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DOCTORAL DISSERTATION

Sulphur and organic sulphur alterations in biodesulphurized low rank coals

Doctoral dissertation is submitted to obtain the degrees of
- *Doctor of Science: Chemistry at Hasselt University*
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KNOWLEDGE IN ACTION

Declaration

Hereby, I declare that the results presented in the dissertation "Sulphur and organic sulphur alterations in biodesulphurized low rank coals" are original. The presented data are also not in violation of the rights or interests of the co-authors in the joint publications and communications.

Sofia *Signature:……..……*

October, 2012 */L. Gonsalvesh-Musakova/*

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"Gratitude turns what we have into enough, and more. It turns denial into acceptance, chaos into order, confusion into clarity...it makes sense of our past, brings peace for today, and creates a vision for tomorrow." – Melody Beattie

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Lenia Gonsalvesh-MusakovaSofia,

October 2012

Table of content

List of abbreviations

S_p – pyritic sulphur

S_s – sulphate sulphur

S_o – organic sulphur

S_t – total sulphur

ar – as received

ad – air dried

db – dry basis

daf – dry, ash free basis

MCL – Molten Caustic Leaching

ABL – Aqueous Base Leaching

MWD – Microwave Desulphurization

S-VOCs – Sulphur containing Volatile Organic Compounds

TF – *Thiobacillus ferroxidans*

TT – *Thiobacillus thiooxidans*

TA – *Thiobacillus acidofilus*

LF – *Leptospirillum ferrooxidans*

SA – *Sulfolobus acidocaldarius*

SS – *Sulfolobus solfataricus*

PP – *Pseudomonas putida*

DBT – dibenzothiophene

HFBT - 3-hydroxy-2-formylbenzothiophene

2-HBP – 2-hydroxybiphenyl

DBTO – DBT 5’ – sulphooxide

$DBTO_2$ – DBT 5’ – sulphone

2-HBPS – 2-hydroxybiphenyl-2-sulphinic acid

RR – *Rhodococcus rhodochrous*

DHBP – 2,2’-dihydroxybiphenyl

Th - thiophene

BzTh – Benzothiophene

DBDS – Dibenzyl disulphide

P – Pirin subbituminous coal from "Pirin" coal mine

IN – Maritza East lignite from "Trajanovo-North" mine

M – Humovitrain Maritza East

B – Beypazari lignite from "Cayirhan" mine

AF – Demineralized samples

APF – Demineralized and depyritized samples

Oxy – Oxidized samples

PC – *Phanerochaeta chrysosporium*

TV – *Trametes versicolor*

TV-1 – Biotreatment with *Trametes versicolor* at 37°C

TV-*2* - Biotreatment with *Trametes versicolor* at 30°C

MC – *Mixed Culture of microorganisms*

MC+BS –*Mixed Culture of microorganisms* in Basal salt

PDb – Potato Dextrose broth

AA – Agar-agar

ATCC – American Type of Culture Collection

Nb – Nutrient broth

Rnm – Raymond nutrient medium

CNo – Number of cells

ASTM - American Society for Testing and Materials

W – moisture

VM – Volatile matter

C_{fix} – fixed carbon

TGA – thermogravimetric analysis

TG – thermogravimetric

BBOT – 2,5 bis (5-tert-butyl-benzoxazol-2-yl) thiophene

HHV – Higher heating value

AP-TPR - Temperature Programmed Reduction at Atmospheric Pressure

pot - potentiometry

AP-TPR-pot – AP-TPR "on-line" interfaced to potentiometry

MS – Mass spectrometry

AP-TPR-MS – AP-TPR "on-line" interfaced to mass spectrometer

TD-GC/MS – thermal desorption gas chromatography/mass spectrometry

AP-TPR-TD-GC/MS – AP-TPR "off-line" coupled with TD-GC/MS

SAOB – Sulphides antioxidant buffer

EDTA – Ethylenediaminetetraacetic acid

S_{vol} – organic sulphur compounds neither reduced in AP-TPR condition nor captured in tar/char fraction

P-APF – demineralized and depyritized Pirin coal sample

M-APF - demineralized and depyritized Humovitrain Maritza East

B-APF – demineralized and depyritized Beypazari lignite

P-APF-PC – *Phanerochaeta chrysosporium* biotreated P-APF coal

M-APF-PC – *Phanerochaeta chrysosporium* biotreated M-APF coal

B-APF-PC – *Phanerochaeta chrysosporium* biotreated B-APF coal

P-APF-SS – *Sulfolobus solfataricus* biotreated P-APF coal

M-APF-SS – *Sulfolobus solfataricus* biotreated M-APF coal

B-APF-SS – *Sulfolobus solfataricus* biotreated B-APF coal

In-PP – *Pseudomonas putida* biotreated Maritza East lignite

In-oxy-PP – Oxidized and *Pseudomonas putida* biotreated Maritza East lignite

AF-PP – Demineralized and *Pseudomonas putida* biotreated Maritza East lignite

AF-oxy-PP – Demineralized, oxidized and *Pseudomonas putida* biotreated Maritza East lignite

APF-PP – Demineralized, depyritized and *Pseudomonas putida* biotreated Maritza East lignite

APF-oxy-PP - Demineralized, depyritized, oxidized and *Pseudomonas putida* biotreated Maritza East lignite

HA – Humic acids

HL – Humus-like material

List of abbreviations

Chapter 1 Introduction

Coal as an abundant natural solid fuel is among the most important and valuable resources used for energy production, as a chemical feedstock for numerous synthetic compounds (e.g., dyes, oils, waxes, pharmaceuticals, and pesticides) and for coke production used in metallurgy. Coal is the major source of energy in the production of electrical power using steam generation. In addition, coal gasification and liquefaction produce gaseous and liquid fuels that can be easily transported and conveniently stored in tanks. However, dealing with coal is not an easy task. Despite the benefits associated with widespread consumption of coal, its exploitation invariably has environmental consequences and cause technological problems.

1.1 Importance of sulphur removal

The sulphur content of coal is one of the greatest obstacles for coal utilization. An important problem associated with direct utilization of coal for whatever purpose (combustion, gasification, coking, hydrogenation, etc.) is the emission of sulphur containing gases, i.e. sulphur dioxide (SO_2), hydrogen sulphide (H_2S), carbonyl sulphide (COS), carbon disulfide (CS_2) and sulphur containing volatile organic compounds (S-VOCs). All sulphur containing gases are poisonous and contribute to air pollution. In addition, sulphur induces supplemental undesirable effects such as operational problems, i.e boiler fouling, corrosion and equipment wear, poisoning of catalysts, and production of metallurgical coke with a low quality for steel manufactory. Nowadays, SO_2 emission into the atmosphere during coal combustion is of serious ecological concern. SO_2 can promote sulphate aerosol formation, resulting in respiratory illnesses [1-2]. SO_2 can react with moisture in the air and cause acid rain or low pH fogs. The acid formed in this way can trigger adverse impact to vegetation, depress the pH of the lakes with low buffer capacity and endanger the marine life [3-4]. Moreover, it is proved that SO_2 emissions can contribute to global climate warming, cooling and drought [5].

Environmental legislation to limit sulphur emissions is already in place in many countries. A number of policies have been implemented within Europe that

either directly or indirectly act to reduce SO_2 emissions. As a result, appreciable progress in reduction of SO_2 emissions have been realized by many countries; EEA-32 emissions of SO_2 have decreased by 74% for the period 1990 - 2008 [6]. This reduction is influenced by emission abatement techniques such as flue gas desulphurisation and the greater use of low-sulphur fuels. Nevertheless, despite the significant reduction, electricity and heat production from public thermal power plants (TPP) remain the main significant source of SO_2.

Across EEA-32, Bulgaria had by far the highest SO_2 emissions intensities in 2008 followed by Romania, Turkey and Greece (Figure 1-1) due to TPP [7]. High SO_2 emissions intensities for these countries are mainly due to an increasingly heavy reliance on high sulphur containing coal and lignite used to generate public conventional thermal power. On the other hand, produced energy has a significant role in the total energy supply.

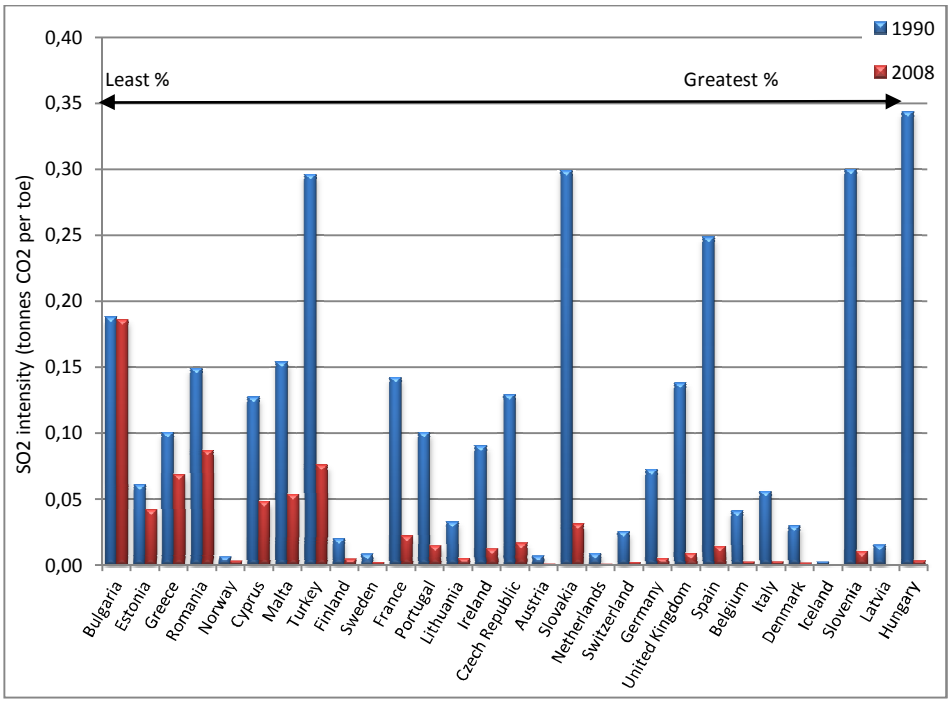


Figure 1-1 Emissions intensity of sulphur dioxide
from public conventional thermal power production [7]

The largest share in the toxic emissions in Bulgaria have the TPPs from "Maritza" energy complex, which emits about 74% of the total emitted sulphur oxides

(Figure 1-2) [8]. According to some international environmental organizations, the three thermal power plants of "Maritza" energy complex, emitted about 566.4 kt SO_2 in 2006 [8], are the largest polluters not only in Bulgaria but in Europe as well. From the foregoing it is clear that the sulphur content of Bulgarian coals is a serious problem for their utilization. However, due to the recent introduction of desulphurization installations in TPP "Maritsa East 3" and to some of the blocks of the TPP "Maritsa East 2", the areas around the plants are considerably improved. Nevertheless, there is still a need for significant further SO_2 reductions to fulfill ecological norms.

In general, two different methods can be used to decrease the sulphur emissions impact from a coal-fired power plant. The first is to remove the sulphur from the coal before it is burned (precombustion), and the second one is to "scrub" the sulphur oxides from the combustion gases after burning (postcombustion) [9]. It is believed that precombustion desulphurization methods offer significant advantages over postcombustion desulphurization ones, since they eliminate simultaneously both environmental pollution and problems associated with the equipment corrosion. However, to use any precombustion desulphurization method effectively, at first it is necessary to determine the sulphur forms present in coal, and second how these forms are affected by various processes. As a sequence, it is necessary to consider how the variations in coal properties can influence the processes, which can be applied to treat it effectively [9].

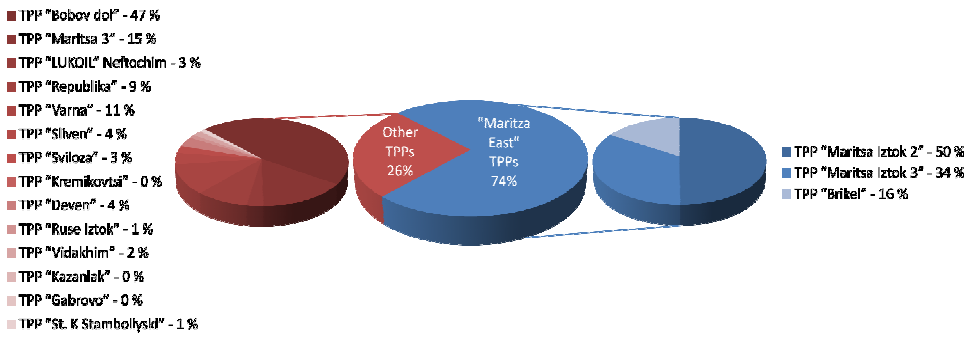


Figure 1-2 Plant-by-plant SO2 emissions in Bulgaria [8]

1.2 Forms of sulphur in coals

All fossil fuels, regardless of their nature and degree of metamorphism, contain sulphur in the form of various compounds. Depending on the deposition environment of the coal, the sulphur content can vary greatly from place to place. It ranges from approximately 0.5 to higher than 11%. The sulphur removal from coal is greatly complicated by the complex chemistry of sulphur. It can occur as inorganic sulphides and inorganic sulphates, and as a wide range of organic compounds, and in some cases even as elemental sulphur and polysulphides.

1.2.1 Pyritic Sulphur (S_p)

The sulphide minerals generally accepted to occur in coal in significant amounts are pyrite and marcasite. Both are frequently referred as "pyritic sulphur" [9]. They have the chemical composition FeS_2, but they are dimorphs (minerals that are identical in their chemical composition, but different in crystalline form).

Pyrite is the predominant sulphide mineral which is ubiquitous in coal. It has a cubic crystal structure with a specific density of 5.0 [10] and occurs in several forms: 1) nodules and partings; 2) disseminated pyrite; 3) thin, platy pyrite in cleats; 4) pyrite veins; and 5) pyrite in permineralized peat or coal balls [11]. It is obvious that pyrite inclusions can take many forms and can have particle sizes ranging from 0.1 μm to 1000 μm. The morphology of the individual pyrite particle varies broadly depending on how they are deposited and on the characteristics of the embedded sediments. As a result, the surface chemistry, density, and degree of locking to coal vary considerably. Consequently physical removal of the smallest pyrite particles is complicated.

Marcasite has been found in lesser amounts compared to pyrite. It is a rhombic crystal with a specific density of 4.87 [10]. Marcasite is characteristic for higher rank coals [12] and coals which are formed in acidic environment [9].

Pyrite is more stable and slightly less reactive than marcasite. The marcasite is slowly converted into pyrite by heating to 450ºC. This change is irreversible at any temperature. Nevertheless, the chemical properties of the two disulphides are similar.

Other sulphide mineral present in coal is the chalcopyrite ($CuFeS_2$). It is syngenetic by origin. It occurs as single well walled crystals with honey-yellow color and lower relief compared to pyrite. Galena (PbS), sphalerite ((Zn,Fe)S) and pyrrhotite ($Fe_{(1-x)}S$ (x = 0 to 0.2)) can be also found in coals, but very rarely and in small quantities.

1.2.2 Sulphate Sulphur (S_s)

Coal contains traces to insignificant amounts of sulphate sulphur, in the form of iron, calcium and barium sulphates. Sulphate minerals such as gypsum ($CaSO_4.H_2O$) and barite ($BaSO_4$) are determined in some fresh coals [11]. Oxidized coals, however frequently contain a good deal of iron sulphates, formed by pyrite and marcasite oxidation [9]. Their concentrations increase with the time of coal exposure to the air [10]. Marcasite, which is particularly reactive, is readily oxidized in air, while pyrite oxidizes more slowly unless it is in an acidic environment and/or certain iron-oxidizing bacteria are present. The following iron sulphate minerals are weathering products of pyrite [11]: szomolnokite ($FeSO_4.H_2O$), rosenite ($FeSO_4.4H_2O$), melanterite ($FeSO_4.7H_2O$), coquimbite ($Fe_2(SO_4)_3.9H_2O$), roemerite ($FeSO_4.Fe(SO_4)_3.14H_2O$), jarosite ($(Na,K)Fe_3(SO_4)_2(OH)_6$) and halotrichite ($FeAl_2(SO_4)_4.22H_2O$). Since the sulphates are mostly water soluble, they are almost completely removable by coal-washing operations. Therefore, they are not considered to be a serious source of sulphur in the clean coal [9].

1.2.3 Elemental Sulphur (S_{el})

Elemental sulphur is not found in freshly mined coals, but only in coals that have been oxidized to some extent [9,13]. When coal oxidizes, S_p sometimes forms S_{el} and sulphur-rich polymers on its surface. The lasts are produced most readily during oxidation of marcasite, but can also be formed by partial oxidation of pyrite, i.e pyrite oxidation by the action of iron-oxidizing bacteria. Like coal, S_{el} is a naturally hydrophobic compound, and it therefore makes the surface of pyrite and marcasite more closely resemble the surface of coal. Since the most effective processes for physically separating very fine particles are based on

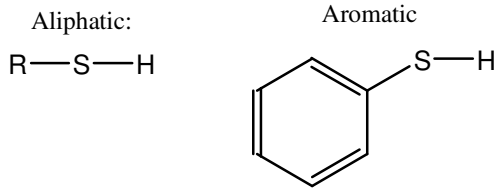
surface chemistry differences, S_{el} can be a serious obstacle for physical separation of fine S_p from coal.

1.2.4 Organic Sulphur (S_o)

The organic sulphur is the sulphur chemically bonded to the hydrocarbon matrix of the coal (coal substances). It can be divided according to the type of functional group in which it appears. There are at least four basic types of structures: (i) mercaptans or thiols; (ii) sulphides or thio-ethers; (iii) disulphides; (iv) and thiophenes. Examples of these structures are illustrated as follow (R and R' are unspecified aliphatic hydrocarbons):

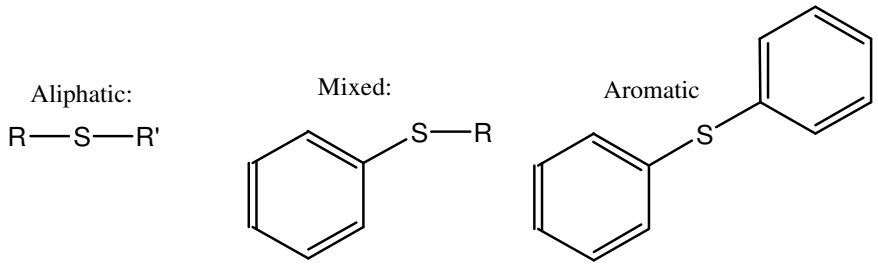
1.2.4.1 Thiols

Thiols are the sulphur-base analogue of alcohols and include both aliphatic compounds (mercaptans) and aromatic compounds (thiophenols):



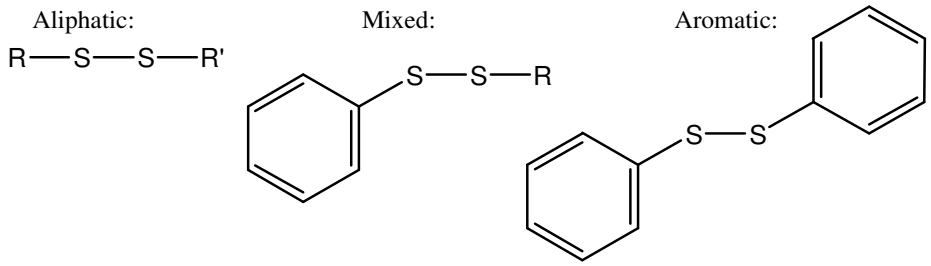
1.2.4.2 Sulphides

The sulphide form of organic sulphur includes the thioethers:



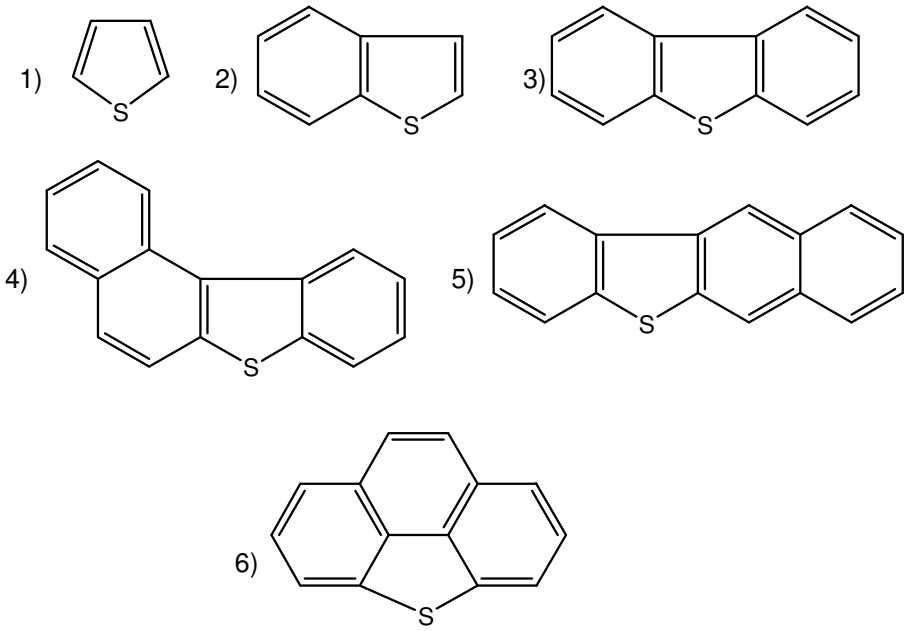
1.2.4.3 Disulphides

Disulphides have a structure superficially similar to the sulphides, except that they contain two sulphur atoms directly bound with each other instead of one sulphur atom:



1.2.4.4 Thiophenes

Compounds including thiophene ring:



1)Thiophene, 2) Benzothiophene, 3) Dibenzothiophene, 4) benzo[d]naphtho[2,1-b]thiophene, 5) benzo[b]naphtho[2,3-d]thiophene, 6) phenanthro[4,5-bcd]thiophene.

There is an evidence that give ground to assume that lignites and high-volatile bituminous coals have a higher content of thiols comparing to low volatile coals [9]. Thiols are considered as secondary products in coals since they are thermally unstable and would not survive the coalification process [10]. The same is valid for disulphide as well. It has also been reported that with the coalification, the proportion of the thiophenic organic sulphur (which is an highly stable form) increases [14].

1.2.5 Total Sulphur (S_t)

The total sulphur is the sum of all sulphur forms encountered in coal. Respectively, $S_t=S_p+S_s+S_o+S_{el}$. S_t content in coal varies from 0.3 up to 15 wt% according to the rank and the genesis of coal. According to the content of S_t, coals are divided into three groups [15]: low-sulphur coals, containing 1 wt% or less sulphur; medium-sulphur coals, containing more than 1 wt% to less than 3 wt% sulphur; and high-sulphur coals, containing more than 3 wt% sulphur. With regard to the distribution of sulphur forms in the S_t, more accurate data can be specified for individual coal fields. However, aside from S_{el}, which is rare and amounts up to 0.3%, the least represented is S_s (10-15% of S_t), and the remainder (90-85% of S_t) is distributed between S_p and S_o.

Sulphur compounds undergo different changes in the technological processing of solid fuels. When coal is carbonised or burnt, part of the sulphur is released with volatile products during carbonization or with flue gases during combustion. The rest of the sulphur remains in the non-volatile residue or in the ash, respectively. These circumstances impose other types of sulphur differentiation, i.e. combustible and non-combustible sulphur as well as volatile and non-volatile sulphur [16].

Combustible sulphur: This is the sulphur that is expelled during the complete combustion of coal. It depends on the type of coal, combustion conditions and chemical composition of the ash. In the case of carbonate and silicate rich ashes, the major part of the resulting sulphur gases (due to combustion) are retained in the ash. This is the case with some lignite from the Sofia bearing province and Maritza West basin. The combustible sulphur, however, includes the total amount of volatile sulphur. When it is present in an appreciable amount

in coal, this combustible sulphur corrodes the metallic structures of boilers. This item concerns the fuel engineers using coal for their different types of boilers and other steam-raising plants. Attempts should, however, be made to recover the combustible sulphur from the products of combustion in varies factories and power-generating stations and may thus be utilized for other purposes.

Non-combustible sulphur: This is the sulphur portion left in the ash after combustion. This sulphur is considered to be inert in its activities.

Volatile sulphur: That is the sulphur portion volatile at 950°C coal carbonisation. It varies with the carbonisation temperature. The volatile sulphur is a hint for the sulphur content of the gaseous and liquid products. When this sulphur content is high, the gaseous and liquid products will have to be further purified to get rid of the sulphur that is in excess of a specified limit. Respectively, this will involve extra cost. It can be mentioned here that a certain amount of volatile sulphur may thus be recovered in the gas and other industries and might subsequently be utilised for sulphuric acid manufacturing.

Fixed (non-volatile) sulphur: It is the amount of the sulphur retained by the coke when the coal is carbonised. This is a very important factor for metallurgists and iron smelters. A metallurgical coke with a high sulphur percentage is not suitable for iron blast furnaces and other high class smelting operations.

1.3 Sources of sulphur in coal

The question of the sulphur compounds origin in coal is of great interest and it is very important for improving coal utilization. Geochemical studies of sulphur in coal provide information about abundance, distribution, and speciation of sulphur in coal. Many of these properties are determined by geological environments and processes of coal formation. Various hypotheses have been proposed to explain the sources and conditions for the formation of sulphur compounds in coal. One of them is the hypothesis for the organic nature of sulphur in coal, offered by Donath. His concept is that the only source for sulphur compounds are plant and animal debris involved in coal formation. According to him, during peatification and early diagenesis, iron bicarbonates are brought by the water to the accumulated plant and animal debris. These

bicarbonates are turned into carbonate by CO_2 removing. Simultaneously, proteins of the organic debris are decomposed with release of H_2S, which reacts with the iron carbonate to form pyrite. Partial oxidation of pyrite results in sulphates formation, while non-reacted sulphur of the proteins has remained in the coal as S_o. However, this hypothesis, although shared by other researchers suffers of drawbacks. For example it cannot explain the high content of sulphur (>0.5%) in some coal characterized by not sufficient sulphur in parent vegetation [17-18]. Furthermore, among coal precursors, the main amount of sulphur is incorporated in the proteins (which are source of coal nitrogen). Therefore, some researchers admit correlation between the nitrogen and S_o contents in coals, but such a relationship is not registered [19-20]. This hypothesis cannot also give relevant explanation for the different sulphur contents in the different areas of the same coal layer, formed from the same precursor.

Other sources of sulphur input in coal might be fresh water, extraneous mineral matter and sea water. However, fresh water contains 0 to 10 ppm sulphur and therefore, even if there is prolonged circulation of fresh water through the peat, it cannot attribute much sulphur to the coal [17]. Similar is the situation with the extraneous mineral matter. It is unlikely that a lot of sulphur in coal can originate from extraneous mineral matter. If the latter one contains sulphides, then these could provide a source of sulphates and hydrogen sulphide to the coal. Nevertheless, these amounts would usually be negligible. With regards to the sea water, some researchers recognised it as the main primary source of sulphur in coal. These authors support the hypothesis for the inorganic origin of the sulphur in coal. They insist that both, S_p and the biggest part of the S_o, are formed by sulphates originating from sea water transgression to the peat.

One of the researchers worked deeply on the origin of sulphur in coal is Yurovski [18]. Although he does not reject the fact that plant proteins have played a significant role in the formation of S_o, he develops in detail and substantiates the hypothesis for the mineral origin of sulphur in coal. Yurovski assumes that the incorporation of sulphur into coal follows a four step mechanism [10,18]: (i) contact between the organic coal substance and sulphates; (ii) ingress (penetration) of ferruginous solution; (iii) formation of iron sulphate and its conversion to hydrogen sulphide; (iv) and finally formation of S_p and S_o.

1.3.1 Contact between the organic coal substance and sulphates

During deposition, plant detritus (involved in coal organic substance formation) are in a systematic contact with sea water. Sulphates in sea water are believed to be the primary source of sulphur in coal as stated above. It is established that sea water contains on the average of about 0.6% sulphates. Taking into account the content of sulphates in sea water and the humidity in peat bogs, Yurovski has calculated that coals with 4-5% sulphur content can be formed. This value might be decreased by washing of the sulphates or increased by re-flooding of the peat bog with salty marine water.

1.3.2 Ferruginous solution penetration

Pyrite in coal typically can be formed from H_2S and Fe in solution. Therefore, Yurovski has recognised iron as the second essential component for the formation of pyrite. H_2S may be formed from sulphates originating from sea water or vegetation but none of these sources provide iron. It is probably derived as a ferric oxide and hydroxides, adsorbed on fluvial clays in fresh water floods during parent substances sedimentationas and at various stages of coal bed formation. However, the access of Fe to organic substances can be limited and determined by the nature of the overlying sediments (the roof rocks). Thus, the ferruginous component might not always be present in excess and might have restricted the formation of coal sulphur at certain beds and zones.

1.3.3 Formation of iron sulphate and its conversation to hydrogen sulphide

According to Yurovski [18] under the conditions of organic matter saturation with sea water and with iron containing fresh water, the following processes are possible:

- Interaction between iron compounds and highly soluble sulphates resulting in iron sulphate formation.

$Fe(OH)_2 + MgSO_4 \rightleftarrows FeSO_4 + Mg(OH)_2$

$2Fe(OH)_3 + 3MgSO_4 \rightleftarrows Fe_2(SO_4)_3 + 3Mg(OH)_2$

- Conversion of iron sulphate to H_2S by the action of sulphate reducing bacteria under reducing atmosphere in alkali medium.

Admittedly, besides these processes, many other processes can occur simultaneously, such as partial reduction of sulphates by carbon, interaction between released during decomposition of plant proteins H_2S with Fe, etc.

1.3.4 Formation of pyrite and organic sulphur

- Pyrite formation

Based on the fact that the predominant secondary sulphur components in the coalification are iron sulphate and H_2S, Yurovski concludes that the three most likely reactions leading to the formation of pyrite are:

$2Fe(OH)_2 + 4H_2S + O_2 \rightarrow 2FeS_2 + 6H_2O$ (1-1),

$2FeSO_4 + 4H_2S + 2CaCO_3 + O_2 \rightarrow 2FeS_2 + 2CaSO_4 + 4H_2O + 2CO_2$ (1-2),

and $2FeSO_4 + 5H_2S \rightarrow 2FeS_2 + 2S_{el} + H_2SO_4 + 4H_2O$ (1-3).

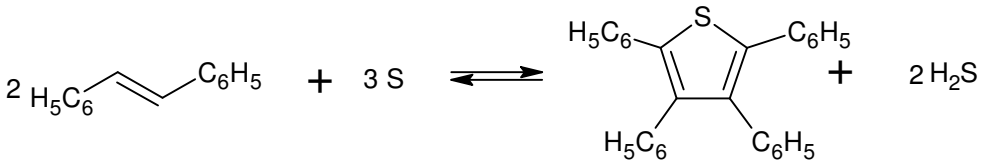
According to Yurovski, first and second reactions are with limited significance for pyrite formation, since they are restricted by the content of dissolved oxygen in the water for reactions (1-1) and (1-2) and the presence of carbonates for reaction (1-2). The author considers that reaction (1-3) is the most likely reaction which has the greatest importance for pyrite formation. In it, along with pyrite, S_{el} and H_2SO_4 are formed as well. S_{el} can further interact with organic matter and can form organic sulphur compounds. On the other hand, H_2SO_4 may limit the reduction of iron sulphate to H_2S by the action of sulphate reducing bacteria, whose vital functions fading in pH<5.

An alternative scheme for the formation of pyrite is proposed. Some authors, reviewed by Chou [11] suppose that during early diagenesis in a reducing environment, ferric oxide is reduced to ferrous, which reacts with H_2S to form iron monosulphide. According to them, if the basic mechanism of pyrite

formation is similar to that in marine sediments, iron monosulphide (FeS) is transformed by reaction with S_{el} through several sulphide phases; from mackinawite ($FeS_{0.9}$) through greigite (Fe_3S_4) to framboidal pyrite. Disseminated pyrite single crystals may be formed by direct precipitation of FeS_2. Plant structure, preserved in coal balls, indicates that pyritic coal ball originate late in the peat stage, but before peat is strongly compacted. Pyrite nodules and partings are formed in the process of transformation of peat to coal. Pyrite in cleats and veins is deposited from migrating solution, after the compaction of peat into coal.

- Organic sulphur formation

Two types of S_o are recognized [10]: i) primary S_o originating from coal parent vegetation (the hypothesis for the organic nature of sulphur in coal), and ii) secondary S_o obtained in the process of coalification. One of the main sources for secondary S_o formation is S_{el} obtained during pyrite formation. S_{el} interacts with coal substances and creates C-S bonds. An example of such an interaction is the formation of thiophenic sulphur in the reaction [10]:



It is generally accepted that organic sulphur compounds are formed in the early stage of the coalification (humification), when plant debris are decomposed under bacterial activity to a premaceral humic substance. H_2S, S_{el} and polysulphides produced by dissimilatory processes may have reacted with organic matter to form organic sulphur compounds. Various studies summarised by Chou [11] have shown that above mentioned compounds can react with organic matter to create organic sulphur compounds, similar to those determined in some coals.

1.4 Sulphur in Bulgarian coals: content, forms and distribution.

Bulgaria has significant but very low-grade coal reserves. The solid fuels reserves amount up to 3 billion tonnes, comprising 88.7% lignite, 10.9% brown coal and 0.4% hard coal [21]. The lignite reserves have a heating value of about 5652 – 7746 kJ/kg, high ash (17-45%, as received (ar)) and moisture (50-60%, ar) contents. In addition, the lignites are characterized by high sulphur contents (2.2-2.8%, ar).

One of the coal provinces with economical significance for the country is the Thracian coal province. It is comprised by three main coal basins: Maritza West, Maritza East, and Elhovo, all extremely immature coals – lignites [12,22-23]. A small coal-bearing deposit in the area of the Gulf of Sozopol also belongs geographically to the Thracian coal province [12]. Since Thracian low rank coals are the main source of energy for electricity production in Bulgaria, their proper characterization is of utmost importance. Sulphur distribution assessment in Thracian lignites has been an object of investigation by numbers of authors. For example, Kostova has established that the coal of Maritza West and Maritza East basins are characterized by high sulphur content [24-26]. The investigation carried out on sixteen lignite samples taken from various layers of the Kipra coal seam, Maritza West basin, shows that the average S_t content is 7.5 wt% on dry ash free basis (daf), ranging from 4.1 wt% to 11.5 wt% [24]. The content of the various sulphur forms calculated on daf basis are as follows: average S_p content of 1.6 wt%, ranging from 0.6 wt% to 3.2 wt%; average S_o content of 4.2 wt%, ranging from 1.8 wt% to 6.0 wt%; and average S_s content of 1.7 wt%, ranging from 0.3 wt% to 2.9 wt%. Kostova study reveals that pyrite and its decomposition products (gypsum and jarosite) account for the most of the inorganic sulphur content in the studied Kipra lignites. They are the main sulphur bearing minerals and vary broadly in the mode of occurrence. The coals from Maritza East deposit are characterized by high S_t content as well. It is revealed that average S_t content for 83 lignite samples from the second layer (seam) of Maritza East coal deposit (Trayanovo North mine and Trayanovo 3 mine) is 5.77 wt%, ranging from 1.53 wt% to 9.16 wt% [25,27]. For these coal samples S_p (average 3.19 wt%, ranging from 0.49 wt% to 5.07 wt%)

predominates followed by S_o (average 2.51 wt%, ranging from 0.43 wt% to 3.98 wt%) and S_s (average 0.07 wt%, ranging from 0.01 wt% to 0.26 wt%). S_{el} is also detected. Pyrite and gypsum are again the main sulphur bearing minerals in the studied samples. With regards to Elhovo basin, the easternmost basin within the Thracian coal province, 45 samples representing the three main lignite seams and originating from three boreholes are investigated by Zdravkov [28]. It is determined that average S_t content calculated on dry basis (db) for Elhovo lignite samples is 8 wt%. The S_t contents range from 5 to 13 wt% for Seam I, from 5 to 11 wt% for Seam II and from 3.8 to 15 wt% for Seam III. Detailed information about abundance of organic sulphur forms and compounds in representative samples from Thracian coal province is also published [29-31]. About 60% of the organic sulphur is assigned to thiophenic structures. A relatively high proportion of disulphides is registered for Elhovo and Maritza East samples, while in the case of Maritza West lignite the content of thiol prevails the content of other aliphatic sulphur compounds.

Coal samples from the Pernik, the sub-Balkan and the Balkan coal provinces are also extensively studied [25,27,32-38]. In the Pernik coal province are concentrated the main reserves of subbituminous coals. This province comprises Pernik, Bobov Dol, Pirin basins and Suhostrel coal field (Late Eocene bituminous coal) [12,22,39-40]. The average S_t content of Pernik coal is 2.36 wt% and ranges from 0.81 wt% to 4.19 wt% [25,27]. The average contents of various sulphur forms, i.e. S_p, S_s and S_o are 1.42 wt%, 0.04 wt% and 0.91 wt%, respectively. Similar to lignites from Thracian coal province, pyrite and gypsum are the most abundant among the sulphur bearing minerals of Pernik coal [34]. However, they are registered as minor minerals in these samples. Marcasite is also detected [27,34]. Similar trend with respect to pyrite and gypsum presence is registered for Bobov Dol coal as well [33]. Its S_t content calculated on dry basis is 1.39 wt% [41]. Limited are the data on sulphur distribution in Pirin coal. However, S_t content can vary from 2.14 wt% to 4.88 wt% [40,42]. Among the sulphur forms, S_o is presented in the greatest amount [42]. Representatives of the sub-Balkan and the Balkan coal provinces are Bourgas and Balkan coal basins, respectively. Although it is in limited volume, the Balkan basin remains the only basin exploiting bituminous coal [25,37]. Its average S_t content is 3.11 wt% and ranges from 1.09 wt% to 8.22 wt% [27]. A slight predominance of

average S_o content over average S_p content is observed for Balkan coal. The average content of S_s is 0.06 wt%. With regard to the sub-Balkan province, it comprises Bourgas and Nikolaevo basins, and Borov Dol field [12,22]. However, the coal from Nikolaevo basin and Borov Dol are depleted. Thus, only Bourgas basin is with industrial significance. Its coal maturity is within the subbituminous stage. The average S_t content for 24 core samples collected from coal seam "A" in the Bourgas deposit is 4.6 wt% and ranges from 3.0 wt% to 11.6 wt% [35]. The average S_o content is 2.8 wt% (ranging from 1.2 wt% to 10.0 wt%), while the average S_p content is 1.6 wt% (ranging from 0.5 wt% to 5.5 wt%). High sulphur amounts in the Bourgas coal are consistent with marine to brackwater environment of the coal formation.

Another coal province containing basins of industrial importance is Sofia coal province situated in West Bulgaria. It comprises Sofia, Chukurovo, Beli Breg, Stanyantsi, Samokov basins and some coalfield, i.e Karlovo, Gabrovitza, Aldomirovci and Kovachevci [12,22-23]. The coals from these basins and coalfields are studied by Markova, Kortenski, Zdravkov, etc. [36,43-47]. In the examination of 17 coal samples from the Chukurovo coal basin and 15 samples from the Beli Breg basin, it is established that Beli Breg coals contain higher amounts of S_t, S_p and S_o, and nearly the same amount of S_s compared to the Chukurovo coal. The content of S_t for Beli Breg coal varies from 1.10 wt% to 3.66 wt% (average 1.86 wt%) [46]. The content of S_p is in the range of 0.34 wt% - 2.10 wt% (average 0.71 wt%). Considerably higher is the content of S_o (0.47 wt% - 2.73 wt%, average 1.10 wt%) as compared to that in Chukurovo coal. The percentage of S_t in Chukorovo coal varies in the range of 0.42 wt% - 1.47 wt% (average 0.78 wt%). Its average S_p and S_o contents are 0.23 wt% and 0.49 wt%, respectively. Identification of the sulphur forms and their distribution in coals from the Sofia (Balsha district) and Stanyantsi basins have been the objective of investigation as well [45]. The coal samples from the Balsha district are drilled from the P-866 and P-899 boreholes and average S_t contents of about 2.8 wt% are registered. For borehole P-866 S_p (average 1.1 wt%) has been found to be the dominant form of sulphur occurrence followed by S_s and S_o. The coals from borehole P-899 are also rich in S_p, whereas S_s and S_o have prevalence in various areas along the coal seam. A similar tendency in sulphur forms distribution has been observed in the coals from the Stanyantsi

basin. However the coals from the last mentioned basin contain higher amounts of S_t (average 3.6 wt%) than the Balsha coals. The Karlovo coal is very low in sulphur (average S_t of 0.75 wt%, db).

1.5 Desulphurization of coals

It has long been recognized that the high sulphur content of coal is a major source of problems associated with air pollution, particularly from electricity production in thermal power plants and industrial boilers. In order to meet the high environmental standards during coal combustion, coal desulphurization has become crucial in recent years. Desulphurization methods can be classified into two main groups depending on their application – precombustion and postcombustion methods. Each of them has advantages and limitations and should not be considered to be in competition. Moreover, they should be considered as complimentary technologies. It is the best to be able to use both types of processes to remove the greatest proportion of sulphur at the lowest cost [9].

1.5.1 Postcombustion desulphurization

In postcombustion desulphurization, the sulphur oxides that are produced during coal combustion are removed from the combustion flue gases in a solid form that can be disposed of by using an absorptive chemical. There are two basic approaches to capture the sulphur: i) Scrubbers technologies or so called Flue Gas Desulphurization – selectively remove SO_2 in combustion flue gases through a scrubber unit that is separate from the combustor; ii) In-Combustor Technologies – minimize the release of sulphur from combustion by direct injection of absorbent along with the fuel [9,48-51].

The most common absorbents are lime (calcium hydroxide) and limestone (calcium carbonate) slurries. Limestone is the preferred absorbent in many modern scrubbers, because of its low cost compared to lime and other absorbents. Lime is also used however, because of its higher reactivity, which allows it to absorb sulphur more rapidly. In both cases the primary solid product is calcium sulphite hemihydrate. Since it has no market value it must be disposed by land filling. Another alternative is to oxidize the sulphite to sulphate.

The solid product is gypsum which can be marketed to make plaster, wallboard, cement and other construction products. In addition to lime and limestone, a number of other absorbents as NaOH, Na_2CO_3, Na_2SO_3 and MgO have also been used in order to improve the efficiency of sulphur removal, to recover the sulphur in a marketable form and to regenerate the used absorbent.

Among In-combustion technologies Fluid-Bed Combustion (FBC) and Integrated Gasification Combined Cycle (IGCC) are commercially applied. In FBC, grounded into small particles coal and mixed with limestone is burnt in a boiler filled with spent bed material (primarily ash, free limestone, and calcium sulphate) which is fluidized by air. In this process limestone absorbs SO_2 during coal burning. Respectively, sulphur is not released with flue gases. Limestone forms a solid sulphate, which is discarded with the ash. Other sulphur absorbents that have been used in FBC application are chalk, dolomite and high-calcium waste dust from cement manufacture.

In IGCC systems coal is not directly burned. It is converted into combustible gases (gasification step), which are subsequently burned to produce power. In the gasification process, over 90% of the sulphur components in the feedstock are converted mainly to hydrogen sulphide (H_2S) because of the reducing condition due to hydrogen presence in the gasifier. The rest is converted to carbonyl sulphide (COS). Compounds such as SO_x and CS_2 are essentially absent in the syngas. In present-day IGCC plants, sulphur compounds in the syngas are removed by two preferred processes: chemical washing, based on aqueous methyldiethanolamine (MDEA), and the Selexol process, based on a physical solvent. Both methods can reduce the total sulphur (H_2S + COS) to levels below 20 ppmv in the cleaned syngas. Further on, removed H_2S must be converted into a chemical product which can be reused. The most common method for that is the Claus process which produces elemental sulphur by sub-stoichiometric combustion with air or oxygen. Different versions of this process are available. The sulphur may be fixed as S_{el} in liquid or solid form or as sulphuric acid.

1.5.2 Precombustion desulphurization

Desulphurization of coal prior to combustion comprises various physical, chemical and microbial methods. While the physical methods aim to separate

the inorganic sulphur, the target of the chemical and biological methods includes removal of the S_o as well. A schematic illustration of the precombustion coal desulphurization techniques is given in Figure 1-3.

One of the main benefits of processing coal to remove sulphur prior to combustion is related to the fact that this treatment, besides sulphur removal, generally gets rid of various ash forming minerals as well. Respectively, fuel with higher quality and increased heating value per ton of coal is obtained. This indirectly reduces the shipping cost of the solid fuel. However, there are some limitations of precombustion desulphurization application as well. It should be mentioned that many high-sulphur coals are not desulphurized prior to combustion since they contain a lot of their sulphur in forms that cannot be economically removed. It should also be stressed that the effectiveness of precombustion desulphurization depends on the characteristics of the coal, and no one precombustion method is universally capable for application to all coals. Nevertheless precombustion desulphurization is effective for coal with a higher presence of coarse pyrite and mineral matter. It is suitable for premium coals production for markets other than coal combustion. It is especially appropriate in coal liquefaction, coking and semi-coking technologies.

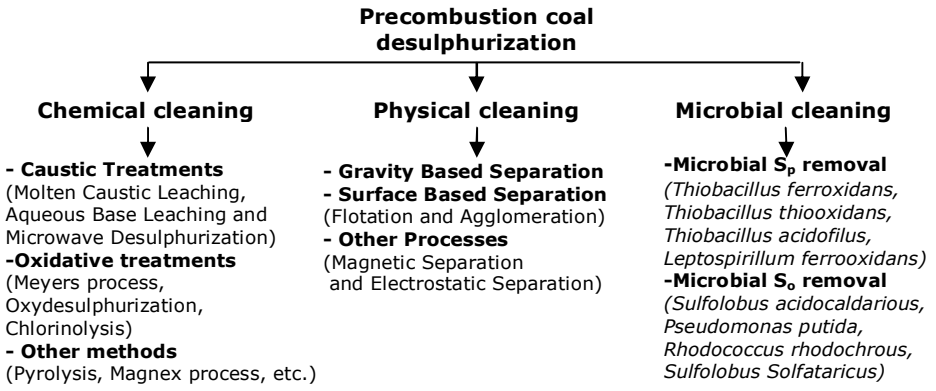


Figure 1-3 A schematic illustration of existing or emerging precombustion coal desulphurization processes

1.5.2.1 Physical Cleaning methods

It is known that physical cleaning methods are successful in removing the large fragments of associated minerals in coal but fail in effectively removing the finer

particle (-0.1mm) [50]. Constituents chemically bound to coal matrix such as S_o cannot be separated.

All of the coal cleaning devices that are in current industrial use, with an exception of froth flotation, use density-based concentration methods [9]. They clean coal based upon the differences in specific gravity of coal and mineral matter. The high density difference between pyrite and coal makes this system an excellent candidate for cleaning treatment. The processes that provide the sharpest separation based on density are the heavy-media separation techniques. They work by immersing the coal in a fluid that has density between the density of coal and minerals. The coal then floats in the fluid, while the minerals sink (Float-Sink Separation). Other than heavy-media devices, there are a wide range of processes that separate particles based on their densities, including the various types of jigs, spirals and tables. While they generally do not make separations that are as sharp as those made by heavy-media processes, they are less expensive to install and operate.

Other technique useful for cleaning fine coal from mineral matter is the froth flotation. Unlike the processes discussed above, froth flotation separates particles based on their surface chemistry. The basis of froth flotation of coal is the difference in the wetabilities of coal and mineral matter. Since coal is composed of organic compounds, it tends to be water-repellent, or hydrophobic, while most of associated mineral matter is easily wetted by water, or hydrophilic. So, if particles of hydrophobic coal are suspended in water, and air is bubbled through the suspension, the coal particle will tend to attach to the air bubbles and float to the surface, while the mineral matter having less tendency to attach to air bubbles will remain in suspension. In general, froth flotation is very effective for separating coal from ash-forming minerals, but it is somewhat less effective for coal desulphurization for the following reasons: i) Pyrite in mildly oxidized coals can, in some circumstances, become naturally hydrophobic, ii) Small particles of pyrite are carried into the froth by entrainment in the water or by flocculation to floating coal particles, iii) Pyrite grains are often locked to coal particles and therefore behave very similarly to coal particles.

Column flotation is a significant improvement over conventional flotation for cleaning and desulphurization of coal. The basic principle of column flotation is

the use of countercurrent flow of air bubbles and solid particles. This is achieved by injecting air at the base of the column, coal feed near the midpoint and addition of wash water at the top of the column. The result is that these types of columns provide improved hydrodynamic conditions for flotation and thus produce a cleaner product while maintaining high recovery and low power consumption.

Selective agglomeration also has a potential for separating pyrite from micronized coals. In this process an immiscible liquid is added to coal water slurry. Upon agitation, the immiscible liquid coats the hydrophobic particles (pyrite particles) and adhere them into agglomerates by capillary forces. Other physical cleaning methods are also available. These are magnetic and electrostatic separation based on differences in magnetic susceptibilities and electrical properties (dielectric constant, electrical conductivity and work function), respectively, of coal and pyrite.

1.5.2.2 Chemical cleaning

The majority of the chemical beneficiation processes are able to reduce ash content, finely disseminated pyrite or organically bound sulphur in coal. While some remove only pyritic sulphur, others remove both forms of sulphur. Chemical desulphurization techniques can be classified according to the mode of involving action to caustic, oxidative and other treatments [9].

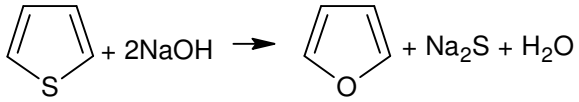
1.5.2.2.1 Caustic treatments

Pyrite reacts through displacement reaction with alkali in either aqueous solution or molten state at temperatures of 200ºC to 400ºC [9-10]. The products are sodium sulphide, thiosulphate and sulphate. The underlying chemical reactions proceed:

$8FeS_2+30NaOH \rightarrow 4Fe_2O_3+14Na_2S+Na_2S_2O_3+15H_2O$,

$35FeS_2+140NaOH \rightarrow 16Fe_2O_3+64Na_2S+64H_2O+Fe_3O_4+6Na_2SO_4+6H_2$.

Organic sulphur can also undergo a displacement reaction with alkali where sulphur atom is replaced by an oxygen atom.



The chemical desulphurization processes based on this type of reactions are Molten Caustic Leaching (MCL), Aqueous Base Leaching (ABL) and Microwave Desulphurization (MWD).

The caustic used most often for MCL is sodium hydroxide, although the more reactive and more expensive potassium hydroxide is sometimes used in place of up to half of the NaOH [9,52]. It is established that the ratio of KOH and NaOH may be critical in removing sulphur from organosulphur moieties in coal and that the reaction temperatures and reaction times might determine which type of organosulphur species is being removed and to what extent [53]. The MCL process, which has been tested on a pilot plant scale, is the Gravimelt process. In it almost all of S_p and about 80% of S_o can be removed by using 10 parts of molten caustic to treat 1 part of coal at 370ºC, followed by washing with dilute H_2SO_4. In addition, the final ash contents are generally less than 1%. An example of ABL treatments is the Batelle Hydrothermal Process. It employs heating of an aqueous slurry of powdered coal in the presence of 10% NaOH and 2-3% $Ca(OH)_2$ at 250-300ºC for 10-30 minutes. Over 90% of S_p and 20-70% of S_o can be removed from coal [9,50]. Heating value losses are between 5-10%. MWD has demonstrated also some good results, but its performance is variable and difficult to reproduce. It has been reported that the use of microwave to heat the alkali/coal mixture reduces the reaction time and the reaction temperature, i.e. increases coal desulphurization rate [54-55]. Microwave irradiation followed by magnetic desulphurization is also reported to enhance sulphur removal [56]. It is due to the fact that microwave irradiation converts pyrite to pyrothite and γ-hematite and thus the magnetic susceptibility is increased.

1.5.2.2.2 Oxidative treatments

Different reagents, i.e. oxygen, metal salts, chlorine gas, nitrogen or sulphur dioxides, peroxides, sodium hypochlorite, ozone, potassium permanganate, etc. have been tested to oxidize the sulphur in coal. Oxidation based processes need to be selective for sulphur, as otherwise oxidation of the coal results in losses of heating value. Among the oxidative desulphurization processes, only Meyers process has been extended to pilot scale tests [9,57-61]. It is an example of a metallic salt sulphur oxidation method. The process removes 83 to 99% of S_p resulting in S_t content reduction of 25 to 80% through chemical leaching of S_p with aqueous ferric sulphate solutions at temperature of 90º-130ºC [61].

The Meyers process is an excellent choice for removing S_p that is too finely dispersed for removal by physical separation. Unfortunately it is not effective for S_o removal. Another example of metallic salt sulphur oxidation method is the treatment with ferric chloride [60,62]. Oxydesulphurization [63-68] and chlorinolysis [69-70] have been widely studied as well. Both processes remove S_p as well as S_o. Other oxidizing agents used for coal desulphurization are nitrogen and sulphur oxides [9], peroxyacetic acid [71-74], peroxides [62,74-75], nitric acid [62,72-74,76-80], potassium permanganate [74]. However, the last mentioned treatments are only used on laboratory scale.

1.5.2.2.3 Other methods

In addition to the processes that use alkali leaching and oxidative treatments, a number of other techniques such as pyrolysis, hydrodesulphurization, Magnex process and Chemical comminution have been tested for coal desulphurization [9].

Pyrolysis can be considered as a suitable method for sulphur removal from coal [81-83]. Under the influence of temperature the sulphur compounds in the coal undergo decomposition. As a result, part of them remains in the coke, while the rest passes to the liquid and gaseous products. During the various types of coal pyrolysis, the mechanisms of sulphur species release and the desulphurization effects are influenced by numerous factors, i.e. forms of sulphur present in coals, coal rank, experimental condition, etc. [81-91]. Hydrodesulphurization of coal is a two step process comprising oxydesulphurization and hydrogenation

treatments [92]. It is claimed to reduce S_t up to 90%. Magnex process is unique in that it converts weakly magnetic pyrite and nonmagnetic mineral matter into paramagnetic material, which can be easily removed by low-intensive magnetic separation [9]. Chemical comminution is a method, which tends to weaken the coal structure by chemical action [93-94]. This concept has been applied to remove mineral matter from coal, to desulphurize coal and to facilitate in-situ coal extraction from underground deposits. Various reagents have been studied for chemical comminution, i.e. pure and aqueous ammonia, NaOH, KOH, Na_2CO_3, $Ca(OH)_2$, etc. and organic solvents. Other laboratory sulphur extraction methods, apart from those described above in oxidative approaches (section 1.5.2.2.2) have been developed as well. They comprise Supercritical Fluid Extraction [95-98], Hydride reduction usually followed by SET/BASE treatments [99-101] and extraction with chlorinated solvents [102-103].

Chemical desulphurization methods are capable to remove simultaneously S_p and S_o, unlike the physical desulphurization techniques attacking only S_p. However, the chemical desulphurization methods suffer from number of limitations. The major ones are that they are too aggressive and corrosive, and decrease heating value. In addition, they are too expensive to be applied at current coal prices. Perhaps this is the reason why coal biodesulphurization became recently to the forefront of coal technology research as a method with high potential toward sulphur removal at mild experimental conditions with no harmful reaction products where the value of coal is only slightly affected.

1.5.2.3 Microbial cleaning (Biodesulphurization)

Microbial desulphurization is a relatively new approach, extensively studied in the recent decades as alternative options to remove sulphur from fossil fuels. Microbial coal desulphurization prior combustion, namely biodesulphurization, have the following advantages over physical and chemical methods [104-105]:

- Biodesulphurization usually requires lower capital and operating cost compared to high cost chemical processes and flue gas desulphurization;
- Unlike physical desulphurization, finely distributed sulphur compounds (S_p and S_o) can be removed by microbial catalysis, without causing any significant energy loss or coal refuse;

- The biodesulphurization operates at relatively low temperatures (25-75ºC) and atmospheric pressures and, therefore, is less energy intensive than chemical processes;
- Since microorganism have a heating value of about 14000 kJ/kg, they could contribute the heating value of biodesulphurized coal;
- Since the organisms oxidizing S_p are autotrophs and utilize carbon dioxide as carbon source, bioprocess provides a useful means for utilization of stack gas as source of carbon dioxide (e.g., CO_2 released from coal combustion can be recycled by the bioprocess).

Other advantages could be added to the above listed [106]:

- The possibility of reducing the ash content;
- The compatibility of the process with hydraulic transport of coal and the coal-water mixture technique.

As well as:

- Since in biological activities, biocatalysis (enzymes) are involved, biodesulphurization would be highly selective [1];
- Combustibility of biodesulphurized coals is not substantially modified by biological treatments [42,107].

There are four basic ways that microorganisms can be used to desulphurize coal [9]. Namely:

- Oxidize and dissolve S_p in coal;
- Selectively attack the molecules that contain coal S_o;
- Coal destruction to liquids, gases or water-soluble compounds to produce liquid or gaseous fuels, so that normal chemical processing methods can be used to remove the sulphur from these fuels;
- Coal or sulphur properties alteration with the aim that other sulphur-removal process will be more effective.

Irrespective of the involved action, biodesulphurization can be accomplished directly by the microorganisms (cellular material) or by chemicals that they produce (acellular material), which are either naturally released into solution or are extracted from the organisms. The accellular materials that are of utmost interest are enzymes acting as highly specific catalysts.

In general different types of bacteria are needed to eliminate S_p and S_o [106]. Those which dissolve pyrite use sulphur as energy source, while those which eliminate S_o do so as a secondary metabolic function. Thus, according to their capacity to eliminate one type of sulphur, microorganisms can be classified in three groups: i) obligate autotrophs, which oxidize only S_p; ii) facultative autotrophs, which oxidize S_p and some compounds of S_o; and iii) heterotrophs, which only oxidize organic compounds.

1.5.2.3.1 Pyritic sulphur bioremoval

Microbial removal of inorganic sulphur from coal has been described in numerous papers [41,108-126]. The most studied microorganism for depyritization is the mesophilic and acidophilic autotroph *Thiobacillus ferroxidans* (*TF*), growing at 30-35°C and pH 2.0-2.5 [9,41,108,113-117,124-128]. Depending on coal quality and experimental conditions, over 90% S_p desulphurization can be achieved [114-115,117,125-127]. The main tested experimental conditions for improving desulphurization rate are pH, pulp density, coal particle size, media composition and contact time. It is established by Acharya et al. [115] that optimal conditions for maximal depyritization of Polish bituminous coal (39.2%) and Rajasthan lignite (99.8%) are: pH=1.5; pulp density 2% (v/v); inoculums 10% (v/v); particle size 45 μm; temperature 35ºC; shaking 140 rpm; duration 30 days. The authors consider that the lignite is the most suitable for microbial depyritization inasmuch as: i) it is a comparatively immature coal and the pyrite in such coals is weakly attached to the coal matrix making the bond easy to break under microbial influence; ii) the investigated lignite is characterised by high S_p and *TF* demonstrates good adaptability; iii) the type of pyrite present in the studied coal, i.e. framboidal form, is most susceptible to oxidation. The fact that the nature of coal and the morphology and distribution of pyrite influence the overall efficiency of the microbial process is confirmed by other authors as well [117]. On other hand, although the temperature of 35ºC is considered as optimal by Juszczak et.al. [127], they do not observed a bacterial growth at pH=1.5. However, it should be mentioned that the measured pH in all studies is the pH at the beginning of the process (initial pH) and it may change during the microbial process. The changes can be in various directions mainly due the different chemistry of the treated

coals. The extraction of pyritic sulphur increases as particle size and pulp density decrease [117,127]. Although the lower pulp densities and particle size make the microbial process by *TF* economically unattractive, this process has progressed to extent of pilot scale tests [129-130].

The ability of *TF* to catalyse pyrite oxidation is well documented [106,129,131-132]. Two mechanisms for oxidation of pyrite by *TF* have been proposed: direct and indirect. The first one requires physical contact between *TF* and pyrite particles and involves biological oxidation of pyrite as represented in the following reaction:

$$4FeS_2 + 15O_2 + 2H_2O \xrightarrow{TF} 2Fe_2(SO_4)_3 + 2H_2SO_4$$

Several attempts have been done to demonstrate the direct attack of *TF* on metal sulphides. It can be considered as a heterogeneous process in which the bacterial cell attaches itself to the sulphide crystal surface and oxidation occurs in a thin film located in the interspaces between the bacterial outer membrane and the sulphide surface. With certain coals, the direct mechanism for oxidation of pyrite may be limited because the microorganisms are too corpulent to penetrate into most of the coal pores. Respectively, pyrite oxidation in coal to a large extent must rely on the indirect mechanism.

The indirect mechanism involves the chemical oxidation of the sulphur in pyrite by ferric iron:

$$FeS_2 + 14Fe^{3+} + 8H_2O \longrightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+$$

The ferric iron is produced by the microorganisms by the oxidation of ferrous iron:

$$Fe^{2+} \xrightarrow[H^+,\ H_2O]{TF} Fe^{3+}$$

The oxidation of ferrous iron in the absence of microorganisms is a slow process. It is considered as a rate limiting step for the oxidation of pyrite with ferric iron. Another option of indirect mechanism is that the ferric iron oxidises the ferrous iron in the pyrite, leaving elemental sulphur behind.

$$FeS_2 + 2Fe^{3+} \xrightarrow{TF} 3Fe^{2+} + 2S_{el}$$

The elemental sulphur which results from the oxidation of pyrite can be oxidized by the ferric iron:

$$S_{el} + 4H_2O + 6Fe^{3+} \longrightarrow SO_4^{2-} + 6Fe^{2+} + 8H^+$$

or by oxygen, in which case the bacteria, which oxidize the sulphur, catalyse the reaction:

$$2S_{el} + 3O_2 + 2H_2O \xrightarrow{TF} 2H_2SO_4$$

Another application of *TF* is for H_2S elimination from gas streams [133-135]. This process involves contact of H_2S-containing gases with a solution of ferric sulphate in an absorber. The solution absorbs H_2S and oxidizes it to S_{el}, while the ferric sulphate is reduced to ferrous sulphate. This is shown in the following reaction:

$$H_2S + Fe_2(SO_4)_3 \longrightarrow S_{el} + 2FeSO_4 + H_2SO_4$$

This reaction goes to completion very rapidly and H_2S is removed efficiently. Ferrous sulphate obtained in the first step is further treated by *TF* in order to oxidize ferrous iron to ferric iron in a bioreactor. The obtained ferric iron is recycled to the absorber for the first step and the cycle is repeated.

There are several factors that play a role in the rate of oxidation of ferrous ions by *TF*. They include ferrous/ferric concentration, cell and oxygen concentration, pH, temperature, etc. The various studies summarised by Daoud and Karamanev [136] have concluded that ferric ions competitively inhibit ferrous ion oxidation by *TF*, an inhibitory effect that can be reduced by increasing cell concentration. The pH 2.0 and temperature of 30ºC are optimal for bacterial operation and oxidation rate are maximal. An important factor that has to be considered during coal microbial desulphurization is the precipitation of ferric hydroxysulphates with formula $MFe_3(SO_4)_2(OH)_6$, where M = K^+, Na^+, NH_4^+, Ag^+, or H_3O^+. These precipitates are known as jarosites and have been found to occur at pH values as low as 1.5. The H_3O^+, K^+ and Na^+ types are the most likely to form in coal processing and the potassium form is the most insoluble [129]. However, since *TF* is grown on 9K medium containing high concentration of NH_4^+ ions, ammoniojarosites ($NH_4Fe_3(SO_4)_2(OH)_6$) are the main jarosites produced during microbial treatment with *TF*.

The extent of precipitation of jarosites may decrease the desulphurization rate in two ways. Firstly, sulphate ions will be in solid state instead of being removed with the water as soluble species. Secondly, the precipitation may decrease the area of unreacted exposed pyrite surfaces available for microorganisms and, therefore, slow down the desulphurization. However, jarosite precipitation can be prevented by keeping moderate temperature and the pH sufficiently low. It is revealed that the optimal pH and temperature combination producing the least amount of jarosite is pH 1.6-1.7 at a temperature of 35ºC [136]. According to some researchers, the most significant factor affecting jarosite formation is the total iron content of unprocessed coal [131]. It may be due to the fact the jarosite is not precipitated but rather produced by the reaction of dissolved sulphate with iron sites on the coal surface.

The ability to oxidize sulphur and/or iron in an acidic environment is not exclusive to *TF* but is also characteristic for other mesophilic microorganisms, i.e. *Thiobacillus thiooxidans* (*TT*), *Thiobacillus acidofilus* (*TA*), *Leptospirillum ferrooxidans* (*LF*) [129]. *TT* and *LF* are autotrophs able to oxidize sulphur and iron, respectively, while *TA* is facultative autotroph. These microorganisms are interesting not only because they can oxidize sulphur and/or iron. They can be used in consortium to enhance pyrite solubilisation. Some of the used mixed cultures are: i) *TF* and *LF* [108]; ii) *TF*, *TT* and *LF* [117]; iii) *TF* and *TT* [137].

Some facultative autotrophs and heterotrophs, i.e. species belonging to the genus *Pseudomonas* and *Sulfolobus*, have been tested for coal inorganic sulphur removal. Garcia et. al. [117] in their comparative study of the biodesulphurization of Spanish coal by *Thiobacillus* and *Sulfolobus* conclude that *Thiobacillus* is more effective than *Sulfolobus* in dissolving pyrite. However, *Sulfolobus* can remove approximately half of the S_p (48%), and unlike *Thiobacillus*, one third of S_o (28%). The thermophilic and facultative autotrophic microorganism *Sulfolobus acidocaldarius* (*SA*), strain 98-3, has demonstrated promise for the removal of sulphur from coal. About 96% of inorganic sulphur has been destroyed within 10 days in coal samples containing 4% S_t and 2.1% inorganic sulphur, which resulted in 50% S_t removal [112]. *SA* has some advantages over the widely used *TF* for microbial desulphurization [106,109,112]. Its extremely thermophilic, acidophilic, aerobic and autotrophic character makes it resistant to contamination. The rates of chemical reactions

between ferric iron and pyrite are increased at high temperature and benefit the process of sulphur removal. The solubility of some mineral sulphates is higher at lower pH and high temperature, which reduces reprecipitation in the process of sulphur reduction. Finally, microbial ability to remove S_o inasmuch as this microorganism is facultative autotroph is very important. However, it should be mentioned that high temperature promotes jarosite formation [129]. *Sulfolobus solfataricus* (*SS*), strain DSM 1616, also demonstrated the ability to reduce S_p with about 71% after optimizing the conditions for microbial growth [123]. However Karlsson et al. [116] revealed that strains of *Sulfolobus* (*SA*, DSM 639 and *SS*, DSM 1616) and *Pseudomonas* (obtained from the Department of Applied Microbiology, University of Lund, Sweden) available in their lab are not capable for oxidizing the pyrite under the recommended conditions for microbial growth. Nevertheless they achieved substantial S_p removal from coal by using *Acidianus brierleyi*. The experiments applying *Pseudomonas putida* (*PP*) should be mentioned as well, as they have been reported to dissolve up to 75% of the S_p [119,138].

1.5.2.3.2 Organic sulphur bioremoval

Certain organisms are found to metabolize some stable sulphur-containing organic compounds like dibenzothiophene (DBT). Actually, initial attention is focused on bioremoval of sulphur in DBT, since it is regarded as a model compound representative for the organic sulphur forms in fossil fuels. Three major pathways for DBT degradation by microorganisms are reported: i) the ring-destructive oxidative pathway; ii) the sulphur-specific pathway; and iii) the completely destructive pathway.

In the ring-destructive oxidative pathway, known as the Kodama pathway, DBT is partially oxidized to water soluble intermediates, but the sulphur of DBT remains intact. In this pathway, DBT is degraded by hydroxylating one of the benzoid rings to DBT-dihydrodiol and DBT-diol, cleaving the ring between the diol substituents, and then producing, in sequence, 4-2-(3-hydroxy)-thionaphthenyl-2-oxo-3-butenoic acid and 3-hydroxy-2-formylbenzothiophene (HFBT), as shown in Figure 1-4 [1,139-142].

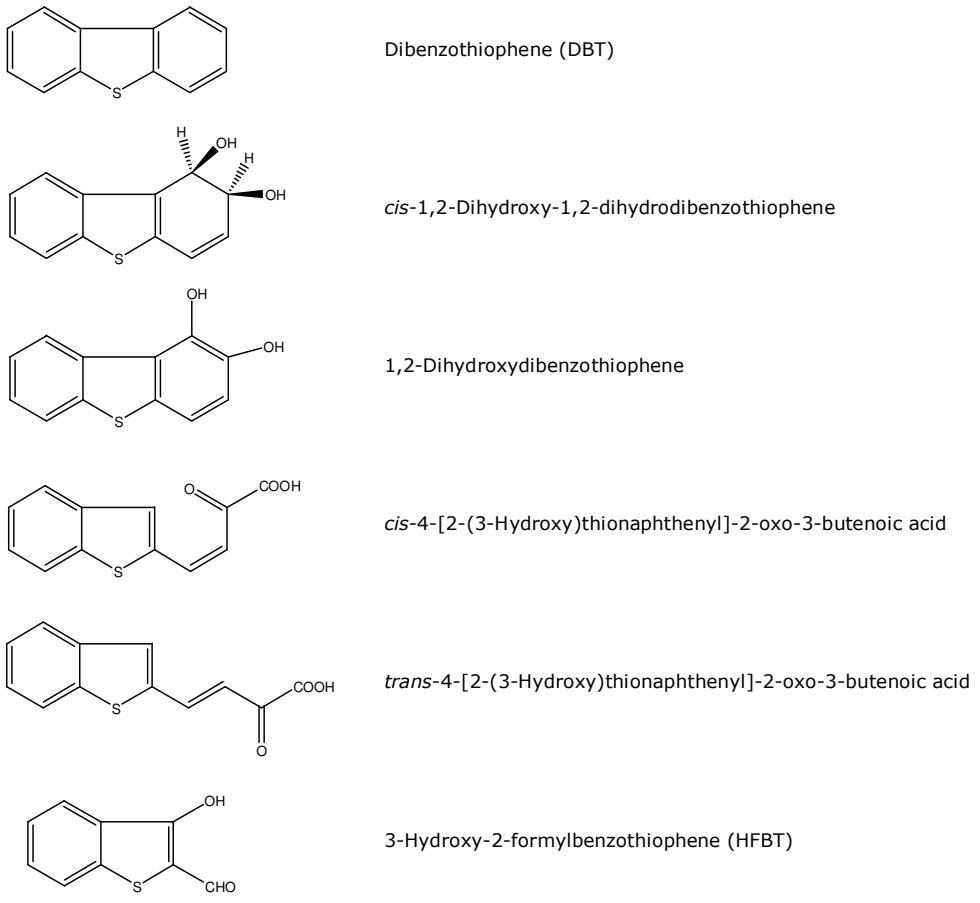


Figure 1-4 Ring destructive oxidative pathway of DBT

Various microorganisms, i.e. *PP*, *Beijerinckia*, *Burkholderia*, *Bacillus circulans*, *Rhodococcus gordoniae* and *Rhizobium* are known to respect this pathway [143-148]. The majority of investigations have been focused on *Pseudomonas* cultures. Monticello et.al. [143] prove that Kodama pathway is plasmid associated in at least two *Pseudomonas* species, namely *Pseudomonas alcaligenes* and *PP*. The product of DBT oxidation is inhibitive for both cell growth and further DBT oxidation. The same plasmid is reported to mediate the biodegradation of other aromatic compounds such as naphthalene and salicylate. The fact that DBT-metabolizing genes from strain of *PP* encode an upper naphthalene catabolic pathway, give ground to Denome et. al. [144] to conclude that the ability of this strain to metabolize DBT is limited to oxidation of the

aromatic ring in a similar manner to the described for the upper naphthalene catabolic pathway. In addition to Kodama pathway, it is claimed that *PP* follows an alternative pathway as well [145]. The organism first metabolizes DBT *via* the Kodama pathway and then transforms DBT to DBT sulphone. Alike is the metabolism of DBT by a *Beijerinckia sp*. [146]. Although HFBT is reported as the end-product of DBT degradation *via* Kodama pathway, there are instances where other possible products of HFBT such as benzothiophenes-2,3-dione and disulphides have been reported [149-150]. It has also been described that HFBT can be degradated to carbon dioxide [149].

Microbial degradation of organic sulphur-containing carbonaceous materials by ring destructive oxidative pathway results in net carbon loss and reduction of calorific value of the carbonaceous fuel. In addition, it has been observed that the pathway for naphthalene metabolism closely resembles the ring destructive pathway, raising the possibility that further undesired metabolism of structurally related non-sulphur-containing fuel components may occur. Therefore it is advisable to follow a microbial degradation pathway with sulphur removal route without carbon destruction. Thus calorific value of the fuel is preserved to a greater extent comparing to ring-destructive pathway.

The **second** pathway for DBT degradation by bacteria is the sulphur-specific pathway. In it, DBT is desulphurized by the selective cleavage of the carbon-sulphur bonds, resulting in the accumulation of 2-hydroxybiphenyl as the end product [1,139-142]. According to this pathway, as shown in Figure 1-5, DBT is metabolized to 2-hydroxybiphenyl (2-HBP) via four different compounds, i.e. DBT 5′ – sulphooxide (DBTO), DBT 5′ – sulphone ($DBTO_2$) and 2′ – hydroxybiphenyl 2 sulphinic acid (2-HBPS). Respectively, the pathway is known as the 4 step (4S). The specific cleavage of the carbon-sulphur bonds is preferred for microbial desulphurization technology so that sulphur is removed, but the carbon and calorific values remain unaltered. This process for sulphur removal is firstly reported for a strain of *Rhodococcus rhodochrous* (*RR*), IGTS8 isolated from the mixed culture IGTS7 [151-154]. Two routes of sulphur-specific metabolism of DBT by IGTS8 are tentatively identified. Under no growing conditions, the DBT is converted to 2-HBP via the 2′-hydroxybiphenyl-2-sulphinate. Under growth conditions, small quantity of the 2′-hydroxybiphenyl-2-

sulphinate is converted to 2-HBP. The most is oxidized to 2'-hydroxybiphenyl-2-sulphonate, and 2,2'-dihydroxybiphenyl (DHBP) as the main product [152-155].

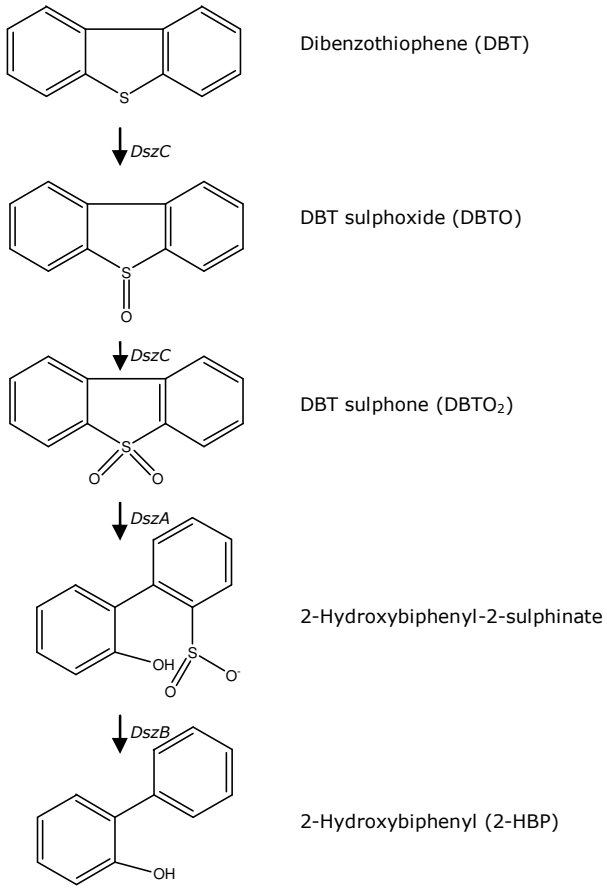


Figure 1-5 Sulphur-specific pathway for microbial DBT desulphurization

Later, identification and cloning of the genes involved in sulphur-specific attack on DBT by *RR* IGTS8 give further weight to these proposed routes [156]. Oldfield et.al. [157-158] also thoroughly investigate and research the *dsz* operon encoded proteins, i.e. DszA, B and C, which are necessary and sufficient to confer the DBT desulphurization phenotype on *RR* IGTS8. However, they establish that whatever the nature of the pathway, desulphurization of DBT to DHBP is quantitatively insignificant compared to 2-HBP under standard assay condition. Furthermore, there is no evidence for growth-dependent metabolic switching between 2-HBP- and DHBP-yielding pathways, as suggested by Gallagher et.al [155]. DHBP formation from DBT is quantitatively insignificant in

both exponentially growing and stationary-phase culture. Additional information on enzymology of desulphurization pathway of *RR* IGTS8 can be found elsewhere [1,141,159-161].

Many research groups have studied the desulphurization of DBT by the sulphur specific pathway, demonstrating the capacity to generate 2-HBP from DBT in *Rhodococcus* ECRD-1 [162], *Rhodococcus erythropolis* D-1 [163], *Sphingomonas subarctica* T7b [164], *Lysinibacillus sphaericus* DMT-7 [165], *Paenibacillus* A11-2 [166-167], *Mycobacterium* X7B [168], *Shewanella putrefaciens* NCIMB 8768 [169], *Corynebacterium* SY1 [170], *Bacillus subtilis* WU-S2B [171], and *Rhodococcus* strains UM3 and UM9 [172], *Arthrobacter* ECRD-1 [173]. The DBT desulphurizing organism last catalogued are isolated by enrichment culture using DBT as sole sulphur source. Some of them readily attack and desulphurize alkylated forms of DBT as well [162,164,171,173-174]. However, a clear preference is shown for unsubstituted DBT. Despite the apparent broad specificity for members of DBT family, some of catalogued isolates seem to be incapable of desulphurizing benzothiophenes (BzTh). In particular, strains IGTS8, *Arthrobacter* ECRD-1 and 10 independently isolated by Oldfield et.al. DBT-desulphurizing *Rhodococcus* strains are not capable of desulphurizing BzTh [158,173,175]. The reported microorganisms that are capable of desulphurizing BzTh are *Rhodococcus* WU-K2R [176], *Paenibacillus* A11-2 [166] and *Sphingomonas subarctica* T7b [164].

The **third** pathway of DBT metabolism is completely destructive pathway in which DBT is mineralized to CO_2, sulphite and water [139,177]. In it (Figure 1-6), DBT serves as the sole source of carbon, sulphur and energy. Only two microorganisms, i.e. *Brevibacterium* DO and *Arthrobacter* DBTS2 are known to respect this metabolic pathway.

The above described microbial cultures are involved in aerobic biodesulphurization of sulphur-containing organic compounds. Anaerobic biodesulphurizations of sulphur-containing hydrocarbons have been reported as well [139,142,178-182]. *Desulfovibrio sp*. anaerobically degrades organic sulphur compounds. A bioelectrochemical process has been developed [182] to deliver electrons through electrochemical cell to *Desulfovibrio desulphuricans* M6 resulting in biphenyl and hydrogen sulphide formation from DBT. Conversion of DBT to biphenyl has been reported to be achieved by other bacteria isolated

from oil field, but the conversion ratio is very low [180-181]. Dibenzyl disulphide (DBDS) is reductively degraded by a methanogenic mixed culture derived from sewage digester [179]. Mixed culture of thermophilic bacteria obtained from crude oil is also capable of anaerobic biodegradation of DBT [178].

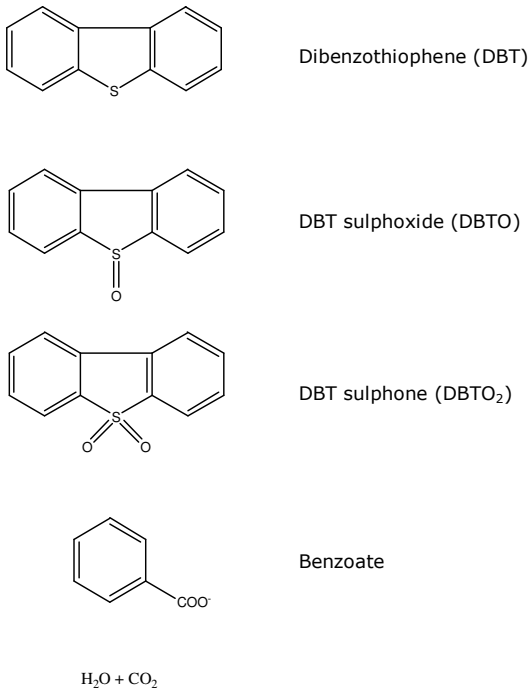


Figure 1-6 Metabolic pathway for DBT degradation by Brevibacterium

Biodesulphurization of other sulphur-containing compounds, especially BzTh also has been intensively studied. It has been reported that *Pseudomonas sp*., can degrade BzTh [183-186]. However, these reports indicate that BzTh and substituted BzTh cannot serve as sole source of carbon and energy for the microorganisms, but these condensed thiophenes can be cometabolized. For example, Fedorak and Grbic-Galic [184] use 1-methylnaphthalene, glucose or peptone, as a growth substrate for *Pseudomonas* strain BT1. They found that cometabolism of BzTh yielded BzTh-2,3-dione, whereas that of 3-methyl-BzTh yielded 3-methyl-BzTh sulphoxide and the corresponding sulphone. Neither of the sulphur heterocyclic compounds support *Pseudomonas* strain BT1 growth,

but they are transformed by the culture growing on 1-methylnaphthalene, glucose or peptone. Eaton and Nitterauer [183] identified two products as a result of BzTh transformation by *PP* RE204 grown on succinate and yeast extract. The first one is recognized as trans-4-(3-hydroxy-2-thienyl)-2-oxobut-3-enoate. It is a result of the oxidation of the homocyclic ring of BzTh leading to a formation of 4,5-dihydroxybenzothiophene *via* *cis*-4,5-dihydroxy-4,5-dihydrobenzothiophene, and followed by cleavage of the homocyclic ring. The second product is recognized as 2-mercaptophenylglyoxalate. It is a result of the oxidation of the heterocyclic ring of BzTh leading to the formation of 2-hydroxy-3-oxo-2,3-dihydrobenzothiophene *via* *cis*-2,3-dihydroxy-2,3-dihydrobenzothiophene, and followed by cleavage of the heterocyclic ring. According to Eaton and Nitterauer, 2-mercaptophenylglyoxalate might be converted to benzothiophenes-2,3-dione, but probably it is a product of transformation that occurs during extraction of the product. Kropp et al. [186] use 6 of the 15 possible isomers of dimethyl-BzTh for biotransformation studies with three *Pseudomonas* isolates. Each of them is grown on 1-methylnaphthalene or glucose in the presence of one of the dimethyl-BzTh izomers. Sulphoxide and sulphones are the commonly found metabolites in the culture extracts from the 2,3-, 2,7- and 3,7-isomers, whereas 2,3-diones, 3 (2H)-ones and 2(3H)-ones are formed from 4,6- and 4,7-isomers. Microbial transformation of BzTh by *Sphingomonas* sp. XLDN2-5, with carbazole as the auxiliary substrate, can also be achieved [187]. The common transformation products for BzTh, 2-methyl-BzTh and 5-methyl-BzTh are the corresponding sulphoxides and sulphones. *Sphingomonas subarctica* T7b can desulphurize BzTh with long alkyl chains [164]. The microorganisms of the genus *Rhodococcus* that are able to desulphurize BzTh and alkyl-BzTh through selective cleavage of carbon sulphur bonds are *Rhodococcus* JVH1 [188], *Rhodococcus* WU-K2R [176], *Rhodococcus erythropolis* KA2-5-1 [189]. *Rhodococcus* WU-K2R and *Rhodococcus* JVH1 [190] are capable of selective cleavage of carbon sulphur bonds in naphthothiophene and alkyl chains, respectively. GC-MS study reveals that *Rhodococcus* JVH1 oxidizes alkyl sulphides to a sulphoxides and then to a sulphone prior to cleaving the C-S bond to form an alcohol and, presumably, a sulphinate from which sulphur could be extracted for growth. Similar is reported to be the dibenzyl sulphide metabolism by white rot fungi [191].

Subsequently to bioremoval of sulphur from sulphur containing model compounds, an increasing attention is paid on the possibility of coal organic sulphur removal by bacteria. However, information on microbial organosulphur removal from coal is rather scanty. Kargi and Robinson [192] publish that part of S_o from inorganic-sulphur free bituminous coal samples can be removed by the thermophilic, facultative autotrophic, sulphur oxidizing organism *SA.* Nearly 44% of initial S_o is removed from 10% coal slurries at 70ºC in about four weeks. Organisms of the genus *Pseudomonas* also have been tested for sulphur removal from coal and it is found that *PP* is more effective. *PP* is claimed to reduce S_p by 75% and S_o by 37.4% from Texas lignites in 5-7 days for particle sizes varying from 74 μm to 295 μm [119]. Experiments with *Acidianus brierleyi* show that this microbial culture is capable of removing S_o [116]. Kilbane [193] performs biodesulphurization experiments using *RR* IGTS8 at a 200 mesh top size coal Illinois Basin Coal sample program (IBC-101). The obtained results for S_o by using different determination techniques demonstrate substantial S_o removal of 35% on average during 3 weeks biotreatment. Later on, *RR* is intensively studied in order to optimize conditions for sulphur removal from various Turkish lignites [121-122,194-195]. The results reveal that the maximum specific growth rate of *RR is* obtained under fermentation conditions of 28ºC, initial pH 6.5, shaking rate 84 rpm and inoculums concentration 8 vol.%. Biodesulphurization experiments performed under these optimum conditions show maximum total and organic sulphur reductions for Mengen lignite of 30.2% and 27.1%, respectively, at 72 h. In these experiments sodium acetate is used as a substrate [194-195]. The effect of the lignite type and particle size on microbial desulphurization by *RR* have been also tested [122]. Turkish lignites, i.e. Mengen, Elbistan, Tuncbilek and Gediz, are selected for biodesulphurization because of their widely varying sulphur contents. The highest decrease in S_t and S_o are registered for Gediz and Mengen lignites, respectively, characterized by the highest S_o contents. Reduction in particle size from 390 μm to 63 μm results in an increase in S_t and S_o removal rates by factor of 2.8 and 19, respectively. *SS* demonstrate comparatively fast desulphurization rates and higher conversion yield for sulphur forms in Turkish lignites. Experiments have been carried out with *SS* to determine the biodesulphurization effect and the effects of biodesulphurization time. The factors affecting growth kinetics of *SS* are under

consideration as well [120,123]. A decrease in all sulphur forms with increasing time is determined for all studied coal. The highest S_t and S_o reductions by this bacterium are observed for Beypazar lignite, 57.1% and 47.8%, respectively. The authors are aware that the lignite types affect microbial sulphur reduction during desulphurization with *SS.*

Some fungi are also tested for coal biodesulphurization since they are able to metabolize and/or modify a broad range of anthropogenic chemicals, hydrocarbons (including polycyclic aromatic hydrocarbons and organosulphur compounds) and even coals through the action of cytochrome P-450 and extracellular enzymes [191,196-197]. Biodesulphurization experiments by using *Trametes versicolor, ATCC 200801,* and *Phanerochaete chrysosporium, ME 446,* are carried out with Tuncbilek lignite, characterized by high sulphur content [198]. The maximum S_t desulphurization effect of 40% is recorded after 6 days without change in calorific value. The optimum condition for microbial growth and maximal removal of sulphur are pH=6, T=35ºC, agitation rate of 125 rpm, particle size 200 μm, pulp density of 5% (w/v). *Aspergillus*-like fungi is also reported as capable of removing sulphur from coal. S_t elimination as high as 70-80% are achieved in 10 days with this fungi from Assam coal [199]. The authors especially underline that the results indicate microbial attack on S_o since sulphur presence in Assam coal is mainly in organic form. However, although biological coal desulphurization of S_o has a deal of promise at laboratory scale, there is a need for additional research to be performed in order to increase the desulphurization rates and effectiveness of the microorganisms. This could promote its future industrial application.

1.6 Research objective

It is obvious that high sulphur content in coal provokes a number of undesirable and serious obstacles related to environmental pollution and technological problems. High sulphur containing coal and lignite use for public conventional thermal power generation is the main reason why Bulgaria along with other Balkan countries, i.e. Greece, Turkey and Romania are the biggest SO_2 polluters in Europe. In order to limit sulphur pollutions it is advisable to apply pre-combustion desulphurization methods. Several of them have been proposed to reduce sulphur content before coal combustion. The applied techniques comprise physical, chemical and biological processes and practically the most of the inorganic sulphur in fossil fuel can be removed easily. However, there is one portion, known as refractory organic sulphur, which is very difficult to be eliminated. The current methods affecting the refractory sulphur, operate under extremely invasive conditions and produce considerable amount of CO_2. This is the reason why biodesulphurization becomes attractive as a method with potential toward organic sulphur removal in coal at mild experimental condition with no harmful reaction products and no significant change in calorific value.

The presented review on biodesulphurization of S_o in coal indicates that the research in this field is rather scanty. Moreover, most of the studies are limited to the optimization of the conditions for biotreatment in order to increase the effect of biodesulphurization and its quantification. However, since there is no standard analytical procedure for the direct measurement of S_o, it is determined indirectly, i.e. by subtracting the sum of inorganic sulphur (S_s+S_p) from S_t. Respectively, in the presence of another sulphur form, such as $S_{el,}$ its quantitative value will be included into the quantitative value of the so called organic sulphur and not correct estimation of the S_o magnitudes and S_o biodesulphurization effects will take place.

Studies on the organic sulphur transformation mechanisms during microbial desulphurization of coal are not available as well. In addition, there are no comprehensive data available on the influence of different characteristics of the coal macromolecule on biodesulphurization. Both are partly related to the limitations of the existing analytical techniques used for coal organic matter characterization and especially for coals organic sulphur forms investigations.

However by upgrading the available analytical tools for coal characterization and by developing analytical methods for determination of sulphur forms, i.e. organic and inorganic, a significant progress in this area could be attained. It is believed that deeper knowledge in organic sulphur transformation in coal during biodesulphurization as well as appreciation of coal matrix influence on biodesulphurization will reflect in better understanding of microbial desulphurization in coal and will bring greater success in microbial sulphur removal. With the acquisition of such knowledge it will be possible to define: (i) which coals are suitable for coal biotreatment; (ii) proper microorganism that can be used for certain coals; (iii) sulphur forms and sulphur functionalities that are preferred by the bacteria; (iv) pretreatment necessary to be performed in order to convert coal and its sulphur forms and functionalities in a form suitable for microbial processing, etc.

The main goal of the present study is tracing the changes that occur with sulphur, its forms and functional species arranged in organic compounds in the process of effective biodesulphurization of coals from different sources. This will contribute to development and better understanding of the coal biodesulphurization. To achieve this purpose the following tasks are addressed:

(i) Biodesulphurization of high sulphur coal with appropriate microbial cultures. Qualitative and quantitative assessment of the changes that occur with sulphur and its forms and functionalities as a result of the biotreatments by the use of pyrolytic, chromatographic and spectrometric methods.

(ii) Biodesulphurization of preliminary demineralised and depyritized coal samples and qualitative and quantitative assessment of the changes that occur with sulphur and its forms and organic functionalities as a result of biotreatments by the use of pyrolytic, chromatographic and spectrometric methods. This task has been imposed in order to focus our efforts on organic sulphur biodesulphurization research and to improve the information obtained by analytical tools used for sulphur assessment.

(iii) Evaluation of elemental sulphur in the samples under consideration by developing and implementing a methodology for its determination. This

will give us ground to attain a better sulphur balances for initial and biotreated samples.

(iv) Desulphurization of technological coal sample with an industrial significance by a combination of chemical and microbial treatments. Qualitative and quantitative evaluations of the sulphur changes that occur as results of the applied treatments.

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Chapter 2 Materials and methods

2.1 Coal Samples

Three Bulgarian and one Turkish low rank coal samples are investigated. The selection of the samples is mainly provoked by their high sulphur content, i.e. organic sulphur, and their significance for the industrial electricity production.

Bulgarian coal samples under consideration are:

- Pirin subbituminous coal from "Pirin" coal mine, R_r = 0.46% [1] – code **P**;
- Maritza East lignite from "Trajanovo-North" mine, R_r = 0.20% [2] – code **IN**;
- Manually picked Humovitrain of Maritza East lignite from "Trajanovo-North" mine, R_r = 0.20% [2] – code **M**.

Turkish lignite under study is:

- Beypazari lignite from "Cayirhan" mine, R_r = 0.38% [3] – code **B**.

Proximimate and ultimate analysis of initial samples are presented in Table 2-1.

2.2 Chemical treatments

Demineralization, depyritization and thermooxidation with atmospheric air are applied to most of the studied samples. All of these treatments are performed to obtain mineral and inorganic sulphur free coals, to promote better microbial processing and to focus our research on the removal and changes in organic sulphur as a result of biodesulphurization.

2.2.1 Demineralization

Demineralization procedure comprises three consecutive treatments with mineral acids [4-5]. The mineral free samples are obtained by intermixing 20 g coal (particle size <0.2 mm) in 500 ml 5% HCl at 60ºC for 1h (first step). The aim of the first treatment is to avoid formation of insoluble fluorides during the second step. After the first acid treatment the slurry is separated by filtration

and subjected to a next acid treatment. The residue is re-intermixed with 500 ml 40% HF at ambient temperature for 1h (second step) to dissolve any remaining non-destructed mineral matter. The filtration residue after the second step is intermixed with 300 ml 36% HCl acid at ambient temperature for 1h (third step). Subsequently it is washed with hot distilled H_2O. Demineralized samples are marked as "AF".

Table 2-1 Proximate and Ultimate analysis of initial samples

Analysis	Samples			
	P	M	B	IN
Proximate (wt.%)				
W^{ad}	6.5	8.1	12.3	8.4
Ash^{ad}	10.4	8.5	29.1	32.1
VM^{daf}	37.2	48.8	40.2	52.6
$C_{fix}{}^{daf}$	62.8	51.2	59.8	47.4
Ultimate (wt.%)^{daf}				
C	70.3	59.7	47	57.9
H	5.6	5.4	4.7	5.9
N	1.7	0.5	1.6	1.2
O^{diff}	16.9	27.3	39.1	25.5
S_t*	**5.5**	**7.1**	**7.6**	**9.5**
Sulphur forms (wt.%)^{daf}				
S_s	**0.54**	**1.54**	**1.23**	**1.65**
S_p	**0.57**	**1.64**	**1.68**	**3.19**
S_o	**4.38**	**3.88**	**4.71**	**4.64**

W - moisture; VM - volatile matter; C_{fix} - fixed carbon; ^{ad} *– air dried base;* ^{daf} *– dry, ash free base;* O^{diff}*=100-(C+H+N+S);*
**determined by ASTM D3177.*

2.2.2 Depyritization

For depyritization, the demineralized samples are treated by diluted nitric acid [4,6-7]. In the treatment about 10 g ash free coal (particle size < 0.063 mm) is intermixed with 250 ml 17% HNO_3 for 3h at ambient temperature and shaking.

Demineralized and depyritized samples are assigned as "APF" (**A**sh and **P**yrite **F**ree).

2.2.3 Thermooxidation with atmospheric air

Several reports indicate that an enhance oxygen content of coal promotes better microbial desulphurization [8-10]. Therefore, prior to biotreatment oxidation is applied. Additional advantage of this preliminary treatment is the expected oxidation of S_p into S_s, which is soluble in water and is not considered as a serious source of sulphur in coal. From the described in the literature coal oxidation methods [11-12], the Rustchev et al. method [13] is selected and slightly modified to achieve our goals. This method relates to solid state oxidation with atmospheric air of lignite with a high content of S_p and is based on the conversion of S_p into S_s. Its advantages are the lack of liquid phase, producing soluble impurities, application of cheap oxidizing agents and simplicity of the equipment.

In our study, thermooxidation with atmospheric air is carried out in an electrical oven. For this purpose, coal sample (particle size < 1 mm) placed in thin layer in quartz plate is oxidized with air flow of 150 ml min^{-1}. For the best S_p reduction during oxidation with atmospheric air under our experimental set up, different temperature and reaction times are tested (see Figure 2-1). The same amount of coal samples with the same particle size are under consideration in these experiments. It is determined that the highest S_p oxidation is achieved for 45h at 150ºC. Therefore, the thermooxidation of the investigated samples is carried out at the following experimental conditions: 150 ml/min air, T=150ºC, 45h duration. Evidence for organic matter oxidation processes occurring during the treatment with atmospheric air is obtained by elemental analysis (further discussed in Chapter 6) and FTIR. Higher presence of oxygen containing groups, most probably aromatic carboxylic groups appear in the spectral region 1730-1670 cm^{-1} with peak maximum at 1700 cm^{-1} as presented in Figure 2-2.

Oxidized samples are assigned as "oxy".

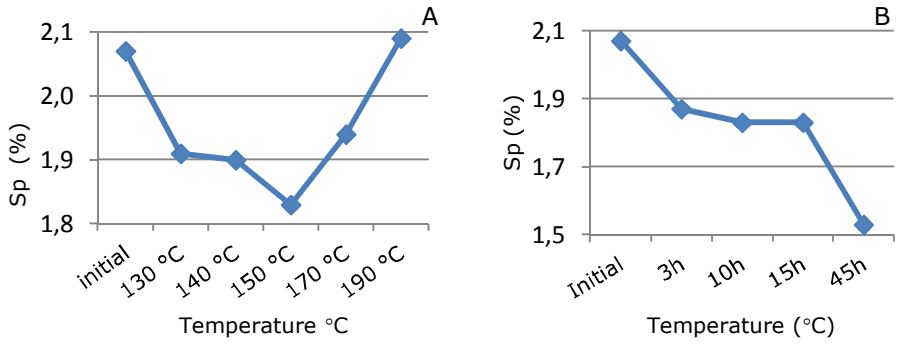

Figure 2-1 S_p decrease by atmospheric air oxidation of IN coal sample in *wt*.%, on dry basis): A) for 10h at different temperatures; B) for different reaction times at 150ºC

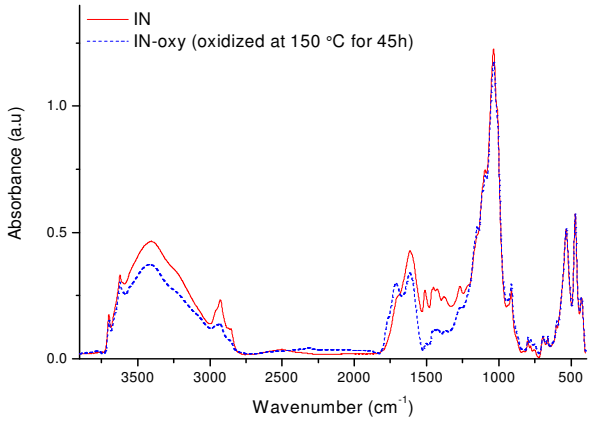

Figure 2-2 FTIR spectra of IN and IN-oxy samples

2.3 Biodesulphurization

Some microorganisms effective towards sulphur compounds, namely *Phanerochaeta chrysosporium*, *Trametes versicolor*, *Mixed Culture of microorganisms*, *Sulfolobus solfataricus* and *Pseudomonas putida* are applied. These microorganisms are selected based on data obtained from previous

studies [14-24] and used at the optimum conditions. Additional information concerning the microorganisms can be found in Table 2-2.

Table 2-2 Microorganisms used in the study

Microorganisms	Code	Organism Type	Metabolic Type	Source
Phanerochaeta chrysosporium	*PC*	white rot fungus	Chemoheterotroph (Saprotroph)	ATCC, No 34541
Trametes versicolor	*TV*	white rot fungus	Chemoheterotroph (Saprotroph)	ATCC, No 200801
Mixed Culture of microorganisms	*MC*	bacteria	n.d.	ATCC, No 39327
Sulfolobus solfataricus	SS	bacterium	Autotroph/heterotroph	ATCC, No 35091
Pseudomonas putida	PP	bacterium	Chemoheterotroph (Saprotrophs)	Sofia University "St.Kl. Ochridski"

ATCC – American Type Culture Collection.

2.3.1 White rot fungi treatments: *PC*, *TV-1* and *TV-2*

The culture growth media for white rot fungi inoculation is prepared by diluting 1.2 g Potato Dextrose broth (PDb) and 1g Agar-Agar (AA) in 50 ml deionized (DI) water under heating and intensive stirring. The prepared hot solution of PDb-AA is distributed into several test-tubes (6 ml in each one with volume of 15 ml) and autoclaved at 121ºC, 1.5 atm, 20 min. In order to get maximum surface area of PDb-AA gel, after sterilization the tubes are placed in a semi-recumbent position and left to cool off. When growth medium is completely cooled down it solidifies and thus is ready for fungi inoculation. Inoculation is done by a needle ending with a circular hollow ear. With its help, spores and mycelia from purchased *PC* and *TV*, respectively, are transferred onto the cooled culture medium and left in a incubator to grow for 6 days at 30ºC (the temperature for optimal growth). This procedure is performed to breed the fungal cultures purchased from ATCC bank. Some of the tubes with grown fungi are used to obtain the supernatant for coal desulphurization while the rest are stored in a refrigerator and kept for further breeding.

The cultural supernatant of *TV* and *PC* used for coal biodesulphurization are prepared by transferring the already grown fungi, i.e *PC* and *TV,* in 100 ml of 24 g/L autoclaved PDb and left in incubator to grow for 6 days at 30ºC. Prior to inoculation, the pH of PDb is adjusted to 4.7 by the use of sterile acetate buffer. After six days, the resulting supernatants are disaggregated, homogenized and used for coal biodesulphurization.

Lab-scale, shake-flask biodesulphurization experiments with *PC* and *TV* are performed at the following conditions: 3% (w/v) coal and 3% (v/v) supernatant in PDb at T=30°C, pH=4.7, agitation rate 125 rpm, 6 days duration. The biodesulphurization experiments with *TV* and *PC* performed at these conditions are marked as *TV*-2 and *PC*, respectively. Biodesulphurization coal experiment at the same experimental conditions but temperature at 37ºC is also performed with *TV*. Laccase enzyme activity of *TV* is found to be the highest at temperature 37°C. Biodesulphurization experiment with *TV* at 37ºC is marked as *TV*-1.

2.3.2 Mixed culture of microorganisms treatments: *MC* and *MC+BS*

The mixed culture of microorganisms (MC) isolated from coal and soil is patented by Stevens and Burgess [19]. They claim that *MC* is capable of reproducible reduction of sulphur, in particular organic sulphur. Even it is not really determined, it is believed that *MC* is comprised of seven aerobic gram negative rods, which are probably one or more of the following: Pseudomonas, Acinetobacter, Azotobacter or Flavobacter. In our study *MC* is purchased from ATCC bank. The used cultural media for its growth is Nutrient broth (Nb) as recommended by the bank. For breeding, the content of the purchased ampoule containing the *MC* is transferred to a test tube containing 5 ml of 8 g/L Nb and left in incubator for one week at 37ºC.

The cultural supernatant of *MC* is prepared by inoculation of 1 ml of the already grown for one week bacteria in 100 ml of 8 g/L autoclaved Nb and left in incubator to grow for another 6 days at 37ºC. Prior to inoculation, the pH of Nb is adjusted to 6 by the use of sterile buffering agent. This pH is maintained in order to avoid the predominance of eventually present acidophilic bacteria and to encourage the growth of soil and coal heterotrophic bacteria [19]. After six days, the resulting supernatant is homogenized and used for coal

biodesulphurization at the following conditions: lab-scale, shake-flask experiments, 3% (w/v) coal and 3% (v/v) supernatant in Nb at T=37°C, pH=6, duration of 6 days. The coal biotreatment with the mixed culture of bacteria is marked as above described for *MC*.

Another type of biotreatment with *MC* is also applied. It is marked as *MC*+*BS*. The experiment is performed at the same condition as *MC***,** but Basal Salt (0.1 g $CaCl_2.H_2O$, 0.1 g $MgSO_4.7H_2O$, 0.002 g ferric citrate in 1000 ml DI water, 0.973 g NH_4Cl, 0.2 g K_2HPO_4, 0.1 g KH_2PO_4) is added to the Nb. It is also used in the US Patent [19].

2.3.3 Sulfolobus solfataricus treatments: *SS*

The content of *SS* in the purchased ampoule is transferred to a test tube containing 5-6 ml of the cultural growth medium (see Table 2-3) recommended by ATCC bank and left in shaker for one week at 75ºC and 40 rpm shaking rate. Since half of the cultural growth medium has been evaporated on the fifth day, new breeding is performed by transferring 1 ml of the supernatant to 100 ml of the cultural growth medium and again left for growth at 75ºC and 40 rpm shaking rate. In addition, a new portion of 3-4 ml cultural growth medium is added to the left in the test tube supernatant.

Table 2-3 Cultural growth medium of *SS*

Compound	Amount (g/L)
Yeast Extract	1.00
KH_2PO_4	3.10
$(NH_4)_2SO_4$	2.50
$MgSO_4.7H_2O$	0.20
$CaCl_2.2H_2O$	0.25
$MnCl_2.4H_2O$	$1.8x10^{-3}$
$Na_2B_4O_7.10H_2O$	$4.5x10^{-3}$
$ZnSO_4.7H_2O$	$0.22x10^{-3}$
$CuCl_2.2H_2O$	$0.05x10^{-3}$
$Na_2MoO_4.2H_2O$	$0.03x10^{-3}$
$VOSO_4.2H_2O$	$0.03x10^{-3}$
$CoSO_4.7H_2O$	$0.01x10^{-3}$

pH adjusted to 4.0-4.2 with 10N H_2SO_4 at room temperature. Autoclaved at 121ºC for 15 minutes.

Although some of the experimental conditions for *SS* growth and coal biodesulphurization by *SS* are already optimized [14-15,21], various conditions to optimize *SS* growth are tested. The pH, $(NH_4)_2SO_4$ and glucose content in the growth medium, shaking rate, and temperature of the growth chamber are varied and evaluated. Final optimized growth conditions for *SS* are determined as 9 ml supernatant inoculum, 3.00 g/L $(NH_4)_2SO_4$ and 10 g/L glucose in the growth culture, pH=4, shaking rate 40 rpm, T=70ºC. Strictly under these optimal conditions lab-scale, shake-flask coal biodesulphurization experiments are carried out by adding 3 % (w/v) coal to the cultural growth medium.

2.3.4 Pseudomonas putida treatments: *PP*

Pseudomonas putida (*PP*) bacterium is also an effective microbial culture capable to decrease coal organic sulphur [22-25]. In the present study *PP* bacterial strain has been isolated by the group of Prof. Groudeva from the Department of General and Industrial Microbiology at the Faculty of Biology, Sofia University "St. Kliment Ochridski" from soils polluted with crude oil. The strain used in our experiments is re-inoculated from test tube containing the strain on slant agar. The re-inoculation is done in a sterile flask containing 100 ml Raymond nutrient medium (Rnm) (Table 2-4). The obtained suspension (cell+medium) is equally divided in two other flasks. To each of them 0.5 ml of oil are added. Incubation is carried out at 28ºC, pH=6.8-6.9 (at ambient temperature) in a shaker for 3 days. After this period, second inoculation and incubation is done by using 50 ml Rnm, 0.5 ml oil and 5 ml suspension from the first inoculation. After 3 day, third inoculation and incubation is made at the above described conditions. However, 5 ml suspension from the second inoculation is used. For coal biodesulphurization experiments the suspension comprises *PP* grown cells and Rnm from the third inoculation is utilized. Laboratory scale, shake-flask biodesulphurization experiments are carried out at pulp density 10% (w/v), inocolumn 10% (v/v), at 28^{o}C, pH=6.8-6.9 (at ambient temperature).

Table 2-4 Raymond nutrient medium

Compound	Amount (g/L)
NH_4Cl	2.00
$MgSO_4.7H_2O$	0.20
KH_2PO_4	2.00
Na_2HPO_4	3.00
$CaCl_2.6H_2O$	0.01
Na_2CO_3	0.10
$MnSO_4.5H_2O$	1ml/L 2% sollution
$FeSO_4.7H_2O$	1ml/L 2% sollution
NaCl	5.00

pH adjusted to 6.8-6.9; Autoclaved at 1atm for 15 minutes;

In order to determine the most appropriate duration of biodesulphurization experiment with *PP*, IN sample is biotreated with *PP* at different experimental durations at the above mentioned conditions. Along with tracking of S_t changes occurring as a result of *PP* biotreatments for different times, inoculation on plates is also conducted in order to determine the number of cells and to monitor growth kinetics of *PP*. For the purpose, the Consecutive dilutions method is applied [26-27]. Decimal dilutions are made, considering that dilutions to 10^{-8} are sufficient. In each recess (they are a total of 10) of the plate, 900 μL Rnm are added. For the first dilution 100 μL suspension of the studied sample is added to Rnm. For each subsequent dilution, 100 μL of the previous is added to Rnm. The growth of *PP* is checked after two weeks of cultivation. A positive result for bacterial growth in all made decimal dilutions is considered if opalescence is detected. The number of cells (CNo) can be determined by the table of Mac Credit depending on the number of the recess in which opalescence is observed. The results for *PP* bacterial growth and S_t changes as a function of biodesulphurization experiment duration are presented in Figure 2-3. Based on the obtained results it is decided to conduct all coal biotreatments with *PP* for 15 days.

2.4 Washing procedure

Biotreated coal samples are separated by filtration and then washed by diluted HCl acid and hot distilled water. Subsequently the coal samples are dried at

106°C and subjected to investigation. Bearing in mind the dry weight of the microorganism at the optimum growth conditions (≈ 0.05 g/l) and the amount of used sample in the experiment (30 g/l), biomass contamination in this procedure is unlikely.

Summarized information on investigated initial and treated samples can be found in Table 2-5.

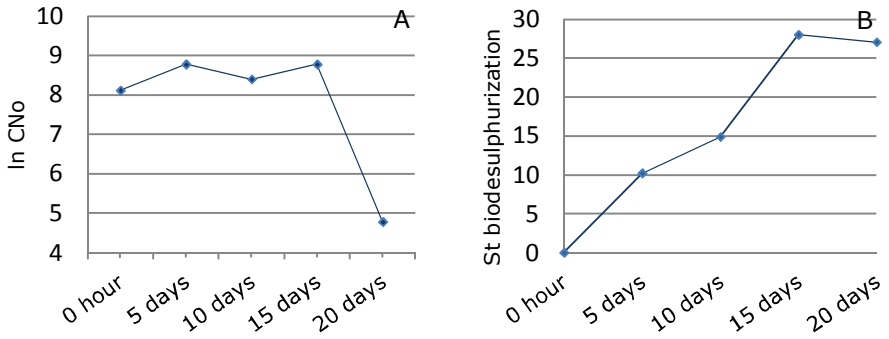

Figure 2-3 Bacterial growth (A) and S_t changes (B) as a function of experiment duration during biotreatment with PP

Table 2-5 Investigated initial and treated samples in the current research

	Chapter III	Chapter IV	Chapter V	Chapter VI
Initial sample	P	P, M, B	P, M, B	IN
Chemical treatments	none	demineralization depyritization	demineralization depyritization	demineralization depyritization oxidation
Chemically treated samples	none	P-APF M-APF B-APF	P-APF M-APF B-APF	IN-oxy AF AF-oxy APF APF-oxy
Applied microorganisms	TV,PC, MC	PC, SS	PC, SS	PP
Biodesulphurized samples	TV TV-1 PC MC MC+BS	P-APF-PC P-APF-SS M-APF-PC M-APF-SS B-APF-PC B-APF-SS	P-APF-PC P-APF-SS M-APF-PC M-APF-SS B-APF-PC B-APF-SS	IN-PP IN-oxy-PP AF-PP AF-oxy-PP APF-PP APF-oxy-PP

2.5 Analyses

2.5.1 Sulphur forms analyses

The ASTM methods for determining sulphur in coal are D3177 and D4239 for measuring S_t and D2492 for determining the quantities of sulphur forms, i.e S_s, S_p, S_o. In these methods, S_t and S_s are determined directly, while S_p is determined by measuring the quantity of pyritic iron present. Organic sulphur is determined by subtracting the quantity of S_s and S_p from S_t. For sulphur forms determination ASTM D3177 and D2492 are used. Since there is no standard analytical procedure for direct measurement of the S_o and latter is calculated indirectly, cumulative errors in determining S_t, S_s and S_p are reflected in the values of S_o. Moreover if S_{el} is present in coal, it will be included into the so called S_o. Therefore, it is of utmost importance to determine S_{el} as well. A procedure for S_{el} is developed in the current study. It is described in details in Chapter 5.

2.5.2 Proximate analysis – Moisture (W), Volatile matter (VM), Fixed carbon (C_{fix}) and Ash (A) content

The coal samples are subjected to thermogravimetrical analysis (TGA) for W, VM, C_{fix} and Ash content determination. DuPont instrument 951 thermogravimetrical analyser is used. The following TG programme is applied [28]: i) heating with 20ºC/min up to 600ºC under nitrogen atmosphere; ii) at 600ºC isothermal hold of the temperature for 10 min; iii) after 5 min of isothermal period, the atmosphere is switched to oxygen; iv) heating with 20ºC/min up to 800ºC under oxygen atmosphere. From the TG curves the following information can be determined: i) moisture content, from ambient temperature up to 150ºC; ii) volatile matter content, from 150ºC up to 600ºC; iii) combustion of fixed carbon in oxygen atmosphere at >600ºC; iv) ash content is the residue at 800ºC. The obtained values are on air dried base.

2.5.3 Elemental analysis – C, H, N, S and O contents

Elemental analysis is performed with a Thermo Electron Flash EA1113 elemental analyzer. By one measurement C, H, N and S can be determined. For the

purpose, the instrument is calibrated using BBOT (2,5 bis (5-tert-butyl-benzoxazol-2-yl) thiophene with the formula $C_{26}H_{26}N_2O_2S$) provided from Thermo Electron corporation. A control sample is periodically analyzed. All samples are analyzed at least in duplicate. The oxygen content is calculated by difference as: oxygen% = 100% - (C + H + N + S + ash).

2.5.4 Energy content – HHV determination

Higher heating value (HHV) is calculated by the formula of Channiwala [29]. The proposed by Channiwala unified correlation comprises computation of HHV from the elemental analysis. The following correlation turned out to be the best in this manner with an average absolute error of 1.45% and bias error of 0.00% with respect to measured values of HHV:

$HHV^{db}(MJ/kg)=0.3491*C+1.1783*H+0.1005*S-0.1034*O-0.0151*N-0.0211*Ash$

The validity of this correlation has been established for fuels having a wide range of elemental composition, i.e. C – 0.00-92.25%, H – 0.43-25.15%, O – 0.00-50.00%, N – 0.00-5.60%, S – 0.00-94.08 and Ash – 0.00-71.4% and HHV – 4.745-55.345 MJ/kg expressed in mass percentages on dry basis.

2.5.5 AP-TPR analyses

The development of new tactics for sulphur compounds removal from coals depends, in part, upon the knowledge of their chemical compositions. A fundamental requirement of any research into desulphurization is an accurate method for assessment the various forms and species of sulphur in coal. Temperature programmed reduction at atmospheric pressure (AP-TPR) and its variation in detection mode has been successfully used for the specification of sulphur functionality species in coal [4,6-7,12,30-49]. The sulphur distribution revealed by AP-TPR is based on the fact that each type of sulphur group under constant heating rate in a reducing (H_2) or an inert (He) gas flow has a characteristic temperature region in which it is reduced/hydrogenated into H_2S. The assignment of the temperature region, in which a certain sulphur functional group is reduced/hydrogenated, is based on the pyrolysis of model compounds [31,33,48,50]. The distribution of sulphur functional groups in coal can be deduced by H_2S evolution as a function of the temperature. The H_2S evolved

during pyrolysis can be detected by potentiometry (AP-TPR-pot) [6,12,31-32,35,37-39,42,44,47,51]. This detection offers qualitative specification of sulphur functionalities in coals and provides information for the reduction/hydrogenation efficiency of coal sulphur distribution into H_2S. Despite of the high reduction/hydrogenation efficiency of total sulphur into H_2S achieved for the most studied coals (70-100%) by AP-TPR-pot [4,6,12,31,37-38], in some cases reduction/hydrogenation efficiency of sulphur into H_2S can be rather low. This is especially in the cases: i) coal samples that contain certain minerals (mainly calcium compounds) as calcium minerals capture the gaseous H_2S, formed under AP-TPR conditions, and produce CaS [12,32,37]; ii) coals that have been subjected to different oxidizing treatments as this can result in formation of oxidized sulphur functionalities, which are not entirely reduced into H_2S but rather evolved as SO/SO_2 [6,12,32,51]. Other explanations for the incomplete reduction of sulphur functionalities might be the formation of reduction/hydrogenation resistant sulphur containing aromatic species which remain in the tar/char fraction and/or the pyrolytic release of volatile organic sulphur compounds neither reduced in the AP-TPR experimental condition into H_2S nor captured into the tar/char fractions [6-7,35,39,47]. In order to obtain more detailed information on total sulphur distribution during TPR pyrolysis and hence more reliable information on sulphur functionalities in coal, the TPR technique is further developed. Variants with mass spectrometric (MS) and gas chromatography/mass spectrometric (GC/MS) detection systems are offered [4,6-7,35,39,47,52]. AP-TPR "on-line" interfaced to mass spectrometer (AP-TPR-MS) enables the monitoring not only of H_2S but also of SO/SO_2 (released during thermal decomposition of oxidized sulphur functionalities) and volatile organic compounds like hydrocarbons and aromatic compounds. Volatile organic sulphur compounds (S_{vol}), neither reduced in the AP-TPR experimental condition into H_2S nor captured into the tar/char fractions, can be quantitatively and qualitatively assessed by AP-TPR "off-line" coupled with TD-GC/MS (AP-TPR-TD-GC/MS). Qualitative and quantitative evaluation of the sulphur incorporated into tar/char residue can be achieved by AP-TPO-MS (pyrolysis in pure O_2) experiments and by oxygen bomb combustion experiments followed by ion chromatography [6-7,35,39,47].

2.5.5.1 AP-TPR-pot

The quantification of reduced/hydrogenated sulphur species in hydrogen atmosphere during AP-TPR experiments is done by AP-TPR-pot. These analyses are performed by using 40 mg of sample and 25 mg of fumed silica placed in a reactor. 100 ml/min flow of pure H_2 is used. A linear temperature program of 5°C min^{-1} from ambient temperature up to 1025°C is applied. The released H_2S during AP-TPR pyrolysis is collected in an aqueous solution of sulphides antioxidant buffer (SAOB) that prevents the oxidation of S^{2-} to SO_2. One litre of this solution is prepared by 80 g NaOH (Merck), 36 g ascorbic acid (ACROS) and 85 g EDTA (ACROS).

For potentiometric system calibration, a standard solution with sulphide concentration of about 10^{-3}M is used. It is prepared by dissolving around 0.095 g Na_2S (Fluka) in 250 ml of SAOB solution. The accurate concentration is established by titration with $Pb(NO_3)_2$ (Janssen Chimica). The formation of PbS (black solid) is perceptible and the change of free S^{2-} is detected by potentiometric measurement. A set of working sulphide solutions are prepared within $1x10^{-3}$ and $1x10^{-7}$ M, by serial dilutions of the previous sulphide standard solution.

The calibration of Silver/Sulphide Electrode should be made frequently to be sure that no alteration happened in the electrode. The calibration is performed with the previously described sulphide standard solutions. According to the Nernst (Equation 1), the potential recorded is linear to the logarithmic concentration of the concerned anion and presents a negative slope.

$$E = E^0 + RT/2F \times \ln C_{S2-}$$

Equation 1. Nernst-Nicolski equation. E: experimental potential value; R: perfect gases constant, 8.3144 J K^{-1} mol^{-1}; T: temperature, K; F: Faraday constant, $9.6484x10^4$ C mol^{-1}; C_{S2-}: sulphide concentration, M; E^0: the experimental value corresponding to the intercept of the equation of calibration, mV.

The H_2S quantification is established by means of sulphide concentration present in solution, which is dependent on its experimental potential value as well as the previous calibration curve.

$$C_{S2-}^{*} = \text{Exp}\ ((E^* - E^0)/\ (RT/2F))$$

Equation 2. The estimation of a sulphide experimental concentration (C_{S2-}^{*}). E*: recorded experimental potential, mV; E^0: intercept of the calibration curve; RT/2F: slope of the calibration curve.

Sulphur recovery (R_{pot}) detected by the potentiometric system can be calculated using the Equation 3:

$$\%\ \text{Sulphur recovery} = \{[(m_s/M_{coal})\ x\ 100]/S_t\}\ x\ 100$$

Equation 3. Calculation of sulphur recovery. m_s: the mass of sulphur hydrogenated/reduced during the AP-TPR experiment; M_{coal}: the mass of the used coal (~40 mg); S_t: the total amount of sulphur on analytical basis in the analyzed sample.

More detailed information on experimental procedure can be found elsewhere [31,53].

2.5.5.2 AP-TPR-MS

AP-TPR coupled "on-line" with mass spectrometry (AP-TPR-MS) in different gas flows (H_2 or He) is used to specify organic sulphur functionalities in coal and to assess the changes after biotreatments. These analyses are performed in the AP-TPR set-up described in Figure 2-4. Applied pyrolysis conditions are the same as for AP-TPR-pot. However, TPR reactor is coupled "on-line" with a mass spectrometer (*FISONS-VG Thermolab MS*) through a capillary heated at 135°C. The mass spectrometer equipped with a quadrupole analyzer is set at an ionizing voltage of 70 eV. The MS signals of ions with *m/z* 10 ÷ 160, are "on-line" monitored.

2.5.5.3 AP-TPR-TD-GC/MS

The above described AP-TPR system can also be used in adsorption/desorption mode in order to study S_{vol}, neither reduced in the AP-TPR condition nor captured in the tar/char fraction. The outlet of the AP-TPR reactor is connected

to a set of ice-cooled tubes containing Tenax (Sigma-Aldrich), a porous polymer of 2,6-diphenyl-p-phenylene oxide as adsorbent. The volatiles are collected in a temperature range from 300°C up to 700°C (temperature range is determined by the results of AP-TPR-MS experiments) in discrete temperature intervals (mostly of 50°C). The Tenax tubes are 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 x 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 0.5s. Each Tenax tube is spiked with 0.5 µg d_6-benzene in methanol solution to quantify results for the target sulphur species.

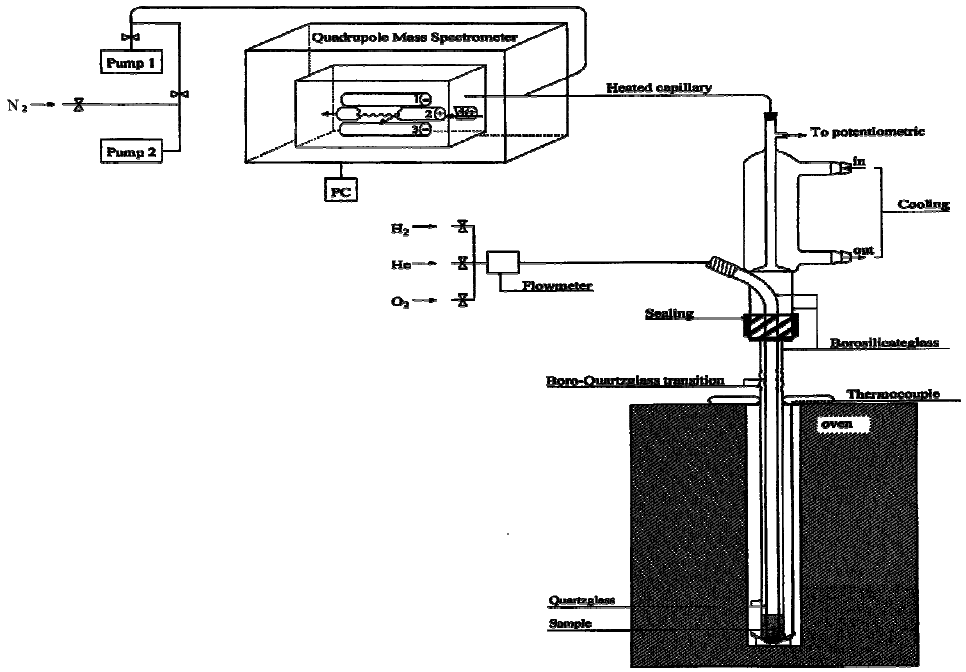

Figure 2-4 Experimental set-up of the AP-TPR-MS apparatus [33]

In order to improve qualitative/quantitative interpretation of S_{vol} released during pyrolysis experiment, the AP-TPR-TD-GC/MS technique is upgraded by replacing

the Tenax tubes by tubes filled with sulphur compounds selective sorbents, and by the use of deuterated sulphur containing organic compound as internal standard. In this case, the outlet of AP-TPR reactor is connected to ice-cooled metal adsorption tubes with SilcoSteel Coating, filled with Tenax/Carbopack B/Carbosieve SIII (Markes) as adsorbents. The volatiles are trapped in the temperature range from 200°C up to 950°C in temperature intervals of 75°C. The adsorption tubes are desorbed systematically and analyzed by a TD-GC/MS. TD-GC/MS apparatus is used with He as carrier gas at 85 kPa at the following conditions:

a) Unity thermal desorber (Marks): primary desorption 20 min at 320°C; Cold trap at - 8ºC, heated at maximum heating rate up to 320ºC, hold time 15 min; flow path temperature 200ºC;

b) Trace GC Ultra-Gas chromatography (Thermo Instruments): capillary column 30 meters ZB 5-MS 0.25 mm x 0.25 µ Phenomenex; temperature program – 3 min at 30ºC, heated 8ºC/min to 100ºC, heated 12ºC/min to 310ºC, hold time 5 min;

c) DSQ-Mass spectrometer (Thermo Instruments): EI spectra; Ionization energy – 70 eV; Scan range – *m/z* 33-480 in 0.4s. Each tube is spiked with 3 µg d_4-thiophene to quantify results for the target sulphur species. NIST library spectra are used for peak identification with special interest to the different sulphur species, liberated or *in situ* formed during the AP-TPR pyrolysis. The formed H_2S gas is not adsorbed by the Tenax tubes, nor by the sulphur selective adsorption tubes.

2.5.5.4 Oxygen bomb combustion followed by Ion Chromatography

The total sulphur content of both tar and char fractions can be determined by using oxygen bomb combustion technique. During each AP-TPR experiment, tar and char fractions are deposited on the reactor walls. They are collected by washing with methylene chloride. Obtained solutions are transferred to the quartz crucible, dried at ambient temperature and later on at 60^{o}C in an oven. The recovered organic material is obtained in a very low quantity. To perform oxygen bomb combustion experiments, sulphur free mineral oil is added to the dried sample. Produced sulphates, after combustion, are transferred quantitatively in volumetric flask by DI water. Sulphur amount (as sulphates) is

determined by ion chromatography instrument Dionex (DX-120), supplied with column (AS4A-SC) and conducting detector on the base of the calibration curve prepared for external standard solutions of SO_4^{2-}. The standards used for the constructing of the calibration curve, are prepared by dilutions of the stock solution of SO_4^{2-} (1000 ppm). The standards are varying in the concentration range of 0.5 to 10 mg L^{-1}. The obtained calibration curve represents the best linear fit to the data. A correlation coefficient of 0.9999 is obtained by the method of least squares. Detection limit of SO_4^{2-} by Ion Chromatography is 0.1 mg L^{-1}.

2.5.6 FTIR

Some of the investigated samples are analyzed by Fourier Transform Infrared Spectrometry (FTIR) [54-55]. Air dried samples are milled (≤ 100 mesh) and dried at 105°C for 2 h. Prior to analysis the samples are additionally ground in an agate mortar for a quarter of hour. Dry KBr is added to the samples and further grinding for half an hour is performed. Coal:KBr (1:100) is used for the pellet preparation.

Infrared spectra of the samples are recorded with FTIR spectrometer (Tenzor-27, Bruker Instrument) by co-adding 250 scans in the range of 400-4000 cm^{-1} at resolution 2 cm^{-1}. Pure KBr pellet is used to obtain a reference spectrum. Semi-quantitative analysis based on the calculation of the ratios between the integrated area of each characteristic band and the integrated area of C=C stretching vibrations at 1600 cm^{-1} (used as a reference) is performed. The absorption of C=C aromatic bonds (representing aromatic structure) is considered as the most stable, respectively most characteristic, for these type of materials. For comparative studies, the applied approach is reliable since features as sample concentration and thickness of the pellets does not affect significantly semi-quantitative interpretation. Thus, comparing the ratios between the integrated area of each characteristic band and the integrated area of C=C stretching vibrations, a semi-quantitative information for functional groups distribution toward aromatic structure can be obtained.

2.5.7 Bitumen extraction and chromatographic separation

As a result of washing procedure on APF-oxy-PP sample, a humus like byproduct (HL) is formed. Methylene chloride soluble portion (bitumen) of HL byproduct is prepared by extraction (1:10 g/ml) at ambient temperature and stirring for 6h. Lipid components (soluble in n-hexane) are obtained after asphaltene precipitation in *n*-hexane (1:100, v/v) and further fractionated on silicagel mini-column. Organic solvents with increasing polarity are used: *n*-hexane – for aliphatic fraction; benzene/dichloromethane - for aromatic fraction and acetone for the nitrogen/sulphur/oxygen (NSO) containing polars. Fractions are studied by gas chromatography-mass spectrometry (GC/MS).

2.5.8 GC/MS

GC/MS study on neutral oil fraction derived from bitumen of HL is performed on HP 6890 GC system plus HP 59763 MS detector operated in electron impact (EI) mode with ionization energy 70 eV. Scan range is from 45 to 750 Da. Capillary column HP-5MS (30 m x 0.25 mm, i.d. x 2.25 μm film thickness) is used with helium as carrier gas and split ratio 10:1. The data are acquired and processed with the HP-Chemstation software.

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Chapter 3 Biodesulphurization of subbituminous coal by different fungi and bacteria

The results discussed in this chapter have been published in a peer-reviewed paper:
Gonsalvesh L, Marinov SP, Stefanova M, Yürüm Y, Dumanli AG, Dinler-Doganay G, Kolankaya N, Sam M, Carleer R, Reggers G, Yperman J. Biodesulphurized subbituminous coal by different fungi and bacteria studied by reductive pyrolysis. Part 1: Initial coal. Fuel 2008; 87:2533-43.

3.1 Introduction

To fulfil the environmental requirement, scientists begin to work intensively in developing effective biodesulphurization methods or upgrading the already existing ones in order to improve coal desulphurization performance. Although much of the work on organic sulphur removal from coal has been attempted with various bacteria [1-8], insufficient work has been carried out with fungi [9-10]. In both cases of coal biodesulphurization, i.e. by bacteria and fungi, information about organic sulphur forms transformation as a result of applied biotreatments is missing. This information is essential since with its help the biodesulphurization of different coal type by various microorganisms can be optimized and better planed.

This chapter describes the biodesulphurization of subbituminous coal by different fungi and bacteria. The aim of this investigation is to assess the changes in sulphur forms and functionalities, i.e. organic and inorganic, that occur in Bulgarian high sulphur subbituminous coal as a result of microbial treatments with bacteria and fungi. The results of this assessment may lead in better understanding of coal biodesulphurization.

3.2 Experimental Section

3.2.1 Coal

Pirin subbituminous coal (P) is subjected to biotreatment with bacteria and fungi. The sample is selected in view of its high organic sulphur content (Table 3-1).

3.2.2 Biodesulphurization

The following microbial experiments for coal desulphurization are performed:

TV-1, *TV-2*, *PC*, *MC* and *MC+BS*.

Additional information about the microorganisms, medium composition and biodesulphurization conditions can be found in Chapter 2.

Table 3-1 Characteristics of initial and biodesulphurized Pirin coal samples

Sample	S content (%)[db]				Proximate analysis (%)[ad]				Caloric value[a]
	S_t	S_p	S_s	S_o	Ash	VM	W	C_{fix}	($MJ.kg^{-1}$)
Initial	4.88	0.51	0.48	3.89	10.4	31.0	6.5	52.2	22.7
TV-1	3.64	0.13	0.12	3.39	8.0	28.7	8.8	54.6	22.3
TV-2	3.94	0.10	0.11	3.73	6.4	29.7	8.7	55.2	22.4
MC	3.87	0.09	0.12	3.66	7.3	29.4	8.1	55.2	22.5
MC+BS	3.63	0.09	0.11	3.43	7.5	30.0	7.4	55.2	22.2
PC	3.66	0.11	0.16	3.39	7.4	30.9	5.8	55.9	22.4

[db]dry basis;[ad]air dried;
[a]calculated on dry basis by the formula of Channiwala [11];
W - moisture; VM - volatile matter; C_{fix} - fixed carbon.

3.2.3 AP-TPR analysis

The identification of the volatile species, i.e H_2S, SO/SO_2 and S_{vol} released during the reductive and inert pyrolysis is done by AP-TPR/pot, AP-TPR-MS and AP-TPR-TD-GC/MS. Quantitative assessment of the released H_2S is done by using AP-TPR/pot analysis, while the quantitative assessment of S_{vol} is done by AP-TPR-TD-GC/MS analysis using adsorption tubes filled with Tenax. In order to improve sulphur balance, sulphur captured in tar/char residue left after TPR

pyrolysis is also determined. The last is done by oxygen bomb combustion followed by Ion Chromatography detection. All of the used analytical techniques for sulphur distribution assessment are described in details in Chapter 2.

3.3 Results and discussion

To select the proper microbial cultures with high desulphurizing effect towards S_t and S_o, different types of microorganisms are applied (Table 3-2). The biodesulphurization by *Trametes versicolor* fungus is held over at two different temperatures. Temperature selection is made considering the fact that 30°C is the optimal temperature of fungi growth (*TV-2*), while 37°C is temperature for the highest "*Laccase Enzyme*" activity (*TV-1*). The results demonstrated that when the temperature is 37°C, a higher level of desulphurization is achieved. For this reason and because the same microorganism is used in both cases, i.e. *TV-1* and *TV-2* systems, the sulphur changes only in *TV-1* system are examined. Concerning *MC* and *MC+BS*, the higher desulphurization effect is achieved for *MC+BS* experiment. Therefore the sulphur changes only in the latter are evaluated. There are investigations on sulphur functionalities changes as a result of biodesulphurization by *PC* as well.

Table 3-2 Biodesulphurization effects on S_t and S_o of Pirin coal, in %

Sample	S_t^{db}	ΔS_t	S_o^{db}	ΔS_o
Initial coal	4.88	-	3.89	-
"*Trametes Versicolor*", (TV-1)	3.64	25.4	3.39	12.8
"*Trametes Versicolor"*, (TV-2)	3.92	19.7	3.73	4.1
"*Mixed Culture"*, (MC)	3.87	20.7	3.66	5.9
"*Mixed Culture+BS* ", (MC+BS)	3.63	25.6	3.43	11.8
"*Phanerochaeta Chrysosporium*", (PC)	3.66	25.0	3.39	12.8

It is found that implemented biotreatments demonstrate maximum S_t and S_o desulphurizations about 26% and 13%, respectively, for the following microbial systems: *TV-1*, *MC+BS* and *PC*. Sulphur changes in solid products provoked by the biotreatments with these microorganisms are investigated in details. In Table 3-1, data for proximate and wet sulphur analyses are listed. It is obvious that for "deeply" investigated biodesulphurized coals a reduction of the ash

content is found, i.e from 10.4% to 7.4%. This observation correlates in part with a decrease in S_p and S_s concentrations in the initial sample (calculated as a part of S_t 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 [11].

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

The H_2S kinetograms of AP-TPR-MS of initial and biotreated coal samples are visualized in Figure 3-1. Because *m/z* 34 (H_2S^+) and *m/z* 33 (HS^+) exhibit the same evolution, only *m/z* 34 ion profiles of investigated samples are demonstrated. Potentiometric kinetograms are very similar to the H_2S^+ MS profiles and therefore are not shown. They are used only for quantitative calculations. There are always two dominant peaks with T_{max} about 400-420°C and about 650°C in AP-TPR-MS (H_2) H_2S^+ profiles of studied samples. The peak at about 400-420°C can be assigned to the presence of di-alkyl, alkyl-aryl and reactive di-aryl sulphides. No unequivocal indications for the presence of thiols or disulphides are obtained according to these profiles, neither in initial nor in treated coals. Peak assignments in H_2S kinetograms are based on the model compound approach and also on AP-TPR-MS profiles of typical aliphatic and aromatic CH-fragments as shown in Figure 3-2 for the initial coal sample as an example for all studied samples. Indeed, for the three treated samples, the same profiles of the above mentioned 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. The typical aliphatic fragments, i.e. unsaturated/saturated CH-fragments (alkenes/alkanes) comprise *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^+ profile (Figure 3-1) refers to the presence of less reactive di-aryl sulphides and more complex thiophenic structures. Also this is based on model compound studies and on the evolution of typical aromatic CH-fragment ions in that temperature range. Figure 3-2B 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 ($C_7H_8^+$) and *m/z* 91 ($C_7H_7^+$); xylene – m/z 91 ($C_7H_7^+$) and *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 biotreated samples almost the same profiles are found in shape, sequence and maximum evolution temperature. Generally, it might be concluded that the biotreatments do not affect significantly the coal matrix.

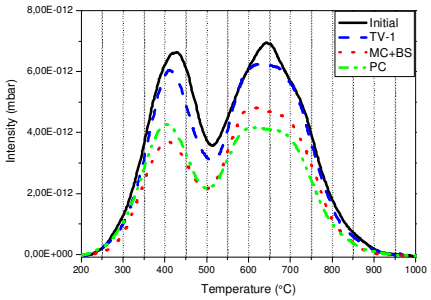

Figure 3-1 AP-TPR-MS (H_2), *m/z* 34 kinetograms of Pirin coal samples: Initial, *TV-1*, *MC+BS* and *PC*

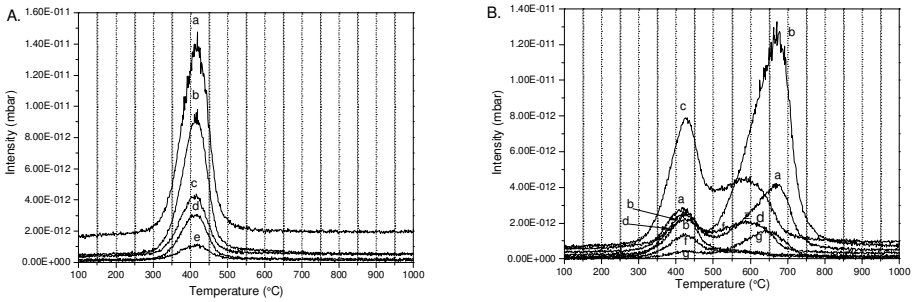

Figure 3-2 AP-TPR/MS (H_2) evolution profiles of initial Pirin coal: A. for saturated and unsaturated CH-chains (alkanes and alkenes); B. for aromatic compounds; Saturated and unsaturated CH-chains: (a) *m/z* = 55 ($C_4H_7^+$); (b) *m/z* = 57 ($C_4H_9^+$); (c) *m/z* = 69 ($C_5H_9^+$); (d) *m/z* = 71 ($C_5H_{11}^+$); (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 ($C_{10}H_8^+$).

For the initial coal compared to the treated ones, the second H_2S^+ evolution peak has a much sharper shape with a well expressed peak maximum. For the treated ones, the shape is more flat and thus may refers to a removal of complex thiophenic sulphur compounds and less reactive di-aryl sulphides. Further the AP-TPR H_2S^+ profiles of all treated samples exhibit almost the same trend, but small differences in shape and height can be pointed out. The presence and bioremoval (- 40%) of pyrite is not detected in the profiles of studied samples (Figure 3-1), first of all mainly due to its low presence in the initial sample. For the *MC+BS* treated sample the first H_2S^+ peak is definitively much lower than the second one, evoking most probably that aliphatic and mixed sulphur compounds are removed to a greater extent than aromatic ones. Other explanation is that due to applied treatment with *MC+BS* aromatic sulphur compounds are altered and thus become more susceptible to reduction/hydrogenation. Obviously, this is not the case for *TV-1* and *PC* treated samples.

Figure 3-3 partly visualizes the evolution of oxidized sulphur functionalities during the AP-TPR experiments in hydrogen atmosphere for initial and biotreated samples. In Figure 3-3A 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 Figure 3-3B both *m/z* profiles are given for the treated samples (for more precise presentation). There is a huge first peak at 260°C for the initial coal sample (Figure 3-3A), which certainly refers to organic sulphonic group decomposition [12]. This signal is almost absent for the treated samples (Figure 3-3B), indicating that for all treated samples this functional group has disappeared and thus has been removed due to applied biotreatments. The large shoulder at 350-400°C for initial coal refers not only to a possible presence of alkyl-aryl sulphoxides but also to CH-fragments, because here *m/z* 64 and *m/z* 48 clearly exhibit different trends. For *MC+BS* treated sample, the difference in the trends of *m/z* 48 and 64 profiles (besides also the small temperature difference) is more evident than in the case of the initial coal sample. For *PC* treated sample the difference in the trends of both profiles is limited. The lower temperature maximum of *m/z* 48 profile compared to *m/z* 64 profile is noticeable for *TV-1* treated sample. All this can be explained as a different

impact of the three treatments on the coal matrix, which reflected in the amounts and in the kind of sulphoxides presence.

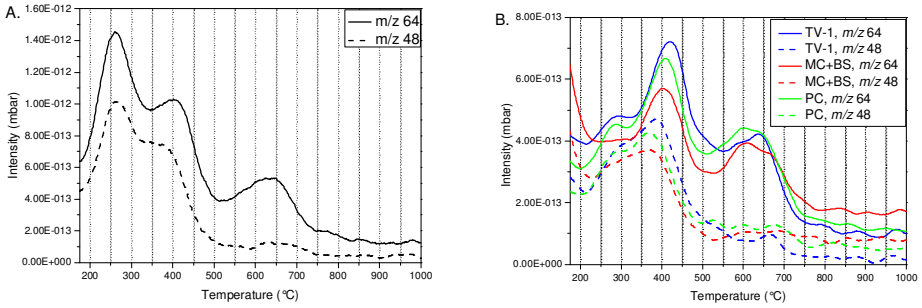

Figure 3-3 AP-TPR/MS (H_2), *m/z* 48 and 64 kinetograms of Pirin coal samples: A. Initial; B. *TV-1*, *MC+BS* and *PC* treated

The less intense second maximum at around 600-650°C in the *m/z* 64 profile of the investigated samples refers rather to CH-fragments and not to the presence of sulphoxides and/or sulphones since *m/z* 48 and 64 clearly exhibit different trends. It can also be seen that the evolution trends of *m/*z 48 and 64 profiles of initial compared to biotreated samples are different. In addition, comparing *m/*z 48 and 64 profiles of biotreated samples to each other some differences in the evolution trends can be depicted. This again could evoke for the different actions of the three studied biotreatments on the coal matrix and/or for the possible presences of different oxidized sulphur functionalities. We should highlight the fact that the registered intensities for *m/z* 48 and 64 profiles are very weak and that the conclusions formulated using Figure 3-3 are perhaps too detailed at this state. 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, additional detection systems are needed to get more detailed information about oxidized sulphur species presence and their transformation as a result of any treatments.

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

In Figure 3-4 AP-TPR/MS evolution profiles of *m/z* 34 in He atmosphere are shown. Now only one broad peak maxima is observed, around 400°C, demonstrating rather the importance of the use of a 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. Obviously biodesulphurization has an effect on the organic coal matrix. The effect most likely is expressed as enhanced coal hydrogen content, which results in a better hydrogenation/reduction in an inert atmosphere of organic sulphur groups still present in the treated samples.

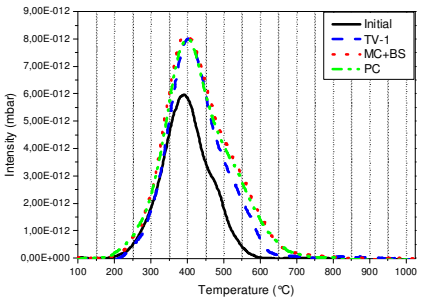

Figure 3-4 AP-TPR/MS (He), *m/z* 34 kinetograms of Pirin coal samples: Initial, *TV-1*, *MC+BS* and *PC*

Additionally, it is believed that in He atmosphere even more information can be obtained for the oxidized sulphur species. In Figure 3-5, AP-TPR/MS kinetograms in He medium for 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 Figure 3-5A and B [AP-TPR/MS (He)] with Figure 3-3A and B [AP-TPR/MS (H_2)] for the initial and the three biotreated 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 (Figure 3-5A) the first peak maximum can still be assigned to organic sulphonic groups decomposition. A new T_{max} appears at 460°C and refers to the reduction of iron sulphate [13]. This maximum completely disappears in a reducing atmosphere (or is not enough developed to be detected) due to SO_2 partly reduction to H_2S for all samples and additionally because of the lower sulphate amounts in the treated samples (Table 3-1). The lower plateau between the two peaks refers to the presence of alkyl-aryl sulphoxides. The shoulder at higher temperature about 580°C on the second maximum (Figure 3-5A) of the initial samples refers rather to diaryl sulphoxides/sulphones. The broad signal in Figure 3-5B with a maximum around 400°C refers to alkyl-aryl sulphoxides again and supports the peak identification that has been already formulated for Figure 3-3B. For *TV-1* the second maximum at 625°C, although with a low intensity, can be attributed to the presence of diaryl sulphoxides/sulphones. It is known that biodesulfurization with white rot fungi can occur via oxidation of dibenzyl sulphide to dibenzyl sulphoxides and dibenzyl sulphones [14]. From the profiles for oxidized sulphur species of biotreated sample (Figure 3-5B) it can be stated again that organic sulphonic compounds are removed due to the applied biotreatments.

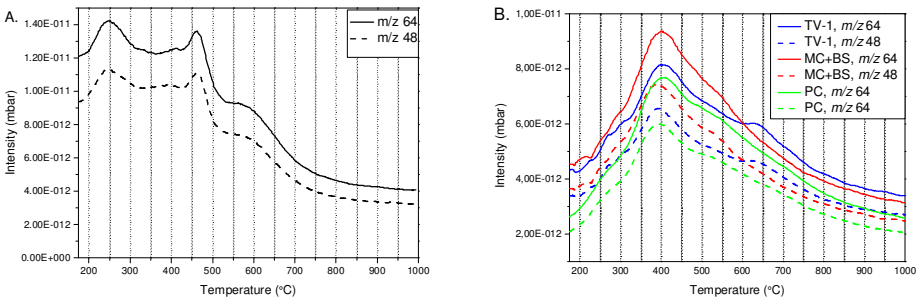

Figure 3-5 AP-TPR/MS (He), *m/z* 48 and 64 kinetograms of Pirin coal samples: A. Initial; B. *TV-1*, *MC+BS* and *PC*

Figure 3-6A and B demonstrate TPR-MS profiles in He atmosphere of typical aliphatic and aromatic CH-fragments for the initial coal sample, respectively. These figures could be considered again as an example for all studied samples.

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 Figure 3-4 only one dominated peak is found for the H_2S evolution for all studied samples. Respectively, less informative H_2S^+ profiles in He atmosphere are obtained demonstrating rather the decomposition reaction of the coal, rather than the decomposition and hydrogenation/reduction reaction as observed in H_2 atmosphere. Nevertheless by the combination of both, i.e. results of the experiments conducted in H_2 and in He atmospheres, supplementary information can be deducted. The less resolved shoulder in Figure 3-4 at the higher temperature range refers (although poorly) to the presence of more complex thiophenic structures.

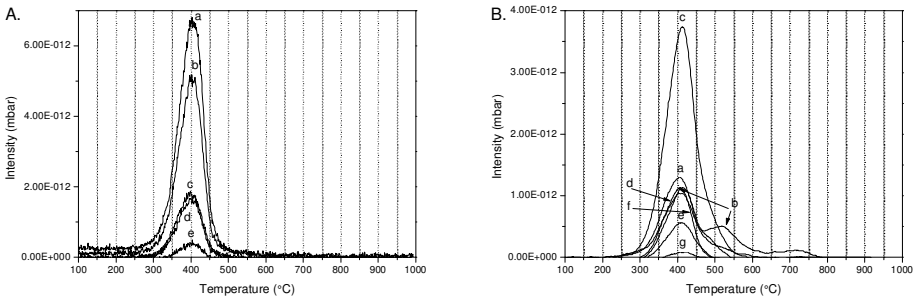

Figure 3-6 -TPR/MS(He) evolution profiles of sample Pirin – Initial: A. for saturated and unsaturated CH-chains (alkanes and alkenes); B. for aromatic compounds; Saturated and unsaturated CH-chains: (a) *m/z* = 55 ($C_4H_7^+$); (b) *m/z* = 57 ($C_4H_9^+$); (c) *m/z* = 69 ($C_5H_9^+$); (d) *m/z* = 71 ($C_5H_{11}^+$); (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 ($C_{10}H_8^+$).

3.3.3 AP-TPR "off-line" TD-GC/MS experiments

Volatile organic sulphur compounds (S_{vol}) released during pyrolysis are quantitatively and qualitatively determined by AP-TPR "off-line" TD-GC/MS experiments in hydrogen atmosphere. For this purpose tubes filled with Tenax are applied. The pyrograms of the samples under investigation are dominated by

typical products of coal pyrolysis, i.e. alkenes/alkanes (nC$_6$-nC$_{13}$), alkylbenzenes (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 are accompanied by their sulphur containing analogues. Organic sulphur species are determined by "off-line" TD-GC-MS technique applying single ion monitoring (SIM):

- m/z 94 for aliphatic sulphides, i.e. dimethyldisulphide (Me-SS-Me) and dimethyl sulphone (diMeSO2);
- m/z 87 +14n, where n is the number of alkyl groups for thiophenes (Th), methyl- (Me-Th), dimethyl- (diMeTh) and trimethyl thiophenes (triMeTh);
- m/z 134 +14n for benzothiophene (BzTh) and methylbenzothiophene (MeBzTh).

GC-MS spectra are quantitatively interpreted by spiking with 0.5 µg d_6-benzene. Typical curves of organic sulphur compounds distributions are illustrated in Figure 3-7, where the profiles are expressed in sulphur compounds *vs*. trapping temperature. Besides the above mentioned sulphur compounds their higher homologues are also detected: like tetra/penta substituted thiophenes and di/three substituted benzothiophenes. Due to their very small amounts, the large numbers of isomers and the strong overlapping signals, the concentrations are not calculated.

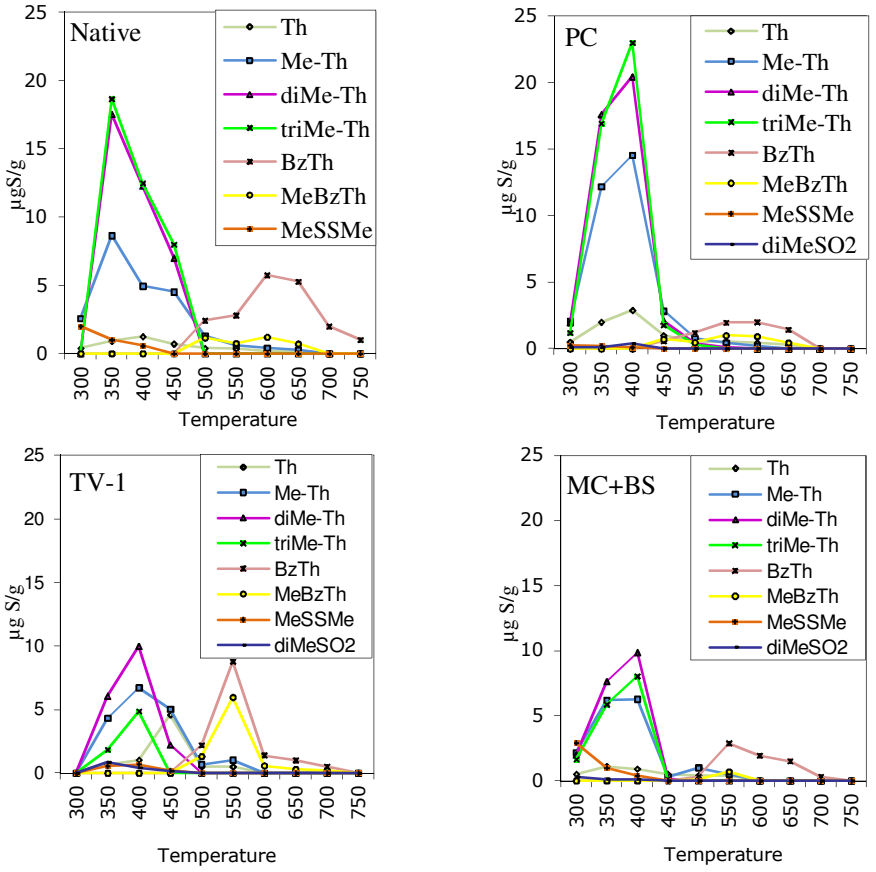

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Figure 3-7 Evolution profiles of determined sulphur compounds by AP-TPR "off-line" GC/MS. (Abbreviations: Th – thiophene; Me-Th – methyl thiophene; diMe-Th – dimethyl thiophene; triMe-Th – three methyl thiophene; BzTh – benzothiophene; MeBzTh – methyl benzothiophene; MeSSMe – dimethyl disulphide; diMeS0$_2$ – dimethyl sulphone.

Based on the above GC-MS quantifications, total content of sulphur compounds under consideration, trapped in the Tenax tubes, are expressed in µg S/g coal (Figure 3-8, Table 3-3).

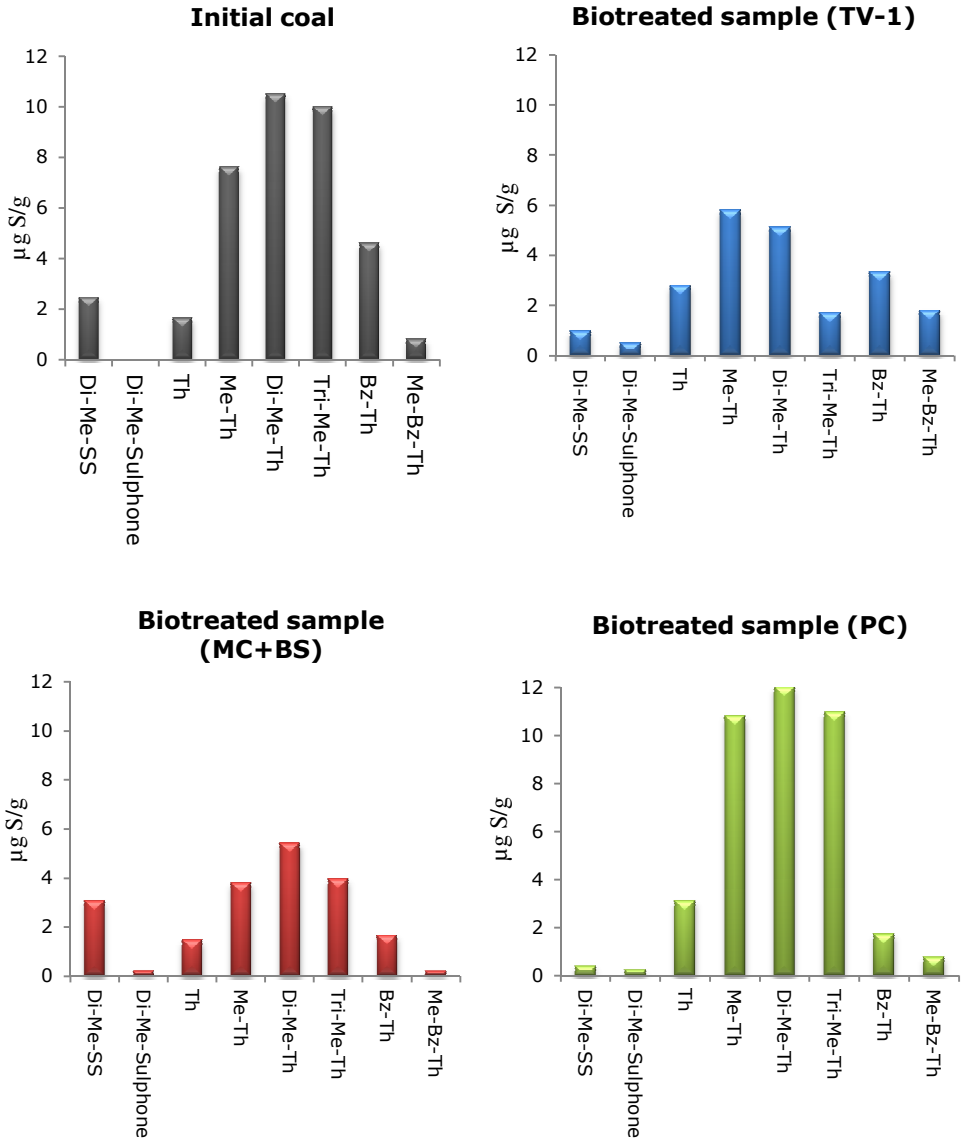

Figure 3-8 Sulphur compounds determined by AP-TPR "off-line" TD-GC/MS, in µg S/g. (Abbreviations: Me – methyl; Th – thiophene; Bz – benzo)

Based on the quantitative data for S_{vol} received by AP-TPR "off - line" TD-GC/MS experiments (Table 3-3, Figure 3-8), it can be concluded that in all biotreated samples dimethyl sulphone is present. This might be explained by aliphatic sulphur oxidation during the biotreatments. Oxidation of organic sulphur compounds during biodesulphurization process with white rot fungi has already

been reported in the literature. For example Hamme [14], in the study of dibenzyl sulphide (DBS) metabolism with white rot fungi (among which *TV* and *PC* are also examined) has established that the C-S bond in the alkyl bridge of DBS is the target bond for oxidation by fungal cultures. The metabolism proceeds from DBS to dibenzyl sulphoxide formation followed by dibenzyl sulphone, prior to C-S bond cleavage. According to this research, ring oxidation and subsequent ring opening occur only after this cleavage. The metabolic conversion of dibenzyl sulphoxide to dibenzyl sulphone seems 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, it is supposed that the increase in the quantity of dimethyl sulphone in the biotreated samples follows a similar mechanism: i.e. oxidation of di-alkyl sulphides to di-alkyl sulphoxide (probably with the catalyzation of extra cellular enzymes) and further oxidation of di-alkyl sulphoxide to di-alkyl sulphone (probably catalyzed by cytochrome P-450). Nevertheless, the confirmation of this pathway needs to be complementary investigated by supplementary techniques.

Table 3-3 Sulphur in organic compounds determined by AP-TPR/GC-MS, in μg/g

S-compounds	Samples			
	Initial	TV-1	MC+BS	PC
Me-SS-Me	2.4	1.0	3.1	0.4
Di-Me-Sulphone	0.0	0.5	0.2	0.2
Th	1.6	2.8	1.5	3.1
Me-Th	7.6	5.8	3.8	10.8
Di-Me-Th	10.5	5.1	5.4	12.0
Tri-Me-Th	10.0	1.7	3.9	11.0
Bz-Th	4.6	3.3	1.7	1.7
Me-Bz-Th	0.8	1.8	0.2	0.8
Total	37.6	22.0	19.6	40.2

(Abbreviations: Me – methyl; Th – thiophene; Bz – benzo)

When comparing contents of thiophene (Th) in untreated coal sample (initial) and biotretaed samples (Figure 3-8, Table 3-3), it can be noticed that for biotreated samples, i.e. TV-1 and PC, the amount of registered Th increases, while in the case of MC+BS the content of Th decreases. It is well known that

fungi are able to metabolize a broad range of hydrocarbons and polycyclic aromatic hydrocarbons, and can even be used for coal solubilization. Several white rot fungi are screened for their ability to solubilize lignites. For example *PC* is found to depolymerize Elbistan lignite with 60% efficiency [15]. Based on comparison of IR spectra of treated and untreated samples, the authors conclude that fungal treatment do not cause demethylation but provokes effective ring opening of the aromatic structure. Therefore it is supposed that the increased Th content of *TV-1* and *PC* samples is partly explained by the breakdown of homocyclic aromatic ring of more complex sulphur organic forms, as BzTh (which quantity decreases in both cases). Moreover, if effective demethylation does not occur during fungi biotretments, then the content of methylated forms of Th should increase as well. However, increase and significant decrease are registered in the content of methylated forms of Th for *PC* and *TV-1*, respectively. Obviously, during the *TV-1* fungi treatment of the studied sample, along with the effective aromatic ring opening, demethylation also proceeds.

MC+BS sample behavior is very different from the above discussed samples. The contents of Th and its methylated forms, i.e Me-Th, Di-Me-Th and Tri-Me-Th parallel with the contents of BzTh and Me-BzTh decrease. In US Patent 4659670 [16], it is described that seven distinct aerobic gram negative rods have been recognized in the microorganism mixture (ATCC No. 39327). It is mentioned as well that mixed cultures ATCC No. 39327 comprise seven aerobic gram negative rods, which are probably one or more of the following: *Pseudomonas*, *Acinetobacter*, *Azotobactor* or *Flavobacteria*. These bacterial species are of great interest in the early success of organic sulphur removal. A genetically modified strain of *Pseudomonas alcaligenes* is shown to be capable of oxidizing thiophenes [17]. Other strain of *Pseudomonas*, isolated for the ability to grow on DBT as source of carbon and sulphur, is used to treat high organic sulphur coal. A decrease of 38-45 % of the total sulphur content is attained in 10 days [18]. *Acinetobacter* strain EP can utilize BzTh and DBT as the only sulphur sources [19]. *Flavobacterium sp*, isolated by enrichment on thiophene-2-carboxylic acid, is reported to release the sulphur as sulphate but it utilizes as a source of carbon the rest of the compound [20]. It is obvious that for making any considerable and definitive conclusions for sulphur transformation caused by the action of the

applied microbial mixture ATCC No. 39327, a more profound and detailed analyses should be made. This is necessary, on one hand, because of the presence of different types of bacteria in the mixed culture, and on the other hand, because of the complexity of the examined product. However, it can be stated that the mixed culture *MC+BS* is capable of organic sulphur removal. Moreover, in our study it turns out to be the most effective microbial system for S_{vol} removal.

3.3.4 Sulphur balance determination

Quantification of sulphur released during pyrolysis as H_2S is possible by AP-TPR experiments with potentiometric detection. In Table 3-4 potentiometric sulphur recovery values (R_{pot}) and potentiometrically determined S_t in the samples (S_I) are listed. Biotreated with PC Pirin sample is characterized by the highest R_{pot} value (76%) while the lowest sulphur recovery is registered for initial coal (64%). The latter can be explained mainly by the high ash content of the initial sample, acting as a H_2S adsorbent. S_{vol}, not determined by AP-TPR/pot, can be quantitatively measured by AP-TPR "off-line" TD-GC/MS device. Apart from volatilization of sulphur species, less volatile and complex thiophenic structures can also be formed due to secondary reaction. These sulphur containing structures are resistant to AP-TPR reduction conditions and can be incorporated into tar/char fraction.

Table 3-4 Sulphur balance, in % (db)

Sample	S_I by TPR/pot	S in Tar/Char		S_{III} Volatiles	*(S_I+S_{II}+S_{III})	R_{pot}
		S_{II} (tar+char)	S tar/S char			
Initial	3.14	0.31	2.226	0.10	72.7	64.3
TV-1	2.71	0.23	2.484	0.06	82.4	74.5
MC+BS	2.51	0.23	2.322	0.05	76.9	69.1
PC	2.79	0.21	1.532	0.11	85.0	76.2

* Sulphur sum (S_I+S_{II}+S_{III}) as a part (%) from the S_t in the samples;

R_{pot}=Sulphur determined as H_2S by AP-TPR as a part of S_t (recovery);

The S_t balance can be calculated as followed:

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

where S_t – total sulphur; S_I – potentiometrically determined sulphur, S_{II} – oxygen bomb determined sulphur; S_{III} – TD-GC/MS "off-line" measured sulphur; S_{res} – sulphur loses. The last comprises: (i) not determined or lost during experiments sulphur compounds, i.e oxidized sulphur species released as SO/SO_2; (ii) sulphur volatiles not captured by the Tenax; (iii) and, calculation errors due to not optimal spiking of the reference, etc.

The following comments can be made: S_{II}-values are all lower for all biotreated samples compared to initial sample. The sample treated with PC has the lowest S_{II}-value. For the same sample, the highest value for the sulphur sum (S_I + S_{II} + S_{III}) is obtained, about 85% (Table 3-4) and thus the highest sulphur recovery (R_{pot}) is registered. S_I-values are also all lower for all biotreated coals compared to initial one. This is most probably related to ongoing oxidation process which lead to increase in oxidized sulphur compounds and thus to increase in released SO/SO_2. As already mentioned, the last are not quantified in the applied experimental set-up.

The sulphur balance for investigated samples is visualized in Figure 3-9. It can be seen that AP-TPR system with its variation in detection mode is a powerful technique for quantitative and qualitative sulphur distribution assessment.

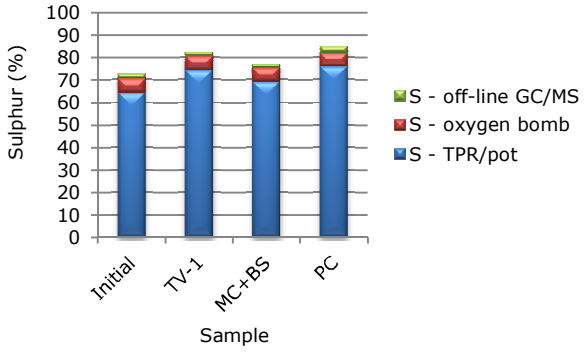

Figure 3-9 Sulphur balance, in %

3.4 Conclusions

Applied biotreatments with *TV-1*, *MC+BS* and *PC* cause biodesulphurization effect of about 26% and 13% concerning S_t and S_o, respectively, without significant change in calorific value. Variety of organic sulphur species present in the studied coal samples are registered by AP-TPR device coupled with different detection systems (potentiometric, MS and GC/MS). Moreover, an attempt to trace organic sulphur forms alteration in the investigated coals due to the biotreatments is made. Most probably the ongoing biodesulphurization mechanisms are oxidative. Certainly, AP-TPR-MS and AP-TPR "off-line" TD-GC/MS study confirm biotransformation process of complex sulphur species into sulphones and sulphoxides accompanied by organic sulphonic group decomposition. In addition, removal of complex thiophenic structures is registered for all biotreated samples. However, aliphatic sulphur is most probably better eliminated during *MC+BS* treatment comparing to aromatic sulphur species. During fungi treatments homocycling ring opening of complex thiophenic structure along with demethylation processes occur. The applied biotreatments affect the evolution of volatile sulphur compounds, which are found to be the highest removed in the case of coal sample treated with *MC-BS*. It is concluded that the biotreatments do not significantly concern the coal matrix. Nevertheless, it is presumed that biodesulphurization has an effect on the organic coal matrix, which results in a better hydrogenation/reduction in an inert atmosphere, of organic sulphur groups still present in the treated samples.

Presented precise balance of the sulphur distribution revealed by AP-TPR coupled with different detection systems gives ground to claim that the technique is reliable for sulphur determination in coal samples.

3.5 References

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Chapter 4 Biodesulphurization of demineralized and depyritized coals: Organic sulphur alteration assessment

The results discussed in this chapter have been published in a peer-reviewed paper:
Gonsalvesh L, Marinov SP, Stefanova M, Carleer, Yperman J. Organic sulphur alterations in biodesulphurized low rank coals. Fuel 2012; 97:489-503.

4.1 Introduction

It is described in Chapter 3 that the white rot fungus Phanerochaeta chrysosporium (*PC*) is capable of removing S_o. However, despite the fact that among applied microorganisms in our initial study (Chapter 3) this fungus shows the highest effect of biodesulphurization towards S_o, this effect is not high enough and satisfying. Therefore, *PC* capability for S_o removal is tested and studied on demineralized and depyritized coal with the idea that this will promote higher S_o bioremoval. Another microorganism, namely Sulfolobus solfataricus (*SS*) effective for S_o removal is also included in this study [1-2]. It should be mentioned that by applying such an experimental strategy, the influence of enrichment in organic matter, i.e. S_o and preliminary chemical treatments on microorganisms performance, can be assessed. Moreover, the information obtained by the AP-TPR concerning organic sulphur functionalities of the coals under consideration will be improved.

The aim of the study summarized in this chapter is to evaluate the desulphurization effect of coals subjected to biotreatments with *PC* and *SS*, and to track the changes that occur with sulphur functionalities. Sulphur forms and species arranged in organic compounds will be of special interest. In order to focus the efforts on S_o biodesulphurization, coal samples are preliminary demineralized and depyritized. In this study, the samples are invesitigated by AP-TPR-MS and AP-TPR "off-line" TD-GC/MS. By special sulphur specific sorbents application, an attempt to enrich the information obtained by AP-TPR "off-line" TD-GC/MS is made.

4.2 Experimental section

4.2.1 Coal

Humovitrain from Maritza East lignites (M), Pirin subbituminous coal (P) and Beypazari lignite (B) are studied. Coal samples are preliminary demineralized and subsequently depyritized according to procedures described in Chapter 2. Demineralized and depyritized samples are assigned as APF (**A**sh and **P**yrite **F**ree). Further on, APF samples are subjected to biodesulphurization.

4.2.2 Biodesulphurization

The microorganisms, i.e *PC* and *SS* are the used microorganisms in the current research under the conditions described in Chapter 2. Proximate analysis values determined by TGA method, S_t content and sulphur forms distribution, as well as higher heating value (HHV) calculated by the formula of Channiwala [3] of initial, APF and APF-PC/APF-SS samples are presented in Table 4-1.

Prior to TPR analysis, APF and biotreated with *PC* and *SS* APF samples are consecutively extracted with chloroform ($CHCl_3$) and cyclohexane (c-He) for the investigation on elemental sulphur changes as a result of applied biotreatments. Figure 4-1 clarifies the experimental strategy.

4.2.3 AP-TPR analysis

AP-TPR coupled "on-line" with mass spectrometry (AP-TPR-MS) in different gas flows, i.e. H_2 and He, is used to specify organic sulphur functionalities in coal and to assess the changes after biotreatments. The AP-TPR-MS analyses are performed as described in Chapter 2.

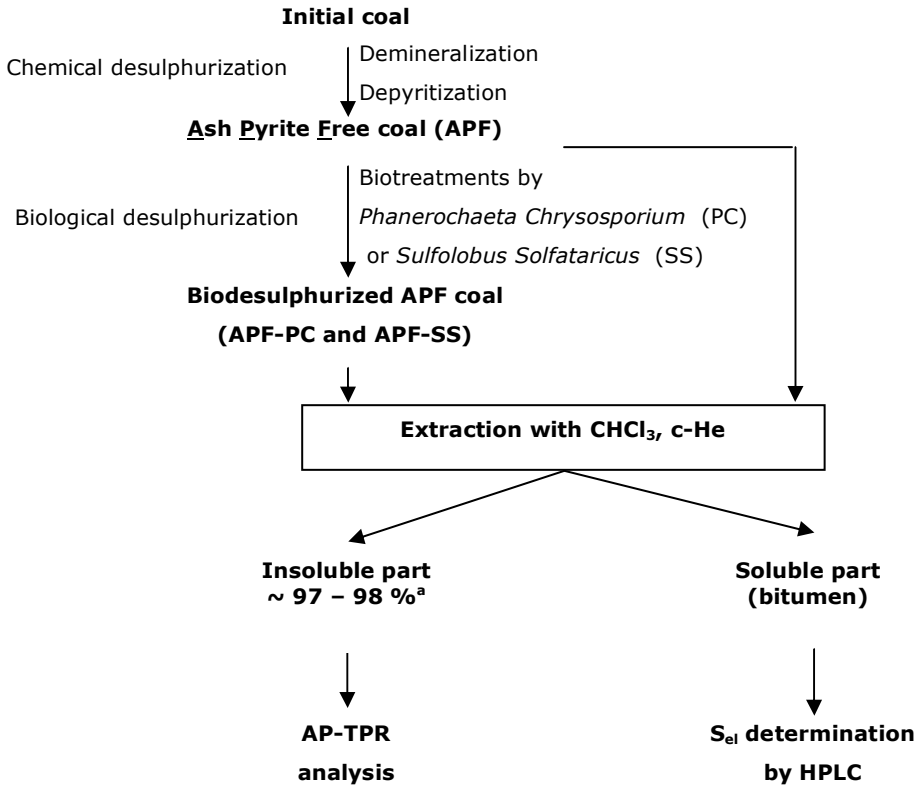
AP-TPR coupled "off-line" with TD-GC/MS is also used to study S_{vol}. The pyrolysis experiments are performed in a reducing (H_2) atmosphere. In Chapter 3 and in previous studies [4-7], the volatiles are trapped by Tenax tubes. In order to improve qualitative/quantitative interpretation of S_{vol} released during pyrolysis experiment, the Tenax tubes are replaced by ones filled with sorbents selective for sulphur compounds. In this study, ice-cooled metal adsorption tubes with SilcoSteel Coating, filled with Tenax/Carbopack B/Carbosieve SIII

(Markes) as adsorbents are used. Details about conditions for volatiles capturing, TD-GC/MS apparatus and its experimental condition used in current study can be found in Chapter 2. GC/MS chromatograms are quantitatively interpreted by spiking the selective sorbents with 3 µg *d4*-thiophene.

Table 4-1 Characteristics of the coal samples

Sample	Proximate analysis (%)				S content (%)^{daf}				HHV*
	Ash^{ad}	VM^{daf}	W^{ad}	$C_{fix}{}^{daf}$	S_t	S_p	S_s	S_o	(MJ kg^{-1})
P-in	10.4	37.2	6.5	62.8	5.49	0.57	0.54	4.38	22.68
P-APF	0.0	42.3	6.2	57.7	4.10	0.23	0	3.87	23.65
P-APF-PC	0.5	42.9	6.6	57.1	3.11	0.16	0	2.95	23.08
P-APF-SS	1.7	41.0	5.3	59.0	3.53	0.20	0	3.33	26.30
M-in	8.5	48.8	8.1	51.2	7.06	1.64	1.54	3.88	19.29
M-APF	1.2	50.8	6.3	49.2	4.01	0.37	0	3.64	19.93
M-APF-PC	0.9	50.5	7.4	49.5	3.04	0.20	0	2.84	20.15
M-APF-SS	1.4	49.9	7.0	50.2	3.87	0.38	0	3.49	24.24
B-in	29.1	40.2	12.3	59.8	7.62	1.68	1.23	4.71	16.51
B-APF	0.6	43.7	6.5	56.3	3.49	0.38	0	3.11	22.03
B-APF-PC	1.0	44.0	6.2	56.0	3.13	0.11	0	3.02	22.58
B-APF-SS	1.1	42.4	6.4	57.6	2.90	0.36	0	2.54	23.00

W – moisture; VM – volatile matter; C_{fix} – fixed carbon;^{ad} – air dried; ^{daf} – dry, ash free;*HHV– higher heating value is calculated on air dry basis by the formula of Channiwala [3].



[a] – Relative % of coal on dry ash free basis

Figure 4-1 Experimental strategy

4.3 Results and discussion

4.3.1 Bulk characteristics

In order to focus our attention on the changes in S_o due to biodesulphurization, samples are preliminary demineralized and depyritized. Effects of the treatments, i.e. chemical desulphurization, biodesulphurization and the desulphurisation of combined action of chemical and biological treatment on the investigated samples, are present in Table 4-2.

Table 4-2 Desulphurization effect (Δ) in %

Sample	Δ S_t			ΔS_p			ΔS_s			ΔS_o		
	Che	Bio	Com	Che	Bio	Com	Che	Bio	Com	Che	Bio	Com
P-in												
P-APF	25.3			59.6			100			11.6		
P-APF-PC		24.1	43.4		30.4	71.9		-	-		23.8	32.6
P-APF-SS		13.9	35.7		13.0	64.9		-	-		14.0	24.0
M-in												
M-APF	43.2			77.4			100			6.2		
M-APF-PC		24.2	56.9		45.9	87.8		-	-		22.0	26.8
M-APF-SS		3.5	45.2		-2.7	76.8		-	-		4.1	10.1
B-in	c											
B-APF	54.2			77.4			100			34.0		
B-APF-PC		10.3	58.9		71.1	93.5		-	-		2.9	35.9
B-APF-SS		16.9	61.9		5.3	78.6		-	-		18.3	46.1

Che – Chemical desulphurization effect;
Bio – Biodesulphurization effect compared to the APF state;
Com – Combined desulphurization effect after chemical and biological treatment compared to "in" state.

As can be seen, chemical treatment mainly attacks inorganic sulphur (S_p and S_s). S_t chemical desulphurization is in the range of 25.3÷54.2%, maximizing at B-APF coal. Maximal S_p desulphurization is achieved for both M-APF and B-APF coals, i.e. 77.4%. However, S_o chemical desulphurization is in the range of 6.2÷34.0%. Despite this S_o reduction, APF samples are "enriched" in S_o in terms of the fact that sulphur left in these samples is mainly in organic form. Demineralized and depyritized coals are subjected to further processing – biodesulphurization. Since the purpose of this study is biodesulphurization, further comment on the effect of the chemical treatment will not be made.

Therefore in this study, the preliminary demineralized and depyritized coals will be considered as initial samples.

Two types of microorganisms are applied: *PC* and *SS*. It is found that biotreatments with *PC* demonstrated extra S_t desulphurization with a maximum of about 24.2% in the case of M-APF-PC coal sample, followed by P-APF-PC coal (24.1%). Maximum extra S_o biodesulphurization with *PC* is achieved for the same coal samples: M-APF-PC (22.0%), and P-APF-PC (23.8%). In the case of B-APF-PC coal, extra S_t biodesulphurization effect is much lower (10.3%) and much more effective toward extra S_p removal (71.1%) than extra S_o destruction (2.9%). However, for *PC* treated P-APF and M-APF samples a relatively high S_p removal is obtained as well. It is obvious that preliminary demineralization and depyritization on Pirin coal sample enhance fungus performance (see Chapter 3). Concerning the biotreatment with *SS*, maximum extra S_t and S_o biodesulphurization of 16.9% and 18.3%, respectively, are achieved for B-APF-*SS* coal. Certainly for M-APF-SS, low extra S_t and S_o desulphurization results of 3.5% and 4.1%, respectively, are obtained in comparison with *PC* treatment. For P-APF-SS sample, achieved extra S_t and S_o removal are 13.9% and 14.0%, respectively. The determined changes in S_p content for *SS* treated samples are in the repeatability limit of 0.05% for S_p determination method.

After biotreatment, the ash content increases in all samples (Table 4-1), except in the case of M-APF-PC. This effect might be contributed to the inoculation/introduction of mineral mass from the culture medium of the microorganisms. Only some insignificant variations in the contents of volatile matter (VM) and fixed carbon (C_{fix}) are registered. Important to mention is the insignificant influence of the *PC* biotreatments on the heating value of the samples (Table 4-1). However, for *SS* biotreated coals in comparison with APF coals, an increase in the heating value in the range of 4.4÷21.6% is found. This increase maximizes in the case of the sample with the lowest sulphur removal results (M-APF-SS) and minimizes in the case of the sample with the highest sulphur removal results (B-APF-SS). Remarkable is the fact that the combined desulphurization treatment (i.e. chemical- and bio-) has always a positive effect on the heating value of the samples. Compared to untreated samples, an increase in the heating value in the range of 1.8÷36.8% for APF-PC samples and of 16.0÷39.3% for APF-SS samples are measured.

4.3.2 AP-TPR experiments coupled "on-line" with MS detection

4.3.2.1 In hydrogen atmosphere

AP-TPR device coupled "on-line" with MS is used to assess S_o alterations as a result of the applied biotreatments. Before analyses samples are extracted by $CHCl_3$ and c-He. Extracted samples are representative for S_o determination by TPR as each of them compose about 97-98% of the corresponding initial sample (before extraction). Unpublished GC-MS results for the neutral oils composition and HPLC data (described in Chapter 5) reveal that there are negligible sulphur amounts in the soluble products. Elemental sulphur (0.01÷0.16 wt.%) and traces of alkyl substituted benzothiophenes are detected.

4.3.2.1.1 H_2S profiles

H_2S kinetograms of AP-TPR-MS in H_2 atmosphere of APF and biotreated APF coal samples are visualized in Figure 4-2A, B and C. As far as *m/z* 34 (H_2S^+) and *m/z* 33 (HS^+) reveal the same temperature evolution, for clearness only *m/z* 34 ion profiles are shown. There are always two dominant peaks for all studied coal samples: the first one with T_{max} about 400ºC and second peak with T_{max} about 600ºC. The peak at the lower temperature can be assigned to the presence of di-alkyl sulphides, alkyl-aryl sulphides and disulphides. It is not also excluded the peak at 400°C to refer to reactive di-aryl sulphides [8]. According to these profiles there is no unequivocal proof for the occurrence of thiols, neither in APF samples nor in biotreated APF coals. Inasmuch as the profiles start at around 200°C, especially in the case of Maritza samples, the presence of thiols can be assumed. Peaks assignments in H_2S kinetograms are based on the model compounds approach and also on AP-TPR-MS profiles of typical aliphatic and aromatic CH-fragments shown in Figure 4-3A and B, respectively. In it, only the profiles for P-APF, M-APF and B-APF are presented, since there are no significant changes in aliphatic and aromatic fragments after biotreatment with *PC* and *SS*. The typical aliphatic fragments, i.e. unsaturated/saturated CH-fragments (alkenes/alkanes) comprise *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 ($C_6H_{11}^+$). There are two dominant peaks in the profiles of these ion fragments (Figure 4-3A). The first one at about 160-180°C most likely can be attributed to aliphatic compounds evolution and represents

desorption of gases formed during coalification, and trapped in the coal by adsorption [9]. Another possibility could be the presence of short-chains hydrocarbon from organic matrix destruction as a result of demineralization and depyritization [10]. The second peak in the profiles could be explained by first-order decomposition reactions [9]. Indeed, for all investigated samples (except Humovitrain Maritza East biotreated by *SS*) the first peak in the profile for H_2S^+ (Figure 4-2) is registered at the same maximum evolution temperature as the second peak in the profiles for typical aliphatic fragments (Figure 4-3A).

In the H_2S^+ profiles (Figure 4-2), the second peak refers mainly to the presence of less reactive di-aryl sulphides and more complex thiophenic structures. This assignment is based on model compounds studies and to the evolution of typical aromatic fragment ions. Figure 4-3B illustrates ion fragments referring to aromatic compounds or fragments as follows: benzene – *m/z* 77 ($C_6H_5^+$) and *m/z* 78 ($C_6H_6^+$); toluene – *m/z* 92 ($C_7H_8^+$) and *m/z* 91 ($C_7H_7^+$); xylene – *m/z* 91($C_7H_7^+$) and *m/z* 106 ($C_8H_{10}^+$); alkyl benzene – *m/z* 105 ($C_8H_9^+$) and naphthalene – *m/z* 128 ($C_{10}H_8^+$). For aromatic compounds, first evolution peaks of typical aromatic fragments at about 400°C can be observed. The simultaneous release of three species at this temperature, i.e H_2S, aliphatic and aromatic fragments, confirms the possible attribution of the first maximum in the AP-TPR H_2S^+ profiles not only to the hydrogenation of di-alkyl sulphides but also to alkyl-aryl and reactive di-aryl sulphides. The second maximum (about 600°C) for the aromatic fragments (Figure 4-3B) of all investigated samples is registered at the same temperature as the second maximum observed in the H_2S^+ profiles (Figure 4-2). No aliphatic fragments can be detected in this temperature region. The maximum of H_2S in this temperature region is therefore attributed to the hydrogenation of less reactive di-aryl sulphides and thiophenic structures. It is known that their degradation involves aromatics formation [4]. However, it should be mentioned as well that part of registered less reactive di-aryl sulphides could be formed during pyrolysis of reactive di-aryl sulphides [8].

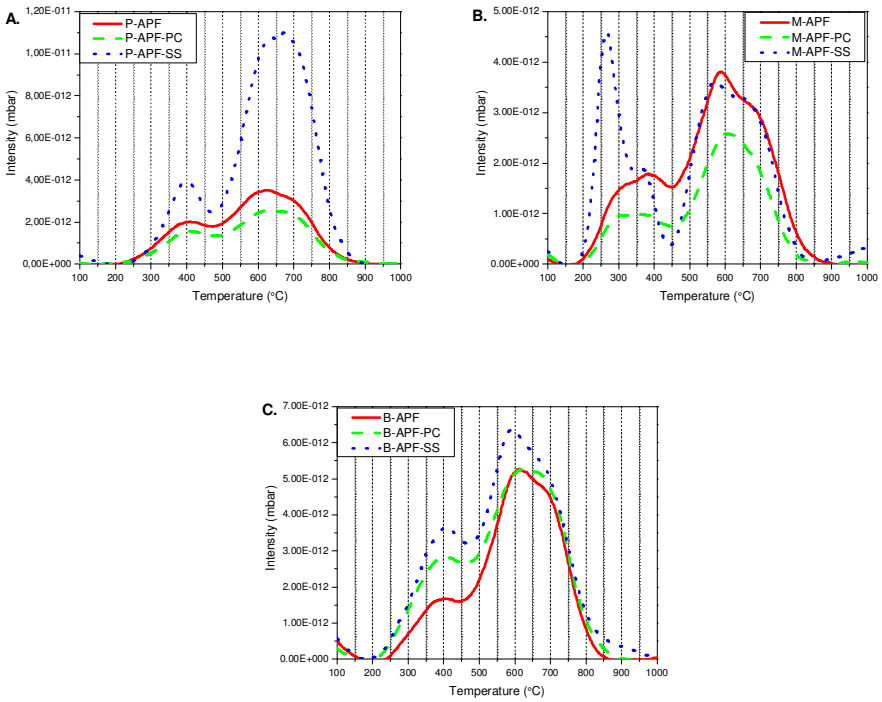


Figure 4-2 AP-TPR-MS (H_2), *m/z* 34 kinetograms of Pirin (A), Maritza East (B) and Beypazari (C) coals

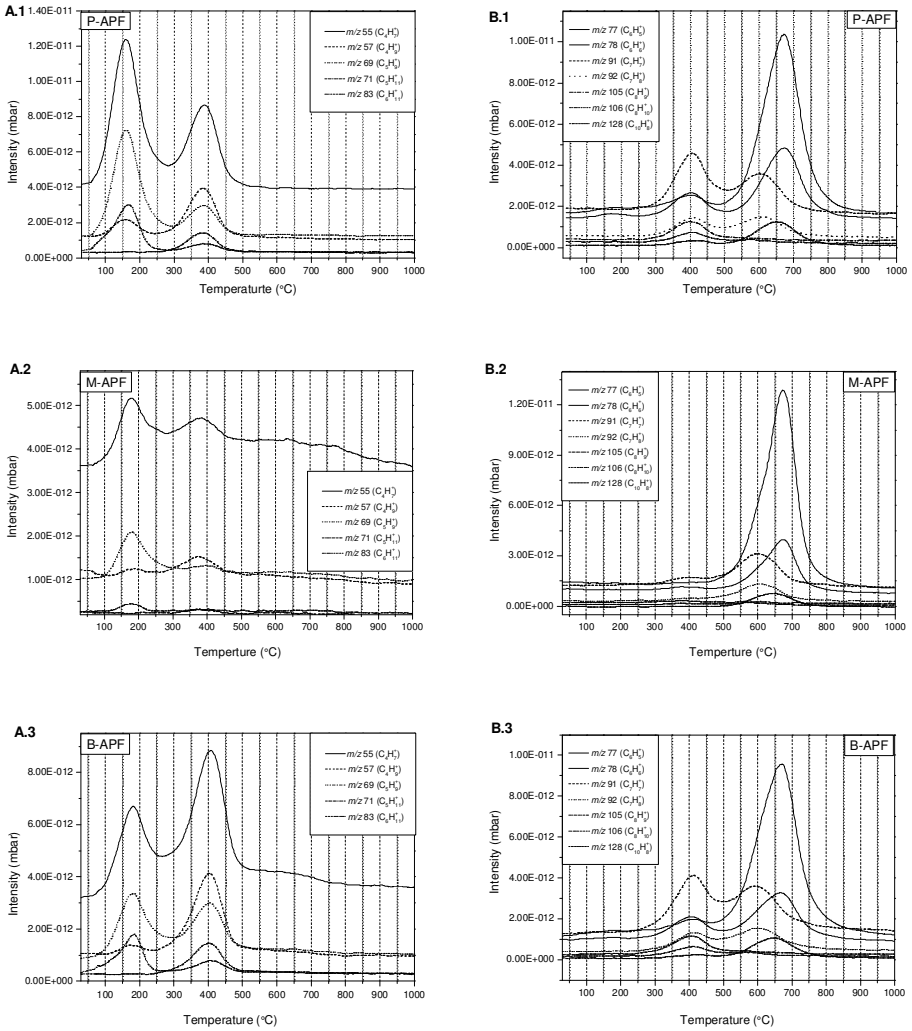


Figure 4-3 AP-TPR-MS (H_2) evolution profiles of P-APF (1), M-APF (2) and B-APF (3) samples: A) saturated/unsaturated CH-fragments (alkenes/alkanes): *m/z* 55 ($C_4H_7^+$); *m/z* 57 ($C_4H_9^+$); *m/z* 69 ($C_5H_9^+$); *m/z* 71 ($C_5H_{11}^+$); *m/z* 83 ($C_6H_{11}^+$). B) aromatic compounds: Benzene: *m/z* 77($C_6H_5^+$); *m/z* 78 ($C_6H_6^+$), Toluene: *m/z* 92 ($C_7H_8^+$); *m/z* 91 ($C_7H_7^+$), Xylene: *m/z* 91 ($C_7H_7^+$); *m/z* 106 ($C_8H_{10}^+$), Alkyl-benzene: *m/z* 105 ($C_8H_9^+$), Naphthalene: *m/z* 128 ($C_{10}H_8^+$).

Table 4-3 Area of the peaks in H_2S profiles of investigated samples

Sample	H_2S area		
	% 1st peak*	% 2nd peak**	2nd peak/1st peak
P-APF	28.5	71.5	2.5
P-APF-PC	31.2	68.8	2.2
P-APF-SS	17.5	82.5	4.7
M-APF	28.1	71.9	2.6
M-APF-PC	21.7	78.3	3.6
M-APF-SS	21.6	78.4	3.6
B-APF	18.8	81.2	4.3
B-APF-PC	29.8	70.2	2.4
B-APF-SS	33.9	66.1	2.0

* – assigned to di-alkyl sulphides, alkyl-aryl sulphides and disulphides;
** – assigned to di-aryl sulphides and thiophenic structures;

Based on the area of each H_2S peak in H_2S profile, information about the ratio between different sulphur forms present in coal can be deduced. Table 4-3 presents the areas of the first (assigned to the presence of di-alkyl and alkyl-aryl sulphides, disulphides, and reactive di-aryl sulphides) and second (assigned to the presence of less reactive di-aryl sulphides and thiophenic structures) peaks expressed as a percentage, as well as the ratio between them. The area of each H_2S peak is determined by means of deconvolution of H_2S profiles using multiple Gaussian functions. It is revealed that the ratio between the areas of the first and second peak is in the favour of the second peak for all investigated APF samples (Table 4-3, Figure 4-2). This inclines us to suppose that di-aryl sulphides and thiophenic structures are more abundant in these samples, but not to the same extend. Thus, clear differences in the type of sulphur functionalities present in the studied samples might be supposed. The same is observed for all *PC* and *SS* treated APF samples. Additionally, some changes in the ratio are depicted as well, indicating which sulphur functionalities might be more affected by the biotreatments. In the case of B-APF-PC and B-APF-SS samples, di-aryl sulphide and thiophenes are better destructed, while in the case of *PC* and *SS* treated M-APF sample, di-alkyl sulphides, alkyl-aryl sulphides and disulphides are preferably attacked. The observed alterations are an indication that organic sulphur changes as a result of *PC* and *SS* treatments of B-APF and M-APF, can be related not only to the type of sulphur functionalities present in

coal but also to structural peculiarities of the samples, i.e. "space accessibility" of the aliphatic and the aromatic sulphur. Noteworthy is the case of M-APF-SS sample. As a result of the biotreatment an intensive peak in H_2S^+ profile appeared. Its maximum can be observed at about 270ºC (Figure 4-2B). Bearing in mind the peak intensity and the fact that in our coal set, elemental sulphur is extracted prior to TPR analysis, the assignment to polysulphides and/or thiols is more likely. Apparently, desulphurization action of *SS* on M-APF sample is different from the one on the other two coal samples (i.e. P-APF and B-APF). Indeed, a similar peak, at about 270°C, is not observed in H_2S^+ profiles of P-APF-SS and B-APF-SS samples.

Different is the situation for P-APF-PC and P-APF-SS samples, in the context that both biotreatments (*PC* and *SS*) do not result in the same alteration in the ratio of sulphur functionalities. After biotreatment with *PC* no significant changes in the ratio can be registered for P-APF-PC coal sample compared to P-APF sample. This is an indication that di-alkyl sulphides, alkyl-aryl sulphides and disulphides as well as di-aryl sulphides and thiophenes are most probably removed in comparable portions. In the case of P-APF-SS, di-alkyl sulphides, alkyl-aryl sulphides and disulphides are preferably attacked and destroyed. These alterations are one more proof for differences in the type of sulphur functionalities and structural peculiarities of the three studied samples. Different preferences towards sulphur functionalities of the used microorganisms might be supposed as well.

4.3.2.1.2 CHS – profiles

The profiles of ion series characteristic for some sulphur-containing organic compounds that may be present in coal or produced during pyrolysis are also tracked. Thus, at some extent, it is possible to confirm the assignment of the peaks in H_2S^+ profiles and to obtain information for the reduction/hydrogenation of sulphur functionalities during TPR experiments. It is known that mercaptanes can be initially present in coal or produced during thermal decomposition of sulphides and disulphides [11]. Sulphides can be present in coal as well and can be produced during thermal decomposition of mercaptanes and disulphides. Therefore sulphur containing aliphatic fragments for thiols and sulphides, $C_nH_{2n+1}S$, n=1, 2, 3, 4 (*m/z* 47, 61, 75, 89) are traced. This ion series and

especially ion fragment *m/z 47* are characteristic for the mass spectra of both classes of compounds [12]. Simultaneously, predominant alkenyl fragments (C_nH_{2n-1}, *m/z* 41, 55, 69,…) and minor aliphatic fragments (C_nH_{2n+1}, *m/z* 43, 57, 71,…) should also be observed in the mass spectra of aliphatic thiols and sulphides. Therefore, if TPR-MS ion profiles of these fragments maximizing at the same temperature, the presence of aliphatic thiol and/or sulphide fragments can be assumed. The profiles of ion series characteristic for aliphatic thiols and sulphides in the case of P-APF, M-APF and B-APF samples are presented in Figure 4-4.

In the TPR profile of *m/z* 47 (aliphatic thiols and/or sulphide fragments) for P-APF and B-APF samples a pronounced peak at about 180°C and a less intensive and broad plateau at about 300-400°C are observed, while in the case of M-APF, *m/z* 47 profile has a well expressed maximum at 310°C. The plateau at about 300-400°C in *m/z* 47 profiles for P-APF and B-APF as well as the peak at 310°C in *m/z* 47 profile of M-APF can be attributed to aliphatic thiol and/or sulphide fragments. Confirmation can be obtained from TPR profiles of other ion fragments in the characteristic ion series of aliphatic thiols and sulphides (i.e. *m/z* 61, 75, 89) maximizing at the same temperature range. In addition, TPR profiles of some alkenyl and alkyl fragments demonstrate a maximum at the same temperature interval (see Figure 4-3). The appearance of aliphatic thiol and/or sulphide fragments in the temperature range of 300 – 400ºC confirms first peak assignment in H_2S^+ profiles (Figure 4-2) of investigated coals to aliphatic thiols, di-alkyl sulphides and disulphides. The peak with high intensity at 180°C can be attributed to desorption of volatile compounds retained by coal porous structure, like cyclohexane and chloroform used for the extraction of elemental sulphur. Indeed, in AP-TPR-MS (H_2) profiles for the most intensive ion fragments from the mass spectra for cyclohexane (*m/z* 41, 69, 84) and chloroform (*m/z* 47, 83, 85, 87) (figures are not shown), a peak which coincides with the peak at 180°C is observed.

Regarding the second peak that appears at higher temperatures around 650-670°C in the TPR profiles of *m/z* 61, 75 and 89 (Figure 4-4), it cannot be attributed to a fragment originating from aliphatic thiols, di-alkyl sulphides and disulphides decomposition. It might be related to a fragments or compounds produced by secondary reactions [13]. However, further research is needed to

confirm this assumption. After biotreatment with *PC* of samples P-APF, M-APF and B-APF significant differences in the TPR profiles of *m/z* 47, 61, 75 and 89 are not found. On the contrary, in the profiles of these fragments for M-APF-SS and P-APF-SS an increase in the content of these two classes of compounds is recognized.

In the MS spectra of saturated aliphatic disulphides, H_2S_2 and its alkyl homologues are the characteristic fragments (*m/z* 66, 80, 94,…). Unfortunately the fragments with *m/z* 66 and 94 can be attributed to phenol as well. Since phenols are highly abundant in low rank coal and can be produced during pyrolysis, the tracking of *m/z* 66, 80 and 94 will not provide additional and unambiguous information for non-reduced disulphides into H_2S. We are faced to similar problems when we try to obtain information for the presence of mixed (alkyl-aryl) and aromatic sulphides and aromatic thiols, neither hydrogenated into H_2S nor retained in tar fraction. For these classes of compounds, $HC{\equiv}S^+$ (*m/z* 45) is characteristic beside the aromatic fragments [12]. Respectively, from *m/z* 45 profile unequivocal information cannot be acquired as it can be related to the fragmentation of disulphide, thiophenes, some oxygen containing compounds, etc.

The ion fragments for thiophene and its substituted homologues (*m/z* 84, 97, 98, 111, 112, 125, 126, 140) as well as the ion fragments for benzothiophene and methyl benzothiophenes (*m/z* 134, 147, 148) are also examined. For all samples, the curves for thiophene and substituted thiophenes are with the same pattern of distribution and maximum evolution temperature at 400°*C*. Apparently, at higher temperature the reduction of the thiophenes into H_2S starts. Concerning the profiles of ion fragments for benzothiophenes, a weak peak at above 600°C only for *m/z* 134 is observed. It does appear in trace quantities in all samples except M-APF, M-APF-PC and B-APF-SS samples.

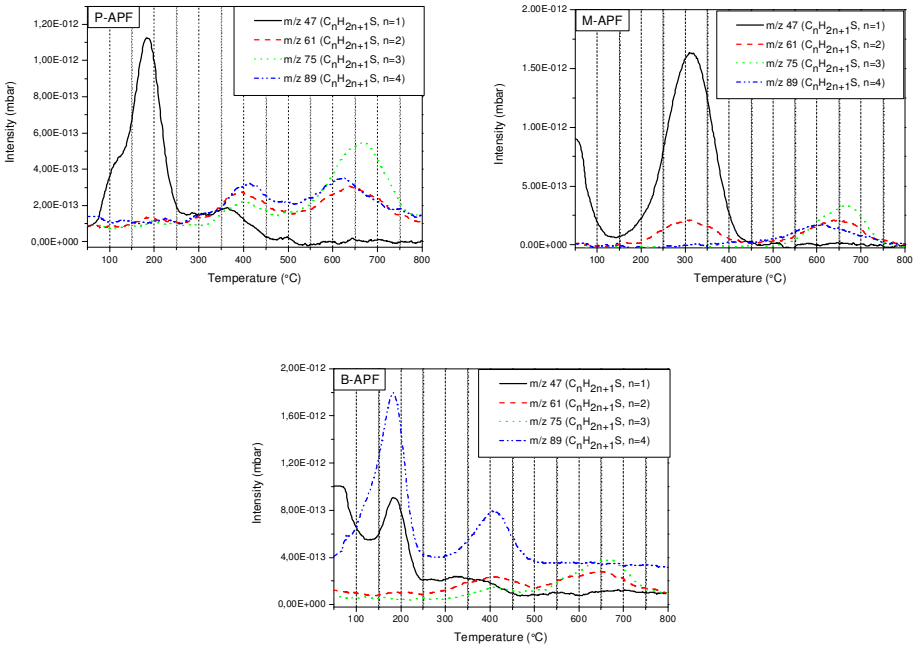


Figure 4-4 AP-TPR-MS (H_2) evolution profiles of P-APF, M-APF, B-APF for aliphatic thiols and sulfides: $C_nH_{2n+1}S$, n=1, 2, 3, 4 (*m/z* 47, 61, 75, 89)

4.3.2.1.3 SO^+/SO_2^+ profiles

It is known that in AP-TPR experiments, the oxidized sulphur forms present in the sample are not entirely reduced into H_2S [14-16]. As a result of thermal decomposition during pyrolysis, the oxidized sulphur species can form SO and SO_2. Depending on the temperature region in which they are released (if they exhibit the same evolution), the sulphur distribution of oxidized sulphur forms in coal can be revealed. Therefore the profiles of both, *m/z* 48 (SO^+) and *m/z* 64 (SO_2^+), are tracked. Figure 4-5 visualizes the evolution of oxidized sulphur functionalities during AP-TPR experiments for APF samples compared to *PC* biotreated APF coals (Figure 4-5A) and APF samples compared to *SS* biotreated APF coals (Figure 4-5B). In general, if *m/z* 48 and 64 profiles have the same temperature evolution and peaks maximizing in the range of 200-300°C, they can be attributed to SO^+/SO_2^+ originating from organic sulphonic groups decomposition. This assumption is based on pyrolysis of model compounds and sulphonated coals [16]. In our study, *m/z* 64 and 48 profiles clearly

demonstrate different trend in the lower temperature range up to 300°C. This observation gives us ground to suppose that *m/z* 48 and 64 profiles, and especially *m/z* 48 profiles of the studied samples correspond not only to SO^+ and SO_2^+. These ion profiles most likely correspond also to other fragments: *m/z* 48 can respond to CH_3SH^+ and $CHCl^+$; while *m/z* 64 to S_2^+ and $C_5H_4^+$ [12]. With respect to *m/z* 48 profiles of the samples under study, they behave differently compared to each other and to their corresponding *m/z* 64 profile. Apparently, *m/z* 48 profiles contain contribution from other fragments. According to our opinion, if CH_3SH^+ fragment contributes to *m/z* 48 (SO^+) profile this will affect *m/z* 48 (SO^+) profile at temperatures higher than 250°C, since thiols and sulphides are released at temperature above 250°C [5-6]. Most likely, this is the case for M-APF, M-APF-PC and M-APF-SS samples, where in *m/z* 48 profile a maximum at about 300°C is observed. In confirmation, for these samples, a signal with maximum at about 300°C is registered in the profiles of the fragments from the ion series characteristic for thiols and sulphides (see Figure 4-4). A similar phenomenon is observed in a previous study as well [5].

In *m/z* 48 profiles of all Pirin and Beypazari samples, i.e. APF and *PC*/*SS* treated APF samples as well as of M-APF-SS sample, a peak or shoulder at about 190°C can be recognized. This peak can hardly refer to a fragment formed during thermal destruction of coal since the temperature is too low. It might be explained by desorption of volatile compounds retained by coal porous structure. As it was already discussed (see section 4.3.2.1.2), in AP-TPR-MS (H_2) profiles of the ion fragments from the mass spectra of chloroform, peaks at about 180-190°C are observed. Therefore, it can be assumed that $CHCl^+$ fragment originating from chloroform fragmentation contributes to *m/z* 48 profiles in the temperature range below 200°C.

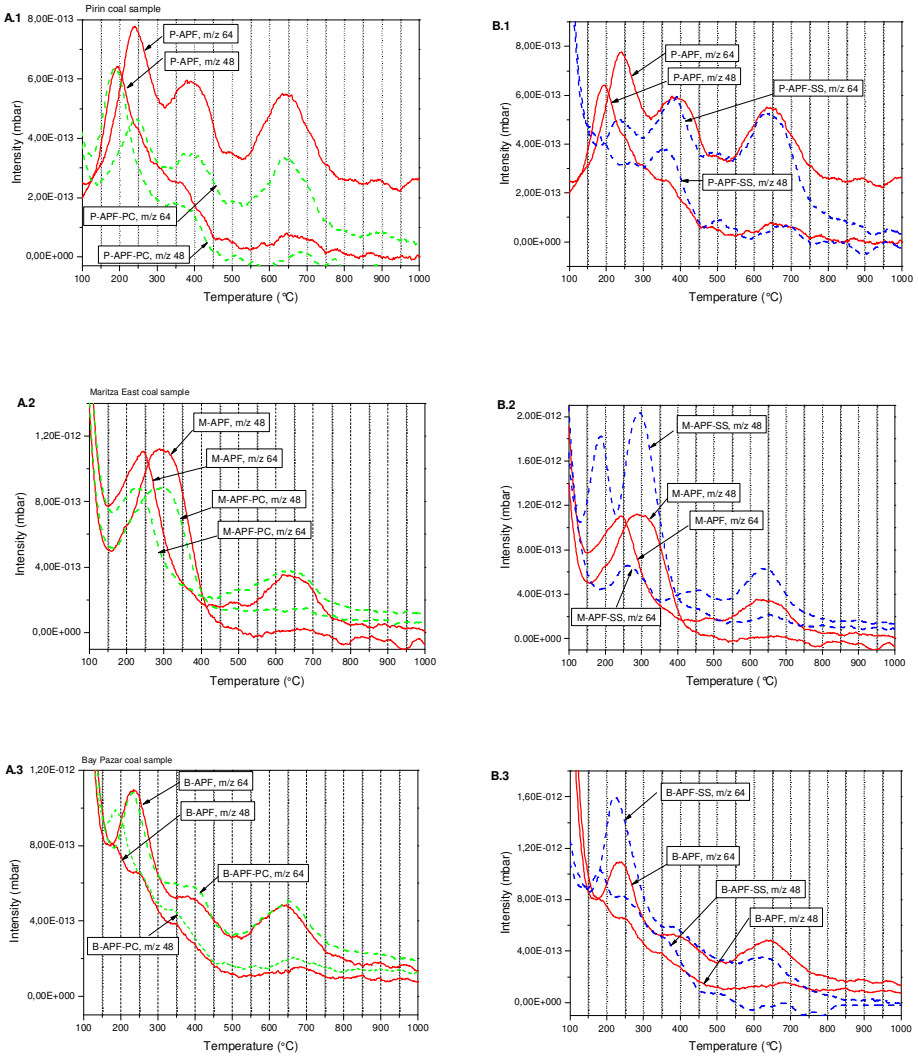


Figure 4-5 AP-TPR-MS (H_2), *m/z* 48 (SO^+) and *m/z* 64 (SO_2^+) kinetograms of Pirin (1), Maritza East (2) and Beypazari (3) coals: A) APF samples compared to APF-PC samples; (B) APF samples compared to APF-SS samples

With respect to *m/z* 64 profiles, a peak with maximum at about 240-250°C is registered for all samples under consideration. For some of them (B-APF, B-APF-PC, B-APF-SS) this peak coincides with a shoulder in the corresponding *m/z* 48 profile. On the other hand, if *m/z* 64 profiles contain contribution from S_2^+ (C-S bridge cleavage) and/or $C_5H_4^+$ (thermal breakdown of styrene structures [16]), these profiles will be affected at temperature above 300°C. Therefore it can be assumed that *m/z* 64 profiles in the temperature up to 300°C refer mainly to SO_2^+. Respectively, despite the differences in *m/z* 48 and 64 profiles in the temperature range up to 300°C due to contribution of CH_3SH^+ or $CHCl^+$ to *m/z* 48 profiles, interpreting *m/z* 64 profiles organic sulphonic groups presence can be assumed in all investigated samples.

Above 300°C, comparison between *m/z* 48 and *m/z* 64 profiles is difficult to be performed because the extremely low intensity of *m/z* 48. In addition, Mullens et al. [16] prove that in the temperature region 400 – 500°C, *m/z* 64 is no longer specific for SO_2^+, as it can also contain a contribution from styrenes destruction. Hence, the peak and the shoulder at 400°C in *m/z* 64 profiles of P-APF and B-APF, respectively, can be attributed not only to a possible presence of alkyl-aryl sulphoxides but also to fragments originating from coal aromatic structure decomposition. The peak at 650°C can be explained by alkyl-aryl sulphoxides, diaryl sulphoxides and/or diaryl sulphones decomposition. This assignment is again based on the pyrolysis of model compounds [15]. The last two mentioned peaks, i.e the peak at 400°C and the peak at 650°C, are registered in *PC* and *SS* biotreated P-APF and B-APF samples as well. With respect to M-APF and M-APF-PC samples, there is no peak at 400°C in *m/z* 48 and *m/z* 64 profiles (Figure 4-5A.2 and B.2). This is an indication that alkyl-aryl sulphoxides are absent in these samples. Thus, the peak at about 650°C can be referred to diaryl sulphoxides and/or diaryl sulphones decomposition. However, based on the evolution of the typical aromatic fragment ions (see Figure 4-3), the breakdown of the aromatic structures and their contribution to the peak at 650°C in *m/z* 64 profiles of all studied samples cannot be excluded. Additional research is needed to confirm these peak assignments.

It should be mentioned also the fact that *m/z* 64 profiles of P-APF, P-APF-PC and P-APF-SS samples exhibit the same pattern of evolution throughout TPR temperature range. The same is observed for B-APF, B-APF-PC and B-APF-SS

coals, as for M-APF, M-APF-PC and M-APF-SS coals. Comparing *m/z* 48 profiles of investigated samples it can be recognized that *m/z* 48 profiles of all APF samples are similar to *m/z* 48 profiles of their corresponding APF-PC sample, and different to *m/z* 48 profiles of their corresponding APF-SS sample. This diversity is especially pronounced in the case of M-APF-SS sample demonstrating again the different impact of *SS* treatment on M-APF coal as already noticed in Figure 4-2B.

4.3.2.2 In helium atmosphere

Pyrolysis in an inert atmosphere can provide additional information for sulphur species. In Figure 4-6A, B and C the AP-TPR-MS evolution profiles in He atmosphere for H_2S^+ for all investigated samples are shown. Differences in AP-TPR-MS evolution profiles for H_2S^+ in He and H_2, demonstrate the importance of the atmosphere used during TPR pyrolysis. In AP-TPR-MS (He) profile for H_2S^+ of P-APF sample, one broad peak is observed with an apex at 400°C, a shoulder at about 480°C and a second shoulder at temperature higher than 525°C. Based on AP-TPR-MS (He) profiles of aliphatic and aromatic CH-fragments, the peak apex can be attributed to the presence of di-alkyl and alkyl-aryl sulphides, disulphides and reactive di-aryl sulphides, while the shoulders at about 480°C and about 525ºC to the presence of less reactive di-aryl sulphides and more complex thiophenic structures. It is obvious that hydrogenation of aromatic sulphur functionalities in an inert atmosphere is strongly limited. For *PC* and *SS* treated P-APF samples the observed peaks in AP-TPR-MS (He) profiles for H_2S^+ are narrower at the higher temperatures range. Manifestly, applied biotreatments provoke some structural rearrangements in coal matrix that influence aromatic sulphur forms susceptibility to hydrogenation in an inert atmosphere. The same tendency is registered for *PC* and *SS* biotreated B-APF samples.

In the case of M-APF and *PC*, and *SS* biotreated M-APF samples again only one broad peak is seen. It is attributed to the presence of di-alkyl and alkyl-aryl sulphides, disulphides and reactive di-aryl sulphides. Outstanding is the fact that for M-APF-SS coal, well expressed differences towards aliphatic sulphur forms and aromatic sulphur forms are found compared to M-APF sample. As a result of the biotreatment, a new peak maximum at about 280°C is observed in AP-TPR-

MS (He) profile for H_2S^+ of M-APF-SS. Most likely it is related to the presence of polysulphides and/or thiols. The same new maximum has been registered in AP-TPR-MS (H_2) profile for H_2S^+ of M-APF-SS but shifted to lower temperature because of more favorable hydrogenation conditions. At higher temperature, one more new peak at about 480°C is observed in the H_2S^+ AP-TPR-MS (He) profile of M-APF-SS coal. Probably it refers to aromatic sulphur functionality, new formed or changed, due to *SS* treatment.

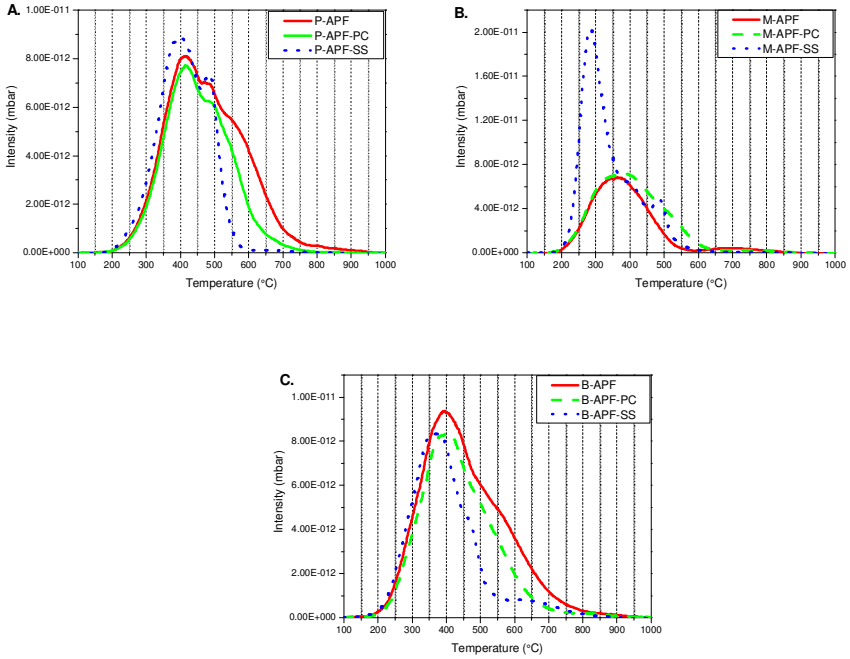


Figure 4-6 AP-TPR-MS (He), *m/z* 34 kinetograms of Pirin (A), Maritza East (B) and Beypazari (C) coals

Additionally, we are convinced that supplemental information for oxidized sulphur species can be obtained by AP-TPR-MS in an inert atmosphere. AP-TPR-MS kinetograms in He atmosphere for *m/z* 48 and *m/z* 64 referring to the presence of some oxidized sulphur compounds are shown in Figure 4-7. Unlike pyrolysis in reducing atmosphere, these profiles exhibit the same trends for APF and biotreated with *PC* and *SS* APF Pirin and APF Beypazari samples. They unequivocally can be attributed to SO^+ and SO_2^+. Concerning the first peak maximum at about 260°C accompanied by its shoulder at about 360°C in AP-

TPR-MS (in He) profiles of SO^+ and SO_2^+ for P-APF and B-APF samples can be assigned to organic sulphonic acids [16]. The peak at 600-650°C can be attributed to di-aryl sulphoxides/sulphones abundance [15]. After bioprocessing with *PC* and *SS* the observed changes are associated with an increase in the content of di-aryl sulphoxides/sulphones, especially pronounced in P-APF-SS and B-APF-SS samples, and a decrease in the content of organic sulphonic acids. It can be mentioned as well that the increased intensity of the peak for di-aryl sulphoxides/sulphones after *SS* treatment of B-APF sample is probably related to the increase in the content of di-aryl sulphoxides. This conclusion is based on the shift of the peak apex to the lower temperatures, at about 500°C.

With regard to M-APF, M-APF-PC and M-APF-SS samples, SO^+ and SO_2^+ profiles in an inert atmosphere clearly demonstrate again different trends in the lower temperature region up to 400°C. A similar phenomenon has been observed during pyrolysis in a reducing atmosphere. In the temperature range up to 400°C, the maximum of the peak in *m/z* 48 profiles of M-APF, M-APF-PC and M-APF-SS samples is again shifted to higher temperature (with about 20°C) compared to the maximum of the peak in *m/z* 64 profiles. Most likely it is due to CH_3SH^+ fragment presence. Remarkable is also the higher intensity of *m/z* 48 for M-APF-SS compared to *m/z* 64 in the temperature range up to 400°C (lower temperature peaks in Figure 4-7B.2), probably confirming again the fact that *m/z* 48 can also refer to CH_3SH^+ fragment, originating from thiols thermal decomposition. In addition, the presence of thiols has already been proved by H_2S^+ profiles (see Figure 4-2B and Figure 4-6B). For M-APF and M-APF-PC samples, the same tendency is registered, but less pronounced. Nevertheless, we still can discuss the peaks in SO^+/SO_2^+ profiles of M-APF, M-APF-PC and M-APF-SS samples. There are two peaks. The first one, at about 260°C, refers to organic sulphonic acids presence, while the second one, at 550-600°C, corresponds to di-aryl sulphoxides/sulphones. After treatments with *PC* and *SS*, changes in di-aryl sulphoxides/sulphones presence are observed.

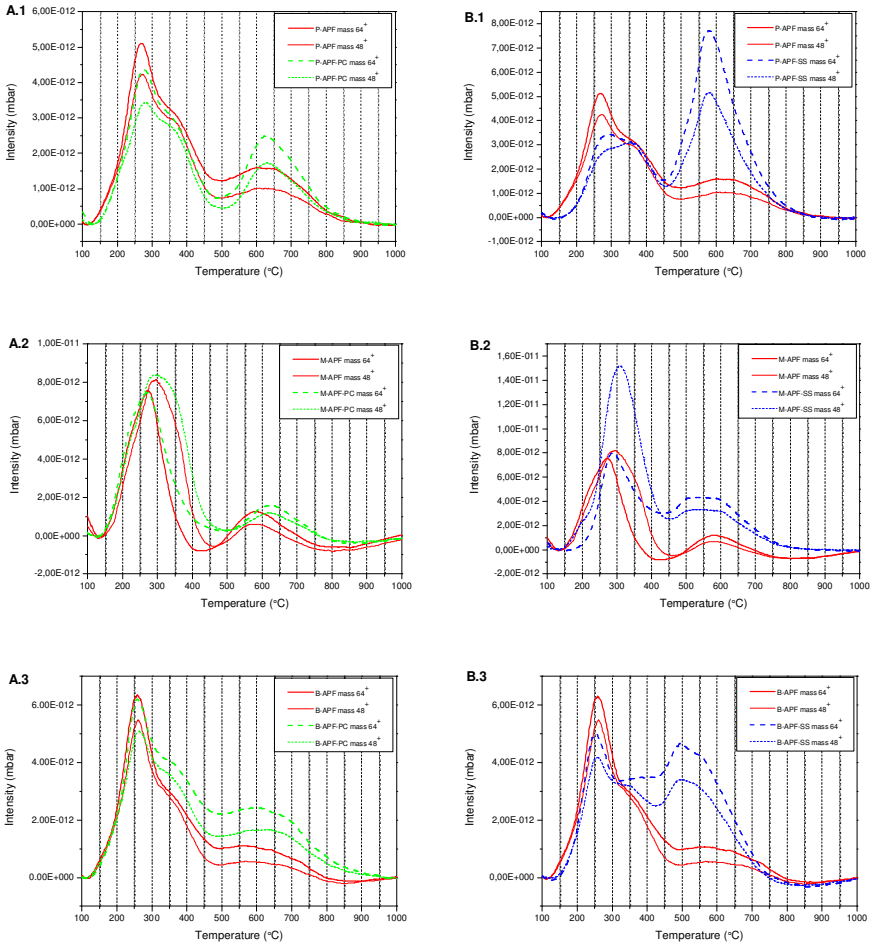


Figure 4-7 AP-TPR-MS (He), *m/z* 48 (SO^+) and *m/z* 64 (SO_2^+) kinetograms of Pirin (1), Maritza East (2) and Beypazari (3) coals: A) APF samples compared to APF-PC samples; (B) APF samples compared to APF-SS samples

Noteworthy is also the fact that the peaks maximizing at 650°C in AP-TPR-MS (He) profiles of *m/z* 64 most probably do not contain a contribution from $C_5H_4^+$ (originating from coal aromatic structure decomposition). This assumption is based on AP-TPR-MS (He) profiles of typical aromatic CH-fragments, in which a peak maximizing at about 400°C is observed for all samples under consideration (figures are not shown). Unlike pyrolysis in reducing atmosphere, there are no second peaks maximizing at 650°C in these profiles.

4.3.3 AP-TPR experiments coupled "off-line" with TD-GC/MS

AP-TPR technique with its extension "off-line" TD-GC/MS is also applied to receive additional information for S_{vol}. In this study the system is upgraded through the use of combination of special sorbents selective for sulphur compounds, namely Tenax/Carbopack B/Carbosieve SIII. Certainly, broader range of S_{vol} as well as SO_2, COS and CS_2 are identified and quantified, by the application of sulphur compounds selective adsorbents. H_2S is not adsorbed by them.

The recovery of the sulphur determined by AP-TPR "off-line" TD-GC/MS is shown in Table 4-4. It is calculated on dry, ash free base by using S_o as reference value. The *wt*% varied in the range of 7.2÷21.5%. With respect to APF samples, the highest presence of S_{vol} in terms of $\mu gS/g^{daf}$ is registered for M-APF sample and followed by B-APF and P-APF samples. This observation correlates with maturity of the coal samples which increases in the same sequence. S_{vol} content is reduced in all *PC* treated samples and B-APF-SS sample, while an increase in S_{vol} content is registered for P-APF-SS and M-APF-SS samples. It can be assumed that *SS* treatment of the last two samples provokes some matrix rearrangements, resulting in improved volatiles release and in "softer", partly and specific oxidation of the sulphur forms.

Table 4-4 Total sulphur in organic compounds determined by AP-TPR "off-line" GC/MS

Sample	S_{vol}	
	$\mu g\ S/g^{daf}$	Re^{So}(wt%)
P-APF	2 768.7	7.2
P-APF-PC	2 505.3	8.5
P-APF-SS	4 453.7	13.4
M-APF	4 917.8	13.5
M-APF-PC	3 641.6	12.8
M-APF-SS	7 495.5	21.5
B-APF	3 575.7	11.5
B-APF-PC	2 376.7	7.9
B-APF-SS	2 247.7	8.8

Results for registered and quantified sulphur containing volatiles are shown in Table 4-5 and Table 4-6, and Figure 4-8. Analyzing the quantitative results received by AP-TPR "off-line" TD-GC/MS experiments, it can be concluded that among the released volatile sulphur compounds, SO_2 is in the highest amount. It originates from decomposition of oxidized sulphur species. Their presence in APF samples is logical since they are treated with diluted nitric acid [14]. The content of SO_2 is approximately equal for all APF samples and decreases in all APF-PC samples. This is an indication for the destruction of oxidized sulphur species during *PC* biotreatment. It is most pronounced in B-APF-PC sample, where SO_2 content decreases with 36.8% compared to B-APF sample. As it is mentioned in Chapter 3 oxidative mechanism for the biodesulphurization of organic sulphur compounds by *PC* has been already described [17]. The metabolism proceeds from DBS to dibenzyl sulphoxides followed by dibenzyl sulphone, prior to C-S bond cleavage. Since the samples in this study are preliminary oxidized (treated with nitric acid), probably C-S bond cleavage is the main process that occurs during *PC* treatment. It favours *in-situ* SO_2 release or soluble oxidized sulphur products formation. This results in a decrease in the amounts of oxidized sulphur compounds present in coal. Respectively, lower content of SO_2 is registered during AP-TPR experiment.

As a result of *SS* biodesulphurization, the content of SO_2 is significantly higher for P-APF-SS and M-APF-SS samples. This is an indication for the progress of oxidation processes during *SS* biodesulphurization of these samples.

In order to confirm attributions of the peaks in *m/z* 48 and *m/z* 64 AP-TPR-MS (H_2) profiles of the investigated samples, evolution profiles of SO_2 monitored by AP-TPR "off-line" TD-GC/MS are also traced in Figure 4-9. The analysis confirms peaks assignment but some peculiarities are depicted. Namely, dissimilarities in the ratio of oxygen-sulphur containing organic forms registered with MS (on-line) and TD-GC/MS (off-line) can be noticed (see Figure 4-5 and Figure 4-9). This is one more proof, that *m/z* 48 and *m/z* 64 AP-TPR-MS (H_2) profiles are not only characteristic for SO^+ and SO_2^+. They can also contain a contribution from other fragments, which are coal pyrolysis products. Therefore AP-TPR-MS (H_2) profiles for *m/z* 48 and *m/*z 64 should be handled with care for the confirmation of the presence of oxidized sulphur forms.

Table 4-5 Sulphur in organic compounds determined by AP-TPR "off-line" TD-GC/MS, in μg S/g^{daf}

Compounds	Structures in Fig. 8	Pirin			Maritza East			Beypazari		
		APF	APF-PC	APF-SS	APF	APF-PC	APF-SS	APF	APF-PC	APF-SS
Sulphur dioxide	I	1 525.9	1 259.8	3 147.1	1 546.7	1 063.5	3 448.2	1 544.4	975.6	1 037.4
Carbonyl sulphide	II	317.6	343.0	190.0	311.3	208.7	152.7	266.3	0.4	120.1
Carbon disulphide	III	68.2	17.0	144.0	624.8	654.7	982.7	402.6	232.0	62.1
Methyl thiocyanate	IV	179.0	170.9	104.0	683.8	351.9	72.0	381.1	408.6	332.9
Methyl isothiocyanate	V	19.6	10.6	12.2	118.2	73.3	5.0	124.6	100.9	94.3
Methyl mercaptane	VI	-	-	-	-	-	424.0	-	-	-
Dimethyl sulphide	VII	-	-	-	245.1	181.9	0.0	7.2	5.4	31.3
Methylene bis(methyl sulfide)	VIII	-	-	-	-	-	15.7	-	-	-
Ethane, 1,1-bis(methylthio)-	IX	-	-	-	-	-	5.6	-	-	-
Dimethyl disulphide	X	38.6	51.0	68.5	834.3	546.2	1 421.8	89.8	73.9	42.5
Methyl ethyl disulphide	XI	5.8	2.4	0.0	30.9	7.3	9.2	6.7	1.4	5.9
Diethyl disulphide	XII	1.7	0.7	0.0	-	-	-	-	-	7.8
Butyl methyl disulfide	XIII	-	-	-	-	-	5.3	-	-	-
Methyl methylthiomethyl disulfide	XIV	-	-	-	-	-	26.0	-	-	-
Dimethyl trisulphide	XV	16.2	17.4	94.2	200.9	236.3	385.4	19.7	26.2	19.6
1,2,4-Trithiolane	XVI	-	-	-	-	-	10.8	-	-	-
Dimethyl tetrasulphide	XVII	-	-	-	29.9	37.5	59.0	13.5	7.0	0.9
Hexathiepane	XVIII	-	-	-	-	-	2.3	-	-	-
Σ of aliphatics		260.9	253.0	278.9	2 143.1	1 434.4	2 442.3	642.6	623.4	535.2
Dimethyl sulphoxide	XIX	-	-	-	-	-	51.3	-	-	-
3-methylthio propanal	XX	-	-	-	-	-	17.1	-	-	-
Methyl methanesulfunate	XXI	-	-	-	10.6	11.8	21.1	1.8	3.4	0.0
Elemental sulphur	XXII	-	-	-	-	-	-	-	1.9	24.3

Table 4-6 Sulphur in aromatic compounds determined by AP-TPR "off-line" TD-GC/MS, in μg S/g^{daf}

Compounds	Sturctures in Fig. 8	Pirin			Maritza East			Beypazari		
		APF	APF-PC	APF-SS	APF	APF-PC	APF-SS	APF	APF-PC	APF-SS
Thiophene	XXIII	68.8	69.5	40.9	30.5	19.4	33.4	41.5	36.1	58.0
Thiophene - C_1		187.2	131.1	219.8	102.2	156.4	103.9	247.0	196.1	164.2
Thiophene - C_2		107.6	150.9	146.0	38.9	25.6	49.9	131.0	125.8	120.6
Thiophene - C_3		21.2	22.8	90.8	2.0	2.6	8.8	39.9	41.0	53.3
Thiophene - C_4		8.2	10.4	21.9	-	-	-	8.6	9.1	12.2
Σ of thiophenes		393.0	384.7	519.4	173.6	204.1	196.0	468.0	408.1	408.3
Benzothiophen	XXIV	131.8	157.2	132.5	70.4	49.8	94.3	129.1	83.1	40.7
Benzothiophen - C_1		59.1	76.9	40.0	31.6	14.6	70.9	92.5	38.0	17.8
Benzothiophen - C_2		12.2	13.7	1.8	5.6	0.0	18.8	26.5	9.7	1.8
Benzothiophen - C_3		-	-	-	-	-	-	1.9	1.1	0.0
Σ of benzothiophenes		203.1	247.8	174.3	107.6	64.4	184.0	250.0	131.9	60.3
Σ of aromatics		596.1	632.5	693.7	281.2	268.5	380.0	718.0	540.0	468.6

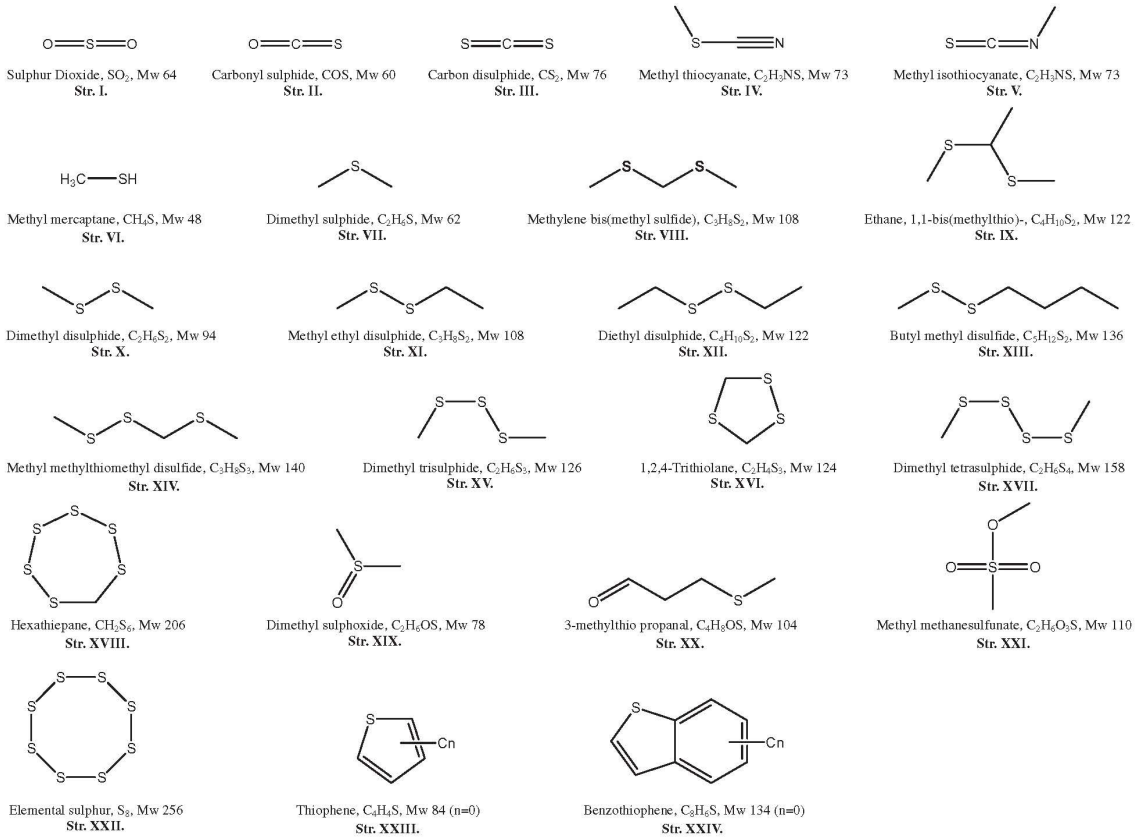


Figure 4-8 Sulphur containing structures of volatiles registered by AP-TPR "off-line" TD-GC/MS

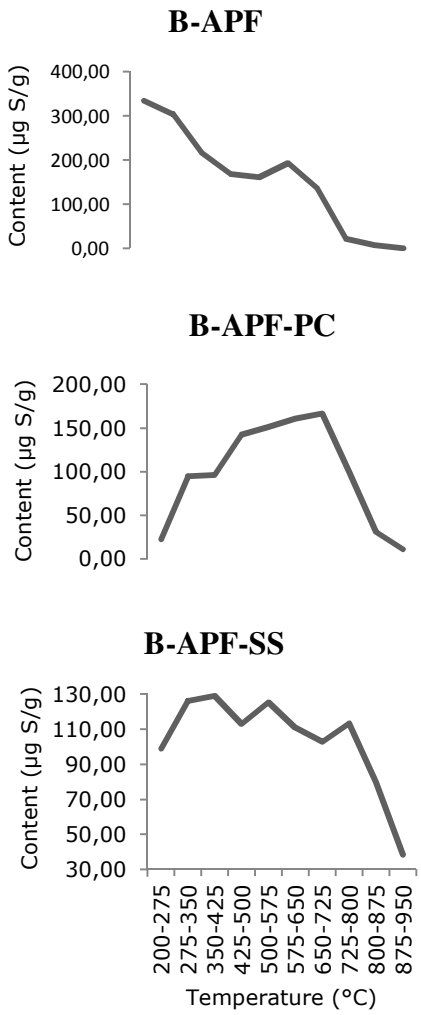


Figure 4-9 Pyrograms of SO_2 registered by AP-TPR "off-line" TD-GC/MS of B-APF, B-APF-PC and B-APF-SS

In the pyrograms of the samples under study, carbonyl sulphide (COS) and carbon disulphide (CS_2) are also registered (Table 4-5). It is known that during coal pyrolysis, they can be produced in small amounts. Since COS is formed by reaction of CO and elemental sulphur, and since considerable amount of CO are generated during coal pyrolysis, it is likely that some elemental sulphur, originally present in coal or formed by pyrite pyrolysis, reacts with CO to COS. Additionally COS can be produced by pyrolysis of organic sulphoxides as well

[13]. Regarding CS_2, it can be formed by reaction of H_2S with coal. Similar performances are observed when H_2S react with methane or with COS [13,18]. During pyrolysis, some sulphur-containing organic compounds may also be involved in the formation of CS_2 and COS [19]. From the foregoing, it is obvious that the sources for the formation of CS_2 and COS during pyrolysis are very diverse. Hence, the registered changes in the content of these pyrolysis products as a result of applied biotreatment cannot be interpreted unambiguously and do not significantly attribute to clarify the biodesulphurization mechanism.

Aliphatic sulphur in the form of thiocyanates, thiols, sulphides, disulphide, trisulphide, tetrasulphide (Table 4-5), etc. as well as aromatic sulphur in the form of thiophene, substituted thiophenes, benzothiophene and substituted benzothiophenes (Table 4-6) are registered in AP-TPR "off-line" TD-GC/MS pyrograms. The highest presence of aliphatic sulphur is found in M-APF followed by B-APF and P-APF (see Table 4-5). This sequence again well mimics with coal maturity. It can be seen that the content of volatile aliphatic sulphur decrease significantly for M-APF-PC sample compared to M-APF sample, while for P-APF-PC and B-APF-PC coals, this decrease is negligible. Aliphatic sulphur is apparently also attacked during *PC* treatment *via* oxidative mechanism. Concerning *SS* treated coals, the content of volatile aliphatic sulphur increases slightly for P-APF-SS and M-APF-SS coals, while in the case of B-APF-SS it decreases. The increase is mainly explained by the increase in polysulphides presence. In the case of M-APF-SS, a significant amount of methyl mercaptane is registered as well. Similar results are obtained from H_2S AP-TPR-MS profiles in H_2 and He atmospheres of M-APF-SS sample. It is worth noting that methyl thiocyanate and methyl isothiocyanate presence is reduced in all biotreated samples compared to APF coals. Probably during *PC* and *SS* biotreatments, nitrogen containing compounds are also attacked and removed.

In general, high content of organic polysulphides in the pyrolysis products is associated with the increased content of elemental sulphur, which reacts with organic matter during pyrolysis and forms these products. In our additional study (see Chapter 5), elemental sulphur in the samples under consideration has been quantitatively determined. It has been established that during biodesulphurization with *SS*, the content of elemental sulphur is reduced to about 52÷54% for P-APF-SS and M-APF-SS coals. From the literature it is

confirmed that such a reduction is possible and it is a result of elemental sulphur oxidation to sulphate [20]. At present, bearing in mind the increased content of polysulphides, the question arises whether a reason for the reduction of elemental sulphur is not an in-situ interaction of elemental sulphur with organic matter under the conditions of the biotreatment with *SS*, i.e temperature of 70 ºC. This item requires further research to be resolved.

For B-APF coal the highest presence of volatile aromatic sulphur in the case of APF samples is registered (Table 4-6). After bioprocessing with *PC* and *SS* of B-APF sample, volatile aromatic sulphur decreases, i.e. thiophenes and benzothiophenes. Different is the case for *PC* and *SS* biotreated M-APF and P-APF coals. For P-APF-PC sample the content of thiophenes decreases and the content of benzothiophenes increases, while for P-APF-SS the content of thiophene increases and benzothiophenes decreases. For *PC* and *SS* biotreated M-APF samples just the opposite tendency is observed. An increase and decrease in the content of thiophenes and benzothiophenes, respectively, is registered for M-APF-PC sample, while a decrease and increase in the content of thiophenes and benzothiophenes, respectively, is recognized for M-APF-SS coal. Hence, at the moment a systematic trend in aromatic sulphur changes, as a result of *PC* and *SS* biotreatments, can not be depicted. However, there is an oposite tendency in the change of thiophenes and benzothiophenes in the case of M-APF-PC, M-APF-SS, P-APF-PC and P-APF-SS samples. If there is an increase in the content of thiophene, then the content of benzothiophenes decreases and vice versa. These observations evoke a supposition that the changes in thiophenes and benzothiophenes for *PC* and *SS* treated P-APF and M-APF samples are interdependent. Noteworthy is the fact that evolution profiles of thiophenes and benzothiophenes, and their substituted homologues monitored by AP-TPR "off-line" GC/MS, have similar pattern of distribution with those obtained with AP-TPR-MS ("on-line") by monitoring the typical ion fragments.

4.4 Conclusions

Total sulphur desulphurization in the range of 25.3÷54.2% after chemical treatments is achieved. Inorganic sulphur (S_p and S_s) is mainly attacked. Registered pyritic sulphur desulphurization effects vary in the range of 59.6÷77.4%.

Biodesulphurization effect for *PC* and *SS* treated APF samples can be determined by comparison with APF samples. Our study demonstrates that higher biodesulphurization effect is attained for APF coal samples treated with white rot fungus "*Phanerochaeta Chrysosporium".* An extra maximum S_t biodesulphurization of 24.2% is found in the case of M-APF-PC coal sample, and 24.1% for P-APF-PC coal. Extra maximum S_o biodesulphurization with *PC* is achieved for the same coal samples: M-APF-PC (22.0%) and P-APF-PC (23.8%). In the case of B-APF-PC coal, extra S_t biodesulphurization effect is relatively lower (10.3%) and is much more effective toward extra S_p removal (71.1%). By *"Sulfolobus Solfataricus"*, extra maximum S_t and S_o biodesulphurization effects of 16.9% and 18.3%, respectively, are determined for B-APF-SS coal sample.

AP-TPR technique coupled with different detection systems (MS and TD-GC/MS) gives the opportunity to be tracked a broad range of organic sulphur species present in studied coal. Organic sulphur alterations as a result of the applied biotreatments have been demonstrated. The information obtained by AP-TPR "off-line" TD-GC/MS is enriched due to special sorbents application. As a consequence, a greater number of sulphur-containing compounds in S_{vol} are identified and quantified.

AP-TPR study inclines us to suppose that degradation processes of complex sulphur species accompanied by oxidation processes take place in a different ways during *PC* and *SS* biotreatments. The observed alterations are related not only to the type of sulphur functionalities and structural peculiarities of the samples, but also to the different preferences of the used microorganisms towards sulphur functionalities.

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Chapter 5 Evaluation of elemental sulphur in biodesulphurized low rank coals

The results discussed in this chapter have been published in a peer-reviewed paper:
Gonsalvesh L, Marinov SP, Stefanova M, Carleer, Yperman J. Evaluation of elemental sulphur in biodesulphurized low rank coals. Fuel 2011; 90:2923-30.

5.1 Introduction

The development of new tactics for sulphur compounds removal from coal depends, in part, upon a knowledge of their chemical constitution. A fundamental requirement of any research into desulphurization is an accurate method for assessment the various forms of sulphur in coal [1]. Besides the fact that the determinations of the different sulphur types in coal concern the most important analytical parameters used to characterize coal, this information is indispensable, both for evaluation of the effects of desulphurization methods and for selection of suitable coals for sulphur species-specific desulphurization process.

It has already been discussed in Chapter 1 that sulphur in coal is recognized as present in three forms: (1) inorganic sulphate (S_s), mainly as ferrous sulphate and gypsum; (2) inorganic sulphides (S_p), generally as ferrous sulphide (pyrites), although sulphides of zinc and lead may occur in some coals; and (3) as organic sulphur compounds (S_o) [2-3]. A forth sulphur form should be mentioned as well - elemental sulphur (S_{el}) is detected in weathered coal.

There are different theories for the appearance of S_{el} in coal. Some of the authors emphasize the importance of S_{el} in the formation of pyritic and organic sulphur compounds and suggest that the S_{el} found in coal today is a primary substance formed during coal formation [3]. However, this view is still under discussion. Duran et al. [4] used extraction and GC analysis to determine S_{el} in a suite of U.S. coals. They found that S_{el} (0.03-0.17%) is present in coal that has been exposed to the atmosphere, but it is absent in pristine samples that have been processed and sealed under a nitrogen atmosphere. According to the same

study, S_{el} is not a natural constituent of coal but rather a product of atmospheric oxidation of pyrite. Beyer et al. [5] prove that, microbial desulphurization in acidic environment results in S_p decreasing and S_{el} increasing with time, whereas the organic sulphur remains unchanged. They suggest that microbial oxidation of pyrite produces ferric sulphate and that the simultaneous inorganic reaction of ferric iron with pyrite produces S_{el} and ferrous iron, as follows:

$2Fe^{3+} + FeS_2$ (pyrite) $\longrightarrow 3Fe^{2+} + 2S_{el}$

There are some consequences when the coal under consideration contains S_{el} [6]. This is related to the fact that there are direct standard analytical procedures for total sulphur (S_t), S_p and S_s determinations. In the absence of standard analytical procedure for direct measurement of the S_o, the latter is calculated indirectly, i.e. by subtracting the sum of inorganic sulphur (S_s+S_p) from S_t. Thus cumulative errors in determining S_t, S_s and S_p are reflected in the values of S_o. Moreover if S_{el} is present in coal it will be included into the so called organic sulphur and overestimation of the organic sulphur magnitude will take place.

Although S_{el} actively participates in many biogeochemical processes, the studies devoted to this species are rather rare, mainly due to the limitations of the few available analytical methods for its determination [7]. There are several attempts for quantification of S_{el} in coal and related material. X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS) and Raman spectroscopy are among the methods used to detect S_{el} at the mineral surfaces [8-9] and coal [5], but accurate quantification of S_{el} with these techniques is rather difficult. Chromatographic methods to solve the problem have been described as well. Generally the procedures for the estimation of S_{el} in coal and environmental samples encompass the extraction of S_{el} and subsequent quantification by spectral or chromatographic approaches. Respectively, appropriate extraction method should be developed. For the purpose Gryglewicz et al. [6] test the effect of different solvents and time on the efficiency of S_{el} extraction. A protocol for S_{el} quantification in coal based on the extraction with cyclohexane for 6 hour with it subsequent quantitative analysis in the extract by GC/MS is proposed. Duran et al. [4] use GC coupled with a Hall electrolytic conductivity detector with a high selectivity to sulphur. Another technique based on perchloroethylene

extraction and subsequent analysis by high-performance liquid chromatography (HPLC) on a C_{18} reverse-phase column with UV detection is used by Buchanan et al. [10]. According to Steudel [11] the so-called C_{18} stationary phase turn out to be the best in the case of sulphur homocycles analysis. The author claims that the best detection for S_{el} is UV absorption at/or near 254 nm as all compounds containing S-S bonds strongly absorb in this wavelength region.

Since our main interest is directed towards quantitative and qualitative specification of organic sulphur variations as a result of biotreatments, elemental sulphur assessment is unavoidable. Therefore, the aim of the study described in current chapter is to apply appropriate analytical procedure for S_{el} determination and to study the effect of biotreatments on the contents of S_{el} in coals. Development of a precise procedure for S_{el} measurement will give us ground to attain a better sulphur balances for initial and biotreated samples.

5.2 Experimental section

5.2.1 Samples under study

Humovitrain Maritza East (M), Pirin sub-bituminous coal (P) and Beypazari lignite (B) are used in this study. The samples are demineralized, depyritized and subsequently biotreated by *PC* and *SS* as described in Chapter 2. The proximate and ultimate analysis of investigated samples, i.e. S_t and sulphur forms are included in Table 4-1. In this table, organic sulphur is calculated by the difference from S_t and the sum of S_p and S_s. Thus, the calculated S_o values comprises S_o and S_{el}.

5.2.2 Extraction procedure

The following extracts are under consideration: (i) cyclohexane (c-He) extracts; (ii) soluble in chloroform part (bitumen) and its fraction of neutral oils; (iii) c-He extracts of the insoluble in chloroform part. The extraction sequence is illustrated in Figure 5-1. Separation and fractionations are included in it as well.

The extractions are performed at the following experimental conditions:

5.2.2.1 Extraction with c-He

A 500 mg coal sample (particle size < 0.063 mm) is extracted in E-flask. The extraction is performed with 50 ml c-He at ambient temperature and stirring with magnetic stirrer for 6 h. 1000 µL perchlorethylene (PCE) is added to the dry extract and S_{el} presence is determined by HPLC. One subsequent extraction with fresh portion of c-He is performed as well. The dry extract is anylized after disolving in 600 µL PCE.

5.2.2.2 Extraction with chloroform and assessment of the soluble part (bitumen)

The extraction is carried out for 6h on a 4 g of coal sample with 50 ml of chloroform reflux at 70ºC and stirring. The obtained bitumen is divided into asphaltenes and maltenes (neutral oils) by asphalthene precipitation (4:1, v/v n-Hexane (n-He):Benzene (Bz)). The soluble part (neutral oils), are fractionated by SiO_2 chromatography into aliphatic (n-He eluent), aromatic (Bz eluent) and polar fractions (Acetone (Ac) eluent).

A half of the aliphatic fraction is dissolved in 600 µL PCE and subjected to S_{el} determination by HPLC. Serial following dillutions with PCE are performed to set the concentration of the sample within the linear range of standard solutions.

5.2.2.3 Subsequent extraction with c-He of the insoluble part, obtained after chloroform extraction

The extraction is performed in a similar way as described in section 5.2.2.1.

5.2.3 HPLC Analysis

HPLC procedure for S_{el} determination has been applied. All analyses are performed on an Agilent HPLC system with 20 µL injection volume and a diode array detector operating at 254 nm with an 8 nm bandwidth. A Varian Chromosphere 5µ C_{18} reversed phase column (4.6 mm x 250 mm) is used with an eluent 95:5 methanol (HPLC grade): water at a flow rate of 1 ml/min.

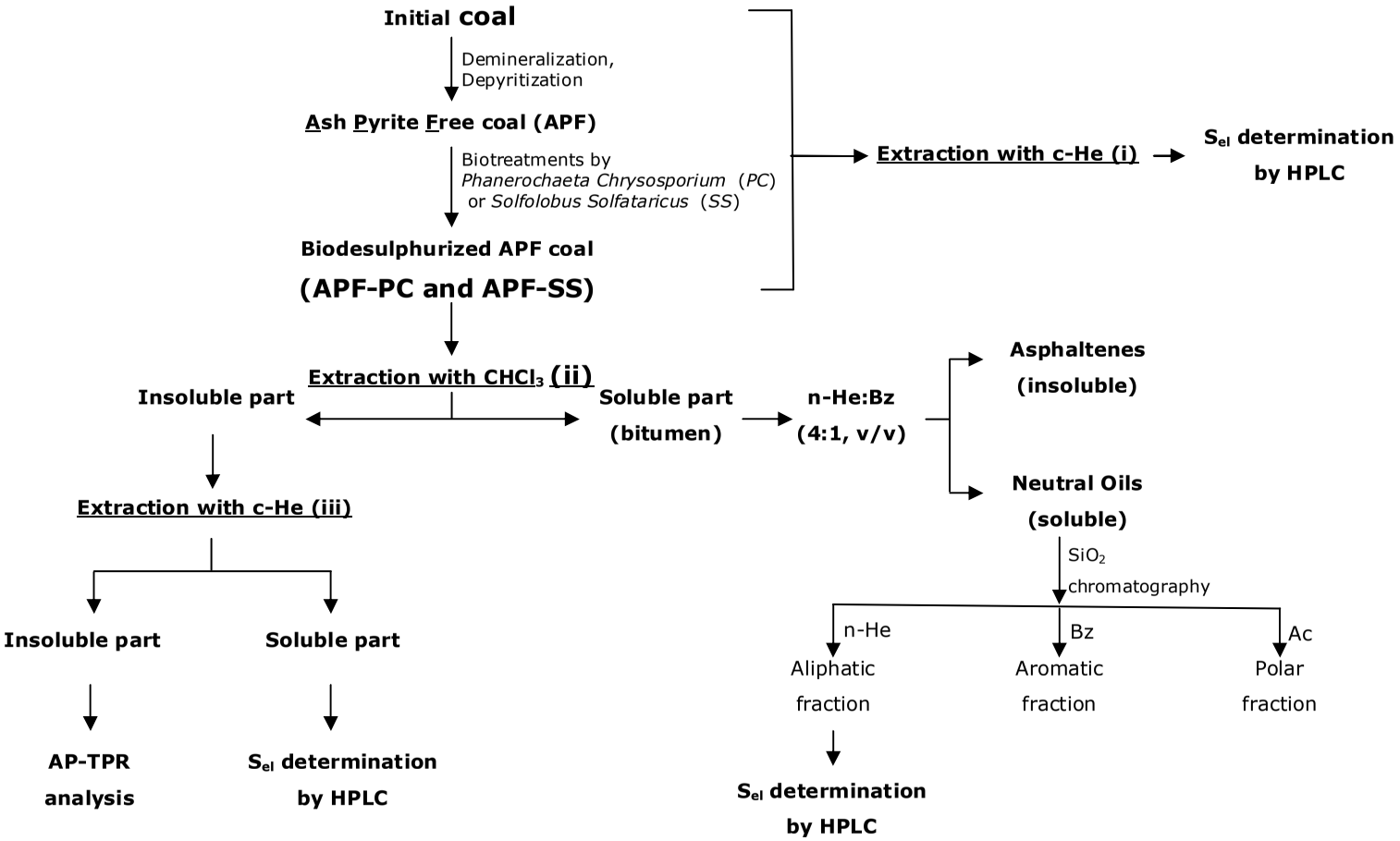

Figure 5-1 Experimental strategy

5.2.4 Elemental Sulphur Standards for HPLC

The external standard is prepared using commercially available S_{el} (Aldrich, 99.998%). Namely, 24 mg S_{el} is dissolved in a beaker containing 20-30 ml of PCE (Across, spectrophotometric grade). The solution is stirred (~1h) and quantitatively transferred to a 100 ml volumetric flask and diluted with PCE to the mark. A final concentration for the stock solution of 240 mg/L is obtained. Subsequent dilutions of the stock solution produce additional standards varying in the concentration range of 0.1÷240 mg/L.

5.2.5 Analysis of extracted solid residue

AP-TPR "off-line" TD-GC/MS technique is used for identification of the volatile species released during pyrolysis of extracted solid residues [12-14]. Pyrolysis experiments are performed in an inert atmosphere. AP-TPR reactor is connected to ice-cooled metalic adsorption tubes with SilcoSteel Coating, filled with Tenax/Carbopack B/Carbosieve SIII (Markes) as adsorbents. They are desorbed systematically and analyzed by TD-GC/MS as described in Chapter 2.

5.3 Results and Discussion

5.3.1 Analysis of standards

The quantity of S_{el} extracted from coal is determined on the base of calibration curve prepared for standard solutions of S_{el} in PCE. Figure 5-2 presents the overlaid chromatograms of series standard solutions of S_{el} in PCE. The peak approximately at 11.4 – 12.1 min is assigned to S_{el}. Its area increases with increasing concentration of S_{el}. The broad peak registered at 4 – 6 min as well as the small peak around 3.6 min are assigned to PCE. The negligible peak appearing around 3.1 min could be attributed to acetone, a rest from glassware washing. No other peaks are observed in the studied range of elution region up to 15 min.

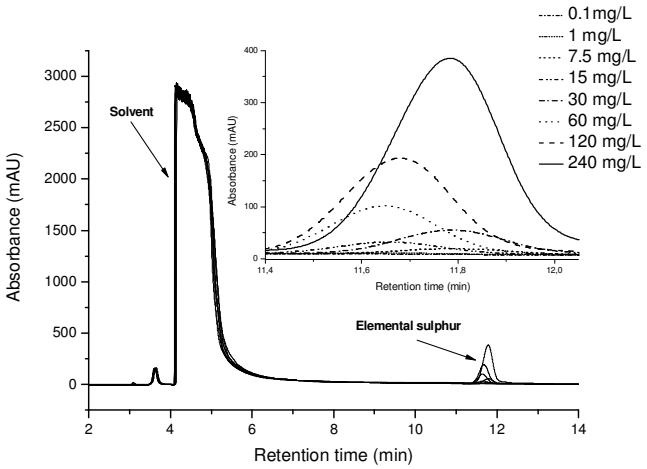

Figure 5-2 Overlaid HPLC chromatograms for a series of standard solutions of S_{el} in PCE with concentration range of 0.1 – 240 mg/L. The inset represents the chromatograms in the retention time window of 11.40 – 12.05 min

Figure 5-3 shows the calibration curve for a series of S_{el} standards. The points represent the area of the S_{el} peak for three series standard solutions (I, II, III) with concentrations in the range 0.1 – 240 mg/L. Linear regression analysis of the calibration points for I, II and III series result in correlation coefficients of 0.99999, 0.99980 and 0.99985, respectively. The drawn lines through all the points represent the best linear fit using the method of least squares and indicate for the high repeatability of HPLC with UV-VIS detection.

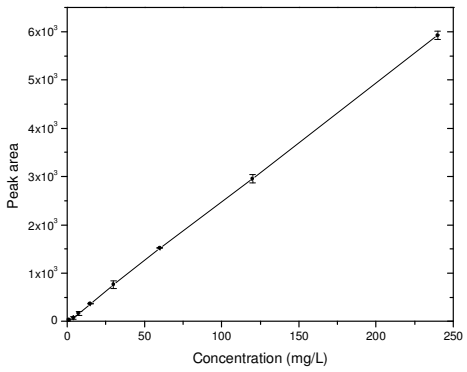

Figure 5-3 Calibration curve for three series of S_{el} standard solutions

5.3.2 Analysis of the extracts

At ambient temperature in non-polar solvents S_{el} dissolves without decomposition [15]. As a rule, the solubility of the sulphur allotropes in organic solvents decreases with molecular size increasing [16]. Carbon disulphide is by far the best solvent, followed by toluene and dichloromethane, while cycloalkanes are suitable for the smaller ring molecules only as far as ambient temperatures are concerned. It should be mentioned that the data cited here refers to non-associated free S_{el}. When it comes to extraction of the S_{el} from environmental samples, presented data for S_{el} extractability of various organic solvents [6,8] are different from those listed for the solubility of S_{el} in organic solvents [15]. Moreover, it is proved that S_{el} extractability with organic solvents depends on S_{el} surroundings [8]. Therefore to use chromatography for S_{el} analysis in any environmental sample an appropriate extraction method should be developed. In the literature several reliable organic solvents are proposed. According to Buchanan et al. [10,17] PCE is an excellent solvent for extracting S_{el} from bituminous coal. Temperature and contact time are important variables for the yield of S_{el} by PCE extraction for the reason of reaction between sulphur and coal. For three different rank Polish coals (lignite to medium volatile bituminous) Gryglewicz et al. [6] test a number of solvents at different extraction times to select the solvent with the highest S_{el} extractability. It is determined that c-He and PCE have demonstrated the highest effectiveness in the extraction of S_{el} for 6h. A prolongation of extraction procedure to 10h results in a lowering of the S_{el} content in the PCE extract whereas for c-He, the value remains the same. According to the same authors this indicates that for the S_{el} extraction, c-He is better than PCE. An extraction time of 6h is enough for S_{el} extraction. Therefore in the present study, c-He is selected as a proper solvent for the extraction of the S_{el}.

The changes in the soluble coal part (bitumen) and its neutral oils are also under consideration. It is well known that S_{el} eluted with the aliphatic fraction of the neutral oil. As a result of aforementioned reasons the investigation strategy illustrated in the outline in Figure 5-1 is applied.

5.3.2.1 Extraction with c-He (i)

S_{el} content in the studied sample extracted for 6h with c-He as well as S_{el} content extracted with the subsequent 4h c-He extraction is presented in Table 5-1. As can be seen, the content of determined S_{el} by HPLC UV-VIS varied in the range of 0.001 – 0.032 wt%, calculated on dry, ash free base (daf). The highest amount of S_{el} is detected in the initial samples. This is probably related to the progress of oxidation processes (weathering), supported by the increased sulphate content (Table 4-1) of the initial samples. According to some authors, a sulphate sulphur content exceeding 0.3% is an indication for the progress of oxidation processes during which part of pyrite sulphur is transferred into sulphate sulphur [18].

Table 5-1 Content of S_{el} in c-He extracts of untreated by other organic solvents coals

Sample	6h c-He extraction	Subsequent 4h c-He extraction	Σ	**S wt%**
	µg S/g ^{daf}	µg S/g ^{daf}	µg S/g ^{daf}	
P-in	27.9	8.6	36.5	0.004
P-APF	8.7	2.7	11.4	0.001
P-APF-PC	7.3	2.4	9.7	0.001
P-APF-SS	7.7	2.3	10.0	0.001
M-in	316.5	6.2	322.7	0.032
M-APF	26.5	2.9	29.4	0.003
M-APF-PC	15.4	2.3	17.7	0.002
M-APF-SS	8.5	0.2	8.7	0.001
B-in	182.3	18.2	200.5	0.020
B-APF	72.8	48.3	121.0	0.012
B-APF-PC	31.6	14.5	46.2	0.005
B-APF-SS	48.8	25.7	74.5	0.007

It is known that S_{el} can be considered as a product of atmospheric oxidation of pyrite by water and oxygen, and in-situ bacteriological action or both. There are two basic reactions proposed [19]:

The first one takes place during microbial oxidation of pyrite;

$2Fe^{3+} + FeS_2 \rightarrow 3Fe^{2+} + 2S_{el}$

The second S_{el} producing reaction from pyrite is the following;

$$4FeS_2 + 6H_2O + 3O_2 \rightarrow 4Fe(OH)_3 + 8S_{el}$$

In both cases, S_{el} is an intermediate product in a series of reactions that ultimately produce sulphate (SO_4^{2-}). Detectable amounts of S_{el} can also be formed on the pyrite surfaces in suitable condition. In our studies, it appears that the amount of detected S_{el} as well as the content of S_s correlates with the content of S_p (Figure 5-4). The highest content of S_{el} is determined in M-initial sample, characterized by the highest content of S_p and S_s, then in B-initial sample and the lowest content in P-initial sample. In consequence of demineralization and depyritization treatments with mineral acid the content of S_{el} is reduced in the range of 40 – 91% related to the oxidation potential of the used mineral acids. Nitric acid, characterized by a strong oxidation potential to pyrite, is widely used for the extraction of pyritic sulphur from coal. The reaction between HNO_3 and pyrite is strongly dependent on the temperature and concentration of the acid. Reaction equations cited in the literature for the HNO_3 – pyrite system are the following [20]:

$$FeS_2 + 4HNO_3 \rightarrow Fe(NO_3)_3 + 2S_{el} + NO + 2H_2O \qquad 5\text{-}1$$

$$2HNO_3 + S_{el} \rightarrow H_2SO_4 + 2NO \qquad 5\text{-}2$$

$$6FeS_2 + 30HNO_3 \rightarrow 3Fe_2(SO_4)_3 + 3H_2SO_4 + 30NO + 12H_2O \qquad 5\text{-}3$$

It is obvious that pyrite oxidation by the HNO_3 produces simultaneously S_{el} and sulphate by separate oxidation processes (reactions 5-1 and 5-3). Produced S_{el} is an intermediate product which is subsequently oxidized to sulphate (reaction 5-2). Respectively "naturally" occurring S_{el} in initial coal is also oxidized by the HNO_3 through reaction 5-2.

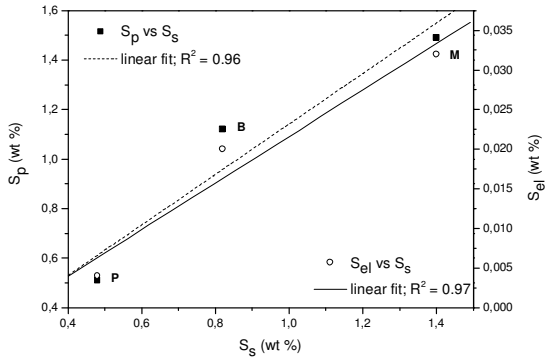

Figure 5-4 Correlations between S_p/S_s and S_{el}/S_s for initial coals (P – Pirin sub-bituminous coal, B – Cayirhan-Beypazari lignite, M – Humovitrain Maritza East)

The results for S_{el} in the c-He extracts of the studied samples gave us ground to assume that due to applied biotreatments the amount of S_{el} decreases in all APF samples biotreated by *PC* and *SS*.

5.3.2.2 Extraction with chloroform and assessment of the soluble part (bitumen), (ii)

The results for separation of bitumens, neutral oils and their fractionation in aliphatic, aromatic and polar fraction are included in Table 5-2. Determined content of S_{el} as a part of the aliphatic fraction is shown as well. It varies in the range of 15 – 76 wt% calculated on dry ash free bases.

HPLC determined S_{el} in the aliphatic neutral oil fraction is higher than the content of S_{el} extracted with c-He. In some cases the amount of S_{el} extracted with chloroform is even 50 times higher than the one in c-He extracts. In Table 5-3 it can be seen that similar to the extraction with c-He (i), the highest S_{el} amount in demineralized and depyritized samples is detected for B-APF coal followed by M-APF. In all biotreated APF samples the content of extracted S_{el} decreases.

Table 5-2 Yields of bitumen, neutral oils and their fractions for APF and biotreated by *PC* and *SS* APF coal samples, in rel.%

Sample	Bitumen[a]	Neutral oils[b]	Neutral oil fractions[c]			S_{el} (%)[d]
			Aliphatic	Aromatic	Polar	
P-APF	1.9	64.7	16.2	30.1	43.6	18.1
P-APF-PC	2.0	71.1	14.3	22.8	48.1	15.1
P-APF-SS	1.3	65.1	12.4	12.4	58.1	15.2
M-APF	1.5	64.2	23.2	46.0	30.0	46.0
M-APF-PC	0.9	52.8	32.1	36.6	34.6	46.8
M-APF-SS	0.6	54.6	24.6	18.7	58.0	61.5
B-APF	2.0	66.9	20.7	24.0	40.5	63.8
B-APF-PC	2.3	74.0	17.1	27.1	43.5	41.4
B-APF-SS	1.2	59.7	23.8	13.0	49.7	76.0

[a] Rel. % of coal.
[b] Rel. % of bitumen.
[c] Rel. % of neutral oil.
[d] S_{el} as a part of aliphatic fraction.

Table 5-3 Content of S_{el} in $CHCl_3$ extracts (aliphatic fraction of the neutral oils) and c-He extracts of already chloroform extracted samples

Sample	$CHCl_3$ extraction	Subsequent c-He extraction	**Σ**	
	µg S/g ^{daf}	µg S/g ^{daf}	µg S/g ^{daf}	S wt%
P-APF	309.5	1.0	310.5	0.03
P-APF-PC	256.7	1.6	258.4	0.03
P-APF-SS	138.1	2.5	140.6	0.01
M-APF	882.1	2.1	884.3	0.09
M-APF-PC	616.4	0.5	616.9	0.06
M-APF-SS	416.1	0.7	416.9	0.04
B-APF	1539.2	17.8	1557.0	0.16
B-APF-PC	1016.0	5.1	1021.1	0.10
B-APF-SS	1112.5	3.2	1115.6	0.11

5.3.2.3 Extraction with c-Hexane of already extracted with chloroform samples (iii)

c-He is a reliable organic solvent for the extraction of S_{el} as it is already mentioned in the previous studies [6]. The initial strategy of our study was: (1) to examine changes that occur with the soluble in organic solvents (in this case chloroform) bitumen, as a result of the applied biotreatments (ii); (2) to determine S_{el} changes by applying subsequent c-He extraction on the insoluble in chloroform residues (iii). However, the obtained results for the quantity of S_{el} in (iii), (see Table 5-3) were extremely low. This fact provoked the question whether part of S_{el} is already completely extracted by chloroform. Our previous GC/MS studies of neutral oils demonstrated that if there was a presence of S_{el} in the bitumen, it would be eluted with aliphatic fraction of the neutral oils. It was therefore necessary to quantify S_{el} amount in the aliphatic fraction of the neutral oils (ii) and in the c-He extracts of none extracted with chloroform samples (i).

The obtained results demonstrate that in our coal set and experimental strategy, chloroform is a better solvent for the extraction of S_{el} comparing to c-He (Table 5-1, Table 5-3). This fact could be attributed to the differences in the polarity of both solvents or eventually, to S_{el} surroundings. It appears that accessibility of S_{el} for various organic solvents is of utmost importance.

5.3.3 Analysis of the solid extraction residue

It is possible that a small amount of S_{el} is retained in the already chloroform and c-He extracted samples. AP-TPR technique with its extension "off-line" TD-GC/MS is applied to receive more detailed information for the presence of S_{el} in the already extracted products. The pyrolysis experiments are performed in inert (He) atmosphere. GC/MS spectra are quantitatively interpreted. Typical products of coal pyrolysis, i.e. alkylbenzenes, alkylnaphthalenes, phenols, etc, are accompanied by their sulphur containing analogues and are clearly detected in the chromatograms of the samples under study. S_{el} is also evidently present. The results for S_{el} are shown in Table 5-4. Registered low quantities of S_{el} in the extracted solid residues are a proof of the effectiveness of the applied extraction procedure with chloroform.

Reactions of sulphur with various organic compounds are also possible [15]. At high temperature, above 180°C liquid sulphur becomes extremely active and can react with aromatic and aliphatic hydrocarbons and their derivatives. This reaction is due to the small sulphur species formed at corresponding temperature and produces organic sulphur compounds similar to those believed naturally occurring in some coals, i.e. sulphides, polysulphides and thiophenes. In the studied samples, the content of sulphides, polysulphides and thiophenes is also traced. In the present study we cannot prove unequivocally how many of these compounds are formed due to reactions of S_{el} with the organic part of the coals during pyrolysis, and how many are naturally present. In order to estimate the part of the S_{el}, which in the process of pyrolysis is liberated as volatile organic sulphur compounds, further detailed work is necessary.

Table 5-4 Content of S_{el} in solid residue after $CHCl_3$ and c-He extraction determined by AP-TPR "off-line" TD-GC/MS

Sample	μg S/ g ^{daf}
P-APF	2.3
P-APF-PC	3.9
P-APF-SS	4.0
M-APF	4.4
M-APF-PC	3.0
M-APF-SS	1.0
B-APF	-
B-APF-PC	3.5
B-APF-SS	7.0

5.3.4 Elemental sulphur balance

Formula for calculation of total S_{el} distribution in the studied samples based on the results for S_{el} content in (i), (ii), and (iii) extracts as well as in the extracted solid residue is proposed. The total elemental sulphur distribution can be calculated taking in consideration:

- S_{el} content in chloroform extract (ii);
- S_{el} content in c-He extract of already chloroform extracted samples;

- S_{el} content in solid residue after (ii) and (iii) determined by AP-TPR "off-line" TD-GC/MS in He atmosphere.

Respectively total S_{el} balance can thus be calculated as follow:

$$S_{el(tot)} = S_{el(ii)} + S_{el(iii)} + S_{el(res)},$$

where $S_{el(tot)}$ – total S_{el} distribution; $S_{el(ii)}$ – S_{el} in (ii) extract; $S_{el(iii)}$ - S_{el} in (iii) extract; $S_{el(res)}$ – S_{el} in solid residue after (ii) and (iii) extractions. The obtained values are summarized in Table 5-5 together with S_{el} content (%) as a part from S_t and S_o. Corrected organic sulphur values by $S_{el(tot)}$ results for investigated samples are included in Table 5-5 as well.

Table 5-5 Elemental sulphur balance and corrected organic sulphur

Sample	Sel(tot)^{daf}				corrected*
	µg S/g coal	wt%	% St	% ^{So}	S_o (wt%) ^{daf}
P-APF	312.8	0.03	0.73	0.78	3.84
P-APF-PC	262.3	0.03	0.96	1.02	2.92
P-APF-SS	144.6	0.01	0.28	0.30	3.32
M-APF	888.7	0.09	2.24	2.47	3.55
M-APF-PC	619.9	0.06	1.97	2.11	2.78
M-APF-SS	417.9	0.04	1.03	1.15	3.45
B-APF	1557.0	0.16	4.58	5.14	2.95
B-APF-PC	1024.6	0.10	3.19	3.31	2.92
B-APF-SS	1122.6	0.11	3.79	4.35	2.42

% St - S_{el}(tot) as a part (%) from the S_t (on dry, ash free basis) in the samples;
% ^{So}- S_{el}(tot) as a part (%) from the S_o (on dry, ash free basis) in the samples;
* S_o corrected by S_{el}.

The highest presence of S_{el} is registered for preliminary demineralized and depyritized coal. It can be explained by the weathered nature of initial coals. The content of S_{el} in the APF samples increases in the following sequence: Pirin < Maritza East < Baypazar. This increase is expected for the first two coals bearing in mind their S_p contents. On the other hand S_p content cannot explain the great difference in total S_{el} distribution for M-APF and B-APF samples. Most likely, this difference could be related to the special characteristics of the coal matrix,

differences in the crystallographic characteristics of the present pyrite, different impact of weathering effects or in-situ different impact of bacteriological action.

Through the implemented biotreatments, the amount of S_{el} is reduced in all samples. More promising biodesulphurization effect toward S_{el} appears in the treatment with *SS*, except for Baypazar. The maximum decrease in S_{el} is 54% and it is achieved for P-APF-SS coal sample. In the treatment with white rot fungus *PC*, the biodesulphurization effect varied in the range of 16 – 34%, with maximum for B-APF-PC sample. Again there is a support for the different characteristics of the Baypazar coals towards sulphur composition.

5.4 Conclusions

A new procedure for S_{el} determination in coal and its fractions is proposed. It includes exhaustive $CHCl_3$ extraction and subsequent quantitative analysis of the extracts by HPLC using C_{18} reversed phase column.

The highest presence of S_{el} is registered in the demineralized and depyritized coals, explained by their natural weathering. The differences in the S_{el} amount for all APF samples could be related to coal maturity and to the special characteristics of the coal matrix. Other explanation might be the S_p content of the initial samples as well as the crystallographic characteristics of the present pyrite. As a result of the implemented biotreatments the amount of S_{el} could be reduced in the range of 16 – 54%. *SS* appears to be a better biodesulphurizing agent toward S_{el}, than the applied white rot fungus *PC*. The maximum reduction is achieved for the most mature coal sample P-APF-SS, with 54%.

The content of S_{el} is also assessed as a part of the total sulphur and organic sulphur. The following range of S_{el} content is measured: 0.01 – 0.16 wt% or 0.3% – 4.6% of total sulphur and 0.3 – 5.1% of organic sulphur. More precised information concerning the content of organic sulphur presence is obtained. Our results clearly demonstrate the significance of S_{el} and that the lack of S_{el} evaluation might be a source of appreciable errors in the magnitude of organic sulphur presence.

5.5 References

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Chapter 6 Biodesulphurized low rank coal: Maritza East lignite and its "Humus-Like" byproduct

The results discussed in this chapter have been published in a peer-reviewed paper:
Gonsalvesh L, Marinov SP, Stefanova M, Carleer, Yperman J. Biodesulphurizaed low rank coal: Maritza East lignite and its "Humus-Like" product. Fuel 2013; 103:1039-50.

6.1 Introduction

It is known that coal biodesulphurization treatments affect not only sulphur functionalities, but also organic coal matrix. Our laboratory scale Maritza East lignite biodesulphurisation by *PP* supplies evidences that biodesulphurization is accompanied by coal organic matter oxidation [1]. Results of Fabianska et al. [2] also indicate that microbial desulphurization by *TF* and *TT* can significantly affect and decompose organic matter of Miocene lignites and embedded sediments. Moreover, it is revealed that some microorganisms, particularly fungi and bacteria, concerned in coal biodesulphurization, are capable of biodegradate and convert some coals into liquid or water-soluble forms [3-9]. The greatest effect in this field is realized for low-rank, highly oxidized coals, chemically close to wood.

In general, the protocol of coal biodesulphurization insists on copious washing of the biotreated material. In the biotreatment process by *PP* of pre-oxidized, demineralized and depyritized Maritza East coal sample, a high volume of dark brown solution is accumulated as a result of the washing procedure. Formation of high quantities of alkaline and water soluble substances during microbial degradation by *PP* bacterial strain of pre-oxidized low rank coals has been already described by Machnikowska et al. [9]. There are some peculiarities in the process of preparation of the water soluble byproduct comparing to the bioextract of the above mentioned paper but globally the same behavior has been observed. However, the work of Machnikowska et al. has been intended to monitor not sulphur alteration but *PP* degradation ability of organic matter of

lignites and subbituminous coals and the effect induced by the biotreatment on the chemical structure of the studied samples.

The aim of the present chapter is to evaluate (qualitatively and quantitatively) the desulphurization effect of Maritza East lignite subjected to chemical and microbial treatments. The changes that occur with sulphur functionalities in the demineralised, depyritized, oxidized and *PP* biotreated lignite (APF-oxy-PP) and in its water soluble byproduct (HL) will be of special interest. For sulphur specification a set of spectral, chromatographic and pyrolytic techniques is applied. Since HL byproduct highly resembles humic substances, it is compared to pristine humic acid (HA) obtained from the same coal sample.

6.2 Experimental section

6.2.1 Coal and applied treatments

Bulgarian lignite from Maritza East deposit (Trajanovo-North mine) is the initial object under consideration in this study. Technological sample (IN) of the freshly mined lignite is air dried, milled and sieved, <0.25 mm. In order to obtain mineral free and inorganic sulphur free coal samples and to focus our research on the removal and changes of organic sulphur caused by biodesulphurization, initial sample is subjected to chemical treatments, i.e. demineralization and depyritization prior to *PP* biodesulphurization as described in Chapter 2.

It is noticed, that an enhanced oxygen content of coals promotes better microbial performance [4,9-10]. In this context, IN, AF and APF coals are additionally thermooxidized with atmospheric air for sufficient oxidation of organic matter. Additional reason for carrying out this process is the fact that it would reduce the amount of pyrite S_p in the case of IN and AF samples. Laboratory scale thermooxidation with atmospheric air is carried out at the conditions shown in Chapter 2.

In the present study *PP* bacterial strain has been applied for biodesulphurization of IN, IN-oxy, AF, AF-oxy, APF and APF-oxy samples. The biotreated samples are separated from the media by filtration and consequently washed with hot distilled water. After biotreatment, *PP* is added to the abbreviation of the corresponding sample. The data characterizing biodesulphurized coal samples,

i.e. proximate and sulphur forms analysis together with combined (chemical and biological) desulphurization effects are given in Table 6-1.

Table 6-1 Characteristics of biodesulphurized coal samples

Sample	Proximate analysis (%)				S content (%), daf				Desulphurization (%)			
	W^{ad}	VM^{daf}	$C_{fix}{}^{daf}$	Ash^{ad}	S_t	S_s	S_p	S_o	ΔS_t	ΔS_s	ΔS_p	ΔS_o
IN	8.4	52.6	47.4	32.1	9.48	1.65	3.19	4.64	-	-	-	-
IN-PP	7.9	52.2	47.8	31.0	6.79	0.21	2.45	4.13	28.4	87.3	23.2	11.0
IN-oxy-PP	5.0	47.8	52.2	35.4	5.74	0.16	2.37	3.21	39.5	90.3	25.7	30.8
AF-PP	5.9	52.2	47.8	3.9	5.32	0.08	2.35	2.89	43.9	95.2	26.3	37.7
AF-oxy-PP	6.2	44.0	56.0	5.6	5.32	0.10	2.30	2.92	43.9	93.9	27.9	37.1
APF-PP	3.4	52.5	47.5	2.4	2.87	0.02	0.31	2.54	69.7	98.8	90.3	45.3
APF-oxy-PP	8.0	39.1	60.9	1.8	2.75	0.10	0.30	2.35	71.0	93.9	90.6	49.4

As a result of washing procedure on APF-oxy-PP sample, a high volume of dark brown solution has accumulated. After its acidification by HCl a voluminous precipitate has formed. It is isolated by filtration and subsequently washed by hot water. Inasmuch as it strongly resembles humus substances prepared from Maritza East lignite it is named "humus-like" byproduct (HL). HL byproduct is compared to pristine HA from Maritza East lignite. The latter are prepared according to the standard procedure [11].

6.2.2 Analyses

AP-TPR technique coupled "on-line" with mass spectrometry and "off-line" with TD-GC/MS apparatus is applied as already discussed in order to specify organic sulphur functionalities in IN, APF-oxy, APF-oxy-PP and HL samples. Similar as described in Chapter 4, in the case of AP-TPR "off-line" coupled with TD-GC/MS, the outlet of AP-TPR reactor is connected to an ice-cooled metal tube with SilcoSteel Coating, filled with Tenax/Carbopack B/Carbosieve SIII (Markes) as adsorbents. However, in this study the volatiles are additionally diluted by adding inert gas to H_2 flow in ratio 10:1 (v/v) in order to prevent overloading of the adsorption tube since the volatiles are trapped in the temperature range from 200°C up to 1025°C. The adsorption tubes are desorbed and analyzed by a TD-GC/MS.

For characterization and analyses of humic substances bitumen extraction, chromatographic separation, GC/MS and FTIR are applied at the conditions described in Chapter 2.

6.3 Results and discussion

6.3.1 Bulk characteristics

An original desulphurization strategy including combination of chemical (demineralization, depyritization, thermooxidation) and microbial treatments by *PP* bacteria toward inorganic and organic sulphur removal in Maritza East lignite (IN) is applied [12]. Maximum combined (chemical and microbial) S_t desulphuriztaion of 71.0%, S_p desulphurization of 90.6% and S_o desulphurization of 49.4% are achieved for APF-oxy-PP sample (Table 6-1). Combined S_t removal for IN-PP and IN-oxy-PP samples is respectively 28.4 and 39.5%. There is no appreciable difference in combined S_p desulphurization effect registered for the last two samples, but in the case of IN-oxy-PP sample, S_o is more attacked. With respect to biotreated and demineralized samples, i.e. AF-PP and AF-oxy-PP, combined S_t removal is 43.9%. Differences in combined S_p and S_o desulphurization for AF-PP and AF-oxy-PP samples are negligible.

The highest combined inorganic and organic sulphur desulphurization effects achieved for preliminary chemically treated Maritza East lignite (demineralized, depyritized and oxidized) and subsequently biodesulphurized by *PP* (APF-oxy-PP sample) provoke current study and our deeper interest toward this sample. In order to clarify chemical and microbial treatments contributions to desulphurization effect registered for APF-oxy-PP sample and to focus our research on organic sulphur functionalities changes due to biotreatment, APF-oxy sample is also under consideration. On the other hand, the appreciable quantity of HL byproduct produced during washing procedure of APF-oxy-PP sample and its external similarity to humic substances incite us to pay special attention to HL sample. The quantity of HL sample is measured as a loss of coal weight and amounts up to 15-18 wt% of APF-oxy-PP sample. Respectively, sulphur leached by water soluble material should be rendered in consideration. Proximate and sulphur forms analysis of IN, APF-oxy, APF-oxy-PP, HL and HA

samples are presented in Table 6-2. For clarity of the presentation in Table 6-2 some repetition of the results are admitted.

It can be seen in Table 6-2 that IN sample is characterized by high sulphur and ash contents, which defines it as a low quality coal. As a result of combined chemical demineralization, depyritization and oxidation, and microbial treatments, sulphur and ash contents are significantly decreased while C_{fix} is increased. These findings suppose that the quality of APF-oxy-PP sample is improved. However, it can be clearly seen from Table 6-2 that the coal quality improvement (taking into consideration proximate analysis values) is mainly due to the chemical treatments. The registered chemical desulphurization effects towards sulphur forms for APF-oxy sample in comparison with IN sample are: ΔS_t = 63.3%; ΔS_s = 95.8%; ΔS_p = 87.1%; and ΔS_o = 35.5%. As a result of *PP* biotreatment the following extra desulphurization (biodesulphurization) effects towards sulphur forms are registered for APF-oxy-PP sample in comparison with APF-oxy coal: ΔS_t = 21.0%; ΔS_p = 26.8%; and ΔS_o = 21.7%. However, the question arises what happens with sulphur and in what form it is removed as a result of the applied desulphurization processes.

Table 6-2 Proximate and sulphur analyses
of IN, APF-oxy, APF-oxy-PP, HL and HA samples

Analysis	Samples				
	IN	**APF-oxy**	**APF-oxy-PP**	**HL**	**HA**
Proximate (wt%)					
W^{ad}	8.4	5.3	8.0	6.6	6.7
Ash^{ad}	32.1	2.4	1.8	1.9	5.4
VM^{daf}	52.6	39.3	39.1	46.0	46.9
C_{fix}^{daf}	47.4	60.7	60.9	54.0	53.1
Ultimate (wt%)^{daf}					
S_t	9.48	3.48	2.75	n.d.	n.d.
S_s	1.65	0.07	0.10	n.d.	n.d.
S_p	3.19	0.41	0.30	n.d.	n.d.
S_o	4.64	3.00	2.35	n.d.	n.d.

n.d.-not determined;

The applied chemical treatments are characterized by high oxidation potential toward pyrite [13-16]. As a result of the oxidation by nitric acid and atmospheric air, S_p is converted mainly into S_s. Depending on the condition of the reaction with nitric acid, elemental sulphur may also be formed [16] but it should be

additionally oxidized to S_s during the thermooxidation treatment with atmospheric air. On the other hand, it is known that *PP* is effective in sulphur removal from coal. Rai and Reyniers [17] attain for S_p a bioremoval of 75% and for S_o a bioremoval of 37.4% from a Texas lignite in 5-7 days. In our previous investigation on biodesulphurization by *PP* of Maritza East lignite a similar result with regard to S_p removal (69%) is obtained [1]. Nevertheless, S_p biodesulphurization by *PP* is a bit astounding since this bacterium is saprotroph (attack and decompose organic matter) and the mechanism of S_p bioremoval is not really clarified at the moment. Therefore it can be assume that in the process of biodesulphurization by *PP*, most probably S_p bioremoval is not a result of direct bacterial S_p utilization. It can be associated with pyrite alteration under the action of metabolites bearing oxidation potential or with aqueous oxidation of pyrite by molecular oxygen (the so called "Chemical oxidation pathway of pyrite") as described in details in the work of Rossi [18]. Both result in S_p transformation into S_s. Manifestly we can conclude that as a result of chemical, thermochemical and biological processing of IN sample, S_p is oxidized to S_s, which together with preliminary present S_s are removed during the washing procedure.

Different mechanisms are proposed with respect to S_o biodesulphurization by *PP* [19-20]. It is found that a strain of *PP* transforms dibenzothiophene (DBT) to DBT sulphone ($DBTO_2$) *via* DBT sulphoxide (DBTO). It also degrades DBT to 3-hydroxy-2-formyl benzothiophene (HFBT) *via* ring-destructive oxidative pathway. Neither $DBTO_2$ nor HFBT are further degraded by this organism. Various studies summarized by Gogoi and Bezbaruah [20] indicate that *PP* strains affect also other heterocyclic and non-heterocyclic sulphur containing hydrocarbons. It should be mentioned as well that nitric acid may also attack S_o. According to Rutkowski [14], as a result of nitric acid treatment, some S_o can be removed due to oxidation into sulphoxides, sulphones, sulphonic acid and even sulphates. However, what really happened with S_o due to applied treatments on IN sample needs additional research and analysis.

Elemental analyses of above discussed samples (IN, APF-oxy and APF-oxy-PP) as well as of HL and HA samples are included in Table 6-3. Atomic ratios of H/C, O/C, S/C and N/C together with higher heating value (HHV) calculated by the formula of Channiwala [21] are also included in it. It can be seen that carbon

content is increased for the products of the applied desulphurization processes, i.e. APF-oxy, APF-oxy-PP and HL samples compared to IN sample. This increase is mainly explained by demineralization of the initial sample as a result of the chemical treatments. The carbon content of HA is also higher compared to IN sample, but lower compared to HL product. As a result of the chemical desulphurization procedure, the hydrogen and sulphur content together with H/C and S/C atomic ratios are decreased for APF-oxy sample compared to IN sample. Insignificant are the registered changes in the last mentioned characteristics in the case of APF-oxy-PP sample compared to APF-oxy sample. However, sulphur content is decreased with about 18% according to elemental analysis for APF-oxy-PP sample in comparison with APF-oxy coal. Lower sulphur content is registered for HL sample compared to IN sample and higher compared to APF-oxy and APF-oxy-PP samples. However, the water soluble product HL is characterized by a higher H/C and S/C atomic ratios compared to APF-oxy and APF-oxy-PP samples.

Table 6-3 Elemental analysis

	Samples				
	IN	**APF-oxy**	**APF-oxy-PP**	**HL**	**HA [22]**
Elem. Analysis (wt%)^{db}					
C	39.3	62.0	62.0	55.7	50.8
H	4.0	3.3	3.4	4.4	4.7
N	0.8	3.6	3.4	3.7	1.1
S	5.9	3.4	2.9	4.1	2.9
O^{diff}	15.0	25.2	26.3	30.1	32.9
Atomic ratios					
H/C	1.21	0.63	0.65	0.94	1.10
N/C	0.02	0.05	0.05	0.06	0.02
S/C	0.06	0.02	0.02	0.03	0.02
O/C	0.29	0.31	0.32	0.41	0.49
HHV* (MJ/kg)	16.7	23.2	23.1	21.8	20.0

^{db} dry basis; O^{diff}=100-(C+H+N+S+Ash), db

*HHV– higher heating value is calculated on dry basis by the formula of Channiwala [21].

The oxygen and nitrogen content together with O/C and N/C atomic ratios are significantly increased for APF-oxy, APF-oxy-PP and HL samples, and maximizing again in the case of HL sample. The increased oxygen content and O/C atomic

ratios for the last samples are mainly due to chemical treatments and are an indication for oxidation processes. The increased nitrogen content is again mainly related to chemical treatment, i.e nitric acid treatment. Similar trend is observed for other nitric acid pretreated lignites [5,9]. Moreover, Machnikowska et al. [9] also observed that during microbial degradation of low rank coals by *PP*, soluble part is characterized by higher nitrogen and oxygen content as well as higher N/C and O/C atomic ratios compared to solid bioresidue. Nevertheless, it should be mentioned that as a result of *PP* microbial treatment oxygen content and O/C atomic ratio are also increased. With respect to humic substances (HA and HL samples), the magnitudes of elemental analysis of HL product are quite reasonable compared to pristine humic substance HA from the same deposit [22]. Nevertheless, some differences in atomic ratios can be depicted for HL sample, i.e. lower H/C and O/C ratios and higher N/C and S/C ratios.

Heating values of the samples under study are also determined (Table 6-3). As a result of desulphurization treatment, positive effects on HHV of 38.9%, 38.3% and 30.5% are registered for APF-oxy, APF-oxy-PP and HL samples, respectively, compared to IN sample. Obviously, the increase in HHV is mainly related to the removal of mineral substance at the pretreatment step prior to *PP* treatment. Although *PP* treatment does not affect negatively the HHV of the coal, it resulted in a weight loss. However, as a sequence of this treatment, an extra positive effect due to the reduction of the amount of sulphur is registered. HHV of the humus like byproduct HL is higher compared to HHV of HA sample.

6.3.2 AP-TPR sulphur distribution assessment

6.3.2.1 AP-TPR experiments coupled "on-line" with MS detection

6.3.2.1.1 In H_2 atmosphere

The H_2S kinetograms of AP-TPR/MS in H_2 atmosphere of samples under consideration are visualized in Figure 6-1A. For clearness of presentation only background subtracted *m/z* 34 ion profile is shown as *m/z* 34 (H_2S^+) and *m/z* 33 (HS^+) demonstrate the same evolution pattern. There are two dominant peaks in H_2S^+ profiles of IN sample: the first one with T_{max} at about 420°C is attributed to di-alkyl sulphides, alkyl-aryl sulphides, disulphides and reactive di-aryl

sulphides; second peak at about 630°C refers to the presence of less reactive di-aryl sulphides and complex thiophenic structures. This assumption is based on the model compounds approach [23] and also on AP-TPR/MS profiles of typical aliphatic and aromatic fragments for IN sample (Figure 6-2A.1 and B.1). Despite of high S_p and S_s contents of IN sample, individual peak corresponding to pyrite and iron sulphate presence is not observed. Nevertheless it is noteworthy that the peak with maximum at about 630°C is asymmetric and starts at about 500°C. This is related to pyrite and/or iron sulphate hydrogenation/reduction, which H_2S peak coincides with H_2S peak of sulphur containing aromatic compounds [24]. Inasmuch as H_2S^+ profile of IN sample starts at about 200°C and a weak shoulder at 320°C appears in this profile, presence of thiols can be assumed. To confirm this, sulphur containing aliphatic fragments for thiols (and sulphides), $C_nH_{2n+1}S$, n=1, 2, 3 (*m/z 47, 61, 75*) are traced. This ion series and especially ion fragment *m/z 47* are characteristic for the mass spectra of both classes of compounds, i.e thiols and sulphides [25]. Simultaneously dominant consecutive alkenyl fragments (C_nH_{2n-1}, *m/z 41, 55, 69*,…) and smaller aliphatic fragments (C_nH_{2n+1}, *m/z 43, 57, 71*,…) should also be observed in the mass spectra of aliphatic thiols (and sulphides). The profiles of ion series characteristic for aliphatic thiols (and sulphides) compared to *m/z* 34 (H_2S^+) and *m/z* 55 ($C_4H_7^+$) profiles in the case of IN sample are presented in Figure 6-3. It can be seen that the profiles for aliphatic thiols (and sulphides) maximize at 320°C and coincide with the shoulder attributed to thiols hydrogenation. Inasmuch as the profiles for aliphatic thiols (and sulphides) maximize at 320°C instead of at 420°C (T_{max} of aliphatic and aromatic fragments, see Figure 6-2A.1 and B.1) inclines us to assume that this maximum should be rather attributed to surface thiols than to aliphatic sulphides (and disulphides) thermal destruction. On the other hand, a shoulder or a plateau appears in *m/z 47, 61, 75* profiles of IN sample in the temperature range of 400-500°C. To some extent it confirms the attribution of the peak at 420°C in *m/z* 34 profile of IN sample to di-alkyl sulphides, alkyl-aryl sulphides and disulphide hydrogenation/reduction. Regarding the second peak that appears at higher temperatures around 600-630°C in the AP-TPR profiles of *m/z* 61 and 75, it cannot be attributed to a fragment originating from aliphatic thiols, di-alkyl and alkyl-aryl sulphides and

disulphides decomposition. It might be related to a fragment originating from secondary reactions. However, further research is needed to confirm this.

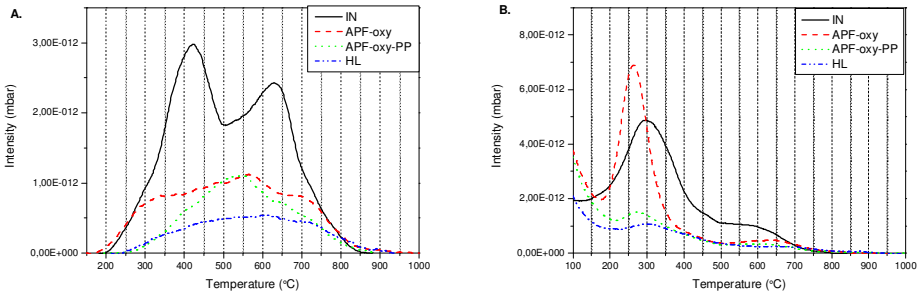

Figure 6-1 AP-TPR-MS (H_2) kinetograms of *m/z* 34 (A) and *m/z* 64 (B) for samples under study

The H_2S^+ AP-TPR/MS (H_2) kinetograms of APF-oxy, APF-oxy-PP and HL samples are also visualized in Figure 6-1A. It can be clearly seen in *m/z* 34 profile of APF-oxy sample that aliphatic, mixed and sulphur containing aromatic compounds are significantly attacked and removed as a result of chemical treatment. Since one broad signal is observed in H_2S^+ profiles of APF-oxy-PP and HL samples starting at higher temperature compared to IN and APF-oxy, it can be assumed that thiols are oxidized due to applied *PP* biotreatment. Indeed, a significant peak is not observed in AP-TPR-MS (H_2) profiles for aliphatic thiols fragments (and sulphides) of APF-oxy-PP and HL samples (figures not shown). It is known that thiols can be oxidized to disulphides under mild air oxidation, while under more severe conditions, they can be oxidized to sulphonates [26]. The maximum of the H_2S^+ signal for APF-oxy-PP sample is clearly at 550ºC. Most probably this maximum could be explained by hydrogenation/reduction of *in situ* formed organic sulphur species during depyritization and biodesulphurization of ash pyrite free coal. Other explanation could be that due to the applied treatments, coal matrix is severely altered. As a result, sulphur compound become better accessible and/or susceptible for reduction/hydrogenation during AP-TPR experiment. The absence of well defined and dominant peaks for di-alkyl and alkyl-aryl sulphides, disulphides, and reactive di-aryl sulphides at about 420°C and for less reactive di-aryl sulphides and complex thiophenic structures

at about 630°C in H_2S^+ profiles of APF-oxy-PP and HL samples as well as in H_2S^+ profile of APF-oxy sample (as already mentioned above) is an indication that these compounds are affected and/or removed due to applied chemical and biological desulphurization treatments.

During AP-TPR pyrolysis, oxidized sulphur species presence can be observed/determined by formed SO (*m/z* 48) and SO_2 (*m/z* 64) fragments [23,27-28]. Therefore both profiles, *m/z* 48 (SO^+) and *m/z* 64 (SO_2^+), are monitored. Depending on the temperature region of their evolution, the sulphur distribution of oxidized sulphur forms in coal can be revealed. Figure 6-1B visualizes SO_2^+/SO^+ AP-TPR evolution in H_2 atmosphere for IN, APF-oxy, APF-oxy-PP and HL samples. Again only *m/z* 64 profiles are demonstrated since they exhibit the same evolution pattern as *m/z* 48 ones. In *m/z* 64 kinetograms of all samples under consideration, a peak at about 270 – 300°C and a plateau at T > 500°C are observed. The peak at lower temperature can be attributed to organic sulphonic acids presence. This assumption is based on pyrolysis of sulphur containing model compounds and sulphonated coals [23]. With regards to the plateau at T > 500°C, it can be explained by diaryl sulphoxides and/or diaryl sulphones decomposition. This assignment is again based on the pyrolysis of model compounds [28]. However, it should be mentioned that aromatic structure breakdown can contribute to the plateau at T° > 500°C in *m/z* 64 profiles of studied samples [23]. Additional research on AP-TPR-MS analysis in an inert atmosphere is needed to confirm or reject the plateau assignment. A dedicated peak for iron sulphate is not observed in *m/z* 64 AP-TPR-MS (H_2) profile of IN sample partly because of SO_2 reduction to H_2S and partly because this compound is converted to troilite, which can be further hydrogenated to H_2S [23,29].

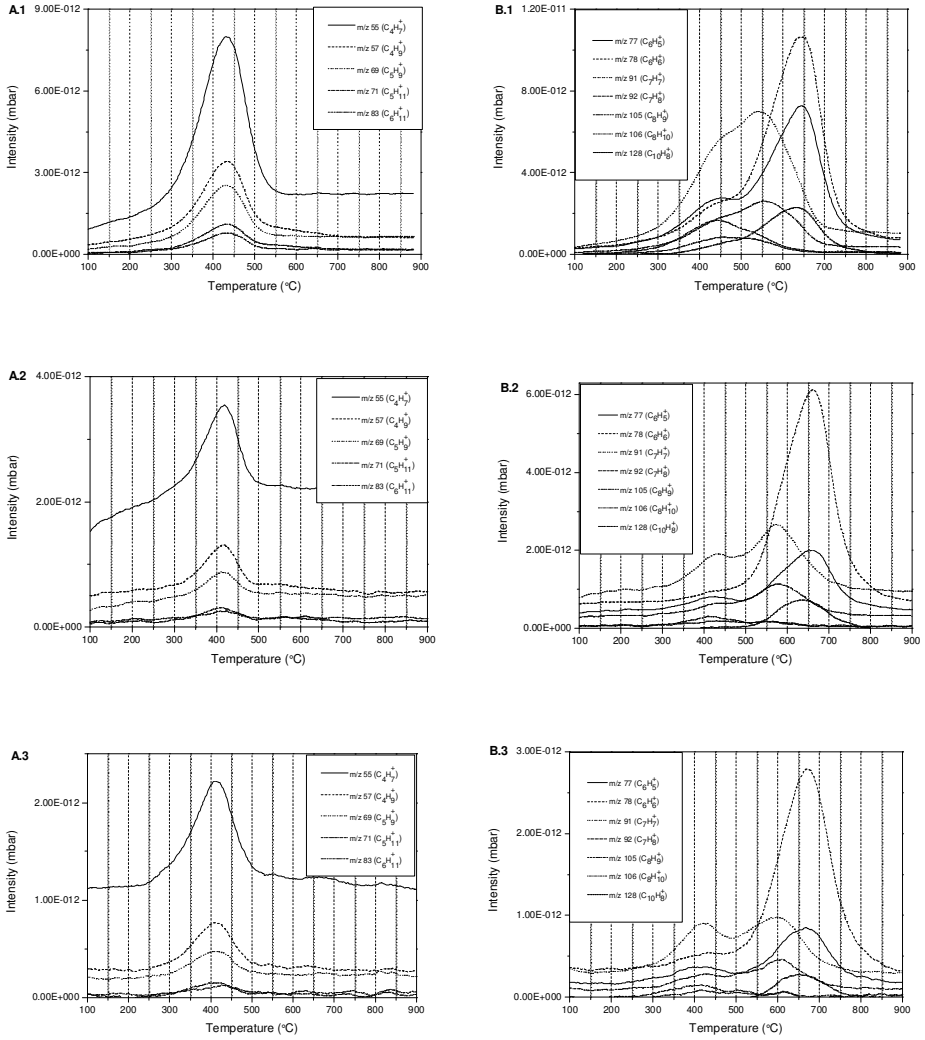

Figure 6-2 AP-TPR-MS (H_2) evolution profiles of IN (1), APF-oxy-PP (2) and HL (3) samples: A) saturated/unsaturated CH-fragments (alkenes/alkanes): *m/z* 55 ($C_4H_7^+$); *m/z* 57 ($C_4H_9^+$); *m/z* 69 ($C_5H_9^+$); *m/z* 71 ($C_5H_{11}^+$); *m/z* 83 ($C_6H_{11}^+$). B) aromatic compounds: Benzene: *m/z* 77($C_6H_5^+$); *m/z* 78 ($C_6H_6^+$), Toluene: *m/z* 92 ($C_7H_8^+$); *m/z* 91 ($C_7H_7^+$), Xylene: *m/z* 91 ($C_7H_7^+$); *m/z* 106 ($C_8H_{10}^+$), Alkyl-benzene: *m/z* 105 ($C_8H_9^+$), Naphthalene: *m/z* 128 ($C_{10}H_8^+$).

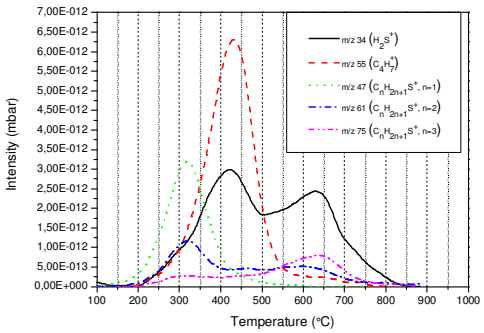

Figure 6-3 AP-TPR-MS (H_2) evolution profiles of IN sample for *m/z* 34, 55, 47, 61 and 75

6.3.2.1.2 In He atmosphere

AP-TPR pyrolysis in an inert atmosphere can give supplemental information on the nature of sulphur species especially on oxidized sulphur forms. Figure 6-4 comprises H_2S (Figure 6-4A) and SO_2 (Figure 6-4B) AP-TPR-MS kinetograms in He atmosphere for IN, APF-oxy and APF-oxy-PP samples. In it, only background subtracted *m/z* 34 and *m/z* 64 profiles are shown since they exhibit the same evolution pattern as *m/z* 33 and *m/z* 48 profiles, respectively. It can clearly be seen for all samples under consideration that the intensity of each H_2S^+ profile in an inert atmosphere (Figure 6-4A) is relatively lower compared to the intensity of its corresponding H_2S^+ profiles in reducing atmosphere (Figure 6-1A). Additionally, hydrogenation of aromatic sulphur functionalities in an inert atmosphere is strongly limited. As a result, a small peak at about 510°C can be observed for IN sample. It can be attributed to pyrite and/or iron sulphate hydrogenation/reduction [24,29]. This peak completely disappears for APF-oxy-PP sample, characterized by low S_p and S_s contents. The peak at about 250°C in H_2S^+ profile in an inert atmosphere of IN sample can be related to surface thiols decomposition followed by hydrogenation through coal supplied hydrogen. Additional evidence for this peak assignment is found in the profiles of ion series characteristic for aliphatic thiols (figure not shown). In H_2S^+ profile as well as in the profiles of ion series characteristic for aliphatic thiols (and disulphide) of IN sample pyrolyzed in reducing atmosphere a similar signal is observed. Obviously mercaptans are easily decomposed and subsequently hydrogenated irrespective

of the applied atmosphere during pyrolysis. In the case of H_2S^+ profiles of APF-oxy and APF-oxy-PP samples, a peak at about 380 – 400ºC can be noticed. Based on the model compound approach [23] and also on AP-TPR-MS (He) profiles of typical aliphatic and aromatic fragments for investigated samples, the expressed peak at ~380°C can be related to di-alkyl and alkyl-aryl sulphides, disulphides, and reactive di-aryl sulphides hydrogenation. A less pronounced peak appearing around 700°C in H_2S^+ profile of APF-oxy-PP sample might be explained by less reactive di-aryl sulfides and complex thiophenic structures hydrogenation. Additionally, the absence of the mentioned peaks (at 380 – 400ºC and at 700ºC) in H_2S^+ AP-TPR-MS (He) profile of IN sample can refer to alteration of di-alkyl and alkyl-aryl sulphides, disulphides, and reactive di-aryl sulphides together with less reactive di-aryl sulfides and complex thiophenic structure which is a result of applied chemical and biological treatments. In H_2S^+ profiles of APF-oxy and APF-oxy-PP samples in He atmosphere thiols presence is not detected.

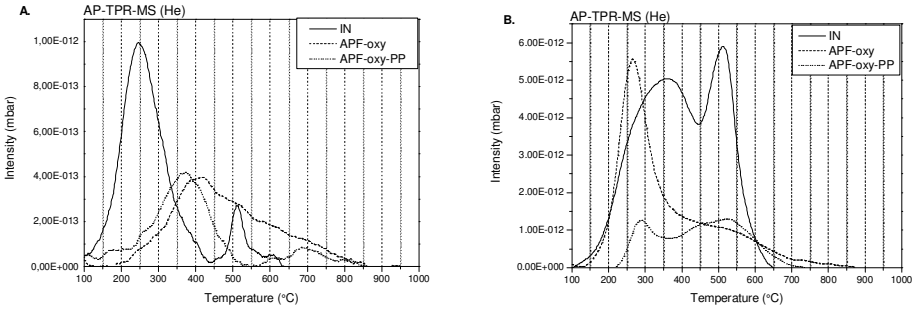

Figure 6-4 AP-TPR-MS (He) kinetograms of *m/z* 34 (A) and *m/z* 64 (B) for IN, APF-oxy and APF-oxy-PP samples.

Two peaks appear in AP-TPR-MS (He) *m/z 64* profile of IN sample (Figure 6-4B). The first broad one at a lower temperature around 360ºC can be attributed to organic sulphonic group decomposition [14,23]. The second sharp peak maximizing at 510ºC can be assigned to iron sulphate and alkyl-aryl sulphones, supporting the conclusion just formulated for the H_2S^+ profile of IN sample in the same atmosphere [28-29]. Presently, additional research is needed to distinguish above mentioned sulphur containing compounds. In the case of APF-

oxy and APF-oxy-PP samples, again two peaks are observed in *m/z* 64 profile. The first one attributed to organic sulphonic group decomposition, in the case of APF-oxy and APF-oxy-PP samples, is maximizing at lower temperature (~ 280°C) compared to the first peak in *m/z* 64 profile of IN sample. However, the presence of organic sulphonic groups is somewhat higher in the case of APF-oxy sample compared to APF-oxy-PP. Inasmuch as treated samples (APF-oxy and APF-oxy-PP) has low content of S_s (Table 6-2), the second broad peak in their *m/z* 64 profiles should be mainly referred to alkyl-aryl sulphones rather than iron sulphate. Based on AP-TPR-MS (He) profiles of typical aromatic fragments (figure is not shown) it is unlikely the last mentioned peak, in *m/z* 64 profiles, to contain a contribution from $C_5H_4^+$ ion fragment (originating from coal aromatic structure).

6.3.2.2 AP-TPR experiments coupled "off-line" with TD-GC/MS

AP-TPR experiments "off-line" coupled with TD-GC/MS are also applied to receive information for the sulphur containing volatile organic compounds neither reduced in the AP-TPR experimental conditions into H_2S nor captured into its tar/char fractions. The pyrolysis experiments are performed in a reducing (H_2) atmosphere and GC/MS spectra are quantitatively interpreted by spiking with 3 μg d_4-thiophene. Results for registered and quantified sulphur containing volatiles as well as their recovery calculated on dry, ash free base by using S_o as reference value are shown in Table 6-4.

Analyzing the quantitative results obtained by AP-TPR "off-line" TD-GC/MS for IN, APF-oxy, APF-oxy-PP and HL samples, from the data in Table 6-4, it can be seen that as a result of applied chemical and biological treatments the amount of S_{vol} is clearly increased. Obviously the increased amount of S_{vol} as a result of *PP* biotreatment (considered as the sum of S_{vol} of APF-oxy-PP sample and S_{vol} of HL sample) is due to the transformation of S_o into water soluble form, which is concentrated in HL byproduct. Among the organic sulphur compounds registered for IN sample, aliphatic sulphur is in the highest amounts followed by thiophenic sulphur and oxygen-sulphur containing compounds. In the case of APF-oxy sample the ratio of registered classes of compounds is changed compared to IN sample. Obviously due to applied chemical treatments and ongoing oxidation processes, the amount of oxygen containing compounds is increased most

probably on behalf of aliphatic compounds and thiophenes. Ongoing oxidation processes occur also during *PP* biotreatment. A significant increase in oxygen containing compounds (sum of APF-oxy-PP and HL) is registered as a result of the biological treatment compared to APF-oxy sample. However, *PP* treatment leads to an increase in aliphatic and aromatic sulphur compounds as well.

Table 6-4 Sulphur in organic compounds determined by AP-TPR "off-line" TD-GC/MS, in μg S/g^{daf}

Compounds	Formula	Mw	Samples				
			IN	APF-oxy	APF-oxy-PP	HL	HA
Sulphur dioxide	SO_2	64	592.8	1862.3	240.2	5882.9	80.0
Carbonyl sulphide	COS	60	-	-	-	29.3	0.0
Σ of O-S containing compounds			592.8	1862.3	240.2	5912.2	80.0
Dimethyl sulphide	C_2H_6S	62	-	-	-	-	86.0
Dimethyl disulphide	$C_2H_6S_2$	94	400.5	23.0	166.7	520.7	84.2
Dimethyl trisulphide	$C_2H_6S_3$	126	335.1	18.2	156.1	344.7	22.7
Dimethyl tetrasulphide	$C_2H_6S_4$	158	47.9	1.2	25.4	342.9	0.0
Σ of aliphatics			783.5	42.4	348.2	1208.3	192.9
Elemental sulphur	S_8	256	26.1	7.3	1.8	71.1	0,0
Methyl thiocyanate	C_2H_3NS	73	-	7.8	2.9	100.5	41.8
Methyl isothiocyanate	C_2H_3NS	73	-	1.1	2.0	122.9	0.0
Thiazole	C_3H_3NS	85	-	-	-	45.0	0.0
Σ of N-S containing compounds			0.0	8.9	4.9	268.4	41.8
Thiophene	C_4H_4S	84	46.1	50.3	71.7	23.3	22.9
Thiophene - C_1	C_5H_6S	98	348.7	103.4	143.5	2623.8	119.2
Thiophene - C_2	C_6H_8S	112	195.3	28.7	113.6	1010.1	46.4
Thiophene - C_3	$C_7H_{10}S$	126	60.2	2.4	20.8	92.4	5.3
Σ of thiophenes			650.3	184.7	349.7	3749.5	193.9
Benzothiophene	C_8H_6S	134	9.6	50.8	44.6	442.2	8.4
Benzothiophene - C_1	C_9H_8S	148	3.2	7.7	5.5	415.9	4.6
Benzothiophene - C_2	$C_{10}H_{10}S$	162	0.0	4.0	3.1	80.6	0.0
Σ of benzothiophenes			12.8	62.5	53.2	938.7	13.0
Σ of all			2065.4	2168.0	998.0	12148.2	521.6
Re^{So} (wt%)			4.5	7.2	4.2	29.6	1.8

Re^{So} is S_{vol} as a part from S_o (on dry, ash free basis);
C_n – carbon number in the substituent;

With regards to water soluble product of APF-oxy-PP sample, i.e. HL sample, a significant increase in S_{vol} content is found in comparison with IN, APF-oxy and

APF-oxy-PP samples. Obviously, the applied *PP* biological treatment on APF-oxy sample transform S_o to water soluble forms, which are highly volatile under applied AP-TPR experimental conditions. All of the registered classes of compound by AP-TPR "off-line" TD-GC/MS technique, i.e oxidized sulphur compounds (registered as SO_2), nitrogen-sulphur containing compounds, aliphatic and aromatic sulphur containing compounds, are in much higher presence in the case of HL sample compared to APF-oxy sample as well as to IN and APF-oxy-PP samples. The increased SO_2 content in HL byproducts compared to APF-oxy sample can be explained by oxidation of organic sulphur compounds into soluble sulphonic acids, sulphoxides and sulphones during *PP* biotreatment. As a result of thermal decomposition during AP-TPR pyrolysis, last mentioned compounds can form SO_2 [14,23,28]. Of a peculiar interest is the increase in the content of Th and BzTh in HL byproducts. The applied biological treatment has a pronounced oxidative character as already mentioned. Respectively, the oxidation of organic sulphur compounds can be carried out at the S-atom and/or at C-atom position. If the oxidation occurs at the S-atom position of Th and BzTh structures, it results in the formation of the corresponding sulphoxides and sulphones. As a consequence, an increased SO_2 and a decreased Th and BzTh contents should be registered by AP-TPR. Obviously this is not the case here. Therefore it can be assumed that as a result of applied *PP* treatment, the oxidation of Th and BzTh most probably occur mainly at the C-atom position. Thus, oxygen containing derivatives of Th and BzTh are formed, which during AP-TPR experiment decompose with Th and BzTh release. A possible reason for the increased content of BzTh can be the DBT degradation by *PP* to HFBT *via* Kodama pathway [20]. However, DBT presence is not typical for low rank coals, especially for lignites, and it is not detected in IN sample and APF-oxy samples. Hence, it cannot be a reason for the increased BzTh content in our case. Mechanisms of biotransformation of BzTh by strains of *PP* are also proposed. Pseudomonas strain BT1 transforms BzTh to benzothiophenes-2-3-dione and 3-methylbenzothiophene to sulphoxide and sulphone [20,30]. Mutant strain of *PP* transforms BzTh to three dihydrodiols: *cis*-4,5-dihydroxy-4,5-dihydrobenzothiophene and *cis*- and *trans*-2,3-dihydroxy-2,3-dihydrobenzothiophene [31]. Eaton and Nitterauer [30] identified two products as a result of BzTh transformation by *PP* RE204. The first one is recognized as

trans-4-(3-hydroxy-2-thienyl)-2-oxobut-3-enoate. It is a result of the oxidation of the homocyclic ring of BzTh leading to a formation of 4,5-dihydroxybenzothiophene *via cis*-4,5-dihydroxy-4,5-dihydrobenzothiophene, and followed by cleavage of the homocyclic ring. The second product is recognized as 2-mercaptophenylglyoxalate. It is a result of the oxidation of the heterocyclic ring of BzTh leading to the formation of 2-hydroxy-3-oxo-2,3-dihydrobenzothiophene *via cis*-2,3-dihydroxy-2,3-dihydrobenzothiophene, and followed by cleavage of the heterocyclic ring. According to Eaton and Nitterauer, 2-mercaptophenylglyoxalate might be converted to benzothiophenes-2,3-dione, but it is rather a product of transformation that occur during extraction of the product. As can be seen from presented literature overview, biotransformation of BzTh by strains of *PP* can indeed occur *via* S-atom oxidizing mechanism and *via* C-atom oxidizing mechanism. Apparently, in the *PP* biotreatment process of APF-oxy sample, similar biotransformation reactions of BzTh take part. However, C-atom oxidizing mechanism should be the dominant one.

With regards to Th, their content in HL sample is also significantly increased compared to APF-oxy sample (as well as to IN sample and APF-oxy-PP samples). This increase is mainly due to an increase in the amounts of mono-substituted and di-substituted homologues. Changes in unsubstituted Th are rather insignificant. However, it is shown that unsubstituted Th is not metabolized by naturally occurring aerobic microorganisms [20]. Despite the lack of information concerning biotransformation of Th by *PP*, based on the proposed mechanisms for biotransformation of BzTh by *PP*, the following assumption can be made: taking the increased content of SO_2 and Th into consideration, it can be supposed that biotransformation of Th proceed *via* oxidation mechanism attacking mainly carbon skeleton of thiophenic structures.

Aliphatic sulphur, registered by AP-TPR "off-line" TD-GC/MS, is in the form of sulphide, di-, tri- and tetra sulphides. Its content increases also for the water soluble product compared to APF-oxy sample (and also compared to IN and APF-oxy-PP samples). The increase is mainly explained by the increase in all registered polysulphides, i.e. di-, tri- and tetrasulphides. However, among registered polysulphides, dimethyl disulphide is in the highest presence for all samples under consideration. Changes in nitrogen-sulphur containing compounds are also determined for HL sample compared to APF-oxy sample.

Even a new compound (thiazole) is registered in the case of HL byproduct. However, the increased contents of water soluble nitrogen-sulphur containing compounds should be somehow related to NH_4Cl presence in the Raymond nutrient medium.

Comparing humus like product (HL) with humic acids from Maritza East lignite (HA), significant differences toward sulphur containing volatiles can be pointed out. The content of the S_{vol} in terms of μg S/g^{daf} as well as the recovery of S_{vol} in wt% determined by AP-TPR "off-line" TD-GC/MS for HA is comparatively low. Variations in the distribution of the different forms of S_{vol} for HL and HA are also noticeable. HA sample is characterized by equal presence of aromatic and aliphatic sulphur, while in the case of HL sample aromatic sulphur presence prevails over aliphatic one. In addition, low content of oxidized sulphur species is registered for HA sample, while for HL sample oxidized sulphur species are rather dominant among registered sulphur volatiles. Briefly, similarity in the registered sulphur species is observed as well as clear differences in their proportions. Previous AP-TPR-MS (H_2) studies of HA confirms these observations [32].

6.3.3 FTIR data

FTIR spectroscopy is applied for semi-quantitative evaluation of structural similarities and peculiarities in HL byproduct and HA. Spectra are visualized in Figure 6-5, as data in Table 6-5. It can be seen that both are characterized by the presence of various oxygen containing functional groups, i.e carboxylic groups, aromatic/aliphatic ether bonds and phenolic, and alcoholic groups. However, the data in Table 6-5 give us ground to list the following peculiarities for HL byproduct in comparison with the pristine HA:

(i) Higher presence of H-bonded OH and NH groups characterized by absorbancies in the spectral region 3600-3200 cm^{-1}.

(ii) Higher presence of aliphatic CH_2 and CH_3 groups characterized by absorbancies in the spectral region 3000-2700 cm^{-1}, maximizing at 2920 $cm^{-1}$and 2850 cm^{-1} and in the spectral region 1530-1350 cm^{-1}, maximizing at 1440 cm^{-1}.

(iii) Significantly higher presence of oxygen containing groups, most probably aromatic carboxylic groups, appearing in the spectral region 1730-1670 cm^{-1} with a peak maximum at 1700 cm^{-1} is registered for HL byproduct.

(iv) The higher presence of variety of oxygen containing functional groups, i.e. carboxylic groups, phenolic and alcoholic groups, aromatic ethers is confirmed by the signals registered in the region 1100-1340 cm^{-1} (maxima at 1260 cm^{-1} and 1210 cm^{-1}).

(v) A new band appears at 1540 cm^{-1} in HL byproduct`s FTIR spectrum. It is attributed to a new formed aromatic NO_2 groups [9,33].

Mineral matter absorption might overlap organic group characteristic peaks. In this respect, strong bands should appear in the region 1100-950 cm^{-1} with a maximum at 1030 cm^{-1} for C-O-C vibrations, anti symmetric Si-O-Si and Si-O vibrations of silicates (clay minerals, kaolinite and illite). Typical kaolinite bands at 3700-3620 cm^{-1}, 1034 cm^{-1} and also centered at 537 cm^{-1} and 476 cm^{-1} [34], are registered only for HA sample.

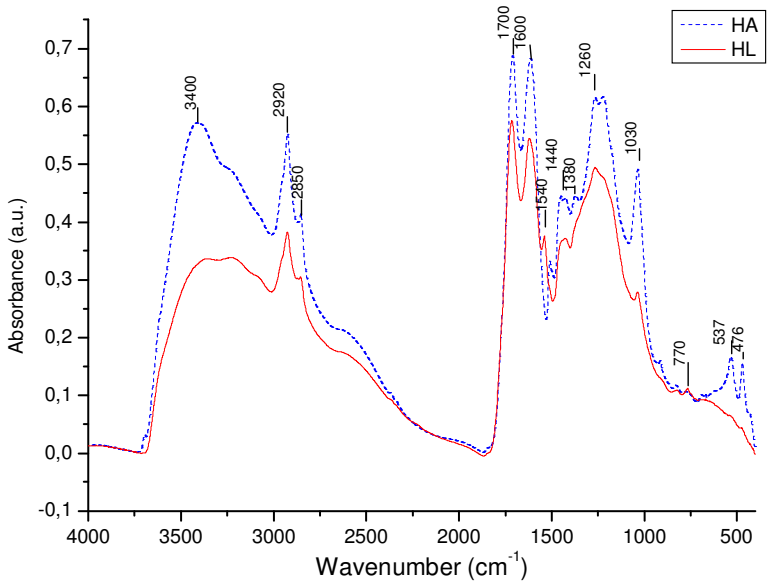

Figure 6-5 FTIR spectra of HL and HA samples

Table 6-5 Results of FTIR study of HA and HL from Maritza East lignite

Region [cm-1]	Assignment	Peak [cm^{-1}]	Absorbance Band Area ratio: A_x/A_{1600}	
			HL	HA
3500-3200	O-H, N-H strechings	3400	5.091	3.617
3000-2700	C-H aliph. stretchings	2920	0.451	0.445
		2850	0.056	0.046
1730-1670	C=O stretching	1700	1.990	1.129
1670-1530	C=C arom. stretching	1600	1	1
1530-1350	C-H aliph. bendings	1440	0.436	0.278
		1380	0.027	0.042
1340-1100	C-O(H), C-O(C) stretchings	1260	0.172	0.116
		1210	0.038	0.036

6.3.4 GC/MS data

The yield of bitumen extracted from HL byproduct is 3.67%. It is distributed in the following fractions, in %: neutrals = 3.7; aromatics = 9.6; and NSO = 73.7. Details concerning bitumen extracted from HA sample and its fractional composition can be found in a previous paper [35].

Results of the GC/MS study of HL byproduct are gathered in Table 6-6. Compounds are grouped in chemical classes. A comprehensive description of all components, listed in Table 6-6, could be found in previous studies [36]. In general, the same components are present in the HA and HL samples. The following peculiarities are observed:

- A striking feature of HA and HL samples is the extremely high content of 16α(H)-Phyllocladane and its unsaturated counterpart;
- Total portion of polar diterpenoids is the highest in HL byproduct and compounds Ferruginol and ketophenol Sugiol dominate in both samples, i.e. HA and HL. Their dehydrogenated counterparts, i.e. Dehydroferruginol and Dehydrosugiol, are abundant as well. New formed compound for HL sample, 7-Keto-totara-5-en-ol, $C_{20}H_{26}O_2$, described in a previous paper [1], is registered.

- Content of alkane-2-ones is doubled for HL byproduct compared to the content in HA sample.
- A particular series of esters of linear fatty acids (FAs) are identified only in HL byproduct. FA esters are depicted in Figure 6-6 by their specific ion, namely *m/z* 88 - for fatty acid ethyl esters (FAEts) and *m/z* 102 - for fatty acids propyl esters (FAPrs). Their quantities in HL sample are calculated bearing in mind the extract`s yield and carbon content. The following values are established: (i) short-chain FAs, propyl esters, nC_{12} ÷ nC_{16} – 66 μg/g C_{org}, 155 μg/kg HL; (ii) Long-chain FAs, ethyl esters, nC_{24}÷ nC_{30} – 124 μg/g C_{org}, 460 μg/kg HL.

Fatty acids are common constituents of humic acids. Their bond linkage have been studied by several researchers in a suite of papers devoted to humic acids in soils, peat, lignite, etc. [37-43]. Except "free" fatty acids, it is possible by pyrolysis in presence of alkylation reagent to obtain strongly "trapped" and esterified FAs. Linear long chain FAs are the main constituents of humic acids from Maritza East lignite, amounting about a half of GC amenable compounds [35]. The distribution pattern presents a long mode maximizing at nC_{28} with strong "even" carbon numbered dominance. Such a distribution indicates a dominant higher plant origin. There are not registered FAEts and FAPrs in pristine humic acids HA from Maritza East lignite. One possible explanation for their presence in HL could be enzymatic bond cleavage in wax esters during biotreatment. It should be emphasized that registered wax esters (Table 6-6) are composed by nC_{14} linear FA esterified by alcohols with different length, nC_{14} ÷ nC_{18}.

FAEts are not unusual as they are found in some coals and geological sediments [44]. Long chain FAs are characteristic for epicuticular waxes of higher plants and ethyl esters presence is attributed to bacterial activity in the deposition environment.

Table 6-6 List of GC/MS registered homologue series and compounds

Chemical class	Homologue	Compound	$M^{+\cdot}$	*m/z* (100%)	Samples HA	HL
Aliphatic lipids						
	n-Alkanes, C_nH_{2n+2}	$nC_{15} \div nC_{35}$, $n\underline{C_{29}}$		85	+	+
	n-Alkan-2-ones, $C_nH_{2n}O$	$nC_{25} \div nC_{35}$, $n\underline{C_{29}}$		58	+	+
	Mid-chain ketone	Nonacosan-10-one, $C_{29}H_{58}O$	422	155	+	+
	Iso-ketone	Trimethylpentadecan-2-one, $C_{18}H_{36}O$	268	58	+	+
	n-Alkohols, $C_nH_{2n+2}O$	$nC_{22} \div nC_{30}$, $n\underline{C_{26}}$		111	+	+
	n-Fatty acids, Et/Pr esters	$nC_{12} \div nC_{30}$ (even homologues), $n\underline{C_{28}}$		88/102	-	+
	Wax esters	$nC_{28} \div nC_{32}$ (even homologues), $n\underline{C_{28}}$		229	+	+
Terpenoids	Diterpenoids					
		16α(H)-Phyllocladane, $C_{20}H_{34}$	274	123	+	+
		Simonellite, $C_{19}H_{24}$	252	237	+	+
		Retene, $C_{18}H_{18}$	234	219	+	+
	Polar diterpenoids					
		Ferruginol, $C_{20}H_{30}O$	286	271	+	+
		Dehydroferruginol, $C_{20}H_{28}O$	284	202	+	+
		Sugiol, $C_{20}H_{28}O_2$	300	285	+	+
		Dehydrosugiol, $C_{20}H_{26}O_2$	298	213	+	+
		7-Keto-totara-5-en-ol, $C_{20}H_{26}O_2$	298	229	-	+
	Ketotriterpenoids					
		Friedelin, $C_{30}H_{50}O$	426	69	+	+
		Amyrone, $C_{30}H_{48}O$	424	218	+	+
		Lupan-3-one, $C_{30}H_{50}O$	426	205	+	+
	Ketohopanes					
		17,21-*Seco*-Pentakisnor-hopan-17-one, $C_{25}H_{42}O$	358	191	+	+
		17a(H)-Trisnorhopane-21-one, $C_{27}H_{48}O$	384	191	+	+
Steroids	Ketosterane					
		Stigmast-4-en-3-one, $C_{29}H_{48}O$	412	124	+	+

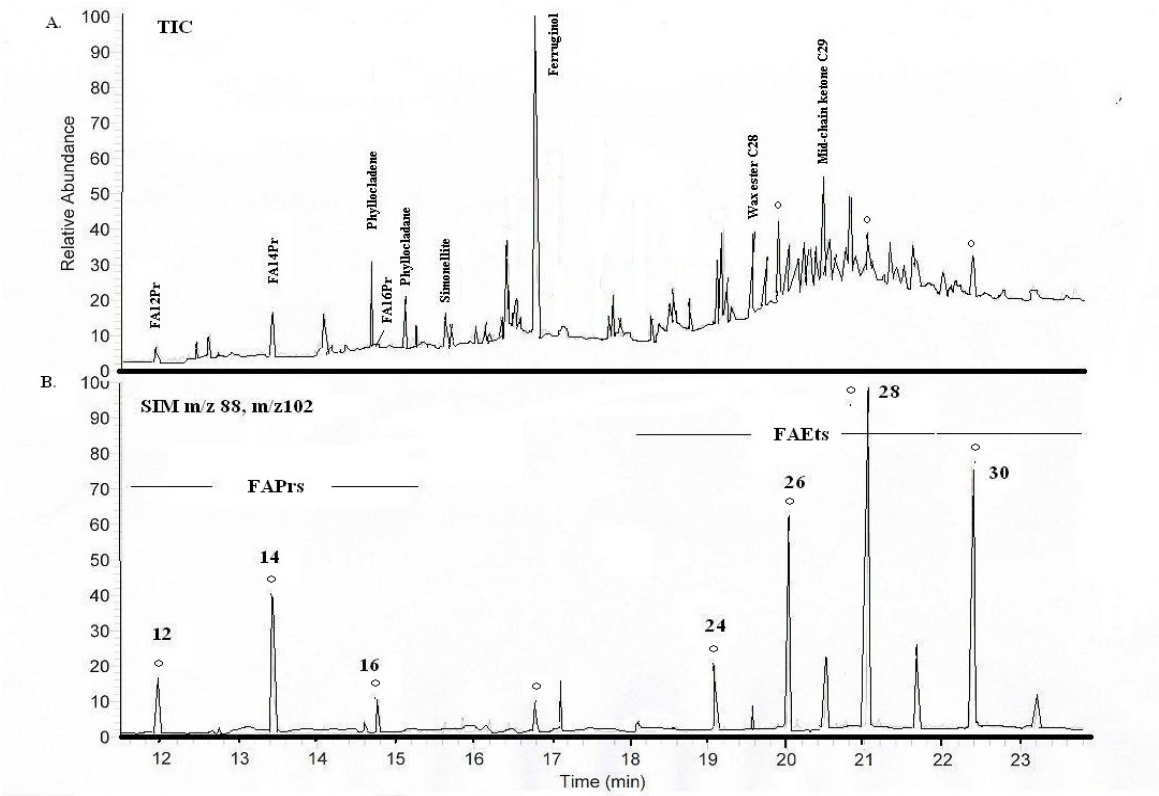

Figure 6-6 TIC of aromatic/polar fraction of HL byproduct (A) and fatty acids ethyl esters (*m/z* 88) and fatty acid propyl esters (*m/z* 102) distribution (B)

6.4 Conclusions

Desulphurization by combinations of chemical and microbial treatments are carried out on Bulgarian lignite from "Maritza East" deposit. The following maximal combined desulphurization effects is achieved for APF-oxy-PP sample: S_t = 71.0%; S_s = 93.9%; S_p = 90.6%; and S_o = 49.4%. The registered chemical desulphurization effects towards sulphur forms for APF-oxy sample in comparison with IN sample are: ΔS_t = 63.3%; ΔS_s = 95.8%; ΔS_p = 87.1%; and ΔS_o = 35.5%. As a result of *PP* biotreatment the following extra desulphurization (biodesulphurization) effects towards sulphur forms are registered for APF-oxy-PP sample in comparison with APF-oxy coal: ΔS_t = 21.0%; ΔS_p = 26.8%; and ΔS_o = 21.7%. By applying AP-TPR technique coupled with different detection systems (MS and TD-GC/MS), organic sulphur alterations as a result of *PP* biotreatment are assessed. It is revealed that both, aliphatic and aromatic sulphur, are affected due to applied treatments. As a result, significant part of S_o is transformed in a water soluble state, which is highly volatile under applied AP-TPR conditions. The ongoing desulphurization mechanism is oxidative. Some pathways concerning oxidation mechanism of organic sulphur compounds transformed by *PP* lignite biotreatment are proposed. Transformation of BzTh and Th during the treatments can occur *via* S-atom oxidizing mechanism and *via* C-atom oxidizing mechanism. However, C-atom oxidizing mechanism should be considered as the dominant one.

Since HL byproduct manifests external similarity to humic substances, it is compared to pristine humic acid HA obtained from the same coal sample. Analysis carried out shows high similarity between HL products and HA. However, some differences are noticed:

- HL sample is characterized by lower H/C and O/C ratios, and higher N/C and S/C ratios compared to HA sample;
- By AP-TPR analyses differences in the proportions of registered sulphur species are observed;

- By GC/MS analysis the following peculiarities are observed for HL sample in comparison with HA sample: (i) content of alkane-2-ones is doubled; (ii) new series of fatty acid appears; (iii) new formed polar diterpenoid is registered;
- By FTIR spectroscopy the following differences are registered for HL sample in comparison with HA sample: (i) higher presence of H-bonded OH and NH groups; (ii) higher presence of aliphatic CH_2 and CH_3 groups; (iii) significant higher presence of variety of oxygen containing functional groups, i.e. carboxylic and phenolic groups, as well as aromatic ethers; (iv) A higher presence of aromatic carboxylic groups is determined; (v) A new formed aromatic NO_2 groups are registered.

6.5 References

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Chapter 7 Contributions and perspectives

Following dissertation contributions can be formulated based on the experimental data and their in-depth interpretation:

- Biodesulphurization effects achieved for all sulphur forms present in the studied coal by a set of different microorganisms are precisely evaluated. By applying an appropriate experimental strategy and adequate analytical techniques, the organic sulphur transformation mechanisms, as a result of applied biotreatments, are explained and clarified at a certain extent.
- A new method for determination of elemental sulfur in coal based on exhaustive $CHCl_3$ extraction and subsequent quantitative analysis of the extracts by HPLC, equipped with a C_{18} reversed phase column is proposed. It results in more accurate sulfur balance.
- Some important improvements are made concerning the AP-TPR technique allowing more reliable analytical use for studying sulphur alterations in coals.
- In the current thesis by the use of original and diversified experimental strategy it becomes possible to determine: (i) suitable microorganism that can be applied for studied coals; (ii) sulphur forms and sulphur functionalities that are preferred by the applied microorganisms; (iii) pretreatments necessary to be performed in order to convert coal, its sulphur forms and functionalities in a form suitable for microbial attack assuring high desulphurization rates and effectiveness of the microorganisms.

Based on the literature overview and analysis of the obtained in the thesis results it is obvious that the known microbial treatments are not yet sufficiently effective as a desulphurization method for organic sulphur removal. Despite the considerable progress in the elucidation of the mechanisms of the biodesulphurization processes of organic sulphur functionalities and the efforts to increase the rates of organic sulphur removal, the registered biodesulphurization effects concerning organic sulphur are low. Therefore, additional study in this field is required. This research can comprise:

- Discovery and selection of more efficient microorganisms towards organic sulfur removal.
- Preparation of mixtures of microorganisms that attack organic and inorganic sulfur and thus increase total sulphur biodesulphurization effect.
- Preparation of mixtures of microorganisms that attack selectively different organic sulfur functionalities and thus increase organic sulphur biodesulphurization effect.
- Genetic engineering in order to modify genes of microorganisms and thus to improve their sulphur removal effect, ability to tolerate toxins, growth rate, etc.
- Combination of microbial treatments with physical and chemical desulphurization methods.

Summary and general conclusions

Sulphur is an undesirable constituent of coal. Technological utilization of sulphur-containing coal, i.e. combustion, dry distillation or hydrogenation, creates air pollution. During combustion of coal, most of the sulphur (pyritic, elemental and organic sulphur) is emitted as SO_2, while during dry distillation and hydrogenation H_2S, CS_2, COS and sulphur-containing volatile organic compounds (S-VOCs) are released. In addition, sulphur in coal induces supplemental operational problems, i.e boiler fouling, corrosion and equipment damage, poisoning of catalysts, and production of metallurgical coke for steel manufacture with low quality.

Environmental legislation norms regarding sulphur emissions from coal combustion is already in place in many countries and is under consideration in others. Therefore in the last years, in order to comply with the strict environmental requirements for coal combustion, desulphurization of fossil fuels has become extremely important. Recently, there is a keen interest on biodesulphurization as a method with high potential toward sulphur removal. It is known that biodesulphurization is relatively new and insufficiently studied method for organic sulphur removal from coal. From the other side, it is a very attractive approach due to the relatively low costs, mild experimental conditions and the lack of additional pollutants. However, the current limitation and disadvantages of microbial treatments hinder their industrial application. In order to promote coal biodesulphurization for future industrial exploitation, there is a need to perform additional research resulting in increased desulphurization rates and effectiveness by the microorganisms.

It is believed that deeper knowledge in organic sulphur transformation in coal during microbial processing as well as appreciation of coal matrix influence on biodesulphurization will reflect in better understanding of microbial desulphurization in coal. This will assure a greater success in microbial sulphur removal. Therefore, the main goal of the present study is tracking the changes that occur with sulphur, its forms and functionalities in organic compounds by biodesulphurization of coals from different sources. To achieve this purpose the following tasks are addressed:

(i) Biodesulphurization of high sulphur coal with appropriate microbial cultures. Qualitative and quantitative assessment of the changes that occur with sulphur and its forms and functionalities as a result of the biotreatments by the use of pyrolytic, chromatographic and spectrometric methods.

(ii) Biodesulphurization of preliminary demineralized and depyritized coal samples and qualitative and quantitative assessment of the changes that occur with sulphur and its forms and organic functionalities as a result of biotreatments by the use of pyrolytic, chromatographic and spectrometric methods. This task has been imposed in order to focus our efforts on organic sulphur biodesulphurization research and to improve the information obtained by analytical tools used for sulphur assessment.

(iii) Evaluation of elemental sulphur in the samples under consideration by developing and implementing a methodology for its determination. This will give us ground to attain a better sulphur balances for initial and biotreated samples.

(iv) Desulphurization of technological coal sample with industrial significance by a combination of chemical and microbial treatments. Qualitative and quantitative evaluations of the sulphur changes that occur as results of the applied treatments.

The current thesis comprises seven chapters. It is a result of cooperation agreement for joint supervision and award of a doctorate between Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences and University of Hasselt, Belgium.

Chapter 1 is an introduction describing the importance of sulphur removal from coal, sulphur forms and their origin in coals and sulphur forms in Bulgarian coals. Special attention is dedicated to desulphurization methods (i.e. postcombustion and precombustion) and particularly biodesulphurization treatments. Simultaneously, an in-depth literature study and an overview is presented. Finally, the research objectives are formulated.

Chapter 2 describes the samples under consideration, used microorganisms and gives detailed information on the applied treatments (chemical and microbial) and used analytical methods.

Chapter 3 describes the biodesulphurization of Bulgarian high sulphur subbituminous coal with the appropriate cultures *Phanerochaeta chrysosporium*, *Trametes versicolor* and *Mixed Culture of microorganisms*. Organic sulphur functionality alterations as a result of applied biotreatments are traced by Atmospheric Pressure-Temperature Programmed Reduction (AP-TPR) technique coupled with different detection systems: Mass spectrometry, Potentiometry, Thermal Desorption-Gas Chromatography/Mass spectrometry (TD-GC/MS).

The following conclusions can be formulated:

- It is determined that implemented biotreatments demonstrate maximum total sulphur and organic sulphur desulphurizations up to 26% and 13%, respectively.
- The ongoing biodesulphurization mechanisms are oxidative. Namely, biotransformation process of complex sulphur species into sulphones and sulphoxides accompanied by organic sulphonic group decomposition are registered.
- Biotreatments do not significantly change the coal matrix. Nevertheless, it is established that biodesulphurization has an effect on the organic coal matrix, resulting in a better hydrogenation/reduction in an inert atmosphere of organic sulphur groups still present in the treated samples.
- Removal of complex thiophenic structures occurs for all biotreated samples. Homocycling ring opening of complex thiophenic structure along with demethylation processes take place during fungi treatments.

Chapter 4 deals with preliminary demineralized, depyritized and subsequently biodesulphurized by *Sulfolobus solfataricus* and *Phanerochaeta chrysosporium* low rank coal samples. The study demonstrates:

- Higher biodesulphurization effect is attained for demineralized and depyritized coal samples treated with white rot fungus *Phanerochaeta chrysosporium.* Total and organic sulphur biodesulphurization effects for coals treated with *Phanerochaeta chrysosporium* vary in the range of

10.3-24.2% and 2.9-23.8%, respectively. By *Sulfolobus solfataricus*, maximum total and organic sulphur biodesulphurization effects of 16.9 % and 18.3 %, respectively, are determined.

- It can be assumed that degradation processes of complex sulphur species by oxidation processes take place in different pathways during *Sulfolobus solfataricus* and *Phanerochaeta chrysosporium* biotreatments.
- The observed organic sulphur alterations are related not only to the type of sulphur functionalities and structural peculiarities of the samples, but also to the different preferences of the used microorganisms towards sulphur functionalities.
- The information obtained by AP-TPR "off-line" TD-GC/MS is improved due to special sorbents application. As a consequence, a greater number of sulphur-containing volatiles are identified and quantified during TPR experiments.

Chapter 5 comprises the experimental work and results related to the development and implementation of a new methodology for elemental sulphur determination in low rank coals. The described procedure is developed in order to specify the changes in the organic and elemental sulphur as a result of the applied biotreatments.

The following conclusions can be formulated:

- A new procedure for elemental sulphur determination based on exhaustive $CHCl_3$ extraction and a subsequent quantitative analysis of the extracts by HPLC equipped with a C_{18} reversed phase column is proposed. Its application gives better sulphur balance.
- The highest presence of elemental sulphur among the investigated samples is registered in demineralized and depyritized coals, explained by their natural weathering.
- The differences in the elemental sulphur amounts for all investigated demineralized and depyritized samples could be related to coal maturity and to the special characteristics of the coal matrix.
- *Sulfolobus solfataricus* appears to be a better biodesulphurizing agent toward elemental sulphur, than the applied white rot fungus

Phanerochaeta chrysosporium. The achieved maximum reduction is 54%.

- The content of elemental sulphur is also assessed as part of the total sulphur and organic sulphur. The results clearly demonstrate the significance of elemental sulphur and that the lack of elemental sulphur evaluation might be a source of appreciable errors in the magnitude of organic sulphur presence.

Chapter 6 describes an original desulphurization strategy including sequence of chemical (demineralization, depyritization, thermooxidation) and microbial treatments by *Pseudomonas putida* bacteria toward inorganic and organic sulphur removal in Maritza East lignite. As a result of the applied combinations of desulphurization treatments: i) maximum of total sulphur desulphurizataion is 71.0%; ii) maximum of pyritic sulphur desulphurization is 90.6%; iii) maximum of organic sulphur desulphurization is 49.4%. These maximal desulphurization effects are achieved for demineralized, depyritized, oxidized and biotreated by *Pseudomonas putida* lignite. Therefore, the changes that occur with sulphur functionalities in the above mentioned sample and in its water soluble byproduct are of special interest. It is revealed that:

- Both, aliphatic and aromatic sulphur, are affected by the applied treatments. As a result, significant part of organic sulphur is transformed in a water soluble form, which is highly volatile under applied AP-TPR conditions.
- The ongoing desulphurization mechanism is oxidative. Some pathways concerning oxidation mechanism of organic sulphur compounds transformed by *Pseudomonas putida* biotreatment are proposed. Transformation of benzothiophene and thiophene during the treatments can occur *via* S-atom and *via* C-atom oxidizing mechanism. However, C-atom oxidizing mechanism seems to be more preferred.
- Analysis carried out shows high similarity between water soluble byproducts and pristine humic acids obtained from the same initial coal. However, some differences are depicted as well.

Chapter 7 comprises the dissertation contributions and perspectives for future investigations.

The following dissertation contributions can be formulated:

- Biodesulphurization effects achieved for all sulphur forms present in the studied coal under the action of a set of different microorganisms are precisely evaluated. By applying an appropriate experimental strategy and adequate analytical techniques the organic sulphur transformation mechanisms as a result of applied biotreatments are explained and clarified at a certain extent.
- A new method for determination of elemental sulfur in coal based on exhaustive $CHCl_3$ extraction and subsequent quantitative analysis of the extracts by HPLC using C_{18} reversed phase column is proposed. It gives the opportunity to achieve accurate sulfur balance.
- Some important improvements are made concerning AP-TPR technique. They allow more reliable analytical tool to be applied for studying sulphur alterations in coals.
- In the current thesis by the application of original and diversified experimental strategy it becomes possible to determine: (i) suitable microorganism that can be applied for studied coals; (ii) sulphur forms and sulphur functionalities that are preferred by the applied microorganisms; (iii) pretreatments necessary to be performed in order to convert coal, its sulphur forms and functionalities in a form suitable for microbial attack assuring high desulphurization rates and effectiveness of the microorganisms.

Резюме и заключения

Сярата е нежелана съставна част на въглищата. Технологичното оползотворяване на сяросъдържащите въглища в т.ч. изгаряне, суха дестилация или хидриране, води до замърсяване на въздуха. При изгарянето на въглищата в котелните инсталации, значителна част от сярата (пиритна, сулфатна и органична) формира SO_2 емисии, докато при сухата им дестилация и хидриране тя се отделя под формата на H_2S, CS_2, COS и сяро-съдържащи летливи органични съединения (S-VOCs). Наред със замърсяването на въздуха, сярата във въглищата създава и редица технологични проблеми като отравяне на катализатори, корозия и износване на инсталациите, както и влошени качества на кокса като металургично гориво, и като суровина за производството на стомана.

С цел да се ограничат вредните серни емисии в много страни са в сила законодателни разпоредби в областта на околната среда, или са в процес на разглеждане в други. Поради тази причина, с оглед спазването на строгите екологични изисквания при изгаряне на въглища, десулфуризацията на изкопаемите горива е от съществено значение. Докато при отстраняването на неорганичната сяра са постигнати високи нива на сероочистка, то при премахване на органичната сяра резултатите са незадоволителни. Напоследък се забелязва повишен интерес към биодесулфуризацията като метод с висок потенциал за отстраняването на органичната сяра във въглищата. От познатите подходи за сероочистка на въглища е известно, че биодесулфуризацията е сравнително нов и недостатъчно изучен метод за десулфуризация. Той е атрактивен поради ниските капитални вложения, "меки" експериментални условия и липсата на отделящи се допълнителни замърсители. Въпреки изброените предимства, биодесулфуризацията има и някои недостатъци, които са възпрепятствали промишленото й използване. С цел насърчаване на индустриалното приложение на въглищната биодесулфуризация, налице е необходимост от допълнителни проучвания, които следва да бъдат извършени с цел повишаване на биодесулфуризационните ефекти и ефективността на микроорганизмите.

Известно е, че по-задълбочени познания за промените, които настъпват с органичната сяра във въглища по време на микробиална обработка, както и по отношение на влиянието на въглищната матрица върху биодесулфуризацията, биха довели до по-доброто провеждане на микробиална въглищна сероочистка. Основна цел на настоящата дисертация е да се проследят промените, които настъпват със сярата, серните форми и функционалности в процес на ефективна биодесулфуризация на различни въглища от нисък ранг. За постигането на тази цел са поставени следните задачи:

- Биодесулфуризация на високо сернисти въглища с подходящи микробиални култури. Количествена и качествена оценка на промените, които настъпват със сярата, нейните форми и функционалности в резултат на биообработките чрез пиролитични, хроматографски и спектрални методи.
- Биодесулфуризация на предварително деминерализирани и депиритизирани въглищни проби. Количествена и качествена оценка на промените, които настъпват със сярата, нейните форми и органични серни функционалности в резултат на приложените биообработки. Тази задача бе поставена с цел да се насочат усилията към по-детайлно изучаване на органичната сярна биодесулфуризация.
- Разработване и прилагане на метод за количествено определяне на елементна сяра в изследваните проби. Това би довело до по-точен баланс на серните форми в изходните и биотретираните проби.
- Десулфуризация чрез комбинация от химични и микробиални обработки на технологична въглищна проба с промишлено значение. Количествена и качествена оценка на серните изменения в резултат на приложените обработки.

Настоящата дисертационна работа се състои от седем глави. Тя е резултат на двустранно споразумение за сътрудничество при ръководене, изпълнение и присъждане на докторска степен между Института по органична химия с Център по Фитохимия, БАН и Университета на Хаселт, Белгия.

Глава 1 е въведение, което описва необходимостта от очистване на сярата във въглищата, видовете серни форми и техния произход във въглищата, тъй както и серните форми в български въглища. В нея специално внимание се отделя на десулфуризационните методи (преди- и след изгаряне) и по-специално на биодесулфуризацията. Едновременно с това е представен задълбочен литературен анализ по темата. Накрая са формулирани целите и задачите на изследването.

Глава 2 описва обектите на изследване, използваните микроорганизми и дава обстойна информация за приложените обработки (химически и микробиални) и използваните методи за анализ.

Глава 3 отразява биодесулфуризацията на български високо сернисти кафяви въглища с подходящите микробиални култури *Phanerochaeta chrysosporium*, *Trametes versicolor* и *Mixed Culture* от микроорганизми. Промените, които настъпват с органичните серни функционалности са проследени чрез използването на "Температурно Програмирана Редукция при Атмосферно Налягане - AP-TPR" техника свързана с различни детекционни системи, в т.ч. масспектрометрия, потенциометрия, термична десорбция-газова хроматография/масспектрометрия (TD-GC/MS).

В резултат на това изследване са формулирани следните изводи:

- Установено е, че приложените биообработки с *Trametes Versicolor*, *Phanerochaeta Chrysosporium* и *Mixed culture of microorganisms* върху изходна въглищна проба "Пирин" има за резултат максимална биодесулфуризация по отношение на общата сяра ~26% и органичната сяра ~13%.
- Приложените биообработки не засягат съществено въглищната матрица и се запазват калоричните показатели.
- Биодесулфуризационният механизъм на протичащите процеси е окислителен. Регистриран е биотрансформационен процес на сулфидни серни функционалности в сулфони и сулфооксиди, съпроведен от деструкция на органични сулфонови групи.
- За всички биотретирани проби е регистрирано очистване на сложни тиофенови структури. При пробите биообработени с гъбите *Trametes*

Versicolor и *Phanerochaeta Chrysosporium* е установено отваряне на хомолитични пръстени в тиофеновите структури, придружено с деметилирането им.

Глава 4 разглежда предварително деминерализирани, депиритизирани и следващо биодесулфуризирани с *Phanerochaeta chrysosporium* и *Sulfolobus solfataricus* въглища нисък ранг. Проведеният анализ показа:

- По-висок биодесулфуризационен ефект е постигнат за деминерализираните и депиритизирани въглища, третирани с гъбичната култура *Phanerochaete Chrysosporium*. Регистрираните биодесулфуризационни ефекти по отношение на обща и органична сяра варират съответно в границите 10.3 – 24.2% и 2.9 – 23.8%. При биообработките с бактерията *Sulfolobus solfataricus* максимално постигнат ефект на биодесуфуризация по отношение на обща и органична сяра е съответно 16.9 % и 18.3%.
- Установено е, че по време на биобработките протичат процеси на деструкция на сложни видове органична сяра. Те са придружени от окислителни процеси и протичат по различни механизми при биообработките с *Phanerochaeta chrysosporium и Sulfolobus solfataricus*.
- Регистрираните промени с органичните серни функционалности в следствие на приложените биообработки са свързани не само с вида на серните функционалности и структурните особености на пробите, но също така и с предпочитанията на използваните микроорганизми към различните серни функционалности.
- Информацията, получена с AP-TPR-TD-GC/MS техника е подобрена чрез употреба на адсорбционни тубички, запълнени с сяро-селективни сорбенти. В резултат на това, по-голям брой летливи в условията TPR пиролиз сяросъдържащи съединения са качествено и количествено определени.

Глава 5 обхваща експериментална работа и резултати свързани с разработването на метод за количествено определяне на елементна сяра във въглища нисък ранг. Той е приложен с цел да се разграничат промените,

които настъпват с органичната и елементната сяра в резултат на приложените биообработки.

В резултат на проведената експериментална работа могат да бъдат формулирани следните изводи:

- Предложена е нова процедура за определяне на елементна сяра във въглища, основаваща се на изчерпателна екстракция с $CHCl_3$ и следващ количествен анализ на екстрактите чрез HPLC с колона с обърната фаза C_{18}. Методиката дава възможност да се постигне по-добър количествен баланс на серните форми.
- Най-високото количество елементна сяра е регистрирано в деминерализираните и депиритизирани въглища, което се обяснява с вероятно протекли природни процеси на изветряне.
- Количествено е доказано, че различията в регистрираната елементна сяра за всички деминерализирани и депиритизирани проби са пряко свързани със зрялостта на изследваните въглища и с особеностите на въглищната матрица.
- По отношение на елементната сяра *Sulfolobus solfataricus e* по-добра биодесулфуризираща култура в сравнение с *Phanerochaeta chrysosporium*. Максималната десулфуризация по отношение на елементна сяра е 54%.
- Съдържанието на елементна сяра е оценено и като част от общата и органичната сяра. Направеният анализ на резултатите доказва, че липсата на количествена оценка на стойността на елементна сяра в изследваните проби може да доведе до значителна грешка в стойността на органична сяра.

В Глава 6 се прилага оригинална десулфуризационна стратегия, която включва последователност от различни химични обработки и микробиално третиране с бактерията *Pseudomonas putida*. Обработките са приложени с цел отстраняване на неорганичната и органична сяра в лигнитни въглища "Марица-Изток". В резултат на това, максимална десулфуризация по отношение на общата сяра (71,0%), максимална десулфуризация по отношение на пиритната сяра (90,6%) и максимална десулфуризация по

отношение на органичната сяра (49,4%) са постигнати за деминерализирана, депиритизирана, окислена и биообработена с *Pseudomonas putida* проба. Поради това подробно са изучени промените, които настъпват със серните функционалности в горепосочената проба, както и в нейния водоразтворим вторичен продукт.

Установено е, че:

- Приложената биообработката с *Pseudomonas putida* повлиява и двата типа сяра, в т.ч. алифатна и ароматна. В резултат, значителна част от органичната сяра се превръща във водоразтворима форма, която е силно летлива в условията на AP-TPR пиролиз.
- Биодесулфуризацията протича по окислителен механизъм. Предложени са механизми на окисление за някои органични серни съединения под действието на бактерията *Pseudomonas putida*. Биоокислението на бензотиофен и тиофен по време на приложената биообработка протича по S-атом и по C-атом окисляващ механизъм, като доминиращ е последният.
- Проведеният анализ установи голямо сходство между водоразтворимият продукт получен след биообработка с *Pseudomonas putida* и хуминови киселини извлечени от същата изходна въглищна проба. Наред с това са регистрирани и някои различия.

Глава 7 представя приносите на настоящата дисертация както и перспективи за бъдещи проучвания.

Формулирани са следните дисертационни приноси:

- Прецизно са оценени биодесулфуризационните ефекти, постигнати под действието на набор от микроорганизми, по отношение на всички серни форми, присъстващи в изследваните въглища. Механизмите на биодесулфуризация на органичните серни съединения в резултат на биообработките са обяснени чрез разработване на подходяща експериментална стратегия и прилагане на адекватни аналитични техники.

- Предложен е нов метод за определяне на елементна сяра във въглища. Той включва изчерпателна екстракция с $CHCl_3$ и последващ количествен анализ чрез HPLC с колона с обърната фаза C_{18}. Приложението на този метод дава възможност да се постигне точен баланс на серните форми.
- Направени са някои важни подобрения по отношение на AP-TPR техниката. Те позволяват да се приложи по надежден аналитичен инструмент за изучаване на серните промени във въглища.
- В настоящата дисертация, чрез прилагането на оригинална и разнообразна експериментална стратегия става възможно да се определят: i) подходящи микроорганизми за биодесулфуризация на изследваните въглищни проби; ii) формите на сярата и серни функционалности, които са предпочитани от приложените микроорганизми; iii) предварителните обработки необходими за трансформиране на въглищата, техните серни форми и функционалности във вид, подходящ за микробиална атака, като по този начин се постига повишаване на биодесулфуризационните ефекти и активността на микроорганизмите.

Samenvatting en algemene conclusies

Zwavel is een ongewenst bestanddeel van steenkool. Technologisch gebruik van zwavelhoudende steenkool: zoals verbranding, droge destillatie of hydrogenatie creëert luchtvervuilingproblemen. Gedurende verbranding van steenkool wordt zwavel (pyriet, elementair en organisch zwavel) vooral uitgestoten als SO_2, terwijl gedurende droge destillatie en hydrogenatie H_2S, CS_2, COS en zwavelhoudende vluchtige verbindingen (S-VOCs) worden vrijgesteld. Bijkomend veroorzaakt de zwavel in steenkool ook operationele problemen: zoals stoomketelverontreiniging, corrosie en apparatuurbeschadiging, vergiftiging van katalysatoren en aanmaak van minderwaardige kwaliteitscoke voor staalbedrijven.

Milieuwetgevingnormen naar zwaveluitstoot afkomstig van de verbranding van steenkool is reeds van toepassing in vele landen of is in overweging. Vandaar dat in de afgelopen jaren, om te voldoen aan de strikte milieunormen rond verbranding van steenkool, voorafgaande ontzwaveling van fossiele brandstoffen zeer belangrijk is geworden. Recentelijk bestaat er ook een bredere interesse om ook bio-ontzwaveling toe te passen als een mogelijke alternatieve methode en dit met hoge ontzwavelingverwachtingen. Bio-ontzwaveling is relatief nieuw en een onvoldoende bestudeerde benadering om zwavel voorafgaandelijk uit steenkool te verwijderen. Anderzijds is dit ook een aantrekkelijke methode gezien zijn lage operatiekost, zachte experimentele condities en afwezigheid van bijkomende polluenten. Door de huidige beperkingen en de nadelen van microbiële behandelingen wordt het industrieel gebruik ervan echter gehinderd. Om toch het toekomstig industrieel gebruik van bio-ontzwaveling van steenkool te promoten is er nood aan bijkomend onderzoek om de ontzwavelingsnelheid en –efficiëntie door micro-organismen te vergroten.

Door een diepgaandere kennis in de organisch zwavel structuurveranderingen gedurende het microbieel proces, naast de impact ervan op de steenkool matrix, zal hierdoor een beter inzicht bekomen worden rond het ontzwavelingmechanisme. Dit zal tot een meer succesvolle microbiële ontzwavelingsmethode leiden. Het hoofddoel van deze studie is dan ook om na te gaan hoe zwavelstructuurveranderingen gebeuren, zowel naar voorkomen als

naar aard, ten gevolge van verschillende bio-ontzwavelingprocessen van steenkool. Om dit te realiseren werden de volgende onderzoekstaken uitgevoerd:

(i) Bio-ontzwaveling van steenkool met hoog zwavelgehalte via wel uitgekozen microbiële culturen. Kwalitatieve als kwantitatieve evaluatie van de optredende zwavelvorm- en zwavelfunctionaliteitveranderingen ten gevolge van de bio-behandeling via pyrolytische, chromatografische en spectroscopische methoden.

(ii) Bio-ontzwaveling van vooraf gedemineraliseerde en pyrietvrije steenkool en de bepaling van de verandering in zwavelvormen en zwavelfunctionaliteiten en dit zowel kwalitatief als kwantitatief via pyrolytische, chromatografische en spectroscopische methoden. Deze studie werd gefocust op de bio-ontzwaveling van organische zwavel in steenkool en het verbeteren en vermeerderen van de informatie verkregen met de gebruikte analytische hulpmiddelen voor zwavel functionaliteitbepalingen.

(iii) Evaluatie van het voorkomen van elementaire zwavel in de monsters door de ontwikkeling en invoering van een bepalingsmethode. Dit leidt tot een betere zwavelbalans zowel voor initiële als bio-behandelde monsters.

(iv) Ontzwaveling van technologische steenkool met een haalbaar industrieel proces via een combinatie van een chemische en microbiële behandeling. Kwalitatieve en kwantitatieve evaluatie van de zwavelvormveranderingen ten gevolge van de toegepaste behandelingen.

De voorgelegde thesis bestaat uit zeven hoofdstukken. Het is een resultaat van een samenwerkingsovereenkomst en onder een gezamenlijke supervisie leidend tot een doctoraat te behalen tussen het Instituut voor Organische Chemie met het Centrum voor Fytochemie, Bulgaarse Academie voor Wetenschappen en de Universiteit Hasselt, België.

Hoofdstuk 1 is in feite een inleiding die het belang van zwavelverwijdering uit steenkool, het zwavelvoorkomen en hun origine, als het zwavelvoorkomen in

Bulgaarse steenkool beschrijft. Bijzondere aandacht wordt besteed aan verschillende ontzwavelingsmethoden (na- en voorverbranding) en specifiek ook naar bio-ontzwaveling. Tezelfdertijd wordt een gedetailleerd literatuur onderzoek voorgesteld als een overzicht gegeven. Tot slot worden de onderzoeksobjectieven geformuleerd.

Hoofdstuk 2 beschrijft de verschillende behandelde monsters, de gebruikte micro-organismen en geeft gedetailleerde informatie rond de gebruikte behandelingen (chemische en microbieel) als de aangewende analytische methoden.

Hoofdstuk 3 bespreekt de bio-ontzwaveling van Bulgaarse subbitumineus steenkool met hoog zwavelgehalte middels geschikte *Phanerochaeta chrysosporium* culturen, *Trametes versicolor* en gemengde culturen van micro-organismen. Organische zwavelfunctionaliteitveranderingen als een resultaat van de gebruikte bio-behandeling worden via Atmospheric Pressure-Temperature Programmed Reduction (AP-TPR) techniek gekoppeld met verschillende detectiesystemen bestudeerd: dit zijn Massa-Spectrometrie, potentiometrie en Thermische Desorptie-GasChromatografie/Massa-Spectrometrie (TD-GC/MS).

Voor dit onderzoek kunnen de volgende conclusies geformuleerd worden:

- De toegepaste bio-behandeling leidt tot een maximale totale zwavel- en organische zwavelverwijdering van respectievelijk 26% en 13%.
- De gevolgde bio-ontzwavelingsmechanismen zijn oxidatief. Namelijk, een bio-transformatieproces van complexe zwavelverbindingen in sulfonen en zwaveloxides te samen met organische zwavelgroep ontbindingen.
- Bio-behandelingen zijn niet specifiek ten opzichte van de steenkool matrix. Niettegenstaande kan toch aangetoond worden dat bio-ontzwaveling een effect heeft op de organische steenkool matrix dewelke in een betere hydrogenatie/ reductie resulteert en/of een betere indringing en ontsnapping toelaat van vluchtige bestanddelen.
- Verwijdering van complex thiofeenstructuren wordt voor alle bio-behandelde steenkool vastgesteld. Homocyclische ringopening van

complex thiofeenstructuren tezamen met een dimethylatieproces grijpt plaats gedurende de bio-behandeling.

Hoofdstuk 4 bespreekt de voorafgaandelijk gedemineraliseerde en pyrietvrije laaggerankte steenkool die vervolgens bio-ontzwaveld werden middels *Sulfolobus solfataricus* en *Phanerochaeta chrysosporium*. Dit onderzoek toont aan dat:

- Een groter bio-ontzwavelingeffect wordt bekomen voor gedemineraliseerde en pyrietvrije steenkool behandelt met *Phanerochaeta chrysosporium.* Totaal en organisch zwavelverwijdering van steenkool behandeld met *Phanerochaeta chrysosporium* varieert respectievelijk tussen 10,3 tot 24,4% en tussen 2,9 tot 23,8%. Voor *Sulfolobus solfataricus* worden voor totaal en organisch zwavelverwijdering respectievelijk een maximum van 16,9% en 18,3% behaald.
- Er kan aangenomen worden dat het degradatieproces van complexe zwavelverbindingen verloopt via een oxidatieproces volgens verschillende reactiewegen en dit zowel voor de *Sulfolobus solfataricus* als voor de *Phanerochaeta chrysosporium* bio-behandeling.
- De vastgestelde organische zwavelfunctionaliteitveranderingen kunnen niet enkel gerelateerd worden met zwavelverbindingsoort als structurele bijzonderheden van de steenkool, maar ook met de voorkeurenverschillen van de gebruikte micro-organismen voor bepaalde zwavelfunctionaliteiten.
- De informatie bekomen met behulp van AP-TPR "off-line" gekoppeld met TD-GC/MS is verbeterd door gebruikt te maken van meer geschikt adsorptiematerialen. Dit resulteerde in een toename van het aantal geregistreerde vluchtige zwavelverbindingen en dit zowel kwalitatief als kwantitatief.

Hoofdstuk 5 behandelt de experimentele benadering en resultaten verbonden met de ontwikkeling en de gebruikte nieuwe methodologie voor de bepaling van elementaire zwavel in laaggerankte steenkool. Het beschrijft de procedure die ontwikkeld werd om de veranderingen in het organisch en elementair

zwavelvoorkomen te specificeren ten gevolge van de toegepaste bio-behandeling zoals beschreven.

De volgende conclusies kunnen geformuleerd worden:

- Een nieuwe procedure voor de bepaling van elementaire zwavel gebaseerd op een doorgedreven $CHCl_3$ extractie en daaropvolgend kwantitatieve analyse van het extract via HPLC, uitgerust met een C_{18} tegenstroom fasekolom. Hierdoor kan een betere zwavel massabalans gerealiseerd worden.
- De grootste aanwezigheid van elementaire zwavel in de onderzochte monsters werd gevonden in de gedemineraliseerde en pyrietvrije steenkool, te verklaren door natuurlijke verwering.
- De verschillen in hoeveelheid elementaire zwavelhoeveelheid van de onderzochte gedemineraliseerde en pyrietvrije monsters kon gerelateerd worden met de steenkool maturiteit en de bijzondere karakteristieken van de steenkool matrix.
- *Sulfolobus solfataricus* is een beter bio-ontzwavelingsmiddel voor elementaire zwavel dan *Phanerochaeta chrysosporium*. De behaalde maximale reductie in zwavel bedraagt 54%.
- De elementaire zwavelinhoud is eveneens bepaald als deel van de totale en organische zwavelhoeveelheid. Het bekomen resultaat toont duidelijk het significant voorkomen van elementaire zwavel en dat bij het ontbreken van deze informatie, dit leidt tot een aanzienlijke fout in het bepaalde organische zwavelgehalte.

Hoofdstuk 6 beschrijft een originele ontzwavelingsstrategie waarbij opeenvolgend een chemische (demineralisatie, pyrietvrij maken, thermo-oxidatie) en microbiële behandeling met *Pseudomonas putida* bacterie voor anorganische en organische ontzwaveling van Maritza East ligniet wordt toegepast. Ten gevolge van de gecombineerde ontzwavelingsbehandeling werd een maximum totaal zwavelverwijdering van 71,0% bereikt, voor pyrietverwijdering werd een maximum van 90,6% behaald en voor organische zwavelverwijdering werd een maximum van 49,4% gerealiseerd. Ten gevolge hiervan was het vrij interessant om de veranderingen van de

zwavelfunctionaliteiten in de hoger beschreven monsters te bepalen alsook de in water oplosbare bijproducten. De volgende vaststellingen werden gevonden:

- Zowel alifatische als aromatische zwavel werden aangetast ten gevolge van de gebruikte behandelingen. Resulterend in een aanzienlijke transformatie van de organische zwavel in een water oplosbare vorm, dewelke vrij vluchtig was onder AP-TPR omstandigheden.
- Het ontzwavelingsmechanisme is van oxidatieve aard. Specifieke oxidatieve reactiemechanismes van organische zwavelverbindingtransformaties door *Pseudomonas putida* van ligniet wordt voorgesteld. Transformatie van benzothiofeen en thiofeen gedurende de behandeling kan gebeuren via S en via C atoom oxidatiemechanisme. Maar het C atoom oxidatiemechanisme lijkt bevoordeligd te zijn.
- Uitgevoerde analyses tonen sterke gelijkaardigheden tussen water oplosbare bijproducten en de oorspronkelijke humuszuren bekomen uit dezelfde initiële monsters. Maar ook enkele verschillen werden vastgesteld.

Hoofdstuk 7 geeft de volgende wetenschappelijk bijdrage als mogelijke perspectieven voor toekomstig onderzoek:

- De resultaten van de bio-ontzwavelingeffecten bekomen voor all zwavelvormen aanwezig in de bestudeerde steenkolen middels verschillende micro-organismen werden gedetailleerd geëvalueerd. Door gebruik te maken van een juiste experimentele strategie en zeer doelgerichte analytische technieken konden de organische zwavel transformatiemechanismen ten gevolge van de toegepaste bio-behandeling beschreven en verklaard worden en dit tot op zekere hoogte.
- Een nieuwe methode voor de bepaling van elementaire zwavel in steenkool gebaseerd op een doorgedreven $CHCl_3$ extractie en daaropvolgend kwantitatieve analyse van het extract via HPLC, gebruikmakend van C_{18} tegenstroom fasekolom, werd voorgesteld. Hierdoor is het mogelijk een accurate zwavelbalans op te stellen.

- Enkele belangrijke verbeteringen werden gerealiseerd met betrekking tot de AP-TPR techniek. Waardoor een meer betrouwbare analytische benadering kan geformuleerd worden voor de studie van de zwavelvormveranderingen in steenkool.
- Deze thesis bewijst dat door het gebruik van een originele en vrij diverse experimentele strategie het volgende kan bepaald worden: (i) juiste selectie van geschikte micro-organismen voor specifieke steenkolen; (ii) welke zwavelvormen en zwavelfunctionaliteiten door specifieke micro-organismen zullen verwijderd worden; (iii) welke voorbehandelingen nodig zijn om de zwavelvormen en zwavelfunctionaliteiten in steenkool optimaal te modificeren zodat voor de microbiële acties een optimale ontzwavelingssnelheid en –efficiëntie kan bereikt worden.

List of publications

The results of this thesis have been presented and published at several international conferences and in peer-reviewed international journal.

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