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Mechanism of pH sensitive flocculation for organic load and colour reduction in landfill leachate

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A B S T R A C T

Landfill leachate has an important fraction of humic substances, mainly humic acids (HAs), which often represent more than half value of COD, specially in liquids proceeded from composting processes of organic fraction of solid wastes. We propose in this article a new method of pH sensitive flocculation for COD and colour reduction in landfill leachate based on the chemical properties of HAs. Landfill leachate with a high content of humic acids can be efficiently treated by pH sensitive flocculation at pH 2.0, reducing COD value in 86.1% and colour in 84.7%. Mechanism of pH sensitive flocculation is based in protonation first of phenolic groups and later of carboxylic acid groups in the HAs molecules, resulting in a reduction of Zeta potential value. For pH over neutrality, carboxylic acid and phenolic groups are ionized and Zeta potential increases in absolute value, maintaining HAs in suspension as colloids and conducting flocculation to be obstructed. Ionized anionic groups (carboxylates) can interact electrostatically with cations abundant in leachate (site binding) aiding to maintain HAs in suspension. Simulation of this situation and ideal visualization of Zeta potential behavior is described in the paper and aggregation of molecules by H-bonds is proposed as the main step in separation of HAs from leachate and reduction of COD value in this complex liquid. CHNS analysis, FT-IR spectrometry and UV–VIS spectrophotometry show chemical elements content in the range of natural and commercial HAs, clear aromaticity and carboxylic acids and phenolic groups presence in the precipitate from landfill leachate.

1. Introduction

Landfill leachate is a complex liquid which is mainly formed by a mixture of metals, organic matter and other components like ammonia, sulphate and chloride (Wiszniowski et al., 2006; Renou et al., 2008). When treatment of this liquid is planned for reduction of toxicity, the nature of organic matter has to be considered in view of application of biological treatment (Zhang et al., 2007; Kheradmand et al., 2010) or combination with flocculation, reverse osmosis or chemical oxidation (Li et al., 2009; Wu et al., 2011; Mahmud et al., 2012).

Organic load in landfill leachates is very diverse and normally low organic loaded leachates (measured in COD units) are easy to be treated by biological treatment (Ding et al., 2001; Wiszniowski et al., 2006; Renou et al., 2008; Chaturvedi and Kaushal, 2018). Problems appear when organic matter is highly present in this liquid and reduction in COD is not performed (Pi et al., 2009; Cotman and Gotvajn, 2010; Martins et al., 2013). The form in which this organic matter is present is related to the origin of leachate and in highly polluted leachates is normally a consequence of composting products of organic fraction of solid wastes (Tamrat et al., 2012; Costa et al., 2018; Domínguez et al., 2019).

Actually, biological treatment combined to flocculation or oxidation processes is considered the adequate way for treatment of landfill leachates (Marañón et al., 2008; Salem et al., 2008; Castrillón et al., 2010; Chemlal et al., 2014). Flocculation appears more useful due to lower operational costs, compared to advance oxidation by ozonation or Fenton oxidation (Marañón et al., 2010; De Torres-Socías et al., 2015; Oloibiri et al., 2017). The difficulties for organic load reduction by biological treatment are logically connected with the nature and biodegradability of these organic substances (Jiang et. al., 2002).

Humic substances (HSs) are known to constitute an important fraction of organic matter in leachate (Kang et al., 2002; Sír et al., 2012). They are responsible of dark yellow and black colours of landfill leachate (Zouboulis et al., 2004; Tong et al., 2015; Oulego et al., 2016; Table 1S

Abbreviations: ABS, absorbance; AHA, Aldrich humic acid; COD, chemical oxygen demand; DOC, dissolved organic carbon; FA, fulvic acid; HA, humic acid; HS, humic substance; RMS, root mean square (molecular radius); SD, standard deviation; WTC, waste treatment centre.

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Table 1
Characterization data of landfill leachate. For nutrients analysis (N, SO_4^{2-}, PO_4^{3-} and Cl^-) 5 samples were collected from the leachate deposit in the WTC during the spring period (March-June). Metal ions were measured once (average relative standard deviation 2%). Cd^{2+} (<3 μg/L) and Hg^{2+} (<0.1 μg/L) were detected in very low concentration. Comparison of concentrations before (concentration) and after pH sensitive flocculation (final conc) at pH = 2.0 of majority metal ions in leachate in order of abundance.

Parameter	Mean ± SD	Metal ion	Concentration	Final conc
pH	7.7 ± 0.1	**Na^+**	3546.1	2423.8
Zeta potential (mV)	−16.8 ± 1.4	**K^+**	2161.9	1983.5
COD	42000 ± 2516	**Ca^{2+}**	582.0	330.3
TS	16410 ± 486	**Mg^{2+}**	162.7	109.3
VS	10076 ± 620	**P(V)**	36.3	30.4
Colour ($ABS_{474\ nm}$)	0.537 ± 0.000	**Al^{3+}**	23.1	1.7
N_{org}	3795 ± 314	**Fe^{3+}**	15.7	4.4
NH_4^+	2484 ± 340	**Zn^{2+}**	2.1	1.8
NO_3^-	417 ± 115	**Mn^{2+}**	1.9	1.4
NO_2^-	11 ± 1	**Cr^{3+}**	1.602	0.490
SO_4^{2-}	223 ± 64	**Cu^{2+}**	1.189	–
PO_4^{3-}	39 ± 18	**Ni^{2+}**	0.607	–
Cl^-	11710 ± 1978	**As^{5+}**	0.353	–
		Pb^{2+}	0.134	–

all concentrations are measured in mg/L, except pH, Zeta potential and colour.

in Supplementary material) and the characteristic odour resembling wood soil, specially present in composting leachate. HSs are considered recalcitrant compounds (Guangxia et al., 2012; Zhang et al., 2016) and normally responsible of low efficiency in COD removal when biological treatment is applied to this liquid. Generally they are abundant in landfill leachates and can represent in some cases more than 60% of organic matter (Christensen et al., 1998; Guangxia et al., 2012).

HSs are subdivided into two fractions with different pH behaviour related to solubility (Graber and Rudich, 2006). Humic acids (HAs) are insoluble at acidic conditions and fulvic acids (FAs) are soluble in the complete pH range (Christensen et al., 1998; Kang et al., 2002; Gomes de Melo et al., 2016; Dia et al., 2018). The insolubility of HAs at low pH is related to protonation of carboxylic acids and phenolic groups and Zeta potencial variation at the full pH range (Motta et. al., 2016). Protonation of carboxylic acids and phenolic groups supposes HAs to be predisposed to formation of H-bonds which can connect two or more molecules and induce flocculation. On the other hand, ionization of carboxylic acids and phenolic groups with increasing pH, increases Zeta potential in the HAs surface because positive ions are generating. Increment of Zeta potential absolute value makes HAs dispersions (colloids) stable because of intramolecular electrostatic repulsion. Carboxylic acids of AHs are ionized from pH 3.5 – 4.0 onwards and phenolic alcohols from pH 5.5 – 6.0 (Álvarez-Puebla and Garrido, 2005). It means, from the theoretical point of view, from pH 6.0 onwards flocculation of HAs will be really difficult if we assume organic matter in landfill leachate to be formed by HAs in a high extent. Electrostatic repulsion forces (high absolute value of Zeta potential) will maintain HAs in suspension under neutral and high pH conditions (Souza and Roca Bragança, 2018).

We assume and prove in this work organic matter in landfill leachate to be formed by biodegradable and recalcitrant compounds, mainly HSs proceeding from composting processes of organic fraction of solid wastes, a common situation in high organic loaded leachates. Biodegradable compounds are biologically treated and recalcitrant compounds are flocculated attending HSs chemical properties related to stability of colloid suspensions. pH sensitive flocculation is considered the crucial step for organic matter reduction of recalcitrant compounds and interesting reflexions are formulated as a novelty in the treatment of this considered toxic residue, from the point of view of the mechanism of flocculation of HAs. By this way, pH sensitive flocculation and biological degradation can be selected as the best combined treatment in the reduction of toxicity of landfill leachates, specially highly polluted in terms of organic matter reduction and economy. Characterization of HAs from landfill leachate is proven by CHNS elemental chemical analysis, FT-IR spectrometry and UV–VIS spectrophotometry.

2. Materials and methods

2.1. Methodology in chemical analysis

For characterization of leachate, pH, Zeta potential, organic load (COD), colour, solids (TS and VS), HAs, nitrogen (N_{org}, N-ammonia, nitrates and nitrites), phosphate, sulfate, chloride and metals were measured in accordance with standard methods (APHA, 2017).

pH and Zeta potential were analyzed by instrumental methods. pH was measured by a pHmeter CRISON MicropH 2000. Zeta potential, mean diameter and polydispersity were measured by triplicate using a Malvern Zetasizer Nano ZS series equipment (Aljerf, 2018).

COD and colour were the selected parameters for the evaluation of landfill leachate treatment. COD was analysed in triplicate in accordance with standard method (colorimetric closed reflux method, 5220D, APHA, 2017). Samples were diluted 8 times because of high concentration of organic matter and pattern calibration line (potassium hydrogen phthalate) was prepared for a maximum COD value of 10000 mg/L. Due to the special characteristics and composition of leachate, and the presence of HS, the spectrophotometric procedure (chromatic method) was used for measuring colour (method 2120C, APHA, 2017).

Solids were quantified by the corresponding standard method (TS: 2540B and VS: 2540E, APHA, 2017).

For N_{org} (organic nitrogen) and N-ammonia analysis, samples were filtered by 0.45 μm pore size filters and measured by Kjeldahl method (4500-NH_3 B and E, APHA, 2017). In N_{org} analysis, samples were digested in H_2SO_4 before Kjeldahl measurement (4500-N_{org} B, APHA, 2017).

Anions were measured after filtration by 0.45 μm pore size filters using an ionic chromatograph Dionex ICS 2000 ISO 10304–1 (nitrate, nitrite, phosphate, sulphate and chloride). Operation parameters of the IC were: eluent flow rate 1.00 mL/min (35 mM KOH), cell temperature: 35 °C, column temperature: 30 °C, applied current: 50 mA and injection volume: 10 μL. Column: IonPac AS19 (4x250 mm), guard: IonPac AG19 (4x50 mm) using software Chromeleon (Dionex) for acquisition of data and calibration.

Metals were analysed by inductive coupled plasma mass spectrometry (ICP-MS) (AGILENT 7800). Due to the high content of organic matter, samples were digested in concentrated HNO_3 in a close Teflon glass for a final sample volume of 50 mL. Mass spectrometer was calibrated with certificated standard solutions of 1000 mg/L (Panreac), by grouping the chemical elements in two multi-element patterns. Operational conditions of mass spectrometer were: plasma gas flux: 15 L/min; auxiliary gas flux: 0.9 L/min; nebulization gas flux: 0.99 L/min and cell gas flux: Helio 4.5 mL/min.

The standard determination of HAs is a suggested method which depends in concentration (5510, APHA, 2017). In natural waters and due to acid properties of HS, the method proposed is a concentration using ionic change columns (diethylaminoethyl cellulose or XAD resins) and analysis of DOC (Abbt-Braun et al., 2004). In liquids with high concentrations of HS expected, HAs are separated by precipitation under acidic conditions, based on the method described by Christensen et al., 1998 (Guangxia et al., 2012; Gomes de Melo et al., 2016). Leachate samples (200 mL) were first acidified to pH = 1.0 with HCl concentrated and placed overnight. The precipitate after 24 h was purified twice with a mixture of 0.5 % (volume) HF and 0.5 % (volume) HCl, 24 h each time with 100 mL wash solution. Supernatant was removed and precipitate was filtered under 0.45 μm Millipore filters, washing 3 times with HCL solution at pH 2.0. After this treatment the precipitate (HAs) was placed in small portions at 35 °C in an incubator during 5 days for drying and

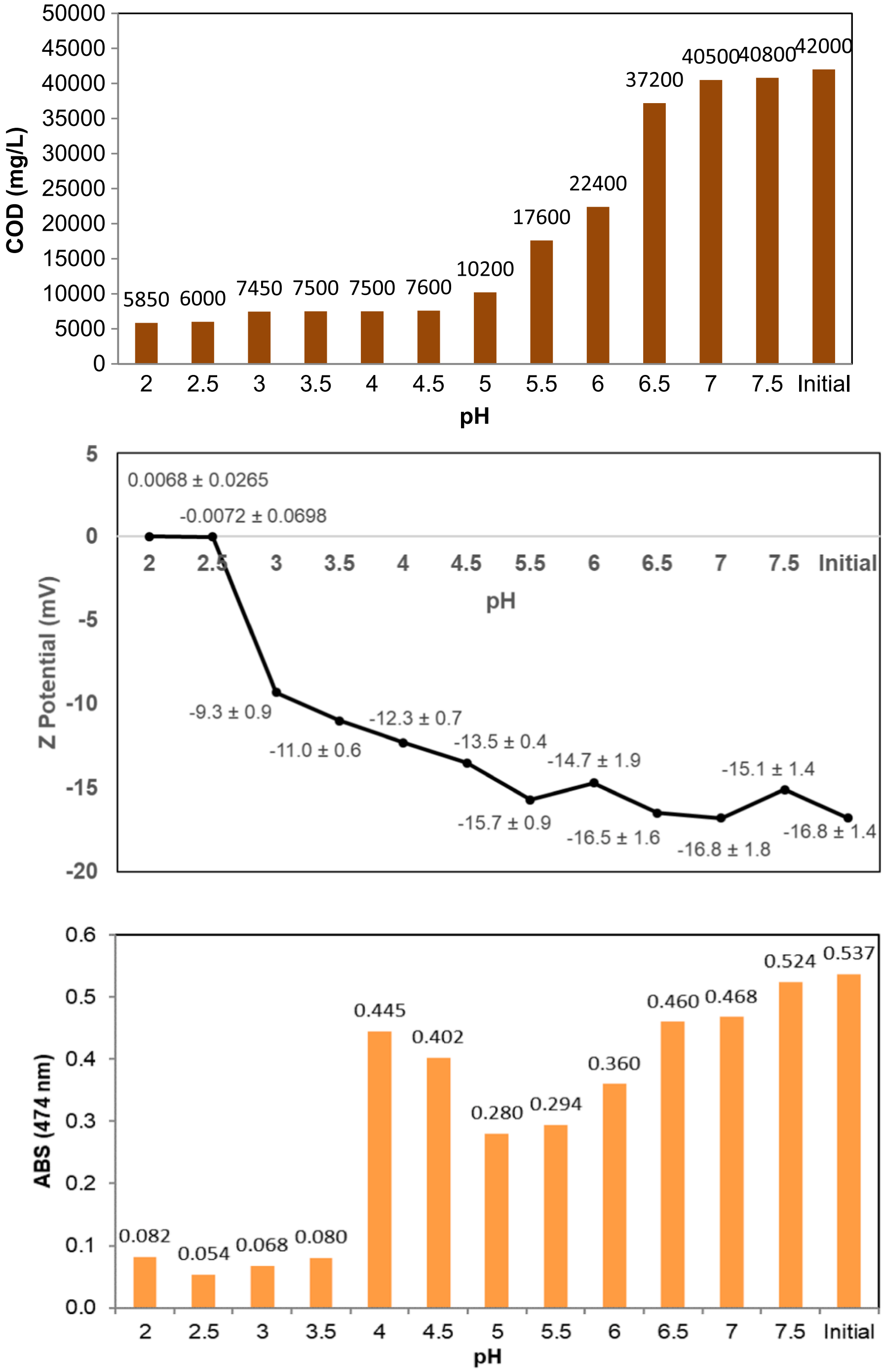

Fig. 1. Results of pH sensitive flocculation for landfill leachate in COD (upper graph), Z potential (middle graph) and colour reduction (lower graph). Nucleation occurs at pH 4.5 (increase of ABS in lower graph).

preparation of further analysis.

HAs from landfill leachate were characterized by elemental chemical analysis (LECO CHNS-932 analyzer), FT-IR spectrometry (Perkin Elmer SpectrumTwo) and UV–VIS spectrophotometry (UV-1800 Spectrophotometer, Shimadzu). IR spectra were recorded preparing samples as pellets of HAs and AHA in KBr and analyzing in the range of 4000 to 400 cm^{-1}. For spectrophotometry analysis, 200 mg/L samples (50 mL) of HAs and AHA were prepared in $NaHCO_3$ solutions at pH 8.0, corrected once in the case of HAs from leachate. The spectra were obtained over a wavelength range of 200 to 800 nm.

2.2. Flocculation

pH sensitive flocculation was assayed using H_2SO_4 9 M for pH reduction of landfill leachate. pH was reduced by the addition of H_2SO_4 and samples were collected every 0.5 unit of pH. COD, colour and Z potential were analysed in samples to follow the chemical stage of the HAs (protonation or ionization of carboxylic acids and phenolic groups).

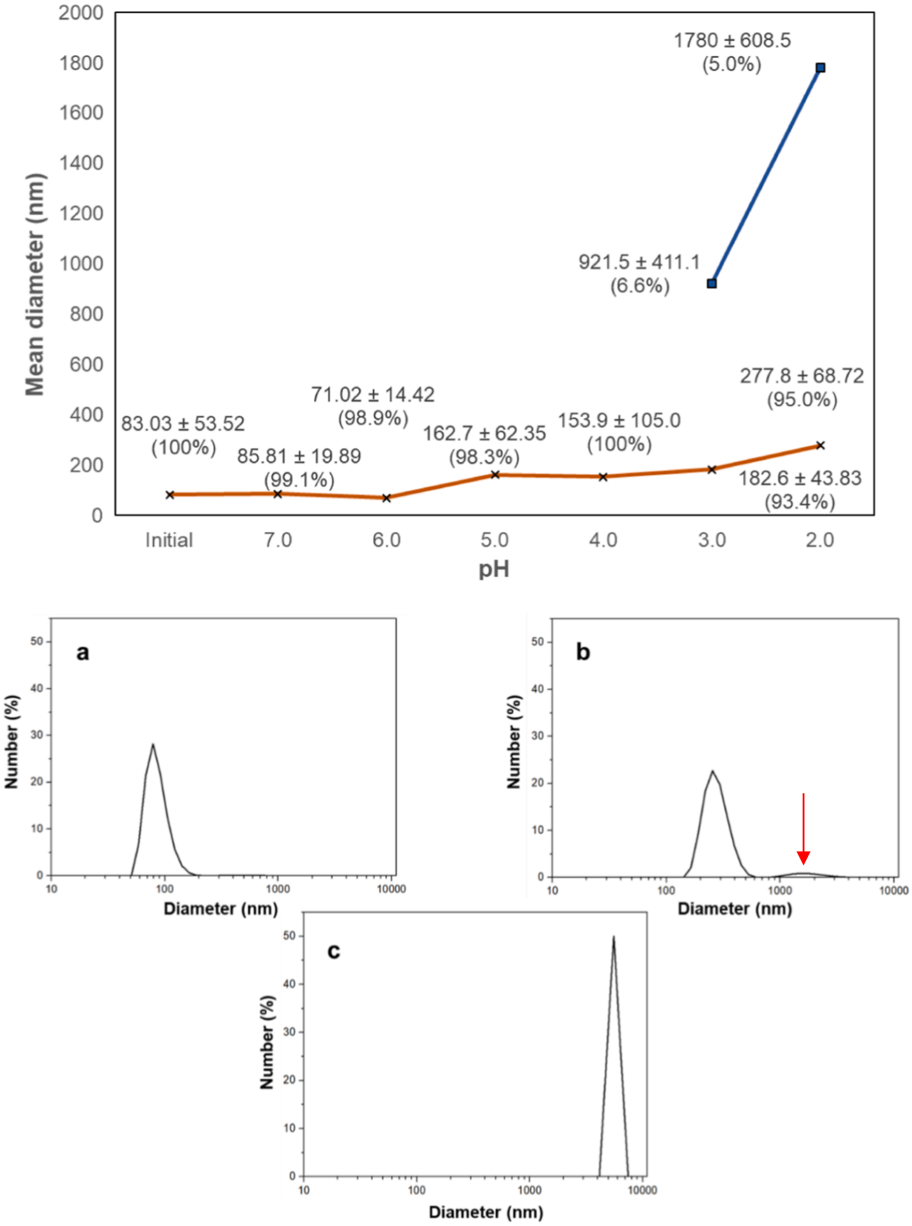

Fig. 2. Mean diameter of suspended particles in landfill leachate during pH sensitive flocculation (upper graph). Size of suspended particles distribution during pH sensitive flocculation at pH 7.0 (**a**), 2.0 (**b**) and precipitate after sedimentation at pH 2.0 (**c**).

3. Results and discussion

3.1. *Characterization of landfill leachate*

Landfill leachate was analysed in the laboratory collecting samples directly from the leachate deposit in the WTC. Table 1 shows most important parameters in the characterization of landfill leachate. COD value is 42000 ± 2516 mg/L, a high value compared with other landfill leachates in literature (Wiszniowski et al., 2006; Renou et al., 2008; Kheradmand et al., 2010), but coincident with high organic loaded leachates (Kang et al., 2002; Lou et al., 2009). The reason of this high organic matter content is because this leachate is the product of percolation from the landfill in which solid wastes are deposited mixed with the liquid originated from composting process of organic fraction of solid wastes (Vaverková et. al., 2020). Normally composting process is regarding the formation of HSs from organic matter transformation, as a result of an aerobic oxidation. In the formation of compost, a high HSs content can be expected (Guo et al., 2019).

Solids are abundant in landfill leachate (TS = 16410 ± 486 mg/L) most of them organics, because of the high value of VS, more than 60%.

Colour analysis of landfill leachate by spectrophotometric method (section 2.1) is shown in Fig. 1S, Supplementary material. Dominant wavelength obtained was 474 nm, which corresponds to dark blue tonalities, and purity 50% obtained both values from the chromaticity diagram. Value of colour is high (ABS $_{474\ \mathrm{nm}}$ = 0.537 ± 0.000, Table 1) and the aspect of this landfill leachate is a strong dark brown coloured liquid.

Nutrients concentration in landfill leachate (Table 1) pointed high concentrations of Cl^-, NH_4^+, NO_3^- and SO_4^{2-}, which are more abundant anions in leachate. PO_4^{3-} and NO_2^- are slightly present in landfill leachate as nutrients. These values are coincident with other authors (Kang et al., 2002; Lou et al., 2009).

With regards to metal ions content, landfill leachate is strongly charged by Na^+ and K^+ and Ca^{2+} and Mg^{2+} are also abundant (Table 1). P(V), Al^{3+} and Fe^{3+} are also remarkable cations present in leachate. Mn^{2+}, Cr^{3+} and Cu^{2+} are present in low concentration in this landfill

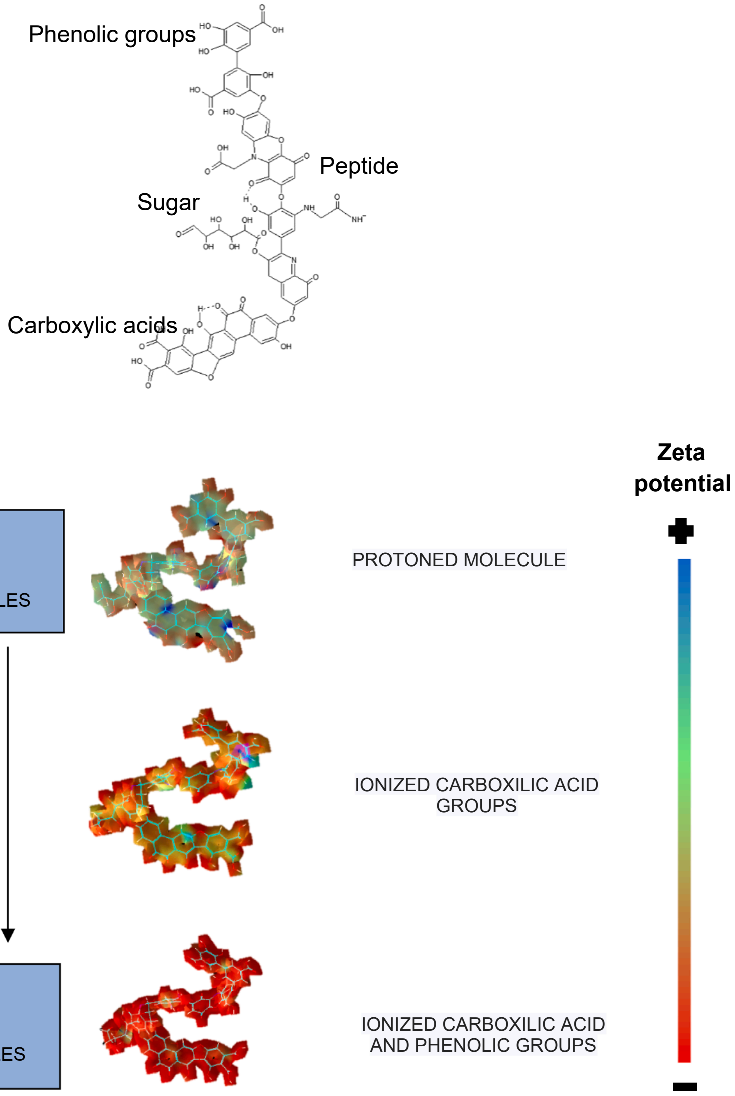

Fig. 3. Zeta potential change versus pH in order of the ionization state of a molecule of a HA. At low pH the molecule is protonated (low absolute Zeta potential value) and over pH = 5.5 the molecule is ionized (high absolute Zeta potential value). In the upper part of the figure, a model structure of a typical humic acid (HA) is presented, showing main functional groups in the molecule: carboxylic acids, phenolic groups, peptide and sugar (based on Manning et al., 2000).

leachate. Other cations are present in concentrations below 1 mg/L. This is a normal distribution of metal ions in landfill leachates (Lou et al., 2009).

3.2. *pH sensitive flocculation of landfill leachate*

pH sensitivity flocculation was assayed modifying pH by the use of H_2SO_4 9 M for acidification. Landfill leachate flocculates significantly at pH 2.0 (Fig. 1), reducing COD value from 42000 ± 2516 mg/L to 5850 ± 923 mg/L (86.1 %).

Z potential was measured in leachate during acidification procedure (Fig. 1). Initially (high pH), Z potential value in leachate is negative (-16.8 ± 1.4 mV) and when pH drops below 5.0, the value decreases to zero. The decay in Z potential value is much more evident below pH 3.5.

A progressive colour reduction is observed during pH sensitive flocculation in Fig. 1 from initial $ABS_{474nm} = 0.537 \pm 0.000$ to 0.082 ± 0.000 (84.7 %). It is remarkable the increase of ABS at pH 4.5 and 4.0 which has been attributed to the initial step of flocculation (nucleation) where flocs formation increases ABS before precipitation occurs. The experiment was repeated 4 times and the same ABS increase was observed at the same time of high amount of foam formation.

Mean diameter and polydispersity were measured during pH sensitive flocculation. Although polydispersity is high in landfill leachate (0.578), due to the high mixture and heterogeneity of the suspended particles, particle size distribution of the organic content of this liquid seems to be not very diverse in accordance with the results obtained, concerning mean diameter distribution in the liquid (Fig. 2, upper graph and (a)). During pH reduction, diameter of suspended organics rises at pH = 5.0 onwards and huge aggregates (blue points in Fig. 2, upper graph and arrow in (b)) begin to appear at pH 3.0 and 2.0. This can only be explained by interaction among several organic molecules (HAs). Precipitate was also analysed for mean diameter, showing to be formed by much bigger particles than those present in the initial landfill leachate (Fig. 2, (c)): polydispersity 0.949, mean diameter 5590 ± 579.8 nm (100%). The size of these aggregates, several micrometers, is in accordance with the size observed by other authors in the aggregation of natural HAs (Baalousha et. al., 2006).

Mechanism of pH sensitive flocculation in landfill leachate can be explained considering a high presence of HAs in the composition of this liquid, specially because an important fraction proceeds from composting leachate. Typical structure of a humic acid is formed by a long aromatic chain in which functional groups are carboxylic acids in the terminal points of the chain accompanied by some phenolic groups and sugar and peptide groups at the centre (Fig. 3). Abundance of protons

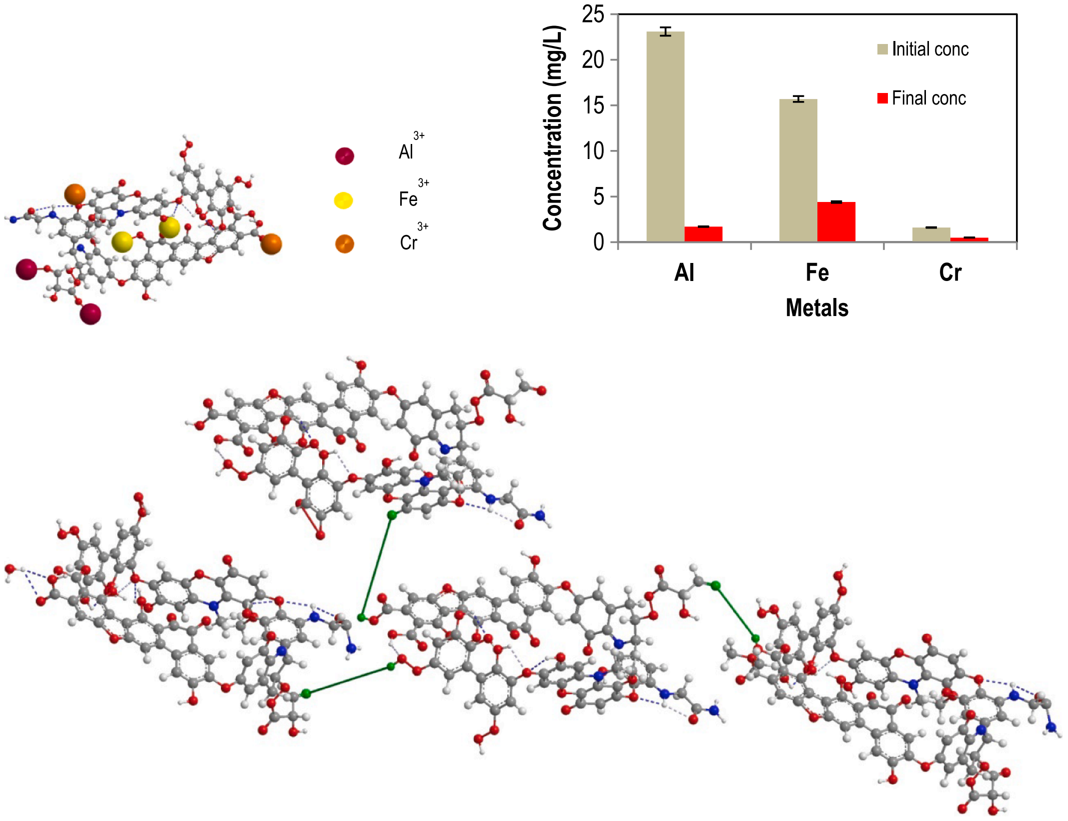

Fig. 4. Simulation of aggregation of four molecules of humic acids protonated, by formation of intermolecular H-bonds (green lines). Intramolecular H-bonds (blue pointed lines) can deform and fold the HAs molecules. In the upper part of the figure, on the left, metal site binding to HAs is visualized. In the upper part of the figure, on the right, a graph with comparative concentrations of most abundant trivalent metal ions in leachate is shown (Al^{3+}, Fe^{3+} and Cr^{3+}) before and after pH sensitive flocculation (values of concentration are in Table 2). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(low pH) means all carboxylic acids and phenolic groups to be protonated, Zeta potential value is reduced because electrostatic charge is reduced in the surface of the molecule and HAs can aggregate by formation of H-bonds (Álvarez-Puebla and Garrido, 2005).

In Fig. 3 simulation of Zeta potential change versus pH and increment due to ionization is showed by different colours in the surface of a HA molecule. At low pH HA is protonated and Zeta potential is low (green and grey colours), at pH over 5.5 carboxylic acid and phenolic groups are ionized and absolute value of Zeta potential increases (red colour). This figure has been originally drawn using a typical structure of a humic acid molecule as a framework and based in the chemical properties of HAs reported by Álvarez-Puebla and Garrido (2005).

On the other hand, and due to the high content of several metal ions in landfill leachate (Table 1), ionized HAs at pH around neutrality will maintain in suspension because of electrochemical reactions between carboxylates (–COO^{-}) and cations (Gomes de Melo et al., 2016), specially trivalent cations, because a higher electric charge and lower atomic radius (Al^{3+}, Fe^{3+}, etc.). This is called site binding, distinct to territorial binding in which the compounds are trapped by large organic structures without specific binding in functional groups (Manning et al., 2000). Complex formation of metal cations and functional groups of HAs (carboxylic acids and phenolic groups) has been described in literature (Zhou et. al., 2005; Gomes de Melo et al., 2016), finding a higher affinity between HAs and metal ions specially for divalent smallest cations and lower affinity for alkali and earth-alkali cations, because its larger radius which makes lower availability for HAs sites. The solubility of cations in the pH range plays also an important roll in the competition of binding with the HAs functional groups.

Table 2
Comparative elemental analysis of HAs from landfill leachate and Aldrich HA and data from literature. HA1 and HA2 are two independent samples of HAs obtained by precipitation at low pH from landfill leachate.

Element/ratio	HA 1	HA 2	Aldrich HA	Data from literature*		
				*Aldrich	*HA aquatic	*HA terrestrial
C	46.37 ± 0.35	47.37 ± 0.35	40.50 ± 0.35	50.3	53–60	54–62
H	5.90 ± 0.30	5.99 ± 0.30	3.65 ± 0.30	5.7	3.7–5.1	2.9–5.8
O	40.37 ± 0.35	39.24 ± 0.35	54.52 ± 0.35	43.6	33.4–40.8	29.5–36.8
N	5.76 ± 0.30	5.85 ± 0.30	1.02 ± 0.30	0.4	2.0–2.1	1.6–4.8
S	1.60 ± 0.35	1.55 ± 0.35	0.31 (<dl)	nd	–	–
H/C	1.53	1.52	1.08	1.35	0.74–1.15	0.56–1.29
O/C	0.65	0.62	1.01	0.65	0.42–0.58	0.36–0.51
N/C	0.11	0.11	0.02	0.01	0.029–0.034	0.022–0.076

nd: not detected, dl: detection limit.

[*] Kang et al., 2002.

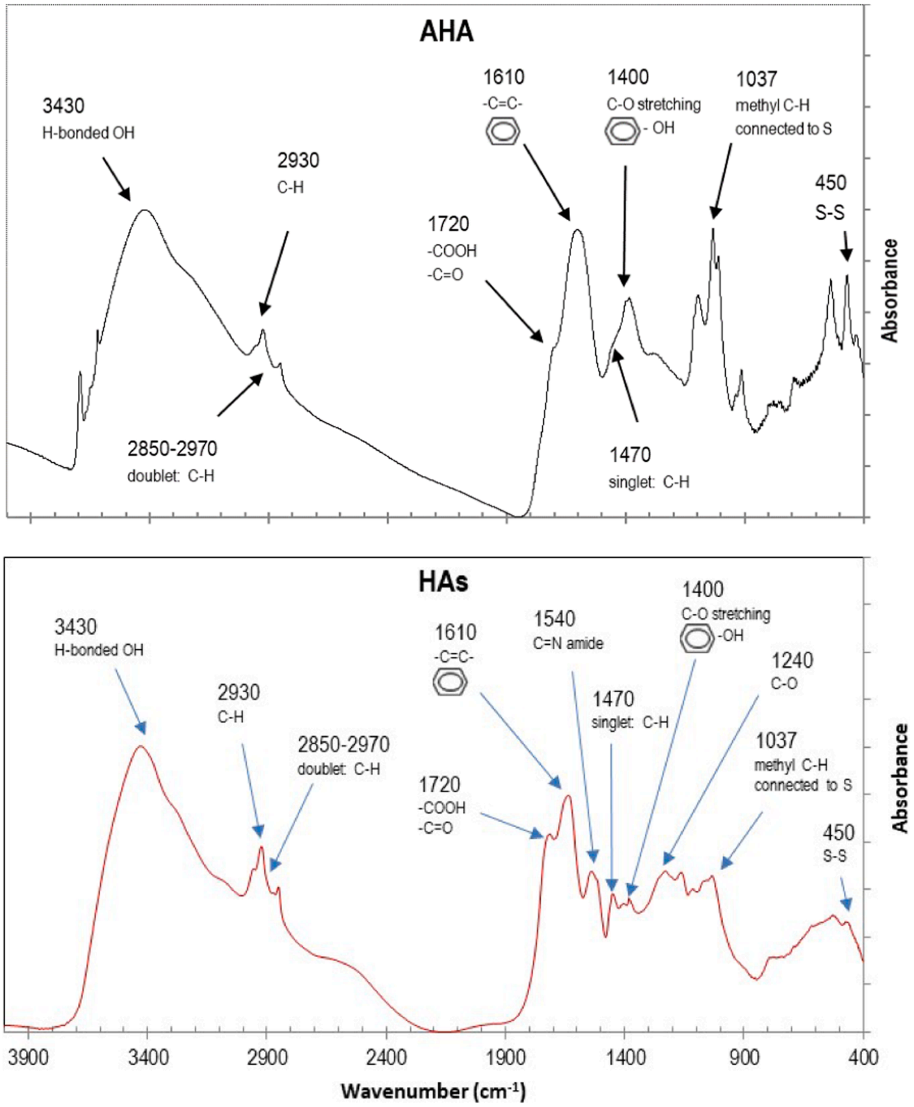

Fig. 5. FT-IR comparative spectra of Aldrich humic acid (AHA) in the upper graph and humic acids from landfill leachate (HAs) in the lower graph. Most representative signals of humic acids are pointed. Signal at 3430 cm^{-1} is assigned to intermolecular H bonds (Guangxia et al., 2012).

After pH sensitive flocculation, concentrations of majority metal ions were measured (Table 1) to prove interactions of humic acids and cations. Due to the high concentration of organic matter in leachate, chromium is considered to be in reduced oxidation state, Cr^{3+} and iron in the form Fe^{3+} because of the presence of oxygen in the liquid. Trivalent cations (Al^{3+}, Fe^{3+} and Cr^{3+}) reduce highly in concentration when pH of landfill leachate is reduced to 2.0 (Fig. 4). We assume trivalent cations are interacting electrostatically with HAs and are trapped in the precipitate when flocculation occurs. This reduction in concentration of trivalent cations is the evidence of interaction with HAs, specially because at low pH (below pH 4.0 for Al^{3+} and Cr^{3+} and below pH 3.0 for Fe^{3+}) the solubility of these metals increases in high extent. Also Ca^{2+} and other divalent cations can induce electrochemical reactions with HAs and participate in their aggregation, although the binding of Ca^{2+} to AHs has been described at high pH, where most of the functional groups of HAs are dissociated, specially carboxylic acids (Christl, 2012; Kloster et al., 2013). Small and high charged metal cations are much more strongly binded to active sites of humic acids (Christl, 2018).

Fulvic acids, which maintain in suspension after pH sensitive flocculation, can be responsible of less reduction than expected in concentration of several cations in Table 1, because of a competitive effect with HAs (precipitated) for binding with these cations (Pinheiro et. al., 1999). It has been suggested in literature two distinct classes of binding sites, been occupied weak sites only after the strong sites are occupied fully, with preference for small and strong charged cations (Sekaly et. al., 1999). This could explain a higher reduction in concentration of trivalent metal ions (Table 1), because they are binded before to HAs sites and not to FAs sites.

Ionic strength and the individual contribution of each ion was calculated for landfill leachate (Table 2S, Supplementary material). Due to the high concentration of Cl^- and monovalent cations Na^+, NH_4^+ and K^+, they are the main contributors to the ionic strength in leachate. Ca^{2+} and Mg^{2+} and trivalents Al^{3+} and Fe^{3+} represent also a visible contribution while other cations do not contribute because of low concentration.

Aggregation of HAs molecules is simulated in Fig. 4. Because of their high average molecular weight (1.2 10^8 g/mol) and RMS radius (436 ± 36 nm) (Manning et al., 2000), HAs tend to form hydrogen bonds when carboxylic acids and phenolic groups are protonated in the molecule. When pH is been reduced, first phenolic alcohols are protonated below pH 5.5 and secondly carboxylic acid groups below pH 3.5 (Álvarez-Puebla and Garrido, 2005). At pH 2.0 all functional groups in the molecule of the HA will be protonated, Zeta potential will be low in

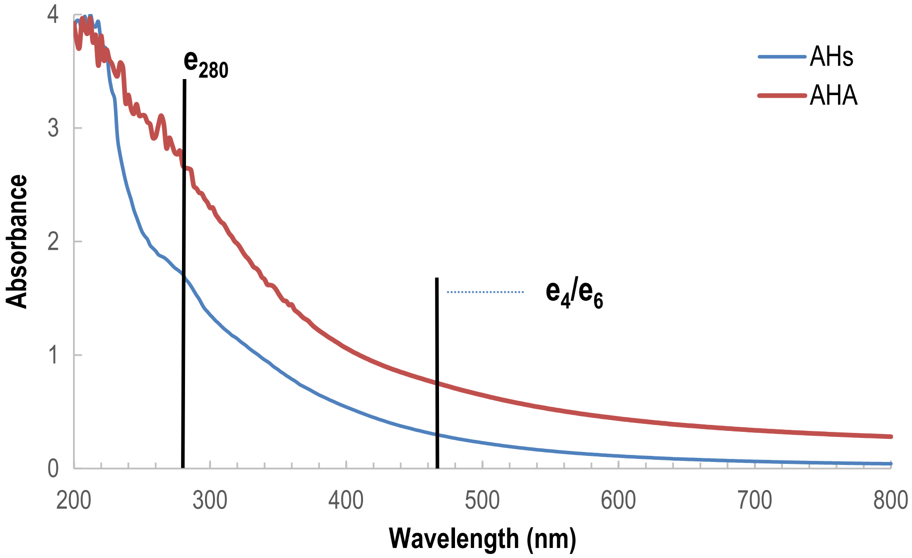

Fig. 6. UV–VIS spectra of Aldrich humic acid (AHA) in the upper line and humic acids from landfill leachate (HAs) in the lower line. Both samples were prepared at 200 mg/L concentration in $NaHCO_3$ solution, pH 8.0.

absolute value and H-bonds will connect molecules increasing the molecular weight of the structure and inducing to precipitate. When precipitation of HAs occurs, some other molecules can be dragged to the precipitate and this can explain why in this leachate pH sensitive flocculation reduces 86.1% of the global COD, more than the expected percentage of HAs in the global COD value (Guangxia et al., 2012).

The appearance of the liquid changes significantly after pH sensitive flocculation. Clarification is evident from dark brown to light brown-yellowish colour and precipitation and formation of foam indicates the aggregation of HAs.

3.3. Chemical and spectroscopic characterization of humic acids

After precipitation at pH 2.0 with HCl (to avoid interference of H_2SO_4 in elemental CHNS analysis), samples were washed in acid medium (section 2.1) and analysed by elemental chemical analysis, FT-IR spectrometry and UV–VIS spectrophotometry.

Table 2 shows the results of CHNS analysis, were two independent samples (HA1 and HA2) and Aldrich HA were measured. Results in percentage were normalized to 100 % of organic components and oxygen content (O) was calculated by subtracting the sum of the other organic components from 100 %. Atomic ratios H/C, O/C and N/C were calculated from element analysis results to obtain more information about HAs. Carbon content in HAs from landfill leachate is very closed to the content present in natural HAs, aquatic and terrestrial, and higher than commercial HA (Aldrich). Nitrogen content is high in HAs from landfill leachate, which is attributed to the presence of amines and amides and the high content of ammonia-N (Table 1) in leachate (Kang et al., 2002; Castro et al., 2018). Oxygen abundance is attributed to carboxylic acids and phenolic groups. Comparative data of sulphur content was not found in literature and result obtained from Aldrich AH sample is under detection limit.

With regards to atomic ratios, H/C ratio clustered around 1.0 reflects a chemical structure based predominantly in aromatic rings (Aldrich HA and natural HAs). A higher value of H/C ratio implies relative abundance of aliphatic functional groups in HAs from landfill leachate. O/C and N/C ratios are over range related to natural HAs, as commented before.

FT-IR analysis of HAs from landfill leachate is shown in Fig. 5 in a comparative graph with Aldrich humic acid (AHA). Signals of the spectra were assigned in accordance to Kang et al., 2002, Tanaka, 2012 and Guangxia et al., 2012.

Most representative signals of HAs are the broad band at 3400–3000 cm^{-1} and the peak at 3430 cm^{-1}, attributed to intermolecular hydrogen bonding and O-H stretching of phenol, hydroxyl and carboxyl, the doublet between 2970 and 2850 cm^{-1} and the singlet at 1470 cm^{-1} due to aliphatic groups and the characteristic peaks of protonated carboxylic acids and ketonic $C = O$ groups (1720 cm^{-1}), and the signal of $C = C$ stretching in aromatic rings (1610 cm^{-1}). The peak at 1540 cm^{-1} is assigned to $C = N$ stretching of amide groups, invisible in AHA because a lower N content (Table 2), but clearly present in AHs from landfill leachate. The singlet at 1470 cm^{-1} is assigned to aliphatic groups and at 1400 cm^{-1} may be due to the deformation of the O-H bond and C-O stretching of phenolic OH. The peak at 1240 cm^{-1} is considered to be due to C-O stretching. The signal at 1037 cm^{-1} can be explained by C-H rocking vibrations connected to S, highly visible in AHA although S measurement was under detection limit in elemental chemical analysis and present in HA spectrum where S has been detected (Table 2). Signal at 450 cm^{-1} is supposed to be due to S-S bonds (Guangxia et al., 2012).

UV–VIS spectra of HAs and AHA (200 mg/L, pH 8.0) are shown in Fig. 6. Basically UV–VIS spectrum in organic substances is a good indicator of unsaturated C content. The absorbance in the UV spectral range (200–300 nm) is attributed to chromophores groups ($C = C$ and $C = O$), related to aromatic structure of the molecule and the abundance of carboxylic acid groups (Aljerf, 2016). Both samples (HAs from landfill leachate and AHA) show high characteristic absorption at 220 nm, and high absorption coefficients at 280 nm in AHA ($e_{280} = 13.3$ L/(g cm)) and HAs from landfill leachate ($e_{280} = 8.5$ L/(g cm)). This absorption coefficient is related to aromaticity in the structure of the components of the sample (Kang et al., 2002), which is concluded to be high in both samples, mainly in AHA, with a higher aromaticity proved by a lower H/C ratio than HAs from leachate (Table 2).

The presence of carboxylic acid groups with relation to molecular weight can be evaluated by the ratio of absorption coefficients at 465 and 665 nm (e_4/e_6). The result of the calculation of these coefficients (HAs from leachate $e_4/e_6 = 4.0$ and AHA $e_4/e_6 = 2.1$) can be interpreted as a higher acidity and content of carboxylic acids in HAs from landfill leachate.

4. Conclusions

Landfill leachate sometimes has negative results when biological treatment is applied for COD reduction, mainly when the origin of this waste is composting processes of the organic fraction of solid wastes. In this paper we present this liquid basically as a mixture of HSs, mainly HAs, biodegradable organic matter and salts. There are other organic components in landfill leachate, but in much lower amount. Because of the chemical behaviour of HAs, protonated at low pH (<3.5) and ionized at pH over 5.5, pH sensitive flocculation is proposed as the best way of treatment for reduction of COD in this liquid. When pH is reduced, phenolic groups in the HAs molecules are protonated ($pH < 5.5$) and carboxylic acid groups later ($pH < 3.5$), resulting in the reduction of Zeta potential value in the surface of the molecules. This situation blocks site binding between ionized carboxylates of the HAs and metal cations abundant in leachate and allows formation of intermolecular H-bonds which conduct to flocculation of HAs. As a result of dragging other organic molecules during precipitation process of HAs, COD and colour can be reduced in high amount (85%).

The formation of huge aggregates of HAs has been followed by measuring mean diameter during pH sensitive flocculation and the precipitate at low pH of landfill leachate has been characterized by Elemental chemical analysis, FT-IR spectrometry and UV–VIS spectrophotometry and compared to commercial HA (Aldrich). Results show composition in C, H, O and S in the range of natural and commercial HAs in elemental chemical analysis, high aromaticity and the presence of carboxylic acid and phenolic groups in FT-IR spectrometry measurements and good indexes of aromaticity and acidity in UV–VIS spectrophotometry assays.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.wasman.2022.03.020.

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