New ways of determining structural groups in brown coals and their bioconversion products by FT IR spectroscopy

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Abstract New methods of determining the structural groups —COOH and —CH2— have been developed. The investigation of carboxyl groups is possible both after derivatization with p-fluorophenacylbromide and by quantitative interpretation of the Fourier transform infrared (FT IR) spectra. There exists a linear relationship between the results of these two methods that is generally valid for the analysis of all brown coal components. The maximum extinction coefficient of the symmetric stretching vibration band of the CH2 groups has been determined using model substances. This allows quantification of this structural group directly from the FT IR spectrum. The results agree with the contents of methylene groups as determined by 13C-cross polarization–magic angle spinning–nuclear magnetic resonance (13C CPMAS NMR) spectroscopy. Using these methods, the COOH and CH2 groups contained in brown coals of the North Rhine region and in their bioconversion products have been quantified.

Introduction

The bioconversion of brown coal is due both to non-enzymatic mechanisms and to enzyme-catalysed cleavage of covalent bonds (Fakoussa and Hofrichter 1999). The enzymatic mechanisms leading to structural alterations can be detected using coal structural analysis methods, i.e., Fourier transform infrared (FT IR) spectroscopy. Furthermore, the determination of functional groups in coals before and after bioconversion is important for the detection of changes in the structure of brown coals during microbial degradation/solubilization.

Conventional methods of determining structural groups in coals require a high input of substance (up to 1 g) (Rast et al. 1982). So far, the quantity of bioconversion products prepared under laboratory conditions has been rather low. Consequently, it was necessary to develop methods which require only a few milligrams of substance.

Using the methods developed, we investigated samples of six German brown coals and additionally the products of one coal from the Rhine area (Bergheim, lithotype A) after bioconversion with the fungus Trichoderma atroviride.

Materials and methods

Coals investigated

We analysed five brown coals from the Rhine area, lithotype A from the Bergheim opencast mine and lithotypes A and B from the Garzweiler and Hambach opencast mines. Lithotype A coals (non-stratified) differ in texture from lithotype B coals (low-stratified). In addition, we investigated one pale lithotype from the region west of the Elbe, from the Schleenhain opencast mine, a so-called Gelbkohle (“yellow coal”). Details of the six coal samples used in this study are shown in Table 1. To calibrate the IR spectroscopic method, another five brown coals (from Dukla and Pres. K. Gottwald, Czech Republic; Espenhain, Germany; Bega-Nuur, Mongolia; and Sieniawa, Poland – all opencast mines) were included in the calibration data.

Fractionation of brown coals

After demineralization of the coals according to the procedure of Kuczynski and Andreják (1961), the demineralized coals were treated with toluene to extract the bitumen (Laske 1961). The humic acids were separated from the debituminized coal by an extraction procedure with 0.5 N sodium hydroxide solution under argon atmosphere according to Verheyen and Johns (1981). The remaining fraction is called matrix.

Products of biosolubilization

The bioconversion products investigated were the residue and the fraction obtained after acid precipitation from the supernatant
solubilized coal) of bioconversion with *Trichoderma atroviride*. Details about this deuteromycelium and the bioconversion conditions are given by Hökler et al. (1995, 1997, 1998). The products were treated with 6 N HCl at 95 °C to remove co-adsorbed constituents of the culture medium and washed in an acid-free bath.

Derivatization of coal carboxyl groups with *p*-fluorophenacylchloride

One milliliter of a solution of 1 mmol triethylamine in 10 ml acetone and 1 ml of a solution of 1 mmol *p*-fluorophenacylchloride in 10 ml acetone were added to 30 mg demineralized coal and stirred for 3 h at 50 °C. Afterwards the reaction mixture was diluted with water. The product was separated using filtering by suction.

IR spectroscopic determination of carboxyl groups

The coal carboxyl groups were converted into their carboxylate form by reaction with barium chloride/Tris(2-hydroxyethyl)ammonium solution (Schafer 1984). IR analysis on the barytated coals were performed using a Nicolet 510 FT IR spectrometer.

Results

Determination of carboxyl groups

The newly developed method on a milligram scale is based on the derivatization of carboxyl groups with a fluorine-containing reagent. Raw coals contain carboxyl (–COOH) and carboxylate (–COO−) groups. Before derivatization, coals are demineralized to convert COO− groups into COOH groups. During the reaction with *p*-fluorophenacyl bromide as derivatizing reagent, fluorine is brought into the coal in stoichiometric relation to the carboxyl groups according to the chemical equations below.

\[ R-COOH + \text{N(C}_2\text{H}_5)_3 \rightarrow [\text{HN(C}_2\text{H}_5)_3] R-COON \]

\[ R-CO^{-} + \text{BrCH}_2\text{CH}_2\text{C} \rightarrow \text{F} \rightarrow \text{F} = \text{C}(\text{CH}_3)\text{CO}-R + \text{HN(C}_2\text{H}_5)_3\text{Br}^{-} \]

The reaction was tested successfully with benzoic acid. Quantification of the fluorine content of the derivatized sample requires its direct combustion under oxygen atmosphere according to Schöninger (DIN 51400). The content of fluoride ions in the digestion liquor is determined by an ion-sensitive electrode. From the fluoride content, the concentration of carboxyl groups in the demineralized sample can be calculated by the following equation:

\[
\% \text{ COOH} = \frac{2.37072 \times \% F}{1 - 0.07166 \times \% F}
\]

The standard deviation of this procedure is within a range of 6.5% relative. The IR spectrum of a derivatized sample shows the successful course of the reaction. The carbonyl band of the ester formed can be observed near 1735 cm−1. The band at 1700 cm−1 is due to the carbonyl group of the derivatizing reagent. The band of the ary group–fluorine vibration and the C–O–C stretching vibration band of the ester formed appear between 1270 and 1100 cm−1 (Fig. 1).

The method developed is practicable for the investigation of demineralized coals and their non-soluble matrix. The carboxyl groups of humic acids and of solubilized coals cannot be determined with this method. The fluorophenacyl derivatives of these components

<table>
<thead>
<tr>
<th></th>
<th>Atomic ratio H:C</th>
<th>Atomic ratio O:C</th>
<th>% COOH</th>
<th>% CH2</th>
<th>% Bitumen</th>
<th>% Humic acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Berghem, lithotype A</td>
<td>0.913</td>
<td>0.308</td>
<td>0.50</td>
<td>10.1</td>
<td>9.0</td>
<td>6.6</td>
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<tr>
<td>Garzweiler, lithotype A</td>
<td>0.808</td>
<td>0.319</td>
<td>0.58</td>
<td>11.0</td>
<td>4.9</td>
<td>3.5</td>
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<tr>
<td>Garzweiler, lithotype B</td>
<td>0.893</td>
<td>0.346</td>
<td>0.52</td>
<td>8.2</td>
<td>4.4</td>
<td>3.6</td>
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<tr>
<td>Hambach, lithotype A</td>
<td>1.004</td>
<td>0.264</td>
<td>0.44</td>
<td>10.4</td>
<td>17.1</td>
<td>14.1</td>
</tr>
<tr>
<td>Hambach, lithotype B</td>
<td>0.855</td>
<td>0.311</td>
<td>0.55</td>
<td>8.3</td>
<td>6.3</td>
<td>4.2</td>
</tr>
<tr>
<td>Gelbkohle</td>
<td>1.192</td>
<td>0.232</td>
<td>0.30</td>
<td>9.4</td>
<td>21.5</td>
<td>20.2</td>
</tr>
</tbody>
</table>

\(^a\text{f}_{\text{a}}\) (aromaticity) = C\text{ar}/C\text{total}, determined by \(^{13}\text{C}\) CPMAS NMR spectroscopy

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**Fig. 1** Fourier transform infrared (FT IR) spectrum of a coal after derivatization with *p*-fluorophenacyl bromide