REGENERATED HUMIC ACIDS OBTAINED BY THE AIR OXIDATION OF SOUTH MORAVIAN LIGNITE. PART. 1. PRODUCTION AND CHARACTERIZATION.

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Abstract

The influence of lignite air oxidation on the yield and chemical/physical character of regenerated humic acids (RHA) has been studied. RHA were produced under different experimental conditions, i.e. both in static and fluid reactor. Oxidation in the fluid reactor brought first a decrease and after a certain period increase in the yield of RHA. The same observation was done in case of static reactor. It has been verified that higher temperature during the oxidation supports the production of RHA. Despite the difference in the yield of RHA (increase up to 75%) their chemical character was in all cases fairly similar. Some tiny differences were revealed using FTIR which showed the increase in number of ester bonds in RHA produced mainly at temperatures above 80°C causing a significant increase in apparent molecular weight as evaluated by HPSEC.

Key words: lignite, regenerated humic acids, air oxidation

1. Introduction

Humic substances are ubiquitous natural products of microbial degradation of dead plant tissues and animal bodies and represent the major biosphere pool of natural organic matter [1]. In the course of their genesis they underwent processes of humification and in case of lignites also partly coalification. Major components of humic substances are considered to be recalcitrance of plants and algae, including materials derived from lignins, tannins, sporopollenins, and large aliphatic molecules such as algaenans, cutans and suberans. From the chemical point of view, they represent a complicated mixture of distinct chemical compound classes such as lipids, waxes, carbohydrates, polyphenols, proteins and nucleic acids derivates [2] Their role in nature is vital for sustainable development of life on the Earth. In soils they help to prevent drying and shrinking, improve moisture retaining properties of soils, permit exchange of gases, stabilize structure, enhance availability of micronutrients to higher plants, increase cation exchange capacity, modify application rate of pesticides for effective control, have a direct effect on plant growth [3]. In this respect, they influence the micro algae growth, seed germination, plant growth and development affecting some metabolic processes as respiration, nutrient uptake and inducing morphofunctional changes in the root architecture. In many cases these effects are similar to those induced by plant growth regulators as auxins, gibberellins and cytokinines [4,5].

Operationally humic substances are divided into three groups according to their solubility, i.e. humic acids (HA) soluble in alkali media, insoluble humin (HU) and fulvic acids (FA) soluble at all values of pH [3].

In the past, HA were posited to consist solely of a system of coiled macromolecules having molecular weights in the range of tens to hundreds of thousands of Da [3]. In basic or low-ionic strength solution they had elongated shapes, but became coils in acidic or high-ionic strength solutions. Later, Wershaw [6] proposed that HA consist of ordered aggregates of amphiphilic molecules, composed mainly of relatively unaltered plant polymer segments possessing acidic functionality and held together by hydrophobic (π-π, charge transfer) bonds and H bonding interactions and the hydrophobic parts of the molecules are in the interior, with the hydrophilic part making up the exterior surfaces. Ordered aggregates of humus in soils were depicted as existing as bilayer membranes coating mineral grains and as micelles in solution.

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Recent research brought a new insight into molecular structure of humic substances. As demonstrated, despite the polymer theory which has never been unambiguously proved, the humic mixture consists of various molecules assembled together via weak interactions (π-π, CH-π, van der Waals and H-bonds) forming aggregates of apparently large molecular weight [7]. Low bonding energy implies the possibility of easy separation of distinctive classes of molecules allowing better characterization followed by a potential technological utilization. Based on the polarity of solvents (among others), the sequential extraction techniques allow the separation of free lipids, bound lipids, saccharidic and aromatic fraction (polyphenols, heterocycles) [8].

Coals of variable degree of coalification possess variable amount of extractable humic acids. Use of oxidation procedures represents an ideal inverse diagenetic process (Fig. 1) resulting in a higher content of extractable humic matter [9]. Oxidation products obtained from coals by pre-treatment with nitric acid, potassium manganate or by air oxidation have been reported as regenerated humic acids (RHA) [9].
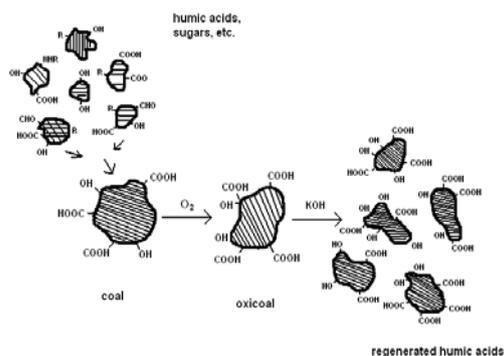
Fig. 1. Coal oxidation as and ideal inverse diagenetic process, adopted from ref. [9]
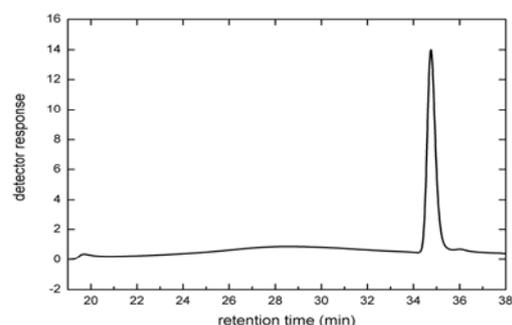
Fig 2. HPSEC analysis of humic acid extracted from parental lignite as detected by RI detector.

Generally, RHA have similar chemical-physical properties as humic substances which were precursors of coal formation. The elemental composition, functional groups content and distribution depends on the conditions of RHA formation and on the chemical properties of parental coal material. In their structure higher content of aromatic structures can be found in comparison with those humic acids extracted from parental coal. RHA also possess more semichinoidal radicals in their structure, i.e. 2×10^{18} spin g^{-1}. That content is minimally twofold higher in comparison with non treated humic samples [3]. Semichinoidal structures are easily oxidizable forming chinones which increases the rate of reduction reactions in soils. Such reactions are responsible for transport of metal ions in soils and support their adsorption by plant roots [9]. Thus technological/agricultural application of RHA bears promising potential.

Air oxidation of coal has already been studied and described in detail [9]. It has been supposed that coal structure contains same "weak" points that can be broken to depolymerize the original structure (Fig. 2). Oxidation at temperatures as high as 180°C was stated the most simplest way of production of RHA [9]. On the other hand, such approach is relatively demanding with respect to the price of the technological performance as well as with respect to the evolution of huge amount of carbon dioxide and other gases.

Since HA as well as RHA represent an interesting raw material useful in technological, agricultural and environmental applications the knowledge on the production is of a great interest [10]. Thus the aim of this work was to investigate the potential of air as a cheap oxidation agent of South Moravian lignite and to assess the chemical-physical character of obtained regenerated humic acids.

2. Experimental

Extraction of HA and production of RHA

Lignite from the Mikulčice locality (Czech Republic) was used in this work. Detailed information on the lignite chemical-physical properties were already reported elsewhere [11,12]. Using several screens, lignite particles were separated according to the particle size into three groups: 1-0.5 mm, 0.5-0,125 mm and fraction <0.125 mm.

RHA were extracted from air pretreated lignite. Lignite was treated by two ways. In the first case, lignite was placed into a container and stream of air made lignite particles "fly" and therefore provided maximal contact with air. Therefore, kind of fluid reactor was used for this purpose. Temperature was

constant either at 25°C or 50°C. The second way of oxidation was carried out in the oven in which the lignite sample was let to be oxidized put on the floor at 85°C for the chosen time period.

Humic substances were extracted from pretreated and parental lignite according to the procedure described earlier[13]. Briefly, a lignite fraction was mixed with the aqueous 0.5 M NaOH and 0.1M $Na_4P_2O_7$ solution (1:10) and stirred for 3 hours. After centrifugation, the supernatant was treated with the concentrated HCl until pH was about 1 in order to precipitate the humic acids (fraction HA). HA were then treated with 0.5% (v/v) HCl-HF solution overnight to remove residual ashes, dialyzed (Spectra/Por® dialysis tubes, 1000 *Mw* cut-off) against distilled water until chloride-free. Obtained products were dried in the oven to constant weight.

All humic samples (50 mg) were suspended in CO_2 free distilled water (60 ml) and titrated to pH 7 with 0.1M NaOH solution employing an automatic titrator as described earlier [14]. The resulting sodium humates were freeze-dried and homogenized in an agate mortar for further HPSEC analyses.

High performance size exclusion chromatography (HPSEC)

The HPSEC system Agilent consisted of a pump equipped with two detectors in series: a UV/VIS detector set to 280 nm for humic analyses, and a refractive index detector (RI). An automatic injector, with a 100 μl sample loop, was used to load HPSEC solutions and a Phenomenex Biosep S2000 (600 × 7.5 mm) column was used for size exclusion separations. The column was preceded by a Biosep Guard column and a 0.2 μm stainless-steel inlet filter. Flow rate was set to 0.6 ml min^{-1} while the HPSEC eluent was a 50 mM $NaH_2PO_4.H_2O$ solution adjusted to pH 7 with 1M NaOH. The buffer concentration was chosen to have a constant ionic strength of 50 mM in order to minimize ionic exclusion or hydrophobic interactions with the column.

All solutions were filtered through quartz filters (Glass Microfibre Filter Whatman International, LTD) before injection. The HPSEC analyses were conducted in duplicate and no significant differences were observed between measurements. For the calculation of weight average molecular weight (*Mw*) following equation was used:

$$M_W = \frac{\sum_{i=1}^{N}(h_i M_i)}{\sum_{i=1}^{N} h_i}$$

where M_i and h_i are the molecular weight and the height of each i-th fraction in the chromatogram, respectively.

Standards of known *Mw* such as polysaccharides (PS) of 186, 100, 23.7 and 12.2 kD, (Polymer Sciences Laboratories, UK) and sodium polystyrenesulphonates (PSS) of 169, 123, 30.9 and 6.78 kD (Polymer Standard Service, Germany) were used for column calibration. Calibration curves were semi-log linear over the range defined by standards and were used to obtain the molecular weights of humic samples.

FTIR spectroscopy

Conventional KBr pellet technique was used. Humic samples previously dried at 105°C for 2 hours and cooled and stored in desiccator were mixed in agate mortar with KBr (1:200 w/w). For this purpose Nicolet Impact 400 was employed.

3. Results and discussion

Production of RHA extracted from South Moravian lignite has been already published [12]. It has been demonstrated that the content of RHA can be increased from 20% to 60% using nitric acid or 45% using hydrogen peroxide. Nevertheless, such approach was associated with a loss of a huge amount of carbon due to the intensive oxidation attack.

3.1 Influence of particle size on the yield of humic acids

In the first step, the attempt was to evaluate the influence of particle size on the yield of extraction of humic acids from lignite. For this purpose 3 particle size fractions mentioned in Experimental were used. Table 1 reports the results of the experiment.

Table 1 Yield of extraction of humic acids from lignite of various particle sizes

Fraction (mm)	1-0.5	0.5-0.125	0.125>
Yield (%)	12.0	23.0	22.0

One can see that the particle size is an important parameter for the production of humic acids. While large particle diameter provided a relatively low yield, particles smaller than 0.5 mm gave yield above 20%. It is logical since lower particle size is connected with larger area and it seems that the extraction agent could not reach to the particles interior and extracted solely the humic material from the particle surface. Additional conclusion can be made considering that humic acids are more abundant in particles with lower diameter. This assumption can be justified by the fact that in the structure of lignite, there can be identified parts of different degree of coalification as well as non-decomposed moieties of parental phytomass having different mechanical properties.

Therefore, in the next experiments, different fractions were used to test the efficiency of suggested oxidation methods.

3. 2 Oxidation in a fluid reactor

This part of the work has been devoted to the evaluation of influence of air oxidation on the yield of RHA extracted from oxidized lignite. For this purpose the fraction 1-0.5 mm was chosen. The obtained data are summarized in Table 2.

Table 2 Oxidation of fraction 0.5-1 mm in the fluid reactor

Oxidation parameters	24 hours at 25°C	72 hours at 25°C	120 hours at 25°C	72 hours at 50°C
Yield* (%)	-15.63	-7.97	75.42	5.46

Increase (+) or decrease (-) with respect to the value given in Table 1

As can be seen in Table 2, the oxidation in fluid reactor did not give rise to the yield increase in all cases. We assume that it is caused by the composition of the particles having diameter 0.5-1 mm. In fluid state particles are supposed to be under the mechanical stress (milling) and thus the particle size is decreasing (verified by a parallel experiment). Such processes should lead to the increase in the yield of humic acids. However, it seems that such way of oxidation preferably supports the oxidation of COOH groups of humic acids responsible for their solubility. Kinetics of oxidation of other structures, resulting in formation of other carboxylic groups is evidently slower which is indicated by the yield after 72 hours at 25 and 50 °C and after 120 hours.

3.3 Oxidation in stationary phase

In this part of the work we have tested the influence of air oxidation on the yield of humic acids. Since oxidation in fluid reactor was not too sufficient for fraction 0.5-1 mm, we used the fraction 0.5-0.125 to see whether in this case the yield increase will be more intensive. Temperature 85°C was chosen with respect to the results reported recently, which indicated that at temperature above 80°C lipid fraction present in humified matter forming a specific domain is melted [15] and, thus, some parts of lignite are better accessible for oxygen diffusion. Again, similar trend as in case of fluid reactor has been observed (Table 3). Oxidation of lignite brought about a decrease in the yield of RHA with the minimum after 120 hours of oxidation. Further, an increase was observed which resulted in 18% abundance after 456 hours. It seems that lignite is relatively resistant to the oxidation attack under these conditions and the kinetics of oxidation of carbon skeleton is relatively slow. Although the increase of HA content using such simple and chemically mild methodology is low, the next part is devoted to the characterization of selected humic acids obtained during above-mentioned procedures.

Table 3 Oxidation of fraction 0.5-0.125 mm in the static reactor

Oxidation period (hours)	72	120	240	456
Yield* (%)	-22.94	-43.65	-5.51	17.98

Increase (+) or decrease (-) with respect to the value given in Table 1

3.4 Characterization of HA and RHA

Selected samples were characterized for their elemental and ash content. Obtained values are reported in Table 4. Values are given in weight %. Chemical properties of humic acids extracted from fraction 1.0-0.5mm did not practically differ from RHA prepared for 24 hours at 25°C, i.e. RHA1, so the latter is used as a reference. Samples produced in fluid reactor were denoted as follows: RHA2 and RHA3 are regenerated humic acids produced for 72 and 120 hours, respectively, and RHA4 stands for regenerated humic acids produced 72 hours at 50°C. Samples RHA5, RHA6, RHA7 and RHA8 were prepared at 85°C in the static reactor for 72, 120, 240, 456 hours, respectively. Sample HA9 represents humic acid extracted from parental lignite.

Table 4 Elemental composition, ash and moisture content of parental and regenerated humic acids, ash content in all samples up to 2.2%, traces of sulphur are included in O content.

	C (%)	H (%)	N (%)	O*(%)	C/H	C/O	Moisture
RHA1	56.07	4.82	1.28	37.22	11.75	1.52	8.34
RHA2	57.37	4.80	1.36	36.47	11.95	1.57	7.63
RHA3	56.62	4.89	1.34	37.15	11.58	1.52	7.85
RHA4	56.81	4.45	1.29	37.45	12.76	1.52	8.16
RHA5	56.91	4.66	1.37	37.06	12.21	1.54	7.61
RHA6	57.21	4.97	1.29	36.53	11.51	1.57	7.98
RHA7	57.72	4.72	1.26	36.30	12.22	1.59	7.03
RHA8	57.42	4.84	1.29	36.45	11.86	1.58	7.48
HA9	57.21	4.62	1.05	37.22	12.38	1.53	n.d.

*calculated by difference

Decreasing C/H ratio appeared to be related to decreasing aromaticity or degree of unsaturation [13]. Basically, the higher C/H value the higher is the content of aromatic structures in humic material. Ratio C/O indicates the proportion between oxygenated carbons and indirectly also the content of COOH groups.

In fact, the elemental content and its ratios did not show any remarkable changes in composition of RHAs. As mentioned previously, the reason can be seen in the kinetics of oxidation processes as well as in the strength of oxidation agent.

Fig. 2 shows a representative HPSEC of humic acid extracted from parental lignite detected by RI detector, Table 5 reports the respective *Mw* of all HA and RHAs based on sodium salt polystyrene sulfonates (PSS) and polysaccharides (PS) calibration. Although the comparison of *Mw* among each other has an importance, the values should be taken as apparental since there is no humic material which can be used for the molecular weight calibration [16]. Further, as mentioned before, humic acids should be considered as a mixture of relatively small molecules and the data reflect more the aggregation properties of humic acids. Aggregates are stabilized by weak interactions (π-π, CH-π and van der Waals and by H-bonds) which depends predominantly on the pH value and ionic strength [17]. In our case, analysis resulted in two distinctive peaks. As demonstrated recently, largest molecular size fractions excluded from column first contain aromatic, alkyl and potentially carbohydrate-like content. The carbohydrate-like and the alkyl chain length seem to decrease with decreasing molecular size. Progressive reduction of aromatic carbon atoms was also observed with decreasing molecular size of the separated fractions. Further, the abundance of hydrophilic molecules have been reported in low molecular fraction [16,18].

Table 5. HPSEC based molecular weight calculated from UV and RI records

	PSS calibration (UV)	PS calibration (RI)
RHA1	9232	9444
RHA2	12646	10760
RHA3	13404	13710
RHA4	7279	8442
RHA5	15213	15030
RHA6	10323	10840
RHA7	12881	13540
RHA8	17460	16940
HA9	9961	nd

In contrast to the elemental analysis the HPSEC analysis revealed better the differences among humic sample (Table 5). Although there is a shift between RI and UV calibrations, the mutual comparison within HA and RHA is the same. Comparison of samples in the first set prepared in the fluid reactor shows the increase in *Mw* with increasing time of oxidation. On the other hand, elevated temperate of oxidation (50°C) brought a significant decrease in *Mw*. It means that the oxidation of lignite progressively liberates the soluble fractions with larger dimensions while temperature can cause their size reduction.

The oxidation in stationary phase at 85°C showed also increasing tendency. The exception is RHA6 which gave a lower value of *Mw* than RHA5, although such value is still higher than that of HA extracted from non-treated lignite. It is noteworthy, that *Mw* of sample RHA8 is almost two-fold larger than the original HA9.
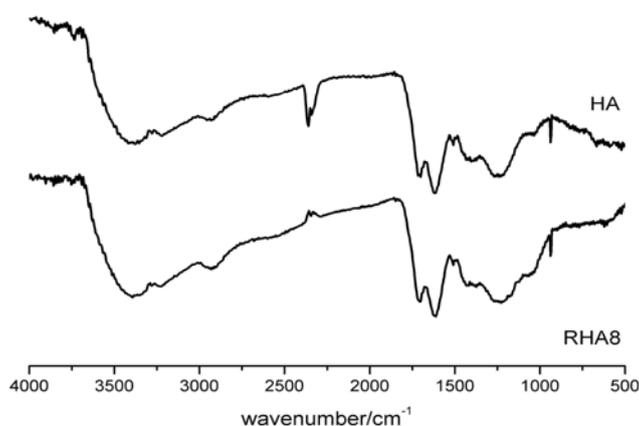
Fig. 3. Comparison of FTIR spectra humic acid extracted from parental lignite (HA) and regenerated humic acid (RHA8)

FTIR spectroscopy measurement of humic acids and RHA showed typical spectra published and described by many authors [3,12]. In this work only the FTIR spectra of HA and RHA8 are reported (Fig. 3). First, some notes to the attribution of peak wave numbers to chemical bonds vibrations. Fig 3 shows an intensive peak within the range 3400–3420 cm^{-1} which can be attributed mainly to the hydrogen bonded –OH; weak peaks in the region 2850–2960 cm^{-1} (aliphatic C–H stretching), strong sharp peaks around 1710 cm^{-1} (C=O of COOH) and 1620 cm^{-1} (C=C stretching, C=O stretching of COO$^-$, ketonic C=O and aromatic C=C conjugated with COO$^-$), a peak around 1510 cm^{-1} (N-H deformation, C-N stretching vibration, C=C aromatic bounds) 1450–1400 cm^{-1} (aliphatic C–H bending, and COO$^-$ asymmetric stretching, and possibly C=C and C=N plane vibrations of heterocycles), peaks around 1250 cm^{-1} (aromatic C, C-O stretch), a weak peak around 1050 cm^{-1} (C-O of polysaccharides and Si-O) were observed. The comparison of relative intensities of aliphaic and aromatic bands gave similar results as those reported for elemental analysis. Further, deeper analysis and comparison of peaks in the area 1710 cm^{-1} revealed the slight increase in abundance of esters in samples with higher molecular weight. Therefore, it can be assumed that oxidation of parental lignite led to oxidative disruption of weak points in lignite resulting in progressive increase in yield of extractable humic material. It is likely that higher temperature together with air atmosphere support the reactions between COOH and OH- alcohol groups, i.e. esterification. The increase in molecular weight, i.e. increase in number of ester is significant especially for samples developed at temperatures above 80°C when lipidic fraction is melted and recombination reactions can occur. Such conclusion is in agreement with the ability of regenerated humic acids to form gels having high water holding capacity [9]. However, in this work, gel formation was attributed to the higher ability of regenerated humic acids to form H-bridges due to the increase in content of COOH groups. To shed light on the influence of ester groups on water holding capacity is beyond the scope of this paper and it will be investigated in a special article.

4. Conclusion

The influence of lignite air oxidation on the yield and chemical/physical character of regenerated humic acids (RHA) has been shown. RHA were produced under different experimental conditions, i.e. both in static and fluid reactor. Oxidation in the fluid reactor brought first a decrease and after a certain period increase in the yield of RHA. The same observation was done in case of static reactor. It has been verified that higher temperature during the oxidation supports the production of RHA. Despite the difference in the yield of RHA (increase up to 75%) their chemical character was in all cases fairly similar. Some tiny differences were revealed using FTIR which showed the increase in number of ester bonds in RHA produced mainly at temperatures above 80°C causing a significant increase in apparent molecular weight as evaluated by HPSEC.

Acknowledgements

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