Chemical and microbiological problems associated with research on the biodesulfurization of coal. A review

Gregory J. Olson* and Robert M. Kelly

Polymer Division, National Institute of Standards and Technology, Gaithersburg, MD 20899, USA
Department of Chemical Engineering, The Johns Hopkins University, Baltimore, MD 21218, USA

(Received January 3, 1990; accepted after revision July 3, 1990)

ABSTRACT


The study of microbial processes for the removal of organic and inorganic sulfur from coals is complicated by the lack of direct methods of measurement for organic sulfur content and the related incomplete understanding of the specific forms of organic sulfur in coal. In addition, the accessibility of specific chemical groups in the coal matrix to microorganisms and their enzymes is uncertain, raising questions about the nature and validity of model compound studies. Thus, interpretation of data from numerous efforts focussed on the microbial removal of inorganic and organic sulfur from coals remains controversial. The discussion here reviews recent developments in the chemical characterization of coal sulfur related to bioprocessing research and describes some recent efforts in involving sulfur transformation by hyperthermophilic archaebacteria.

INTRODUCTION

Although a considerable amount of study has been conducted to determine the forms of sulfur in coal, there remains some disagreement and incomplete understanding on this matter, especially concerning the “organic” fraction of coal sulfur. Also, since many methods of coal sulfur analysis require transformations of sulfur species to forms that can be discerned, anomalies often create confusion in the interpretation of results. For example, uncertainties in the disposition of inorganic sulfur species in coal can effect the information derived from experiments involving transformations or speciation of organic sulfur forms.

The difficulties associated with direct measurements of sulfur speciation in

*Author to whom correspondence should be addressed. Present address: US Department of Energy, Pittsburgh Energy Technology Center, Pittsburgh, PA 15236-0940, USA.
coal cause difficulties in designing and testing of microbial coal cleaning processes. Those involved in microbial coal desulfurization must contend with an, as yet, incomplete understanding of coal sulfur chemistry as well as microorganisms whose physiology and genetics are only beginning to be unraveled. Nonetheless, it is becoming increasingly clear that substantial progress in this area will come about as a result of efforts that integrate advances in coal sulfur chemistry with progress in elucidating the relevant metabolic characteristics of organisms effective in beneficiation. In this regard, several relevant aspects of coal sulfur chemistry, both old and new, are reviewed here. Through work with hyperthermophilic archaebacteria, illustrations of how biological and chemical aspects might be considered together are presented.

FORMS OF SULFUR IN COAL

Inorganic sulfur

Numerous studies employing techniques such as X-ray diffraction and Mössbauer spectroscopy report that pyrite (cubic FeS₂) is the most significant form of mineral sulfur in coal [1,2]. Illinois No. 6 and Indiana No. 5 lignite coals studied by X-ray diffraction show no evidence of other minerals forms such as pyrrhotite (Fe₁₋ₓS) or marcasite (orthorhombic FeS₂) (Fig. 1). However, not all pyrite is identical. Smith [3] noted variations in electrical conductivity and other properties of pyrites from several sources, attributing these differences to both sulfur deficiencies and lineage differences, indicative of crystal lattice imperfections. Furthermore, coal pyrite differs in many ways from pyrite found in ores, and coal pyrites themselves vary significantly, depending on rank of the coal. Temple and Koehler [4] found “sulfur ball” pyrite concretions in coal varied in susceptibility to oxidation. Additionally, Leathen and co-workers [5] found that “sulfur ball” concretions in coal were much more readily attacked by bacteria than was museum grade pyrite. Esposito et al. [6] studied pyrite from several coal sources and an ore pyrite and also found coal pyrite more reactive (Table 1).

As is evident from Table 1, coal pyrites were found to differ from the ore pyrite in several properties including surface area, semiconducting properties, morphology and reactivity toward ferric ions and oxygen. However, Esposito et al. [6] concluded that the measured differences in reactivity among pyrites were not sufficient to account for ore and anthracite pyrite retaining their luster for years, whereas pyrite from bituminous coals rapidly oxidizes in a humid atmosphere. These authors attributed the lower rates of reactivity

Fig. 1. X-ray diffraction spectra of Indiana No. 5 (top) and Illinois No. 6 coal (bottom). Vertical lines at the bottom of the spectra denote standard quartz (bold) and pyrite (thin).
TABLE 1

Properties of coal pyrite compared with mineral pyrite. (From ref. [6].)

<table>
<thead>
<tr>
<th>Property</th>
<th>Coal pyrite</th>
<th>Ore pyrite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flotation</td>
<td>More refractory</td>
<td>Less refractory</td>
</tr>
<tr>
<td>Surface area</td>
<td>Higher</td>
<td>Lower</td>
</tr>
<tr>
<td>Temperature of formation</td>
<td>Lower</td>
<td>Higher</td>
</tr>
<tr>
<td>Semiconductor type</td>
<td>p</td>
<td>n</td>
</tr>
<tr>
<td>Morphology</td>
<td>Varied-porous grains,</td>
<td>Smooth</td>
</tr>
<tr>
<td></td>
<td>plant imprints, frambooids,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>granular masses</td>
<td></td>
</tr>
<tr>
<td>Surface properties after</td>
<td>Variable-ranged from crust</td>
<td>Hard, retaining</td>
</tr>
<tr>
<td>grinding</td>
<td>formation in hours to</td>
<td>luster after grinding</td>
</tr>
<tr>
<td></td>
<td>staying bright for years</td>
<td></td>
</tr>
<tr>
<td>Oxidizability by ferric ions</td>
<td>Low to high</td>
<td>Low</td>
</tr>
</tbody>
</table>

in the laboratory to formation of passivating surface layers including iron hydroxides, elemental sulfur and/or unidentified sulfur species.

The differences in chemical and physical properties among coal pyrites and coal pyrite compared to ore pyrite may account for discrepancies in the literature regarding the mechanisms and rates of pyrite weathering, especially in the formation of elemental sulfur from the reaction of pyrite with ferric ions. In this connection it is noteworthy that Spanish mineral pyrite with no measurable impurities produces elemental sulfur on reaction with ferric ions [7]. Garrels and Thompson [8] suggested that $S^0$ forms during oxidation of pyrite by ferric ions under near equilibrium conditions between $Fe^{2+}$, $Fe^{3+}$ and $FeS_2$. If excess ferric ions are present, the sulfur is oxidized to sulfate.

Differences in rate of microbial attack of coal pyrite compared to ore pyrite could also be attributed to differences in semiconducting properties between ore pyrite (n-type) and coal pyrite (p-type). Next to solubility product, the concentration of broken bonds or holes (electron vacancies in the valence band) on the sulfide surface is the most important parameter affecting bacterial activity on metal sulfides. Given that two metal sulfides have similar solubility products but differ in semiconductor properties, the release of negatively charged sulfur ions from surfaces of p-type semiconductors is more easily achieved [9,10]. Consequently, coal pyrite, described by Esposito et al. [6] as a p-type semiconductor, would be expected to be more readily oxidized by bacteria than ore pyrite.

Pyrite in coal is formed by relatively low temperature processes involving the reaction of iron minerals with sulfide generated by bacterial sulfate reduction. The formation of pyrite under low temperatures and pressures occurs by at least two routes. Non-framboidal pyrite is formed by the reaction of ferrous ions with aqueous polysulfide ions as discussed by Rickard [11]:
Fe$^{2+} + S_x S^{2-} + H S^- \rightarrow Fe S_2 + S_{x-1} S^{2-} + H^+$  \hspace{2cm} (1)

The polysulfides are formed by nucleophilic attack of HS$^-$ ions on S$_8$ rings, the molecular form of sulfur at low temperature, opening the sulfur ring to form sulfanes of varying lengths.

Framboidal pyrite, appearing as tiny, clustered spheres, is produced at low temperatures by reaction between greigite (Fe$_3$S$_4$) and ferric ions [12]. Framboidal pyrite is more reactive than massive pyrite [13]. At higher temperatures (100°C), pyrite is produced rapidly by a solid state reaction between FeS and S$^0$ [11].

**Organic sulfur**

According to the ASTM definition [14], organic sulfur is sulfur unaccounted for after the subtraction of sulfatic (extractable in dilute hydrochloric acid) sulfur and pyrite sulfur (iron content of the coal extractable in dilute nitric acid and multiplied by the ratio of iron to sulfur in pure pyrite) from the total sulfur content of a coal. This organic sulfur fraction consists of sulfur bonded to carbon (true organic sulfur) as well as other forms of sulfur not measured as sulfate or pyrite. For example, metal sulfides other than pyrite could degrade to form H$_2$S during the pyrite and sulfate extractions steps and thus be lost from the system. Additionally, elemental sulfur is measured as organic sulfur [15].

True organic sulfur in coal is thought to consist of sulfur bound in ring forms (thiophenes), as thiols and disulfides [16]. About 40–60% of the organic sulfur in bituminous coals was estimated to be thiophenic [16]. However, recent work by Narayan et al. [17] and Lee et al. [18] have shown that extraction of coal with perchloroethylene (PCE) yields extracts containing significant S$^0$ and coal with lower organic sulfur content. Narayan et al. [17] and Lee et al. [18] proposed that coal organic sulfur may contain substantial amounts of sulfur occurring as polysulfides. Conversely, Buchanan and Chaven [19] provided evidence from sulfur isotope ratios that sulfur in PCE extracts is derived from the pyritic sulfur. Thus, there is wide disagreement on the source of sulfur in coal extracted by PCE. Recent attempts to understand the forms of organic sulfur in coal by Palmer et al. [20] involves oxidation of coal with peroxyacetic acid (PAA). These authors state that PAA oxidizes organic sulfur functional groups in coal to sulfoxides, sulfones and sulfonic acids. Products of these reactions were characterized by gas chromatography.

**MICROBIAL ACTIVITY TOWARD COAL SULFUR SPECIES**

In view of the above discussion concerning coal sulfur chemistry, it is clear that uncertainties in this regard have a bearing on understanding microbial
coal desulfurization. Furthermore, since many of the organisms used in desulfurization studies (i.e. *Thiobacillus ferrooxidans*, *Sulfolobus acidocaldarius* etc.) have not been well characterized in terms of their physiology, biochemistry and genetics, interpreting experimental results can be problematic. A similar situation exists when mixed cultures of unknown composition are used. Decoupling biotic and abiotic phenomena occurring during microbial coal desulfurization evaluations is difficult at best and likely leads to much of the existing controversy.

*Sulfur reduction by hyperthermophilic archaeabacteria*

One of the tactics taken to study sulfur speciation in coal and sulfur removal involves the use of hyperthermophilic, sulfur-metabolizing archaeabacteria. These organisms typically reduce forms of sulfur to sulfide as a consequence of their metabolism. Bacteria that reduce sulfur to volatile products have not received as much attention as sulfur-oxidizing bacteria for the desulfurization of coal. Since the hyperthermophilic archaeabacteria have been identified in the past ten years, their activity to coal sulfur must be studied simultaneously while elucidating aspects of their metabolism.

Thus far, work has focused on two microorganisms, *Pyrodictium brockii* and *Pyrococcus furiosus* [21,22]. Both organisms were described as being able to reduce elemental sulfur to sulfide. *P. brockii* is an autotroph (uses CO₂ as major carbon source) and oxidizes H₂. *P. furiosus* grows heterotrophically, utilizing S⁰ as a "dump" for excess electrons produced during catabolism. Since *P. brockii* and *P. furiosus* reduce elemental sulfur to H₂S, they could be useful in removing sulfur from such coals, or be useful probes of the occurrence of S⁰, or perhaps other sulfur species, in coal.

Recent papers (see above) have suggested that elemental sulfur, polysulfide sulfur, or some form of organic sulfur convertible to S⁰ by perchloroethylene at 120°C are important forms of organic sulfur in coal [17,18]. However, bioassay with *P. furiosus* showed that among four coals, only a refuse coal contained significant levels of S⁰. These results confirmed reports of others that little or no free elemental sulfur occurs in unweathered coals [19,23]. The Indiana refuse coal contained about 1.2% S⁰, and *P. furiosus* could be grown in continuous culture on the coal substrate, rapidly removing sulfur from the coal (Fig. 2). This suggests a potential application of such organisms in cleanup of such coals. In addition, the use of these organisms in connection with other coal cleaning methods which produce S⁰ may be possible. Meyers [24] described in detail a process whereby pyritic sulfur could be removed from coal by oxidation with ferric ions at 100°C. Under these conditions, the pyritic sulfur is oxidized to S⁰ and sulfate. Removal of elemental sulfur would require a separate step such as distillation. *P. furiosus* and *P. brockii* grow optimally near 100°C and it is tempting to speculate that combining a chem-
Fig. 2. Growth of *P. furiosus* on refuse coal during batch and continuous processing.

Fig. 3. Production of elemental sulfur in coal by ferric ions at 100°C.

The chemical process like that of Meyers with hyperthermophiles might be an efficient method for removing pyritic sulfur from coal. In addition, Narayan et al. [17] reported formation of amorphous elemental sulfur and sulfates from an Indiana coal under mild oxidizing conditions. Consequently, some of current studies of the present authors involve accelerated weathering and oxidations of coals in attempts to produce $S^0$ from coal sulfur.

There is inconsistency in the literature as to rate and extent of $S^0$ formation during treatment of pyrite with ferric ions. This is due in part to varying reaction conditions which affects formation of $S^0$ as described by Garrels and Thompson [8]. Upon oxidation of Illinois No. 6 (1.20% pyritic S) and Indiana No. 5 coals (1.83% pyritic S) with 1.0M ferric sulfate at 100°C, relatively small amounts of $S^0$ were produced (Fig. 3). Other recent experiments examining milder oxidizing conditions have shown mixed results. Weathering Illinois No. 6 and Indiana No. 5 coals in humid atmospheres and low
temperatures (35 to 75°C) did not result in significant formation of S°. However, mild oxidation of Illinois No. 6 coal in aqueous media (at 70 and 100°C) resulted in the transformation of pyritic sulfur to S°. Bioassays have shown that S° or polysulfides that can be reduced to H₂S by *P. furiosus* are present in samples of coal weathered in water. Additional study is warranted to understand the variables affecting formation of S° during coal oxidation.

It has not yet been determined if polysulfide sulfur in the coal matrix, as envisioned by Narayan et al. [17], would be susceptible to attack by *P. furiosus*. These organisms do not need to attach to S° for metabolic activity, since the specific form of sulfur reduced by them is a soluble polysulfide [25]. *P. furiosus* was able to produce H₂S in bottles where it was separated from S° held in dialysis bags (pore size 6500 daltons). Abiotic experiments were also conducted with dialysis bags filled with radioactive elemental sulfur and incubated at 100°C in water containing nucleophilic agents. The most rapid appearance of radioactivity outside the bags occurred with HS⁻ ion (Fig. 4). Nucleophilic agents such as HS⁻, OH⁻ and CH₃⁻ are known to attack the S₈ ring, opening it to form polysulfides, S-S₇-S. These polysulfides are reduced to H₂S by the organisms. In addition, three organic sulfur compounds are metabolized by *P. furiosus*: methyl trisulfide, cystine and cystine trisulfide. Cystine is a disulfide and apparently reacts at high incubation temperatures or with sulfide ions to produce a polysulfide.

The production of hydrogen sulfide and methyl mercaptan from methyl trisulfide by *P. furiosus* suggests that the organism recognizes the central sulfur atom in the zero oxidation state:

\[
\text{CH}_3\text{–S–S–S–CH}_3 \rightarrow \text{CH}_3\text{S}^- + S^{2-}
\]  

(2)

Sulfur in organic and inorganic polysulfides occurs in two oxidation states: zero (termed polysulfide or excess sulfur) and minus two (sulfide sulfur) [26]. The polysulfide form of sulfur is expected to be the form attacked by *P. furiosus*.

---

![Figure 4](image-url)

Fig. 4. Release of ³⁵S-elemental sulfur from dialysis bags (pore size 6000 daltons) in artificial sea water (ASW) containing nucleophilic agents.
*furiosus*, since the organism reduces sulfur. If polysulfide sulfur exists in coal and is accessible, it is expected that *P. furiosus* reduces the polysulfide sulfur to H₂S. However, a key question (applicable to all studies on microbial degradation of organic structures in coal) is the extent to which microbial enzymes could recognize polysulfides bound in the coal matrix as opposed to simple model compounds, such as methyl trisulfide. The next step will be to study reduction of model polysulfide compounds with bulky terminal groups and polysulfide polymers.

The point to the above discussion is that to understand the activity of microorganisms to coal sulfur species, both the relevant coal chemistry and characteristics of the organism(s) must be addressed. There will no doubt be significant coupling of abiotic and biotic phenomena to be considered. Obviously, because both the biology and chemistry are not well understood in most instances, this presents a particular challenge.

**QUESTIONS THAT NEED TO BE ADDRESSED**

(1) How should the differences among coal pyrites and between coal pyrite and mineral pyrite be taken into account in evaluating the effectiveness of an organism(s) for inorganic sulfur removal?

(2) Since many biological removal schemes are carried out under conditions that promote significant coal weathering, how can biotic and abiotic contributions be separated?

(3) Given the complex structural nature of coal, can model compounds effectively be used to evaluate microbial activity to organic sulfur?

(4) Are polysulfides significant intermediates in the transformation of either organic or inorganic coal sulfur?

(5) Do elevated temperatures present significant advantages and/or disadvantages for microbial coal desulfurization?

(6) Can bacteria or their constitutive biomolecules be useful probes for coal sulfur species?

(7) What is the intrinsic maximum rate at which organisms could be expected to transform sulfur in coal and is this rate economically viable?

**ACKNOWLEDGEMENTS**

This work was supported in part by the Electric Power Research Institute and the National Science Foundation. The authors would like to thank I.I. Blumentals, T.L. Peeples, R.N. Schicho and S.H. Brown for the use of their experimental results.
REFERENCES


