWB Technologies Ltd., UK).

Two parameters affecting HA extraction efficiency, i.e., alkali type and solution concentration, were evaluated and performed in three replications. The extraction method described by Swift (1996) was used. Treatments included 0.5, 1 and 2 M KOH prepared using nano-pure water and vinasse solution instead of water in identical concentra- tions. The ratio of extractant volume to filter cake weight was 10:1 (50 ml:5 g).

The extraction of HA was performed under a nitrogen gas atmosphere contained in a basic glove box. After 24 h of shaking, the samples were centrifuged at 5000 rpm for 15 min, the supernatant was collected and 6 M HCl was added to lower the pH to 1.5 to precipitate the HA. The precipitate was purified by being washed twice with HCl ? HF (5 ml concentrated HCl ? 5 ml concentrated HF, in 1L nano-pure water) solution and once with nano- pure water. The ratio of solution to extracted HA was 10:1. Then, the samples were dialyzed using a cutoff membrane. In order to determine the content of pure humic acid in different treatments, the samples were frozen at − 80 °C and then freeze-dried. Extractant pH was measured at all stages of the extraction of HA in alkaline and acidic solutions. The dried HA was weighed to determine the extraction efficiency.

Table 1 Chemical characteristic of filter cake and vinasse

	pH	N	P	K	OC
Filter cake	5.31	1.16 (%)	0.95 (%)	0.51 (%)	50.81 (%)
Vinasse	4.42	0.33(%)	90 (mg L^{-1})	33,500 (mg L^{-1})	3.83 (%)

OC organic carbon

Second Experiment

The chemistry of the HAs extracted from filter cake was investigated using a 0.5 M KOH extractant. The extraction, washing, dialysis and drying steps were performed as described in the first experiment. Functional group analysis for the extracted HAs was performed using Fourier transform infrared (FTIR) from the recorded spectra using a Bruker Tensor 27 spectrometer on KBr pellets over the range of 400–4000 cm^{-1}. Aliphatic and aromatic structures in HAs were studied using a ^{13}C NMR spectrophotometer.

All solid-state ^{13}C NMR measurements were conducted at 298 K using a Bruker Avance II spectrometer, operating at 11.74 T (13C Larmor frequency of 125 MHz). Samples were ground and packed into 4 mm zirconia rotors. The rotors were spun at the magic angle speed of 5 kHz, and 13C spectra were collected using the cross-polarization, total suppression of sidebands (CPTOSS) pulse sequence provided by Bruker. The 1H 90° pulse width was 2.95 ls. A contact time of 1 m s^{-1} and a recycle delay of 2 s were used in the experiments. For each CPTOSS spectrum, 5600 scans were collected and processed with 100 Hz line broadening. Carbon chemical shifts were referenced with respect to TMS by substitution to carbonyl peak of alpha-glycine at 176.5 ppm.

The carbon, nitrogen, hydrogen, sulfur and oxygen contents of each HA sample were measured using a Costech-ECS 4010 CHNSO elemental analyzer. The E4/E6 ratio of HAs dissolved in 0.05 M NaHCO$_3$, measured at 465 nm and 665 nm, was determined with a HACH, DR-6000 spectrophotometer. Absorbance at 400 nm and 600 nm was measured for determination of D log k (Gio-vanela et al. 2010).

Results and Discussion

The chemical properties of the extraction solutions and the recovery of HAs extracted in different treatments are shown in Table 2. The pH changes were minor in solutions prepared with water, while the solutions prepared using vinasse in concentrations of 0.5 M and 1 M had a lower pH than other treatments, both in all of the concentrations of water-based solutions and in 2 M vinasse-based solution.

Therefore, the use of vinasse instead of water at low concentrations of the extractor solution resulted in a decrease in final pH of the solution. However, at the concentration of 2 M in vinasse-based extraction solution, the reducing effect of vinasse on pH was eliminated. The results showed that the concentration of extraction solution increased the percentage of extracted HA. These increased rates for the 1 and 2 M concentrations prepared based on water, compared with 0.5 M, were 4.6% and 8%, respectively, while the increases in vinasse-based extraction were 35% and 73%, respectively (Table 2).

Preparation of extraction solution with vinasse at a concentration of 0.5 M reduced the extraction percentage compared to other treatments. Reduction in pH of the extractant may explain the decrease in the amount of extracted HA. The highest amount of extracted HA was measured in the 2 M concentration of the extraction prepared with vinasse (7.1% HA). Another reason for this increase in the vinasse-concentrated solutions (1 and 2 M) is related to the content of HA in the vinasse which was determined to be about 1% (v/v). This effect in vinasse at 2 M concentration requires more research in the future.

The results of elemental analysis of HA samples are shown in Table 3. Humic acids extracted from filter cake (0.5 M KOH prepared in water or vinasse) are compared to HAs such as the standard HA and the HA extracted from leonardite (Velasco et al. 2004; Busato et al. 2010).

These results indicate that changing the base extractant solution altered the percentage of elements N, C, O, but the percentage of hydrogen in both HA samples did not show any significant change. According to the results, the N and C in HA extracted based on vinasse solution increased by 28.76 and 4.63, respectively. Oxygen in this sample, as compared to the HA extracted with the solution based on water, decreased to 12.22 percent. The increase in these element contents could be connected to the composition of vinasse (Table 1). The fact that vinasse is rich in organic matter, particularly carbon, can explain the element increase (Anonymous 2013).

A significant decrease in oxygen can also be attributed to COD and BOD in vinasse. COD and BOD of vinasse are very high compared with water, and BOD and COD in sugarcane vinasse are usually about 39 g L^{-1} and 84 g L^{-1}, respectively. For water, these parameters are

Table 2 Efficiency of extraction of HA in different treatments of basic solution

Extractant	pH	%Extraction efficiency
0.5 M KOH	13.37	5.45
0.5 M KOH ? Vinasses	10.01	4.1
1 M KOH	13.554	5.7
1 M KOH ? Vinasses	12.46	5.55
2 M KOH	13.74	5.9
2 M KOH ? Vinasses	13.76	7.1

Table 3 Elemental analysis of HAs extracted from different sources

Humic acid	Type	C (%)	H (%)	N (%)	O (%)	C/N	H/C	O/C
Soil IHSS standard[a]	Soil	58.13	3.68	4.14	34.08	14.04	0.06	0.59
Leonardite[a]	Coal	63.81	3.70	1.23	31.27	51.88	0.06	0.49
Sample 1 (prepared in vinasse)	Filter cake	58.72	4.65	3.76	32.89	15.63	0.12	0.56
Sample 2 (prepared in water)	Filter cake	56.12	4.02	2.92	36.91	19.22	0.07	0.66

[a]Velasco et al. (2004)

usually less than 1 mg L^{-1} (Anonymous 2013). Comparisons of HAs extracted from filter cake with other HA samples, and especially the standard HA, show that these samples have good qualities. Furthermore, the sample of HA based on vinasse showed better results in terms of organic carbon, nitrogen percentage and carbon-to-nitrogen ratio when compared with HA extracted based on water.

The C/N of HA (sample 1) was 19% lower than the HA extracted based on water solution (sample 2), and it was similar to that of the standard HA. The lower ratios show the higher levels of products that can be obtained from protein degradation and thus can show a higher degree of humification. The H/C ratio in HA based on vinasse solution was more than the H/C ratio of HA based on water solution, but this ratio in sample 2 was close to the standard humic acid and leonardite. Higher H/C ratio can be attributed to higher aliphaticity and smaller aromatic structures (Da Silva et al. 2018). O/C ratio in HA based on vinasse solution was more than leonardite and close to the standard HA. The O/C is indicative of aromatic structures and increases with the degree of humification (Da Silva et al. 2018).

The results of functional group analysis by Fourier transform infrared (FTIR) are shown in Figs. 1 and 2. Both of the HA samples extracted from filter cake are similar over the absorption range, and their most absorption bands were seen at 3400 cm^{-1}, 2900–3000 cm^{-1}, 1600–1700 cm^{-1} and 1000–1100 cm^{-1}, which is consis- tent with Busato et al. (2010) and the reference HA. The
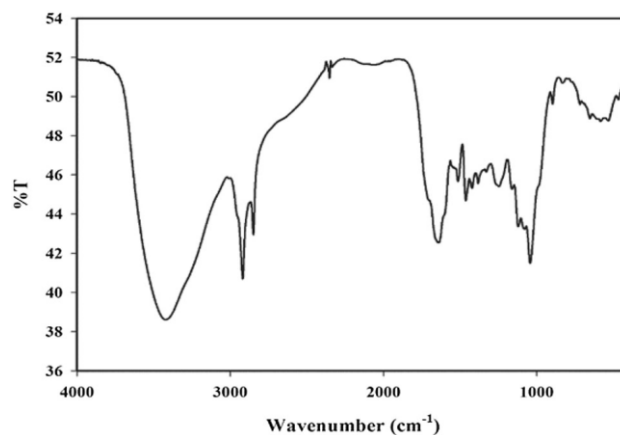

Fig. 1 FTIR spectra of HA extracted from filter cake using KOH based on water

strongest band was at 3400 cm^{-1} which is caused by stretching of alcohols, phenols and N–H stretching of amines or amides and, as suggested by Busato et al. (2010), carboxyl acids. The absorption range of 2900–3000 cm^{-1} belongs to the asymmetric and symmetric stretching of CH bonds of methyl group. The absorption band at 1600–1700 cm^{-1} is attributed to the symmetric stretching of C=O of COOH group (Busato et al. 2010). The bond at 1000–1100 cm^{-1} is due to C–O stretching of polysaccharides.

The ^{13}C NMR spectroscopy spectra were completely similar in both HA samples. Differences and similarities between them and the reference sample were also observed (Table 4). According to Zara et al. (2017), there are five
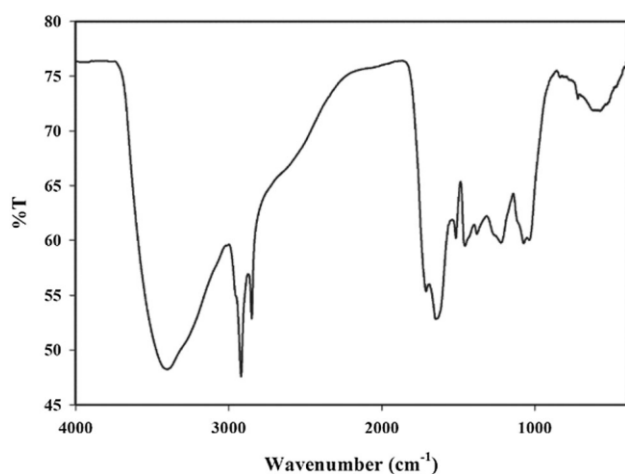
Fig. 2 FTIR spectra of HA extracted from filter cake using KOH based on vinasse

different spectral regions including 0–50 ppm, 51–110 ppm, 111–160 ppm, 161–190 ppm and 191–200 ppm for aliphatic, carbohydrate, aromatic, car- boxyl and the carbonyl groups, respectively. The most intense peaks were observed in the range of 30–32, 34–56 and 74 ppm, in the aliphatic carbohydrate region. The spectra for both the HA samples and the reference sample show resonances between 15–16 and 23–25 ppm but the HAs extracted from filter cake and the reference HA showed intense peaks at 32 ppm and 48 ppm, respectively. Peaks from 48 ppm to 23 ppm were placed in methylene group region and, in general, in aliphatic region (Zara et al. 2017). According to Al-Faiyz (2017), peaks near 15 ppm were correlated with aliphatic (CH and CH2) groups (Table 4, Figs. 3 and 4).

The most intense peaks for the HA samples were at 56 ppm to 75 ppm, and the IHSS standard had a strong peak at 66 ppm. This range corresponded to carbohydrates (Zara et al. 2017; Al-Faiyz 2017). The spectra of the filter cake HAs were similar to those of the reference HA which showed strong peaks near 105 and 107 ppm, respectively, and correlated with the anomeric carbon atoms of carbo- hydrates (Robert et al. 1998) and aliphatic carbon (Gamble et al. 1996). All three samples showed relatively good

peaks in the aromatic region 111–160 ppm. According to Gonzalez-Vila et al. (1983), peaks in the HA samples and the reference spectra at 115 ppm and 123 ppm were, respectively, correlated with aromatic C–H groups which are ortho to an aromatic C–O group. The spectra near 130 ppm in HA samples and 123 ppm in the reference corresponded to ring carbons and an alkyl aromatic carbon substituted by N or O (Al-Faiyz 2017).

In the aromatic region, all three samples showed peaks between 145 and 148 ppm that correlated with the aromatic contents of theses samples which can be related to the functional groups such as OH and NH2 (Zara et al. 2017). According to Poutanen and Morris (1985), the peaks near 155 ppm can be correlated with aromatic carbon substi- tuted by various functional groups (OH, NH2 and OCH). The reference spectrum contains an intense peak at 166 ppm but the peaks of HA samples were at 172 ppm and their peaks were weaker than those of the reference. The peaks of reference and filter cake HA samples corre- lated with carbonyls of carboxylic acids or carboxylate salts esters or peptides (Robert et al. 1998; Helal et al. 2011; Xu et al. 2017).

E4/E6 ratio

The ratio of the absorbance at 465–665 nm (E4/E6) can be connected to some structural features of HA such as the degree of aromaticity (Busato et al. 2010). The E4/E6 ratio is used as an indicator to explain the degree of humifica- tion. A low ratio can be an indicator for high degree of the condensation of aromatic constituents, and a high ratio may indicate the presence of relatively more aliphatic structures (Stevenson 1994). According to Fong et al. (2006), the E4/E6 ratio has an inverse relationship with molecular weight. The E4/E6 ratios in HAs extracted based on water and vinasse were 3.7 and 6.14, respectively, (Table 5). In the first sample, the E4/E6 was low and was consistent with the standard HA samples. The second sample had a ratio of near 6, close to the standard range, and these results are consistent with those of Busato et al. (2010). According to Velasco et al. (2004), Busato et al. (2010) and Stevenson (1994), the E4/E6 ratio has an inverse relationship with

Table 4 Major bands of ^{13}C NMR of HAs

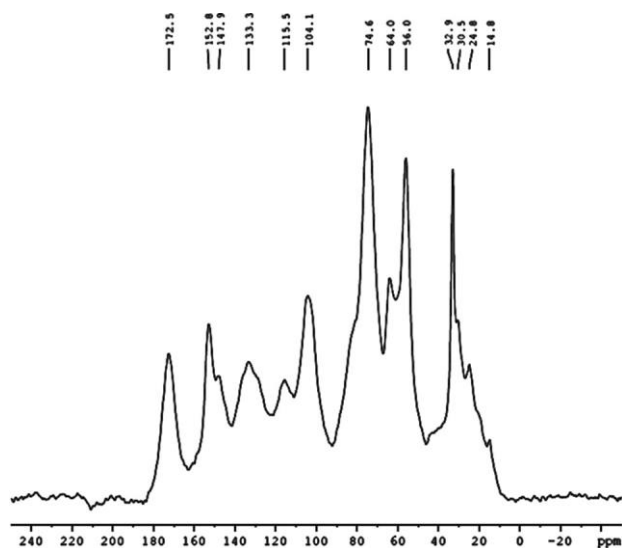| Samples (HA)	Carbonyl shift (191–220 ppm)	Carboxyl shift (161–190 ppm)	Aromatic shift (111–160 ppm)	Carbohydrate shift (51–110 ppm)	Aliphatic shift (0–50 ppm)
HA (KOH based on water)	–	172.5	115.3, 133.3, 147.9, 152.8	56.0, 64.0, 74.6, 104.2	14.8, 24.8, 30.5, 32.9
HA (KOH based on vinasse)	–	172.3	115.9, 132.0, 147.4, 152.8	55.9, 62.8, 73.5, 105.1	14.8, 24.6, 31.1, 32.9
Reference[a]	–	166.6	145.5, 123.2	66.0, 107.2	16.1, 23.2, 48.3

[a]Zara et al. (2017)

Fig. 3 ^{13}CNMR spectra of HA extracted from filter cake (based on water)
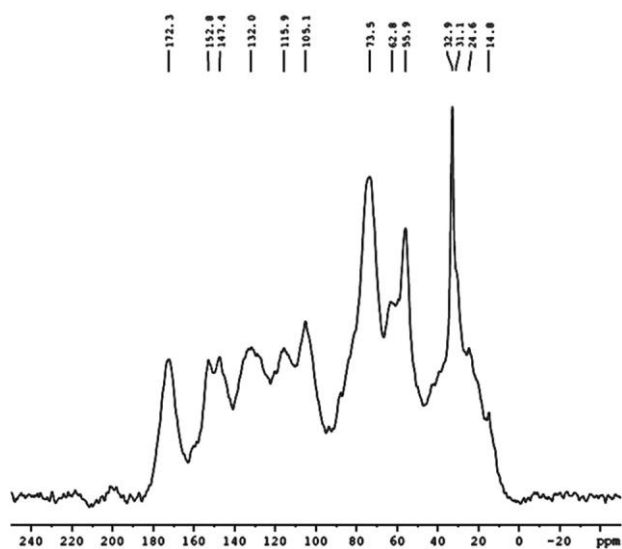

Fig. 4 ^{13}CNMR spectra of HA extracted from filter cake (based on vinasse)

humification degree and so the degree of humification in water-based HA was more than that in vinasse-based HA, HA-1 and HA-2 samples due to their low E4/E6 ratio (Table 5). The higher E4/E6 ratio in vinasse-based HA can be related to the color effect of vinasse on absorbance reading in the spectrophotometer. The HA (based on water) which has the lowest E4/E6 ratio compared with other HA samples may have a higher degree of aromatic condensa-tion and a relatively lower aliphatic structure, as suggested by Velasco et al. (2004).

The value of D log k coefficient (log absorbance at 400 nm − log absorbance at 600 nm) can be used to describe the degree of humification of organic matter (Kumada 1985). According to Fong et al. (2006), HAs based on D log k coefficient can be divided to three types of A, B and Rp, with D log k coefficient values up to 0. 6, 0.6–0.8 and 0.8–1.1, respectively. HAs extracted from filter cake based on water solution and based on vinasse solution can be placed in types B and Rp, respectively. According to Giovanela et al. (2010), the degree of condensation of HA increases by decreasing D log k values. Based on these findings, it appears that the HAs extracted from filter cake have degrees of condensation and humification which are similar to those of standard humic substances.

Conclusion

In this study, the use of filter cake as an available and inexpensive input source for the extraction of HA was examined. To increase the potential of the extraction rate of HA, the solution made from vinasse was used, with various changes in the concentration of the alkaline extraction solution. The results of this study showed that HA can be extracted from filter cake and that the use of vinasse increased HA extraction efficiency. The extracted HAs (especially the HA extracted by using basic solution based on vinasse) had similar chemical characteristics, such as elemental composition, C/N, O/C, H/C and E4/E6 ratios, as compared to the standard HA. Although this research suggests the use of filter cake as a raw ingredient for humic acid extraction, further research is required to investigate

Table 5 E4/E6 ratio and D log k in HA samples

HA sample	E4/E6	D log k
HA (based on water)	3.73	0.67
HA (based on vinasse)	6.14	0.86
HA-1[a]	7.80	0.77
HA-2[a]	5.87	0.72

[a] Giovanela et al. (2010)

the effects of the applications of the humic acid extracted from filter cake in field soils.

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