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Biodesulphurized subbituminous coal by different fungi and bacteria studied by reductive pyrolysis. Part 1: Initial coal

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Abstract

One of the perspective methods for clean solid fuels production is biodesulphurization. In order to increase the effect of this approach it is necessary to apply the advantages of more informative analytical techniques. Atmospheric pressure temperature programming reduction (AP-TPR) coupled with different detection systems gave us ground to attain more satisfactory explanation of the effects of biodesulphurization on the treated solid products.

Subbituminous high sulphur coal from "Pirin" basin (Bulgaria) was selected as a high sulphur containing sample. Different types of microorganisms were chosen and maximal desulphurization of 26% was registered. Biodesulphurization treatments were performed with three types of fungi: *"Trametes Versicolor"* – ATCC No. 200801, *"Phanerochaeta Chrysosporium"* – ME446, *Pleurotus Sajor-Caju* and one Mixed Culture of bacteria – ATCC No. 39327. A high degree of inorganic sulphur removal (79%) with Mixed Culture of bacteria and consecutive reduction by ~13% for organic sulphur (S_{org}) decrease with *"Phanerochaeta Chrysosporium"* and *"Trametes Versicolor"* were achieved.

To follow the S_{org} changes a set of different detection systems i.e. AP-TPR coupled "on-line" with mass spectrometry (AP-TPR/MS), on-line with potentiometry (AP-TPR/pot) and by the "off-line" AP-TPR/GC/MS analysis was used. The need of applying different atmospheres in pyrolysis experiments was proved and their effects were discussed. In order to reach more precise total sulphur balance, oxygen bomb combustion followed by ion chromatography was used.

Keywords: Coal; Biodesulphurization; Sulphur functionalities; Pyrolysis; Mass spectrometry

1. Introduction

The instability of the liquid fuel prices on the world market and observed tendency for their continuous rise pose again question for rationality utilization of solid fuels. It should not be forgotten that all fossil fuels contain different amounts of sulphur. The major sulphur content in coal is in inorganic form like pyritic and sulphatic sulphur. On the other hand some coals also contain a considerable amount of organically bound sulphur. During combustion, the sulphur is emitted as SO_x and is responsible for air pollution. Therefore the coal ecological exploitation is of utmost importance for the energy producing industry.

The most of the applied technologies of coal utilization are focused on sulphur removal before, during or after combustion processes. It is believed that the best methods to limit the amount of sulphur oxide emissions are based on preliminary sulphur decrease [1]. The applied techniques include physical, chemical and biological processes.

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The desulphurization of coal before combustion by means of biotechnological processes should represent an alternative to the costly intensive flue desulphurization which abates SO_2 emission after combustion [2]. Biological techniques are based on degradation of sulphur compounds by microorganisms. They offer many advantages over the conventional physical and chemical processes, namely low costs, easy experimental conditions and little or no energy loss after treatment. Microbial cultures may remove easily the inorganic sulphur up to 80-90%, but elimination of organic sulphur is more difficult [3]. Desulphurization of dibenzothiophene (DBT) was selected as sulphur containing model compound for coal treatment, since DBT was a typical organic sulphur compound in fossil fuels. There were several reports on the isolation of DBT desulphurizing bacteria [4]. Although much of the work on organic sulphur removal from coal has been attempted with various bacteria [5,6], relatively little work has been carried out with fungi [7]. Extra cellular enzymes produced by white rot fungi have the potential to be an effective biocatalyst, due to their broad specificity and ability to attack high molecular weight substrates [8]. Although *Laccase* activity (0.15 U/ml) was detected in the *Trametes* culture, more work is required to determine the major enzymatic system involved in organic sulphur metabolism. Hence, a possibility was found in this natural fungal strain to remove the organic sulphur from high sulphur coals [7].

The evaluation and comparison of different biotreatments and used microorganisms in general require an adequate and unambiguous determination of the sulphur distribution in the sample. First of all identification of organic sulphur in the coal still causes many problems [9].

Temperature programmed reduction at atmospheric pressure (AP-TPR) still proves to be an effective and fast technique for the specification of organic sulphur functionality species in coal [10]. The AP-TPR device, with potentiometric detection of the formed H_2S as S^{2-} ion using an ion selective Ag_2S-electrode, gives quantitative data concerning the presence of different sulphur species [11]. Identification of other evolving gases by extended detection techniques makes it possible to obtain more quantitative and qualitative information concerning sulphur distribution using variants with mass spectroscopy (MS) and gas/chromatographic/mass spectroscopic (GC/MS) techniques, coupled "on-line" and "off-line", respectively [12–14].

The total sulphur amount of less volatile sulphur species incorporated in tar and char fractions is possible to be measured by oxygen bomb combustion. Combination with AP-TPR potentiometric analysis data of the amount of H_2S fraction plus the contents of volatile and incomplete hydrogenated/reduced sulphur functionalities measured by off-line AP-TPR/GC–MS device, a more adequate manner of the total sulphur balance for the reductive pyrolysis of the samples can be determined.

The aim of the present study is to assess the change in sulphur functionalities in a high sulphur subbituminous coal after biotreatment by some proper high desulphurizing microorganism towards the initial total and also organic sulphur species. In part 2 the influence of biotreatment on deashed and pyrite free samples will be discussed in view of its effects and yield on only organic sulphur compounds.

2. Experimental section

2.1. Coal

A subbituminous coal sample from "Pirin" basin, Bulgaria was selected in view of its high organic sulphur content (Table 1).

2.2. Biodesulphurization

Selected microorganisms, effective towards organosulphur compounds, were used according to the previous methods applied [8,15]. Pure cultures of microorganisms were obtained from a microbial bank of ATCC (American Type Culture Collection). Coal samples (<0.063 mm) were added to the selected microorganism in the ratio of 3 g coal: 100 ml microorganism medium. The following six microbial systems were used:

- *Trametes Versicolor* (ATCC No. 200801), (TV-1)[1], white rot fungi. The experiment was performed for 6 days at a temperature of 37 °C and pH = 4.7. The *Laccase* activity was 5 U/ml.
- *Trametes Versicolor* (ATCC No. 200801), (TV-2)[2], white rot fungi.
- *Pleurotus Sajor-Caju*, (PSC), fungi.
- *Phanerochaeta Chrysosporium* (ME446), (PC), white rot fungi. The above mentioned three experiments were performed for 6 days at a temperature of 30 °C and pH 4.7.
- Mixed Culture of microorganisms (ATCC No. 39327), (MC), bacteria. The experiment was carried out for 6 days at a temperature of 37 °C and pH 6.
- Mixed Culture of microorganisms (ATCC No. 39327) + basal salt, (MC + BS), bacteria. The experiment was performed at the same condition as MC, but basal salt was added to the nutrient broth. It was also used in the US Patent [15].

The coal sample was not washed before the treatment with microorganisms. Biotreated coal samples were separated by filtration from the media and then washed by 0.1 M HCl acid and hot distilled water. After that, the coal samples were dried at 106 °C and analyzed for total sulphur content by the Eshka method together with other sulphur forms [16]. Bearing in mind the dry weight of the microorganism at the optimum growth conditions (≈0.05 g/l) and

[1] The highest Laccase enzyme activity was at temperature 37 °C.
[2] The optimal temperature of fungi growth was at 30 °C.

Table 1
Characteristics of the coal samples

Sample	S content (%), db				Proximate analysis (%)				Caloric value ($MJ \cdot kg^{-1}$)
	Total	Pyritic	Sulphate	Organic	Ash	Vol. mat.	Moisture	Fix C	
Initial	4.88	0.51	0.48	3.89	10.36	30.97	6.45	52.22	22.680
TV-1	3.64	0.13	0.12	3.39	7.98	28.65	8.80	54.57	22.278
TV-2	3.94	0.10	0.11	3.73	6.42	29.72	8.67	55.19	22.350
MC	3.87	0.09	0.12	3.66	7.33	29.38	8.12	55.17	22.500
MC + BS	3.63	0.09	0.11	3.43	7.49	29.95	7.41	55.15	22.198
PSC	3.82	0.10	0.11	3.61	6.99	31.03	6.98	55.00	22.450
PC	3.66	0.11	0.16	3.39	7.41	30.92	5.77	55.90	22.391

the amount of used sample in the experiment (30 g/l), biomass contamination in this procedure is unlikely.

2.3. AP-TPR/MS analysis

The identification of the volatile species released during the reductive pyrolysis of the samples under study was done by AP-TPR/MS experiments [10]. Briefly, a sample was mixed with fumed silica in order to prevent clothing and to guarantee optimal contact between the sample and the carrier/hydrogenation pure hydrogen gas. The mixture was placed in the quartz reactor and subjected to a linear heating of 5 °C/min from 25 up to 1025 °C. Hydrogen gas was passed into the reactor and the sample with a flow of 100 cm^3/min. AP-TPR reactor was coupled "on-line" with a mass quadruple spectrometer (FISONS-VG Thermo lab MS) through a capillary heated at 170 °C, operating at an ionizing voltage of 70 eV within the mass range 12–200 amu.

2.4. AP-TPR analysis with potentiometric detection (AP-TPR/pot analysis)

The quantification of reduced/hydrogenated sulphur species in hydrogen atmosphere during AP-TPR experiments was possible by potentiometric detection system. Briefly the procedure was as followed [10]. The H_2S, from the exit gas of the TPR reactor, as discussed in Section 2.3, was collected in an aqueous solution of sulphide antioxidant buffer (SAOB) and converted into HS^- and S^{2-} ions [17]. S^{2-} ion was measured at constant pH, using a S-ion selective electrode.

Sulphur recovery (R_{pot}) detected by the potentiometric system can be calculated using the following equation: % Sulphur recovery $= \{[(m_s/m_{coal}) \times 100]/S^a_{total}\} \times 100$, where m_s, m_{coal} and S^a_{total} are, respectively, the mass of sulphur hydrogenated/reduced during the AP-TPR experiment, the mass of the coal used (40 mg) and the total amount of sulphur on analytical basis in the analyzed sample.

2.5. Gas chromatography/mass spectrometry

The above described AP-TPR system can also be used in adsorption/desorption mode. The outlet of the AP-TPR reactor was connected to a set of ice-cooled tubes containing tenax, a porous polymer of 2,6-diphenyl-*p*-phenylene oxide as adsorbent. The volatiles were collected in the temperature range from 300 °C up to 700 °C in discrete temperature intervals of 50 °C. The tenax tubes were desorbed systematically and analyzed by a Perkin Elmer GC/MS apparatus using He as carrier gas at 48 kPa at following conditions: (a) Thermal desorber ATD 400: desorption temperature and time, respectively, 275 °C and 5 min, outlet split: 14 mL/min; (b) GC Auto system XL with capillary column ZB1 15 m × 0.32 mm and film thickness of 3 μm: initial temperature at 35 °C during 1 min, ramp rate 20 °C/min till an end temperature of 260 °C during 3 min; and (c) MS TurboMass Ver.4.1.1: *m/z* 25 = >300 in 0.5 s. Each tenax tube was spiked with 0.5 μg benzene-d_6 methanol solution to quantify results for the target sulphur species. SIM monitoring, using clusters of selected mass ions to identify individual organic sulphur compounds, allows a dedicated detection of these analyzed compounds. NIST library spectra were used for peak identification with special interest to the different sulphur species, liberated or in situ formed during the AP-TPR pyrolysis experiment. The following ions were selected: *m/z* 94 for dimethylsulphide and dimethylsulphone (different retention times), *m/z* 84 + 14*n* for thiophenes (with *n* = number of CH_2 groups), *m/z* 134 for benzo[*b*]thiophene, and *m/z* 148 for methyl benzo[*b*]thiophene.

2.6. Sulphur balance determinations

The total sulphur content of both tar and char fractions was determined by using oxygen bomb combustion technique. During each AP-TPR experiment, tar and char fractions were deposited on the reactor walls. They were collected by washing with methylene chloride. Obtained solutions were transferred to the quartz crucible, dried at ambient temperature and later on at 60 °C in an oven. The recovered organic material was obtained in a very low quantity. To perform oxygen bomb combustion experiments, sulphur free mineral oil was added to the dried sample. Produced sulphate, after combustion, was transferred quantitatively in volumetric flask by bi-distillated water. Sulphur amount (as sulphate) was determined by ion chromatography instrument Dionex (DX-120), supplied with column (AS4A-SC) and conducting detector.

Apparatus for elemental analysis Flash EA 1112 of Thermo Electron Corporation to calculate S-tar/S-char ratio was used.

3. Results and discussion

To select the proper microbial cultures with high desulphurizing effect towards total and organic sulphur in the studied samples, different types of microorganisms were applied (Table 2). The biodesulphurization made by "*Trametes versicolor*" fungi was held over at two different temperatures. The choice was made based on the fact that 30 °C was optimal temperature of fungi growth (TV-2) while 37 °C was temperature for the highest "*Laccase Enzyme*" activity (TV-1). The results demonstrated that when the temperature was 37 °C, a higher level of desulphurization was reached. For this reason and because the same microorganism was used in both cases with TV-1 and TV-2, the sulphur changes only in TV-1 were examined. Concerning MC and MC+BS, the higher desulphurization effect was achieved for MC+BS experiment. Therefore the sulphur changes only in this case were examined. There were investigations over the sulphur functionalities after biodesulphurization performed by "*Phanerochaeta Chrysosporium*" as well. Experimental data for the biodesulphurization by "*Pleurotos Sajor-Caju*" were not included in the text because of the lower biodesulphurization effect of the treatment.

It was found that implemented biotreatments demonstrated maximum total sulphur and organic sulphur desulphurizations around 26% and 13%, respectively, to the following microbial systems: "*Trametes versicolor*" – ATCC No. 200801 (TV-1), "*Mixed Culture*" + basal salt – ATCC No. 39327 (MC + BS) and "*Phanerochaeta Chrysosporium*" – ME446 (PC). Sulphur changes in solid products provoked by the biotreatments with these microorganisms were investigated in details. In Table 1, data for proximate and wet sulphur analyses are listed [16]. It is obvious that also a reduction of the ash content is found from 10.4% to 7.4%. This observation correlates in part with a decrease in pyritic and sulphate concentrations in the initial sample (calculated to total sulphur presence) from 20% to 6–7% after biotreatments. Applied biotreatments decrease the heating value of the samples by 300–500 kJ kg^{-1}, calculated according to the formula of Channiwala and Parikh [18].

Table 2
Biodesulphurization effect on S_{tot} and S_{org}, in %

No	Sample	S_{tot}, db	ΔS_{tot}	S_{org}, db	ΔS_{org}
0	Initial coal	4.88	0	3.89	0
1	"*Trametes Versicolor*", (TV-1)	3.64	25.4	3.39	12.8
2	"*Trametes Versicolor*", (TV-2)	3.92	19.7	3.73	4.1
3	"*Mixed Culture*", (MC)	3.87	20.7	3.66	5.9
4	"*Mixed Culture + BS* ", (MC + BS)	3.63	25.6	3.43	11.8
5	"*Pleurotos Sajor-Caju*" , (PSC)	3.81	21.9	3.61	7.2
6	"*Phanerochaeta Chrysosporium*", (PC)	3.66	25.0	3.39	12.8

S_{tot}, db and S_{org}, db – S total and S organic, respectively, on dry basis. ΔS_{tot} and ΔS_{org} – S total and S organic differences.

3.1. AP-TPR experiments coupled "on-line" with potentiometric and MS detection in hydrogen atmosphere

The H_2S kinetograms of AP-TPR by MS of initial and biotreated coal samples are visualized in Fig. 1. Because $m/z = 34$ (H_2S^+) and $m/z = 33$ (HS^+) exhibit the same evolution, only the sum of both ion profiles is shown. Potentiometric kinetograms are very similar to the H_2S/HS MS profiles and are therefore not shown and only used for quantitative calculations. There are always two dominant peaks with T_{max} around 400–420 °C and 650 °C. The peak at around 400–420 °C can be assigned to the presence of dialkyl and alkyl-aryl sulphides [10]. No clear indications for the presence of thiols or disulphides are according to these profiles found, nor in initial and nor in treated coal. This conclusion is based on the model compound approach and also on AP-TPR–MS profiles of typical aliphatic and aromatic CH-fragments as shown in Fig. 2A and B for the initial coal sample, respectively, and as an example for all samples studied. Indeed, for the three treated samples, the same profiles for the above fragments are found as well as in shape and as in sequence, as at the same maximum evolution temperature. Therefore these figures are not shown. For typical aliphatic fragments, they included unsaturated CH-chains (alkanes and alkenes) of ion fragments with m/$z = 55$ ($C_4H_7^+$), $m/z = 57$ ($C_4H_9^+$), $m/z = 69$ ($C_5H_9^+$), $m/z = 71$ ($C_5H_{11}^+$) and $m/z = 83$ ($C_6H_{11}^+$).

The second peak maximum in the H_2S/HS profile (Fig. 1) refers to the presence of di-aryl and more complex thiophenic structures. Also this is based on model compound studies and based on the evolution of typical aromatic CH-fragment ions in that temperature range. Fig. 2B shows ion fragments referring to aromatic compounds as follow: benzene – $m/z = 77$ ($C_6H_5^+$) and $m/z = 78$ ($C_6H_6^+$), toluene – $m/z = 92$

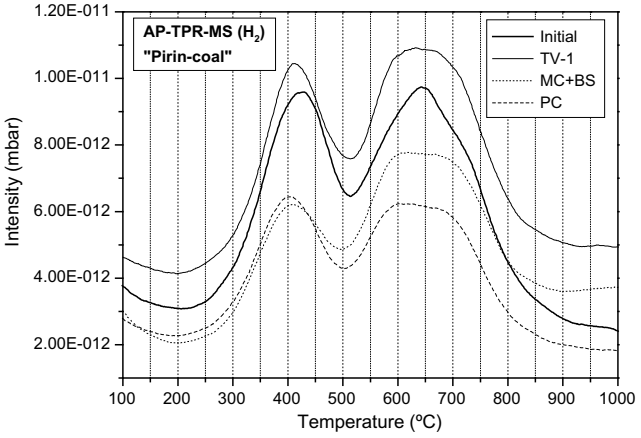


Fig. 1. AP-TPR/MS (H_2), $m/z(33 + 34)$ kinetograms of Pirin coal samples: initial, TV-1, MC + BS and PC.

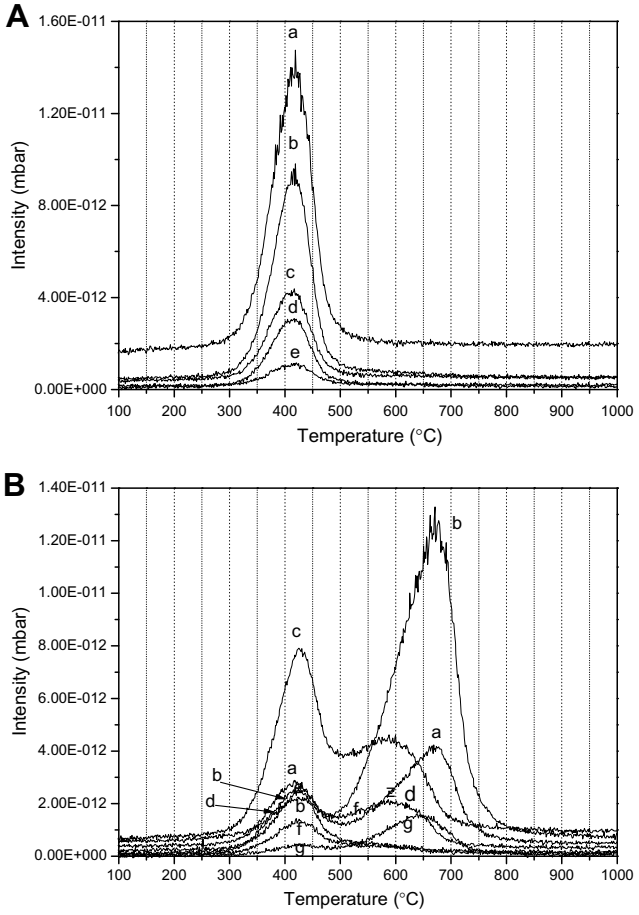


Fig. 2. AP-TPR/MS (H_2) evolution profiles of sample Pirin – initial: (A) for saturated and unsaturated CH-chains (alkanes and alkenes); (B) for aromatic compounds; saturated and unsaturated CH-chains: (a) $m/z = 55$ ($C_4H_7^+$); (b) $m/z = 57$ ($C_4H_9^+$); (c) $m/z = 69$ ($C_5H_9^+$); (d) $m/z = 71$ ($C_5H_{11}^+$); (e) $m/z = 83$ ($C_6H_{11}^+$). Aromatic compounds: benzene: (a) $m/z = 77$($C_6H_5^+$); (b) $m/z = 78$ ($C_6H_6^+$), toluene: (c) $m/z = 92$ ($C_7H_8^+$); (d) $m/z = 91$ ($C_7H_7^+$), xylene: (e) $m/z = 106$ ($C_8H_{10}^+$), alkyl-benzene: (f) $m/z = 105$ ($C_8H_9^+$), naphthalene: (g) $m/z = 128$ ($C_{10}H_8^+$).

($C_7H_8^+$) and $m/z = 91$ ($C_7H_7^+$), xylene – $m/z = 106$ ($C_8H_{10}^+$), alkyl-benzene – $m/z = 105$ ($C_8H_9^+$) and naphthalene – $m/z = 128$ ($C_{10}H_8^+$). Also here for the treated samples almost the same profiles are found in shape, sequence and maximum evolution rate temperature value. Generally, one must thus conclude that the biotreatments does not affect the coal matrix.

For the initial coal compared to the treated ones, the second H_2S evolution peak has a much sharper shape with a clear peak maximum. For the treated ones, the shape more flatted and refers thus to a removal of the same class of a more complex thiophenic sulphur compounds. Further the AP-TPR H_2S kinetogram of all treated samples exhibit almost the same trend, but small differences in shape and height can been seen. The removal effect of pyrite (−40%) [19], due to its low presence in the initial sample, is not detected in the profiles (Fig. 1). For the MC + BS treated sample the first H_2S peak is clearly lower than the second one, meaning that aliphatic sulphur compounds are more removed than aromatic ones. For TV-1 and PC treated samples this seems not the case.

It is known that oxidized sulphur forms present in the sample, are not entirely reduced in AP-TPR experiments into H_2S [20]. Their SO_2 and SO fragments can also be identified and so their sulphur functionalities using AP-TPR with MS detection.

Fig. 3a and b visualize partly the evolution of oxidized sulphur functionalities during the AP-TPR experiments for initial and biotreated samples. On Fig. 3a profiles of both $m/z = 48$ and 64 for initial coal are shown, as the signal output for these ions exhibit different evolution trends. For the same reason in Fig. 3b both m/z profiles are given for the treated samples (for clearness of presentation). There is a huge first peak at 260 °C for the initial coal sample (Fig. 3a), which certainly refers to organic sulphates. This signal is almost absence for the treated samples (Fig. 3b), meaning that for all treated samples this functional group has disappeared and thus has been removed by the biotreatments. The large shoulder at 350–400 °C refers not only to a possible presence of sulphones but mostly to CH-fragments, because here m/z 64 and m/z 48 clearly exhibit different trends. For MC + BS treated sample, the difference in shape (besides also the small temperature difference) is more

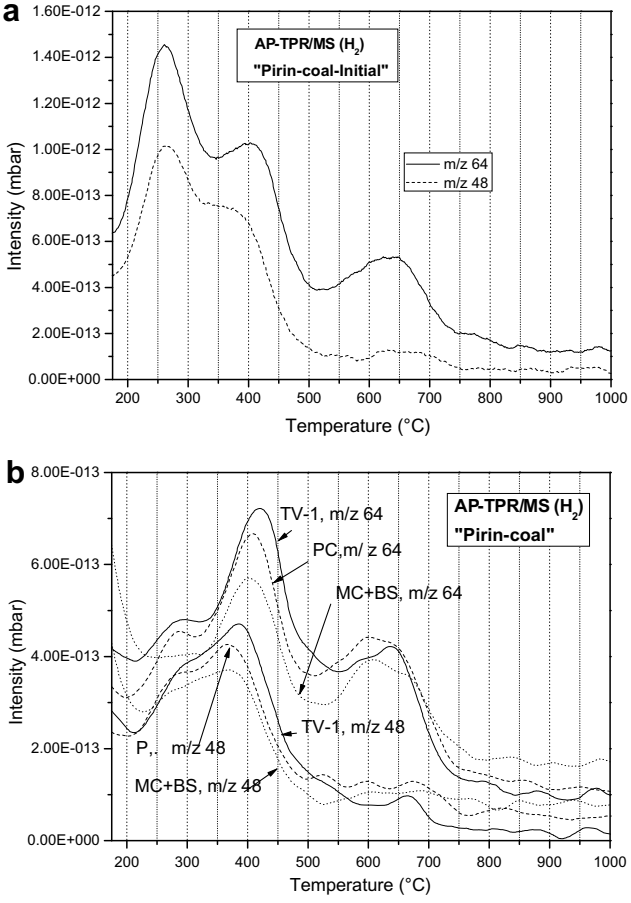


Fig. 3. AP-TPR/MS (H_2), m/z 48 and 64 kinetograms of Pirin coal samples: (a) initial; (b) TV-1, MC + BS and PC.

evident than in the case of the initial coal sample. For PC treated sample the difference in trend of both profiles is limited. For TV-1 treated sample the lower temperature range on the peak is also clearly different. This can be explained as a different impact of the three treatments on the coal matrix, which is reflected in the amounts and on the kind of the possible presence of sulphones.

The less intense second maximum at around 600–650 °C in the m/z 64 profile of the treated samples refers rather to CH-fragments and not to the presence of sulphoxides. Also here we see that for the m/z 64 profiles of initial and for biotreated samples different trends can be observed. For MC + BS and PC these trends look similar and are different from the TV-1 treated sample. This again could point to the different actions of the three studied biotreatments on the coal matrix and/or possible presences of some sulfoxides. We should highlight the fact that the measured intensities are very weak and that the conclusions formulated using Fig. 3a and b are perhaps at this state too detailed [21]. The complexity of the coal matrix compared with the pure model compound approach could induce differences in the m/z 64 and 48 profiles. This effect is under study. Nevertheless, as a first attempt to get more confirmation towards this conclusion, additional AP-TPR experiments are performed in a different atmosphere, i.e. He. Further on, other detection systems are needed to get more certainty about what pathways of the oxidized sulphur compounds and if they are original present in the coal samples. Also this is under study. But because biotreatment reaction mechanisms are going via an oxidative way, a more detailed discussion is important.

3.2. AP-TPR experiments "on-line" with MS detection in inert atmosphere

Pyrolysis in an inert atmosphere can give additional information concerning sulphur species. In Fig. 4 AP-TPR/MS evolution profiles in He atmosphere for H_2S are shown as a sum of ion fragments with $m/z = (33 + 34)$. Now only one broad peak maxima is observed, around 400 °C, demonstrating rather again the importance of the use of different atmosphere, in general, by performing AP-TPR experiments, and the importance of H_2 as carrier gas and its increasing capacity towards hydrogenation or reduction of sulphur compounds at higher temperature. The intensity increase found for the treated samples compared to the initial one should be handled with care. Indeed, the MS detection system is only a semi quantitative technique. Furthermore, the treated samples clearly show a much broader signals compared to the initial one. Biodesulphurization has also a clear effect on the susceptibility of the organic coal matrix towards incoming and outgoing volatiles. This should result in a better release/hydrogenation/reduction of organic sulphur groups still present in the treated samples. Additionally, we believe that even more information can be obtained for the oxidized sulphur species. On Fig. 5a and b AP-TPR/MS kinetograms in He medium for

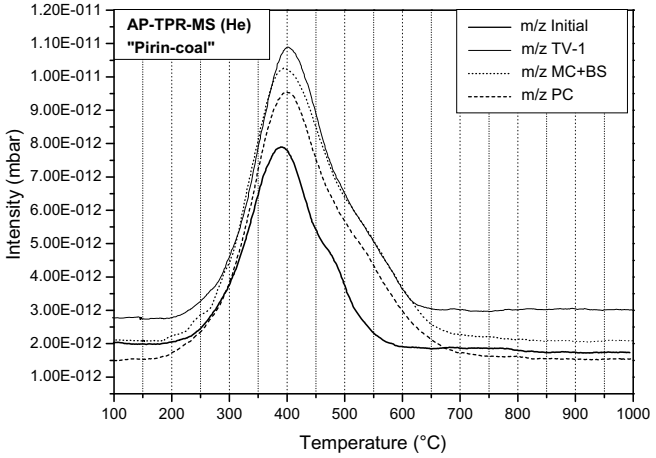


Fig. 4. AP-TPR/MS (He), $m/z(33 + 34)$ kinetograms of Pirin coal samples: initial, TV-1, MC + BS and PC.

ion fragments with m/z 48 and 64 are shown referring to the presence of some oxidized sulphur compounds. Since the profiles now exhibit the same trends, they must be attributed to SO_2 and SO. Comparing Fig. 5a and b [AP-TPR/MS (He)] with Fig. 3a and b [AP-TPR/MS (H_2)] for the three treated samples, the same conclusion, as formulated above, must be formulated. It is obvious that the atmosphere influences both the intensity and the profile. Concerning the initial coal kinetogram (Fig. 5a) the first peak maximum can still be assigned to organic sulphates. A new T_{max} appears at 460 °C and refers to the decomposition of iron sulphate [14]. This maximum completely disappears in a reducing atmosphere due to SO_2 partly reduced to H_2S for all samples and additionally because of the lower sulphate amounts in the treated samples (Table 1). The lower platform between the two peaks refers to the presence of sulphones. The shoulder at higher temperature about 580 °C on the second maximum (Fig. 5a) of the initial samples refers rather to diarylsulphoxides. The broad signal in Fig. 5b with a maximum around 400 °C refers to sulphones again. For TV-1 the second maximum at 625 °C, although with a low intensity, can be attributed to the presence of diarylsulphoxides. It is known that biodesulphurization with white rot fungi TV-1 occurs via oxidation of diarylsulphoxides to diarylsulphones [8].

Fig. 6A and B show TPR–MS profiles of typical aliphatic and aromatic CH-fragments for the initial coal sample, respectively. These figures give also prove for the assignments of the presence of certain sulphur functionalities towards lower temperature than in the case of working in pure H_2 atmosphere. These figures act again as an example for all samples studied. For treated samples, the same profiles are found in shape, trend, temperature and sequence, and therefore again are not shown. These profiles also prove why in Fig. 4 only one dominated peak profile is found for the H_2S evolution for all studied samples and thus less information can be conducted. But combined with the results of the H_2 atmosphere experiment, supplementary information can be deducted. The less resolved shoulder

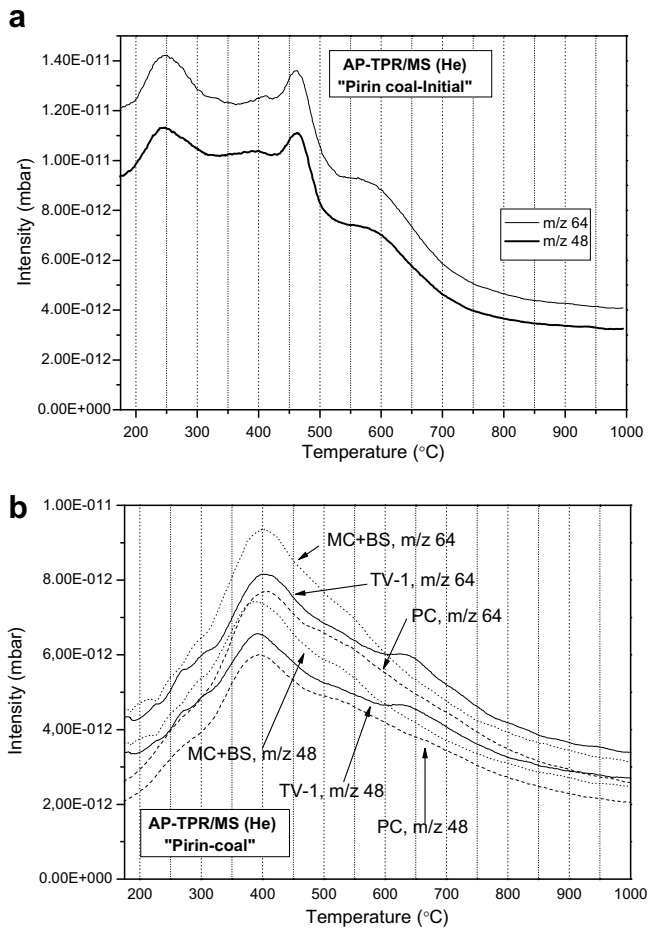


Fig. 5. AP-TPR/MS (He), *m/z* 48 and 64 kinetograms of Pirin coal samples: (a) initial; (b) TV-1, MC + BS and PC.

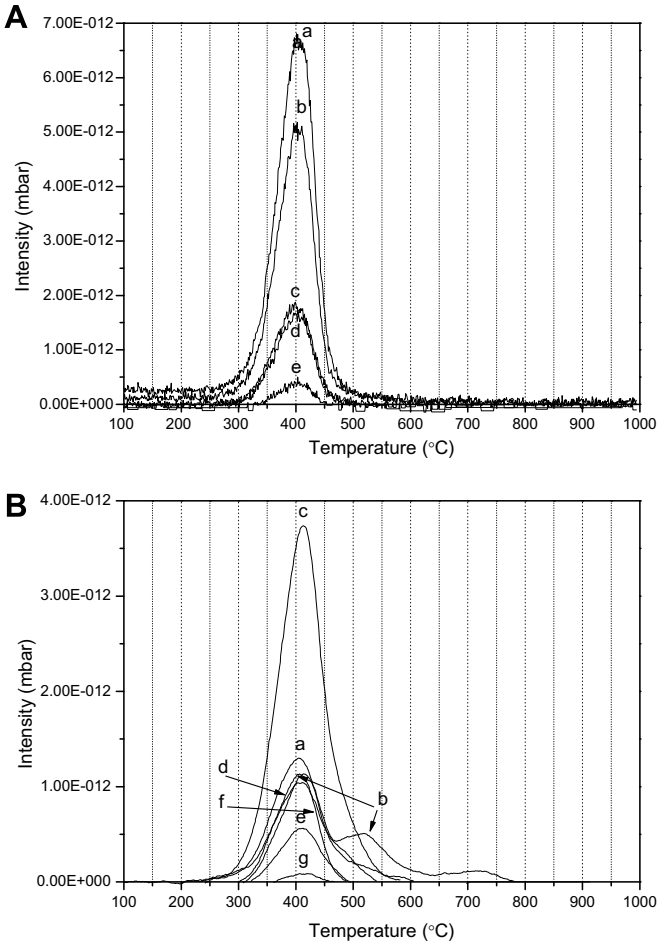


Fig. 6. AP-TPR/MS (He) evolution profiles of sample Pirin – initial: (A) for saturated and unsaturated CH-chains (alkanes and alkenes); (B) for aromatic compounds; saturated and unsaturated CH-chains: (a) $m/z = 55$ ($C_4H_7^+$); (b) $m/z = 57$ ($C_4H_9^+$); (c) $m/z = 69$ ($C_5H_9^+$); (d) $m/z = 71$ ($C_5H_{11}^+$); (e) $m/z = 83$ ($C_6H_{11}^+$). Aromatic compounds: benzene: (a) $m/z = 77$($C_6H_5^+$); (b) $m/z = 78$ ($C_6H_6^+$), toluene: (c) $m/z = 92$ ($C_7H_8^+$); (d) $m/z = 91$ ($C_7H_7^+$), xylene: (e) $m/z = 106$ ($C_8H_{10}^+$), alkyl-benzene: (f) $m/z = 105$ ($C_8H_9^+$), naphthalene: (g) $m/z = 128$ ($C_{10}H_8^+$).

at the higher temperature range refers to the presence of more complex thiophenic structures.

3.3. AP-TPR "off-line" GC/MS experiments

Minor amounts of volatile organic sulphur compounds are neither reduced in the AP-TPR condition into H_2S nor captured in the tar fractions. But these volatiles can be adsorbed on tenax tubes. Afterwards they can be thermally desorbed and qualitatively and quantitatively determined by GC/MS. This set-up is defined as the AP-TPR "off-line" coupled with GC/MS device [22].

The pyrograms of the samples under investigation were dominated by typical products of coal pyrolysis, i.e. alkenes/alkanes (nC_6–nC_{13}), alkyl-benzenes (C_6–C_{10}), alkylnaphthalenes (C_{10}–C_{14}), biphenyls (C_{12}–C_{14}), phenols (C_6–C_{10}), nitriles, etc. All compounds at a certain extent were accompanied by their sulphur containing analogues. Organic sulphur species were determined by "off-line" GC–MS technique applying single ion monitoring (SIM):

- *m/z* 94 for aliphatic sulphides, i.e. dimethyldisulphide (Me-SS-Me) and dimethyl sulphone ($diMeSO_2$);
- *m/z* $87 + 14n$, where n is the number of alkyl groups for thiophenes (Th), methyl- (Me-Th), dimethyl- (diMe-Th) and trimethyl thiophenes (triMe-Th);
- *m/z* $134 + 14n$ for benzothiophene (Bz-Th) and methylbenzothiophene (Me-Bz-Th).

GC–MS spectra were quantitatively determined by spiking with 0.5 μg benzene-d_6. Typical curves of organic sulphur compounds distributions are illustrated in Fig. 7, where the profiles are expressed in sulphur compound *vs.* trapping temperature. Besides the above mentioned sulphur compounds their higher homologues were also detected: like tetra/penta substituted thiophenes and di/three substituted benzothiophenes. But because of their very small amounts, the large numbers of isomers and the strong overlapping signals, concentrations were not calculated.

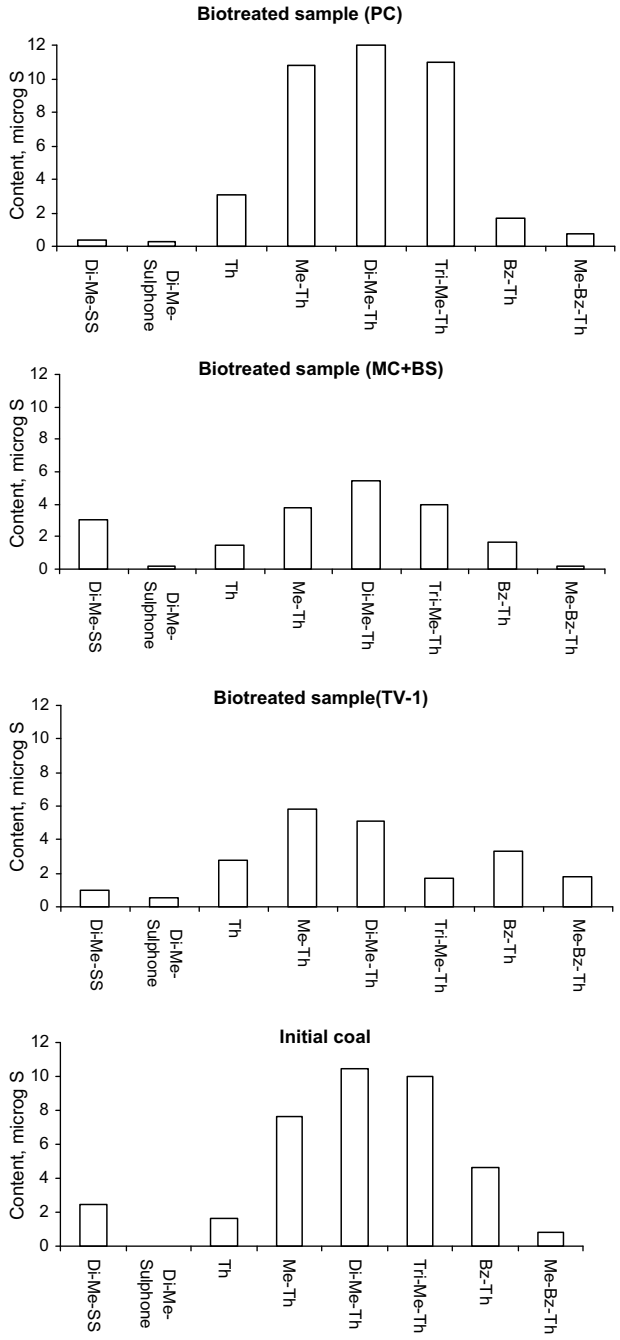


Fig. 7. Sulphur compounds determined by AP-TPR "off-line" GC/MS, in μg/g. (Abbreviations: Me – methyl; Th – thiophene; Bz – benzo.)

Based on the above GC–MS quantifications, the determination of the total content of sulphur compounds under consideration, are expressed in (μg sulphur)/(g coal) found in the tenax tubes (Table 4, Fig. 8).

Analyzing the quantitative results for the volatile organic sulphur compound, received by AP-TPR "off-line" GC/MS experiments (Table 4, Fig. 8), we can conclude that in all biotreated samples dimethyl sulphone is present, which is related with aliphatic sulphur oxidation during the biotreatments. In specialized literature this oxidizing mechanism of the biodesulphurization process of organic sulphur forms can be found. For example, van Hamme et al. [8] studying dibenzylsulphide (DBS) metabolism with white rot fungi (among which TV-1 and PC are also examined here) has found that the C–S bond in alkyl bridge of DBS is the target of oxidation by fungal cultures and the metabolism proceeds from DBS to dibenzyl sulphoxides followed by dibenzyl sulphone, prior to C-S bond cleavage. According to this research, ring oxidation and opening occur only after this cleavage. The metabolic conversion of dibenzyl sulphoxide to dibenzyl sulphone shows to be mediated by cytochrome P-450, while extra cellular enzymes or combination of extra cellular enzymes and cytochrome P-450 may mediate the initial oxidation of DBS. Keeping in mind these data, we can suppose that the increase in the quantity of dimethyl sulphone in the biotreated samples can be based on a similar mechanism. The oxidation of di-alkyl sulphides to di-alkyl sulphoxide (probably with the catalyzation of extra cellular enzymes) and the further oxidation of di-alkyl sulphoxide to di-alkyl sulphone (probably catalyzed by cytochrome P-450). Nevertheless, the confirmation of this mechanism needs to be investigated with complementary and/or supplementary techniques.

By comparison between the quantity of thiophene (Th) in untreated coal samples (initial) and biotreated samples, one can notice that in those biotreated samples with white

Table 3
Sulphur balance, in %

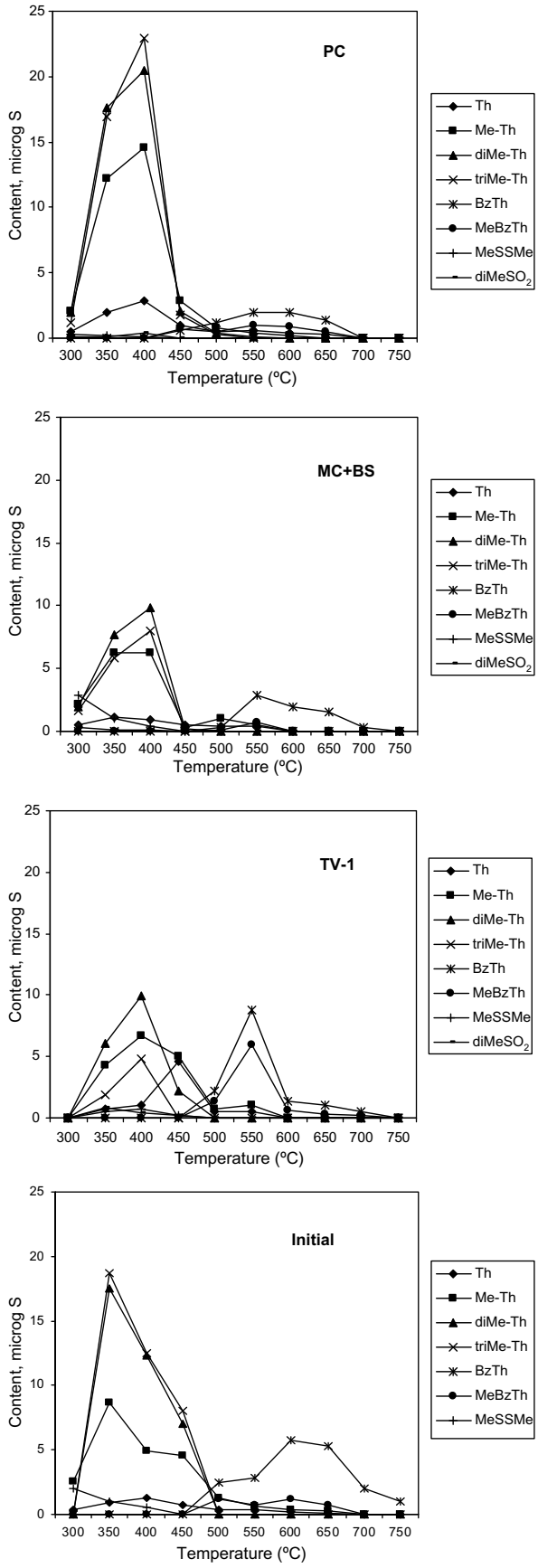
Sample	S_I by TPR/pot	S in tar, char/AP-TPR		S_{III} volatiles	$(S_I + S_{II} + S_{III})$[a]	R_{pot}
		S_{II} (tar + char)	S-tar/S-char			
Initial coal	2.94	0.292	2.226	0.094	72.8	64
TV-1	2.47	0.214	2.484	0.055	82.5	74
MC+BS	2.32	0.211	2.322	0.049	76.8	69
PC	2.63	0.194	1.532	0.099	84.7	76

R_{pot} = sulphur recovery as determined by AP-TPR-pot.
[a] Sulphur sum $(S_I + S_{II} + S_{III})$ as a part (%) from the S_{tot} (on analytical basis) in the samples.

Table 4
Sulphur in organic compounds determined by AP-TPR/GC-MS, in μg/g

No.	S compounds	Sample			
		Initial	TV-1	MC + BS	PC
1	Me-SS-Me	2.43	0.98	3.05	0.38
2	DiMe-Sulphone	0	0.50	0.19	0.24
3	Th	1.62	2.78	1.45	3.10
4	Me-Th	7.60	5.81	3.78	10.81
5	DiMe-Th	10.50	5.12	5.40	12.00
6	TriMe-Th	10	1.70	3.94	10.97
7	Bz-Th	4.60	3.32	1.65	1.71
8	Me-Bz-Th	0.80	1.80	0.18	0.77
	Total	37.55	22.01	19.64	40.15

Abbreviations: Me – methyl; Th – thiophene; Bz – benzo.



rot fungi TV-1 and PC the amount of Th increases, although limited in content to MC + BS. It is well known that fungi are able to metabolize a wide range of hydrocarbons and polycyclic aromatic hydrocarbons, and can even be used for coal solubilization. Several white rot fungi were screened for their ability to solubilize lignites. For example *Phanerochaete Chrysosporium* (PC) was found to depolymerize Elbistan lignite with 60% efficiency [23]. Based on comparisons of IR spectra of treated and untreated samples, the authors conclude that fungal treatment did not cause demethylation but provoke the effective ring opening of the aromatic structure by fungus. This is the reason why we suppose that the increase in the Th concentration found during the AP-TPR experiment in the biotreated samples with fungi may be caused by a firstly breakdown of the aromatic ring structure in more complex sulphur organic forms, as for Bz-Th (which quantity decreases in both cases, Table 4). As a result, the methylated forms of Th in treated coal samples with white rot fungi the quantity of Me-Th, Di-Me-Th and Tri-Me-Th in TV-1 decreases significantly (mostly in Tri-Me-Th). With PC the increase in concentration of these compounds is not so obvious. Probably for the white rot fungi TV-1, along with the effective opening of the aromatic ring, also demethylation occurs.

The case with MC + BS is very different for the latter discussed compounds. The amount of Th and its methylated forms along with Bz-Th and Me-Bz-Th decreases. In US Patent 4659670, it is described that seven distinct aerobic gram negative rods have been recognized in the microorganism mixture (ATCC No. 39327). Also here is mentioned that ATCC No. 39327 is comprised of seven aerobic gram negative rods which are probably one or more of the following: Pseudomonas, Acinetobacter, Azotobactor or Flavobacteria. These bacterial species were of great interest in the early success of organic sulphur removal. A genetically modified strain of Pseudomonas alcaligenes was shown to be capable of oxidizing thiophene [24]. Other strain of Pseudomonas, isolated for the ability to grow on DBTh as source of carbon and sulphur, was used to treat high organic sulphur coal. A loss of 38–45% of the total sulphur content was reached after 10 days [25]. Acinetobacter strain EP can utilize Bz-Th and DBTh as the only sulphur sources [26]. Flavobacterium sp, isolated by enrichment on thiophene-2-carboxylic acid, was reported to release the sulphur as sulphate but it utilized the rest of the compound as a source of carbon for growth [27]. It is obvious that for making any significant and general conclusions for the mechanism of biodesulphurization in the mixed culture MC + BS, a more profound and detailed analyses should be made: on one hand, because

Fig. 8. Evolution profiles of determined sulphur compounds monitored by AP-TPR "off-line" GC/MS. (Abbreviations: Th – thiophene; Me-Th – methylthiophene; diMe-Th – dimethyl thiophene; triMe-Th – threemethyl thiophene; Bz-Th – benzothiophene; Me-Bz-Th – methylbenzothiophene; Me-SS-Me – dimethyldisulphide; diMeS0$_2$ – dimethyl sulphone.)

of the presence of different types of bacteria in the mixed culture ATCC No. 39327, and on the other hand, because of the complexity of the examined product, in our case coals. However, it can be stated that the mixed culture MC + BS turned out to be the most effective one in cleaning the examined volatiles sulphur compounds.

3.4. Sulphur balance determination

Sulphur quantification was possible by AP-TPR experiments with potentiometric detection. In Table 3 potentiometric sulphur recovery values (R_{pot}) and potentiometrically determined total sulphur in the samples (S_I) are listed. Biotreated sample with PC has the highest R_{pot} value (76%) while the lowest sulphur recovery is found for initial coal (64%). The differences in recovery can be explained not only by the presence of inorganic sulphur (pyrite and sulphate), but moreover by the highest ash content of the initial sample, acting as a H_2S adsorbent [28]. Volatile sulphur species, not determined by AP-TPR/pot, can be quantitatively measured by AP-TPR "off-line" GC/MS device. Apart from volatilization of sulphur species, more complex thiophenic structures can be formed (secondary reaction), while sulphur compounds can also be incorporated in tar fraction. The more complex thiophenic structures are very resistant to AP-TPR reduction conditions and they will remain in the char fraction.

The total sulphur distribution can be calculated taking into account the sulphur contents in the tar and char fractions obtained in AP-TPR experiment (S_{II}). The total sulphur balance can thus be calculated as followed:

$$S_{tot} = S_I + S_{II} + S_{III} + S_{res}$$

where S_{tot} – total sulphur; S_I – potentiometrically determined sulphur, S_{II} – oxygen bomb determined sulphur; S_{III} – GC/MS "off-line" measured sulphur; S_{res} – sulphur rest, comprising lost sulphur compounds (i.e. not captured by the tenax tube and/or not optimal spiking reference for sulphur compounds: both under investigation).

Total sulphur in the collected tar and char fractions was thus determined by oxygen bomb combustion (S_{II}). The obtained values are summarized in Table 3. S_{II}-values decrease for all biotreated samples. The sample treated with PC has the lowest amount. For the same sample, the sulphur recovery (R_{pot}) was the highest. The equation for sulphur balance incorporated also the rest sulphur fraction (S_{res}) corresponding to the not condensed volatile sulphur species (apart from H_2S), like SO_2 and SO. These sulphur compounds are released in the lower temperature region where the reduction efficiency of hydrogen and the coal matrix are low. For biotreated sample with PC, the highest value for the sulphur sum ($S_I + S_{II} + S_{III}$) (on analytical basis) was obtained, i.e. around 85% (Table 3). The sulphur balances are visualized in Fig. 9.

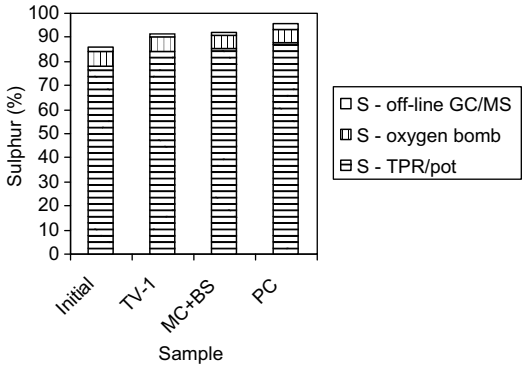


Fig. 9. Sulphur balance, in %.

4. Conclusions

AP-TPR device coupled with different detection systems (potentiometric, MS and GC/MS) gave us the opportunity to quantify almost all organic sulphur species present in the biotreated coal. For the studied subbituminous coal biodesulphurization by selected fungi and mixed bacterial culture system can eliminate the organic sulphur functionalities up to 13% and inorganic sulphur up to 79%. For the biotreated samples, the decrease in heating value was negligible. Additionally, AP-TPR "off-line" GC/MS study confirmed biodegradation process of complex sulphur species into sulphones and sulphoxides. The applied biotreatments predominantly affect the evolution of volatile sulphur species to be the highest with PC fungi.

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References

[1] Prayuenyong P. Coal biodesulfurization processes. Songklanakarin J Sci Technol 2002;24(3):493–507.
[2] Güllü G, Durusoy T, Özbaş T, Tanyolaç A, Yürüm Y. Biodesulphurization of coal. In: Yürüm Y, editor. Clean utilization of coal. Netherlands: Kluwer; 1992.
[3] Acharya C, Kar RN, Sukla LB. Bacterial removal of sulphur from three different coals. Fuel 2001;80:2207–16.
[4] Cray KA, Mrachko GT, Squires CH. Biodesulfurization of fossil fuels. Curr Opin Microbiol 2003;6:229–35.
[5] Kilbane II JJ. Microbial biocatalyst developments to upgrade fossil fuels. Curr Opin Biotechnol 2006;17:305–14.
[6] Durusoy T, Özbaş T, Tanyaolaç A, Yürüm Y. Biodesulfurization of some Turkish lignites by *Sulfolobus Solfataricus*. Energ Fuels 1992;6:804–8.
[7] Acharia C, Sukla LB, Misra VN. Biological elimination of sulphur from high sulphur coal by *Aspergillus-like* fungi. Fuel 2005;84:1597–600.
[8] van Hamme JD, Wong ET, Dettman H, Gray MR, Pickard MA. Dibenzyl sulfide metabolism by white rot fungi. Appl. Environ. Microbiol. 2003;69:1320–4.

[9] Olson GJ, Kelly RM. Chemical and microbiological problems associated with research on the biodesulfurization of coal. A review. Resour Conserv Recy 1991;5:183–93.
[10] Yperman J, Maes II, Van den Rul H, Mullens S, Van Aelst J, Franco DV, et al. Sulphur group analysis in solid matrices by atmospheric pressure–temperature programmed reduction. J Anal Chim Acta 1999;395(1–2):143–55.
[11] Maes II, Mitchell SC, Yperman J, Franco DV, Marinov SP, Mullens J, et al. Sulphur functionalities and physical characteristics of the Maritza Iztok basin lignites. Fuel 1996;75:1286–93.
[12] Marinov SP, Tyuliev G, Stefanova M, Carleer R, Yperman J. Low rank coals sulphur functionality study by AP-TPR/TPO coupled with MS and potentiometric detection and by XPS. Fuel Process Technol 2004;85:267–77.
[13] Marinov SP, Stefanova M, Stamenova V, Carleer R, Yperman J. Sulphur functionality study of steam pyrolyzed "Mequinenza" lignite using reductive pyrolysis technique coupled with MS and GC/MS detection systems. Fuel Process Technol 2005;86: 523–34.
[14] Mullens S, Yperman J, Carleer R, Bozdemir T, Durusoy T, Yürüm Y. Sulfur analysis of Bolu-Mengen lignite before and after microbiological treatment using reductive pyrolysis and gas chromatography/mass spectrometry. Energ Fuels 2005;19:1029–36.
[15] Stevens Jr, Stanley E, Burgess Wilella D. Biological desulfurization of coal. US patent 4 659 670; 1987. p. 1–8.
[16] ASTM standard D2492; 1984.
[17] Majchrowicz B, Yperman J, Mullens J, van Poucke L. Automated potentiometric determination of sulfur functional groups in fossil fuels. Anal Chem 1991;63:760–3.
[18] Channiwala SA, Parikh PP. A unified correlation for estimating HHV of solid, liquid and gaseous fuels. Fuel 2002;8:1051–63.
[19] Gryglewicz G, Wilk P, Yperman J, Franco DV, Maes II, Mullens J, et al. Interaction of the organic matrix with pyrite during pyrolysis of a high sulfur subbituminous coal. Fuel 1996;75(13):1499–504.
[20] Van Aelst J, Alvarez Rodrigues R, Yperman J, Clemente Jul C, Franco DV, Mullens J, et al. AP-TPR investigation of the effect of nitric acid leaching on the sulphur distribution in coal. Fuel 2000;79:537–44.
[21] Van Aelst J, Yperman J, Franco DV, van Poucke LC, Buchanan III AC, Britt PF. Study of silica immobilized sulphur model compounds as calibrant for AP-TPR study of oxidized coal samples. Energ Fuels 2000;14:1002–8.
[22] Marinov SP, Stefanova M, Stamenova V, Gonsalvesh L, Carleer R, Yperman J. Sulphur analysis of household briquettes using MS and GC/MS detection systems after reductive pyrolysis. J Fuel Chem Technol 2006;34(3):257–64.
[23] Gokcay C, Kolankaya N, Dilek F. Microbial solubilization of lignites. Fuel 2001;80:1421–33.
[24] Hartdegen F, Coburn J, Roberts R. The biodesulphurization of petroleum. AIChE 1983 Annual Meeting, Washington, DC, 10/30-11/4/83 Prep N. 84B 28P.
[25] Chandra D, Mishra A. Removal of sulphur from Assam coals by bacterial means. In: Wise DL, editor. Bioprocessing and biotreatment of coals. New York: Marcel Dekker; 1990.
[26] Marzona M, Pessione E, Martino S, Giunta C. Benzothiophene and dibenzothiophene as the sole sulphur source in Acinetobacter: growth kinetics and oxidation products. Process Technol 1997;52:199–205.
[27] Amphlett M, Calley A. The degradation of 2-thiophenecarboxylic acid by a Flavobacterium species. Biochem J 1969;112:12–5.
[28] Jorjani E, Yperman J, Carleer R, Rezai B. Reductive pyrolysis study of sulfur compounds in different Tabas coal samples (Iran). Fuel 2006;85:114–20.