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Biological coal desulfurization

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Abstract

Sulfur removal from coal before combustion is of considerable interest to avoid emission of oxides of sulfur(SO_X). Chemical and physical methods have been developed for removing the inorganic sulfur component in coal. This component is generally associated with pyrites(FeS2). However, no commerical methods have been developed for the removal of the organic sulfur conponent. This study describes microbial methods for removing organically bound sulfur from coal. The specific goals of this study were to ascertain whether a robust biologically active population such as activated sludge biomass can remove organic sulfur from coal and to devise sulfur analytical methods that are less cumbersome than the accepted standards for screening research results. Dibenzothiophene is used in isolating microorganisms that use sulfur as the sole source for growth. It was found that about 55% sulfur can be removed by activated sludge in shaker flasks from a coal which was previously treated to remove inorganic sulfur(IBC-108). A no cycling leaching reactor with activated sludge removed 50% sulfur from the same type of coal. Almost 72% sulfur is removed by A-1, S-D1, T3-2 and Ar-1 mixed cultures. It was also found that about 25 to 29% sulfur can be removed by different cultures from a commercially prepared washed coal in which the pyritic and organic sulfur have not been altered. The application of ion-chromatography and atomic spectrometry in analyzing sulfate, pyritic sulfur, organic sulfur and total sulfur following ASTM extraction can achieve easier and quicker sulfur determinations without sacrificing accuracy and reproducibility.

BIOLOGICAL COAL DESULFURIZATION

BY
MING LE

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I dedicate this paper to my wife, Yong-ping Lu,

for her continued patience, understanding,

inspiration and love

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I. Introduction and Literature Review

Coal combustion causes atmosphere emissions such as particulates, NO_X and SO_X, which, if not controlled, are harmful to the environment. This is a consequence of the composition of coal which contains sulfur, nitrogen, and inorganic ash. Sulfur-containing gas (mainly SO_2), emitted into the atmosphere from the direct combustion of coal, has been shown to have adverse effects on animal and plant life, and contributes to the acid-rain problem.

Coal contains, apart from pyritic sulfur(FeS2), traces of sulfates and elementary sulfur, which is a considerable amount of organically bound sulfur. Organic sulfur is found in chemical coal structures as thiol, disulfide, sufide or thiophene.[1,2] Sulfur occurs in inorganic forms as gypsum ($CaSO_4$),iron sulfates (Fe_2SO_4, $Fe_2(SO_4)$ and free elemental sulfur. Occasionally, metal sulfides such as chalcopyrite ($CuFeS_2$), bornite (Cu_5FeS_2), sphalerite (ZnS) and galena (PbS) may be present in minor quantities.[3]

The percentage of sulfur in coal varies widely, from less than 0.5% to over 11%. Western U.S. coals are generally low in sulfur and are pedeminantly strip mined. Typical total sulfur contents being 0.6% to 1.8%. Eastern U.S. coals tend to be higher in sulfur, averaging 2% to 3.5% and are obtained from deep mines. Table 1. summarizes sulfur content in coals from vanous global regions.[4]

Table1. Sulfur Content of Coal from Various Global Regions

Source	Range of total sulfur (%)
France	0.8-0.4
Netherlands	1.0-3.0
West Germany	1.3-1.5
Belgium	0.5-4.5
Poland	0.5-2.8
India (Assam province)	1.0-3.6
Great Britain	6-8
Eastern US	0.2-7
Western US	0.2-1

Almost all coals contain sulfur in varying quantities and its presence constitutes both metallurgical and environmental problems. Accordingly, it is desirable to reduce the sulfur in coal to acceptable levels prior to utilization. Increased emphasis is being placed on producing clean coals to satisfy stringent environmental requirements recently enacted in the Clean Air Act of 1990. U.S. new source performance standards(NSPS) define "compliance coal" as coal that produces no more than 1.2 lb SO_2/million Btu head input.

Various physical and chemical methods have been developed for partial desulfurization of coal. Some microorganisms have also been shown to solubilize pyrite and marcasite. It has been suggested that these organisms may be useful in a biological treatment process for sulfur removal from coal.

Nonbiological desulfurization of coal

A number of nonbiological methods for coal desulfurization have been developed that are more or less effective in removing pyritic sulfur from coal[5]. The most effective techniques involve the physical cleaning of coal, e. g., heavy media washing, froth flotation, air tables, upward current classifiers, etc. These techinques are based on the significant difference in density of coal (1.1-1.3 g/cm^3) and pyrite (4.8-5.3 g/cm^3). Other, less developed technologies rely on the paramagnetic properties of pyrite (high gradient magnetic separations) or selective agglomeration with oil to achieve separations. Physical treatment techniques can remove, or reduce significantly the level of pyritic sulfur in coal. However, they have no effect on organic sulfur. A major disadvantage of physical cleaning methods is the energy loss associated with removing the fraction of the coal in contact with finely distributed pyrite.

Chemical desulfurization techniques are often energy-intensive. Oxidative methods involve oxidation with air; sulfur is eliminated by conversion to volatile products (primarily sulfur dioxide) and soluble sulfates that are removed with subsequent water washing. Reductive methods, such as hydrodesulfurization, achieve reduction of sulfur moieties to volatile hydrogen sulfide. Solvent refining utilizes pulverized raw coal, slurrying with a coal derived process solvent, and treatment with hydrogen gas at high pressure (10 MPa) and temperature (450°C). Other chemical processes involve the reaction at high temperatures of carbonates, bicarbonates, or hydroxides of alkali metals (lithium, sodium, or potassium) with the coal to produce soluble sulfates. Ferric sulfate leaching utilizes the conversion of pyritic sulfur to soluble sulfate and elemental sulfur. In recent times, a number of chemical methods for the removal of both pyrite and organic sulfur from coal have been advocated. The

Molten Caustic Leaching Process [6] is effective in removing nearly all of the sulfur and ash-forming minerals; however, the process is energy intensive and is highly sensitive to process chemistry and operating conditions.

The chemical desulfurization methods, have two major drawbacks, namely, (1). they are expensive and energy intensive, and (2). they destroy the caking properties of coal. These factors have discouraged the commercial adoption of these processes on an industrial scale, although they may be suitable for special applications.

Microbial desulfurization of coal

Microbiological processes are known to remove most of the pyritic sulfur, as well as some of the organic sulfur, and do not affect the caking properties of caking coal. Microbiological methods of coal desulfurization offer significant advantages over physical and chemical methods. they are low energy process, have low operating costs and do not reduce the heating value of the product coal. Biodesulfurization of coal could be especially promising where coal contains very finely distributed pyrite that is generally not removable by mechanical techniques. Apparent problems with microbial coal desulfurization are relatively long bioprocessing times (days to months) and the production of acidic, and corrosive leaching effluents. However, for future applications, microbial methods are considered to present desirable alternatives for coal beneficiation that are not achieved by other technologies. Consequently, research is warranted in this area.

A number of researchers have shown that coal biodesulfurization can be achieved in the laboratory for both inorganic and organic forms of sulfur. Most research has involved microbial oxidation of pyrite to soluble sulfates. Two groups of microorganisms are

involved in the removal of pyritic sulfur from coal. One group of microbes functions at near room temperature (18-40°C) while the other group is strictly thermophilic, (i.e., they function at elevated temperatures (60-90°C).[7,8]) The microorganisms that are active at near room temperature comprise Thiobacillus ferrooxidans (also called Ferrobacillus ferrooxidans), Thiobacillus thiooxidans, and other related thionic bacteria. The thermophiles comprise Sulfolovus acidocaldarius, Sufovacillus thermosulfidooxidans, Sulfolobus brierlegi, and Sulfolovus sulfataricus.[9,10] Such organisms, obligatively or facultatively, strip the electrons needed for carbon dioxide fixation from inorganic sulfur compounds, such as elemental sulfur, sulfides, thiosulfate, and polythionate. The important organism Thiobacillus ferrooxidans can also access the electrons found in ferrous iron. Members of the archaebacterial genus Sulfolobus are Thermophilic and are well adapted to survive at the elevated temperatures found in geothermal springs and vents.

The microbial removal of pyritic sulfur from coal by the chemoautotrophic microorganism Thiobacillus ferrooxidans has been studied by many investigators.[11,12] T.ferrooxidans flourish in low pH environments and is capable of synthesizing cellular material exclusively from inorganic substrata and obtaining energy via the oxidation of ferrous iron and sulfide. A. S. Myerson[13] employed crushed coal in batch or continuous stirred reactors and reported pyrite removals of 60-98% in time periods of 4-10 days.[14] A percolation bioreactor with T.ferrooxidans was used and resulted in a 75% removal of pyrite within 70 days.[15] Detz and Barvinchak[4] compared the desulfurization capabilities of the mesophilic T. ferrooxidans and a mixed culture of the thermophilic microbes (S. acidocaldarious and ferrollobus). The results of the studies with T. ferrooxidans showed that 86 wt% pyrite was removal after leaching at pH 2-2.4, 28°C, and 20 wt% solid concentration. Essentially the same results on microbial removal of pyritic sulfur from coal by T. ferrooxidans have been reported by several investigators.[16,17,18,19] T. ferrooxidans cultures accumulate metabolic by-products that

inhibit further growth and pyrite oxidation[20].

Some investigators used a mixed culture of T. ferrooxidans and T. thiooxidans for more effective desulfurization of coal[17]. A mixed culture yields sulfur removal rates higher than those obtained by pure cultures of T. ferrooxidans. Mixed cultures of these bacteria were able to remove 97% (wt) of pyritic sulfur from the coal sample (with 4.6% (wt) total sulfur) after 5 days. In F. Kargi's studies, the rate of inorganic sulfur removal was significantly improved by using a concentrated cell suspension of T. ferrooxidans and an external supply of CO_2 and air in a well agitated vessel.[18] One of the problems associated with the use of Thiobacillus in coal desulfurization is low rate of sulfur removal and therefore the process requires large reactor volumes.[21] Also, Thiobacillus species can only remove pyritic sulfur and are ineffective in attacking organic sulfur.

Sulfolobus acidocaldarius has been evaluated in pure culture for the removal of sulfur from coal.[22,23] The bacteria are chemoautotrophic and thermophilic, thriving at temperatures up to 80°C. These organisms are reported immune to the inhibitory effects of organic components present in the coal slurry. This species may prove useful in accelerating the rate of desulfurization by operating at higher temperatures.[23,24,25] Recently, many investigators reported that S.acidocaldarius can oxidize dibenzothiophne (DBT) into sulfate.[17,19]

The microbial-mediated removal of organic sulfur has been demonstrated with sufficiently encouraging results to justify accelerated research. Members of the genus Pseudomonas and species of related genera, are able to release organic sulfur from coal as inorganic sulfate.[26,27] Strains of the genera Rhodoccus, Pseudomonas, Bacillus, and Brevibacterium have been isolated which produce O,O'-biphenol or monohydroxybiphenyl or biphenyl upon exposure to DBT. Table 2. lists some microorganisms that have

substancially removed inorganic sulfur from coal.

Table 2. Summary of Inorganic Microbial Desulfurization[4]

Culture(s)	pH	Temperature °C	Coal			Inorganic sulfur		
			Source	Size (um)	Slurry (in water)	Initial Percentage	%Removed	Time (day)
T.ferrooxidans ATCC 19859	2.5	25	Eastern US	43-74	<20%	4.9	90-98	8-12
T.ferrooxidans isolated from acid mine waters	2.0	35	Ohio	<37	25%	4.1	77	NR
	2	35	New Mexico	<37	25%	1.95	83	NR
S.acidocaldarius (strain 98-3)	2.5	70	Pennsylvania	100-149	5%	2.1	30	10
S.acidocaldarius	1.5	70	Pennsylvania	<48	20%	2.1	90	NR
S.acidocaldarius	1.5-2.0	28	Illinois#2	<74	20%	0.98	90	14
Ferrolobus spp.	1.5-2.0	60	Illinois #2	<74	20%	1.89	90	6
T.ferrooxidans	2.5	25	West Virginia	<74	20%	0.89	90	14
T.thiooxidans	2.0-2.5	"ambient"	--	74-300	20%	3.1	97	5

Mechanism of Microbial Desulfurization of Coal

Microbial pyrite oxidation. The role of bacteria in the oxidation of insoluble iron sulfide (pyrite) and other metal sulfides to soluble ions is well established, although the mechanism of pyrite solubilization is still not completely understood. Pyrite solubilization has been most thoroughly studied in the gram-negative chemolithotroph Thiobacillus ferrooxidans, which utilizes either ferrous iron or reduced inorganic sulfur compounds (including elemental sulfur) as a sole energy source [16]. The reactions involved in pyrite solubilization have been characterized as either direct or indirect mechanisms for oxidation of the substrata by the bacteria. The direct mechanism may require the physical attachment of

the bacteria to pyrite particles, resulting in the localized oxidation of pyritic sulfide to sulfate, and ferrous iron to ferric as outlined below:

$$FeS_2 + 7/2\ O_2 + H_2O \longrightarrow FeSO_4 + H_2SO_4 \qquad (1)$$

$$2FeSO_4 + {}^1/_2\ O_2 + H_2SO_4 \longrightarrow Fe_2(SO_4)_3 + H_2O \qquad (2)$$

Reaction 2 is the rate limiting step in the overall pyrite oxidation and acidity forming process. Ferric ions react with additional pyrite:

$$FeS_2 + Fe_2(SO_4)_3 \longrightarrow 3\ FeSO_4 + 2\ S^\circ \qquad (3)$$

Other metal sulfides (MS) are also attacked by ferric-sulfate solutions:

$$MS + 2\ Fe^{+3} \longrightarrow M^{+2} + 2\ Fe^{+2} + S^\circ \qquad (4)$$

Thus, in the indirect mechanism, the role of bacteria is to reoxidize ferrous to ferric iron. Ferrous ions, although generally considered as an energy source for the bacteria, may also serve an additional purpose. Elemental sulfur, which might be expected to accumulate, is metabolized by T. ferrooxidans, T. thiooxidans, and other acid-tolerant bacteria, to generate sulfuric acid:

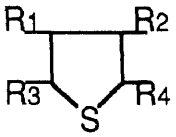
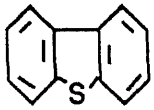
$$2\ S + 3\ O_2 + 2\ H_2O \longrightarrow 2\ H_2SO_4 \qquad (5)$$

This reaction is important because it is believed to prevent a layer of elemental sulfur from accumulating on pyrite surfaces inhibiting further reactions.[28] In general, the overall reaction often used to illustrate bacterial solubilization of pyrite is:

$$4\ FeS_2 + 15\ O_2 + 2\ H_2O \longrightarrow 2\ Fe_2(SO_4)_3 + 2\ H_2SO_4 \qquad (6)$$

Microbial removal of organic sulfur from coal. Both aromatic and aliphatic sulfur groups are part of the molecular structure of coal.[29] Their interaction with the complex aromatic structure of coal is quite complicated and varies depending on the type and source of coal.[30] Table 3 shows the major organic sulfur groups in coal.

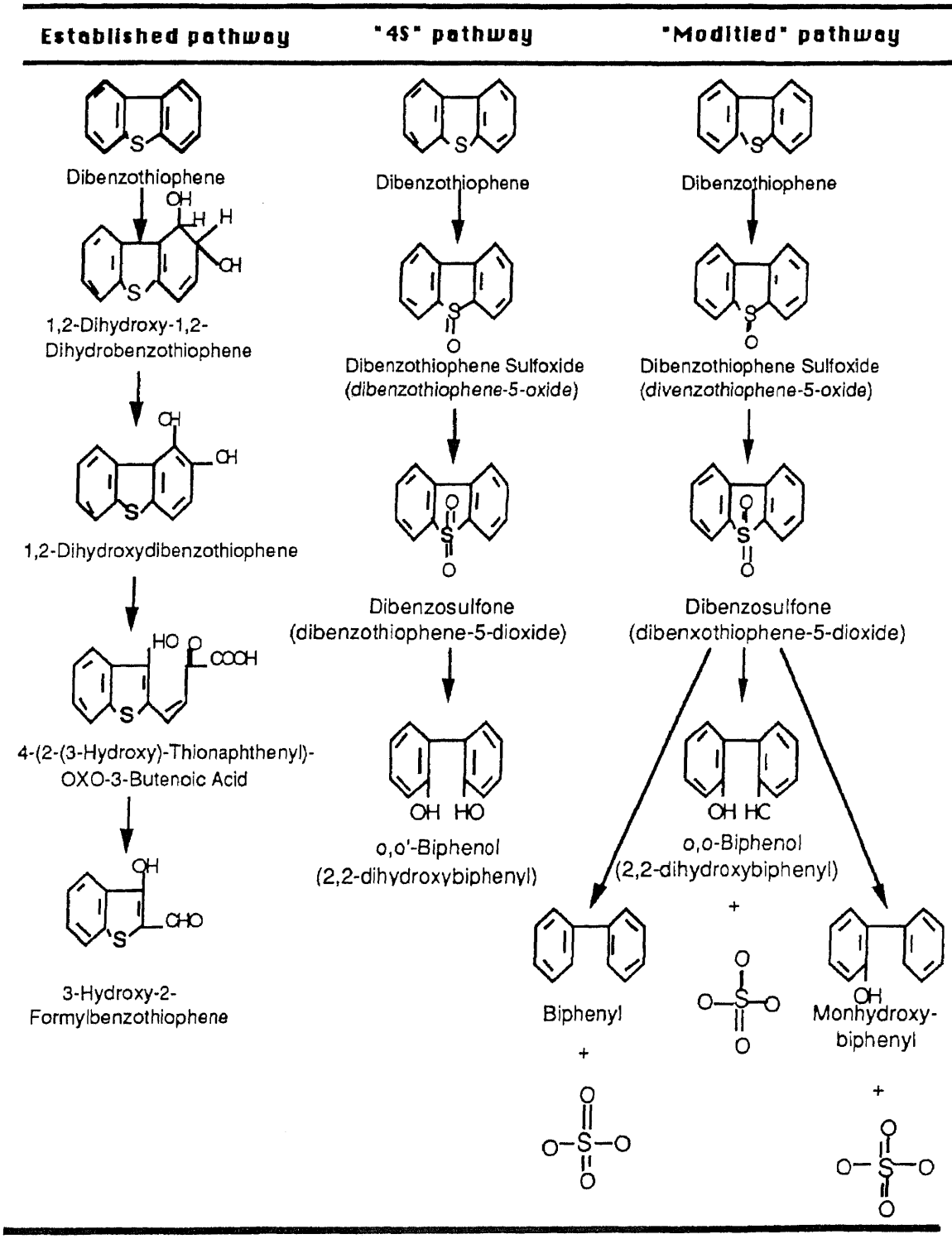
Table 3. Major Organic Sulfur Groups in Coal[31]

Compounds		Sample
Sulfide	R1-S-R2	C2H5-S-C2H5
Disulfides	R1-S-S-R2	C2H5-S-S-C2H5
Tiols	R-SH	C2H5-SH
Thiophenes		R: groups are either hydrogen,alkyl group or aromatic groups,e.g. dibenzothiophene 

Dibenzothiophene (DBT) has been widely used as a model compound in desulfurization studies[23,32,33]. Because it represents the abundant and refractory thiophinic sulfur in coal, there have been few studies on other organic sulfur compounds such as thiols or disulphides.

Work at the University of Mississippi[34] has focused on studies of the "4S" pathway (the postulated sulfoxide/sulfone/ sulfonate/ sulfate "4S" pathway produces 0,0-biphenol from DBT and releases sulfur as sulfate) of microbial removal of organically-bound sulfur from the model compound dibenzothiophene and from depyritized Illinois #6 coal. Steve Kraweic at Lehigh University[33] is conducting studies to assess the 4S-pathway potentials of newly isolated bacterial strains. The pathways of dibenzothiophene degradation by organisms is summarized in Figure 1.

Figure1. The Pathways of Dibenzothiophene Biodegradation



Reports of microbial removal of organic sulfur from coal have only recently appeared in the literature. Most of these studies have been very preliminary in nature. Whether the above mechanisms are involved in other organisms is unknown. In addition, the accessibility of the surface and interior of coal particles for organic sulfur solubilization by microorganisms and their enzymes remains to be determined.[35,36] In addition, little information is available on the loss of coal heating value associated with biological coal desulfurization.

Development of Desulfurization Technology

In May, 1990, the First International Symposium on the Biological Processing of Coal was held in Orlando,Florida.[37] Many investigators reported new results on biological desulfurization of coal. Several studies focused on the scale-up of microbial depyritization, and several process configurations have been suggested. Using some model systems to evaluate the biological and economic constraints on the application of microbial desulfurization to large-scale bioprocessing of coal, they concluded that for many large-scale applications, development of a commercially viable process was not necessarily limited by biological constraints.

In the Symposium, many reports on microbial-mediated removal of organic sulfur were presented with sufficiently encouraging results to justify accelerated research. Finding organisms that can desulfurize coal or model organo-sulfur compounds is an ongoing activity. Workers at Louisiana Tech[37] used a 40-gallon reactor and two 10-gallon batch reactors to "depyritize" slurries of 200 mesh Illinois #6 coal. Inoculum for the above reactors was composed of ATCC cultures Thiobacillus ferrooxidans, Thiobacillus thiooxidans. About 90% pyrite removal was obtained in 10 to 14 days. This laboratory-scale coal desulfurization processes is being scaled up to pilot plant size.[34,40] Arctech constructed a small pilot plant

to use microorganisms to remove organic sulfur from 2,500 pounds of coal a day. The bioreactor was a simple stirred, aerated tank. Economic studies indicated that microbial removal of sulfur from certain coals would be economically feasible[38]. It was estimated that the cost per ton of sulfur dioxide removed varied from $480 to $340. These costs are lower than the alternative flue gas desulfurization that have costs estimated at 1300 to 1600 $/ton. [39] Because the microbiological processes have the potential to address both the organic and inorganic aspects of coal desulfurization, the biodesulfurization may offer its most immediate and significant application to coal processing technology. Bioprocess variables in sulfur removal are summarized in Figure 2.

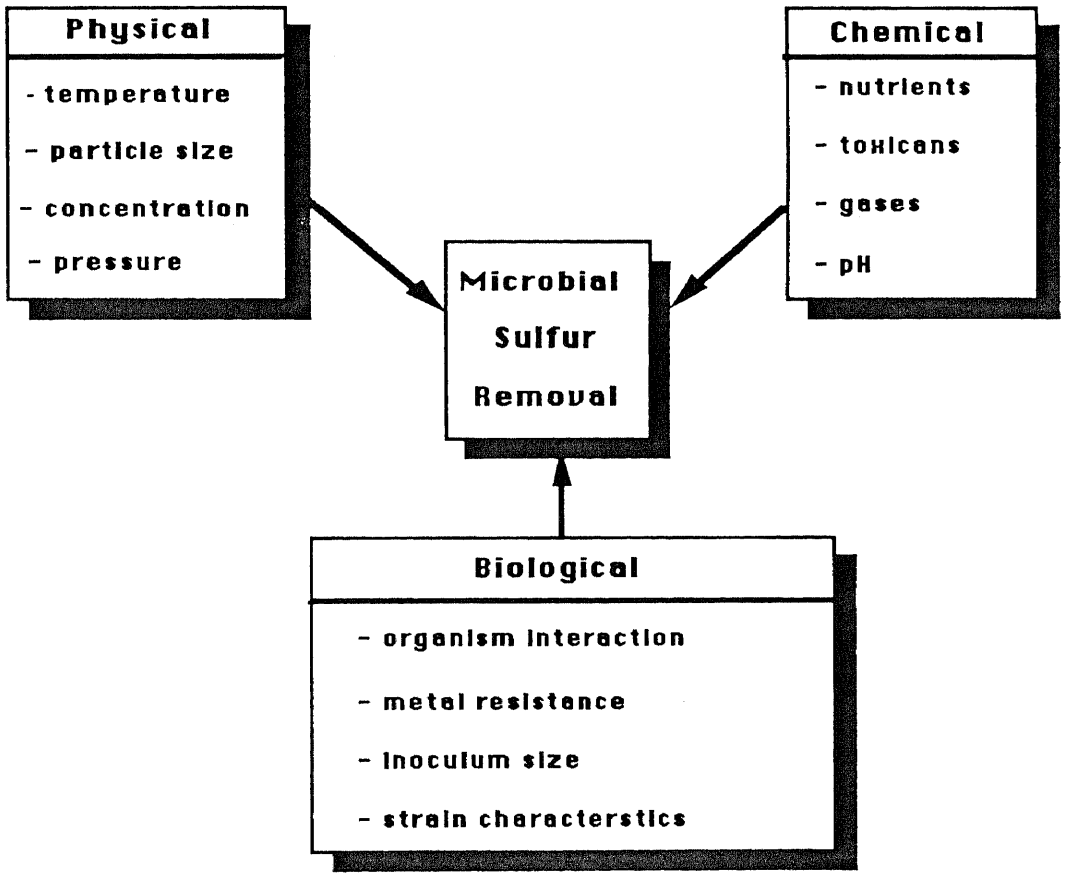


Figure 2. Bioprocess Variables in Sulfur Removal

A review of the studies conducted in past few years on microbial coal desulfurization shows that almost all the investigators focused on pure cultures. The objective of this investigation is to enhance the knowledge of how robust mixed cultures such as activated sludge attack coal sulfur. Activated sludge is a man-made microbial population as complex as any yet described, and selected for its versatility and resistance to fluctuating conditions. Consequently, with activated sludge it is possible avoid the problems associated with temperature and pH sensitivity of any one organism.

The type of organisms in activated sludge stand an excellent chance of rapidly removing not only inorganic, but also removing organically bound sulfur in coal. Activated sludge biomass as a starting material for coal desulfurization has obvious advantages:[42]

1) The genera of bacteria that have been described as being able degrade inorganic and organic sulfur in coal, such as Pseudomonas, Bacillus, Phodoccus, Brevibacterium and Acinetobacter are well represented in activated sludge.
2). It has food handing properties.
3). It is continually produced in very large quantities throughout the world.
4). It currently represents a disposal problem for the treatment plants.
5). It is an extraordinary mixture of aerobic and facultating chemical environment of inflowing sewage.
6). If it is possible to remove sulfur from coal by activated sludge, then this process can be implemented near a sewage treatment plant on a large scale.

The purpose of this project is to answer whether the organisms in activated sludge possess the ability to attack the inorganic and

organic sulfur in coal. The studies included selecting from activate sludge organisms that can remove organic sulfur from coal by using DBT as a surrogate compound. In the study, we intent to establishe methods for the quantitative analysis of inorganic sulfur, pyritic sulfur, organic sulfur and total sulfur in coal samples. Finally, we will investigate the ability of activated sludge to remove sulfur from coal in shaker flasks/incubators and different lab-sale reactors. Appendix 1. summarizes the literature survey of biological coal desulfurization according to 10 key search topics. Figure 3. describes the flow of the anticipeted research.

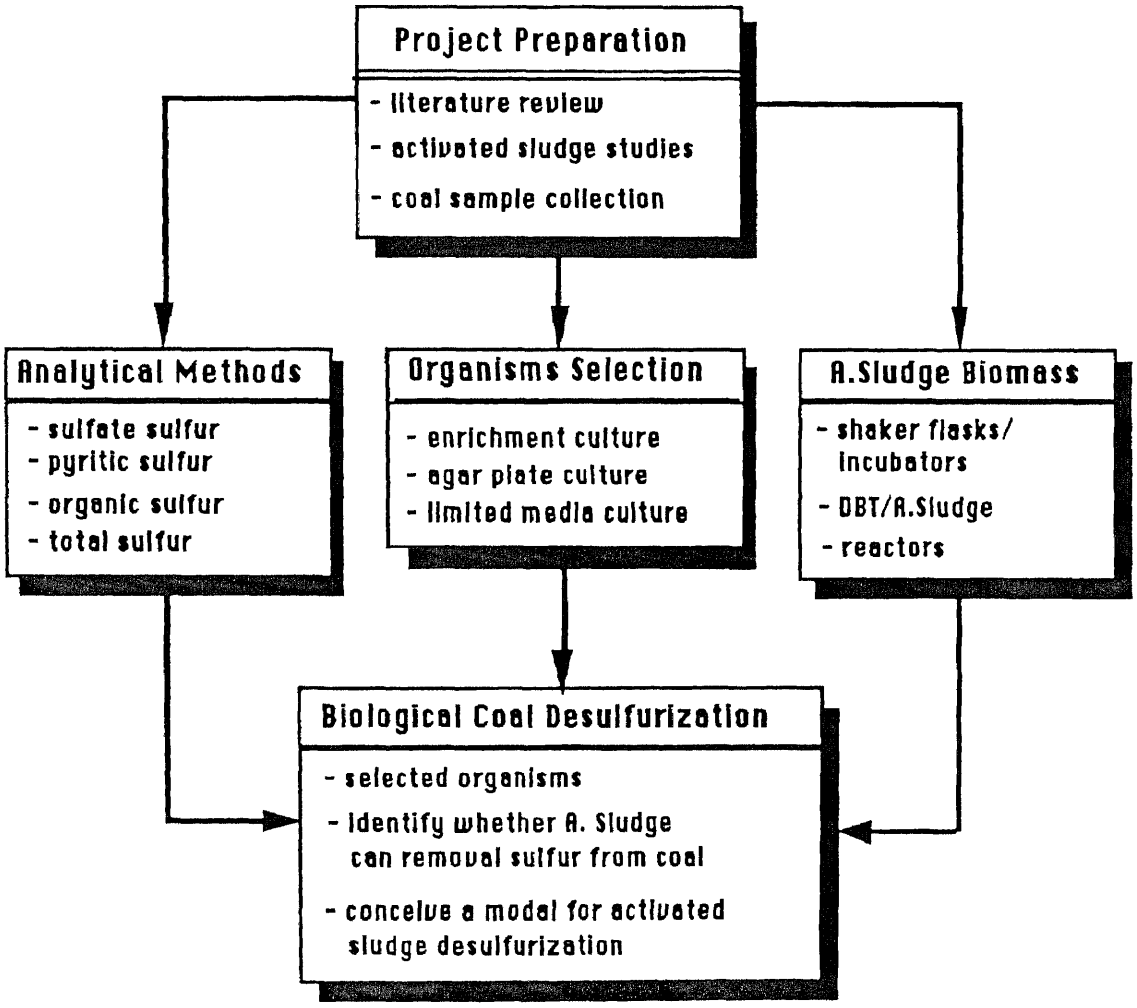


Figure 3. The Project Scope

II. Material and Methods

Chemicals:

Acetonitrile HPLC grade
Barium chlorid ($BaCl_2$)
Bromine water (saturated)
Dibenzothiophene (DBT) purchased from Aldrich Chemical Company Inc.
Dimethylformamide ($HCON(CH_3)_2$)
Ethanol, reagent grade, denatured
Eschka Mixture - Thoroughly mix 2 parts by weight of light calcined magnesium oxide (MgO) with 1 part of anhydrous sodium carbonate (Na_2CO_3)
Hydrochloric acid, 12N (sp gr 1.19)
Hydrochloric acid, 4.8N (2+3) - mix 2 volumes of concentrated aqueous HCl with 3 volumes of water
Hydrochloric acid (1+1)
Hydrochloric acid (1+9)
Hydrogen peroxide (30%)- Concentrated hydrogen peroxide (H_2O_2)
Iron standard for Atomic Absorption (1000 ppm)
Lanthanum solution for Atomic Absorption
Nitric acid (1+7)- Mix 1 volume of concentrated aqueous nitric acid (HNO_3) sp.gr 1.42 with 7 volumes of water
Standard sulfate solution
Sodium carbonate solution

Coal sample:

Juliana 880119-13, total sulfur 0.903 wt%. Brooks Run 890912-1, total sulfur 0.838 wt%. Geo-chemicals 890912-1, total sulfur

0.976 wt%. All three coal sample were obtained from Public Sevice Electric and Gas Company Coal Research Laboratory in Harrison, NJ.

IBC-108 (1001 & 1202) -- State-of-the-art. physically-cleaned blend of Herrin (Illinois #6) and Springtield (No. 5) coal (80% and 20% respectively). It is a micronized coal with low pyritic sulfur. Different kinds of sulfur in coal are shown in Table 4.

IBC-101 -- Herrin (Illinis #6) coal obtained in 1983 from a commercial preparation plant in west central Illinois. It has the highest organic sulfur content of any coal in the program and one of the lowest pyrite sulfur values for a coventionally-washed coal. Different kinds of sulfur in coal are shown in Table 4.

These two samples were obtained from the Illinois Basin Coal. Sample Pragram(IBCSP), Illinois State Geological Survey, 615 E Peabody Drive, Champaign, IL 61820.

	IBC-108 Coal Sample (Total sulfur 2.63%)	IBC-101 Coal Sample (Total sulfur 4.32%)
Sulfate sulfur:	0.01%	0.055
Pyritic sulfur:	0.37%	1.27%
Organic sulfur:	2.25%	3.00%
C/H/N/O:	97.87%	95.68%

Table 4. Kinds of Sulfur in IBC-101 & IBC-108

Instruments and analytical methods:

The detection of the sulfate sulfur was accomplished by turbidimetric method (Method 9038), gravimetrical method (ASTM D2492-84)[43] and **Ion Chromatography.** Pyrites (FeS_2) in coal are deteminated by Atomic Absorption (ASTM D2492-84). The total sulfur is detected by precipitate method (ASTM D 3177-84)[44] ,Ion

Chromatography and X-Ray spectrometer. For the ashing coal sample, the **Bomb Washing Method** and **Eschka Method** are used in total sulfur determination. Organic sulfur is determined after extraction of sulfate sulfur and pyritic sulfur by the same method as the total sulfur or calculated by substracting sulfate and pyritic sulfur from the total sulfur.

Description of Method 9038: Sulfate ion is converted to a barium sulfate suspension under controlled conditions. The resulting turbidity is determined spectrometrically and compared with a curve prepared from standard sulfate solutions.

Description of ASTM D 2492-84: Sulfate sulfur is determined by extracting the coal with dilute hydrochloric acid and determining the sulfur in the extract gravimertrically. Sulfates are soluble in hydrochloric acid, but pyrite and organic sulfur forms are not. Pyrites are extracted quantitatively by dilute nitric acid. The extracted iron is determined by atomic absorption spectroscopy.

Description of ASTM D3177-84: 1. Eschka method-A weighed sample of coal and Eschka mixture are intimately mixed and ignited together. The sulfur is dissolved in hot water and then precipitated from the resulting solution as barium sulfate ($BaSO_4$). 2. Bomb Washing method- Sulfur is precipitated as $BaSO_4$ from oxygen-bomb calorimeter washings and the precipitate is filtered, ashed, and weight.

Ion Chromatography is used as a substitute for the precipitation method after the soluble sulfur extraction or when sulfur is dissolved in hot water. All the samples of culture fluid were prepared by centrifugation at 3,000 rpm for 10 minutes to remove particles and cells. The procedure of sulfate, pyritic and total sulfur analysis has been described on Appendix 2. All analytical instruments used in this study are listed in Table 5

.

Table 5. Analytical Instruments

List of the Analytical Instruments

Instrument	Model	Company
Atomic Absorption	*Smith-Hieftje 11/12™*	Thermo Jarrell Ash Coporation
Ion Chromatography	*a. Advanced Chromatography Module* Column: *IC-Anion-Guard 16537* *IC-Anion-PW 10825*	Dionex
Ion Chromatography	*b. Waters 600E System Controller* *Waters ROM, Reagent delivery Module* *Waters 431, Conductivity detector* *Waters 484, Tunable absorbance detector* *Waters 715 ULTRA WISP* *Sample Processor* Column: *IC-Anion-Guard* *Catolog:127-0056 Serial 16532* *IC-Anion-PW* *Catolog:127-0062 Serial 10917* *IC-Pak A HC* *Waters Millitrap™ H^+ Membrane* *Cartridge*	Waters
UV-V. Spectrophotometer	*DMS 300*	Varian
Electrode Muffle Furnace	*Type10500 Furnace* *(0 -- 2200 °C)*	Thermolyne
Oxygen Bomb Calorimeter	*Calorimeter model 1341* *Oxygen Bomb Model 1108*	Parr Inst. Co
X-Ray Spectrometer	*3030*	Rigaku

- Eluents of ion chromatography is listed in Appendix 3.

Activated sludge sources:

Activated sludge was obtained from an aerobic tank in Livingston Sewage Treatment Plant, Livingston, NJ. (Tele: (201) 377-7050) and Parsippany Sewage Treatment Plant, Edwards, NJ. (Tele: (201) 428-7593.)

Media:

For the selection of organisms from activated sludge, a limited medium is used, in which, the organic sulfur compound DBT is added as the only sulfur source. DBT is not water soluble, but can be dissolved in dimethylfermamide ($HCON(CH_3)_2$). The required amount of organic sulfur sources was put into culture tube/flasks from a stock DBT/dimethyformamide solution. Medium II with very little inorganic sulfur is also used for organism selection. There is limited inorganic sulfur in the medium and more sulfur is needed from organic sulfur compounds. For the coal culture, medium III, is used to enrich organisms that only can grow by using sulfur in coal. All the culture tubes and flasks were sterilized at 121°C for 15 minutes. The DBT is added after media sterilized. The composition of media are listed in below:

Medium I:	K_2HPO_4	2 g	NH_4Cl	4 g
	$MgCl_2$	0.2 g	$CaCl_2.2H_2O$	0.14 g
	$FeCl_3.6H_2O$	10 mg	Trace diement solution	1%
	per liter water		pH 7.2	
Medium II:	K_2HPO_4	2 g	NH_4Cl	4 g
	$MgCl_2$	0.2 g	$CaCl_2.2H_2O$	0.14 g
	$FeSO_4.7H_2O$	10mg	Trace element solution	1%
	per liter water		pH 7.2	
Medium III:	K_2HPO_4	2 g	NH_4Cl	4 g
	$MgCl_2$	0.2 g	$CaCl_2.2H_2O$	0.14 g
	Vitamin solution	0.1%		
	per liter water		pH 7.2	

(components of vitamin can be see on Appendix 4.)

III. Results and Discussion

The initial phase of the experiments were designed to isolate organisms that can remove organic sulfur from coal using organic sulfur compound dibenzothiophene(DBT) from activated sludge and wastes. In the second phase, new analytical methods for sulfate sulfur, pyritic sulfur, organic sulfur and total sulfur in coal were investigated and compared with the standard methods used in coal analysis. The third phase of the research investigated the removal of sulfur from coal with activated sludge and organisms selected form a mixed culture of sludge. Finally, different types of bioreactors were used to study how to desulfurize coal on a lab-scale.

Isolation and Characterization of Organisms

Activated sludge from Livingston and Parsippany sewage treatment plant, oil sand, medical sludge and several waste sources were used as microorganism sources. Medium I added different percentages of DBT, 0.01%,0.05% and 0.1%; medium II with 0.01%, 0.03% and 0.05% DBT and medium III with 6.7% Juliana coal, 6.7% Brooks Run coal and 6.7% Geo-chemical coal sample and 1% vitamin solutions were used as enrichment cultures. These cultures are able to grow with DBT or sulfur from coal as the sole sulfur sources and DBT or carbon compounds in coal as the sole carbon sources. Without a sulfur source, strong bacterial growth was obtained from shaker flasks/incubators. All mixed cultures were further separated by numerous isolation steps. These steps include:

a. directly transfer of 0.1ml mixed culture into medium I with a different percentage of DBT agar plates and culture at 30°C,

b. transfering 1ml mixed culture into 10ml of medium I with DBT liquid culture tube, after culturing 48 hours, scoring on DBT agar plates and culturing at 30°C,

c. scoring mixed cultures on medium III with 1.5% different coal sample agar plates and culturing at 30°C, and

d. scoring mixed culture on DBT and coal samples agar plates with different percentage of yeast extract.

After much isolating work, three-species community(A-1, A-2 and Ar-1) were obtained from the mixed cultures of activated sludge, and two species communities, S-D1 and S-d' were obtained from mixed culture of oil sand waste, and one specie community, T3-2, was obtained from mixed culture of aerobic tank. These organisms could be distinguished by the morphology of their colonies on different agar plates. The growth characteristics of these organisms on different agar plates are shown in Table 6.

The results showed that A-1, S-D1 and T3-2 can grow on 0.1% DBT in dimethylformamide agar plate. The organism A-1 can grow well in medium I with 0.1% DBT in dimethylformamide and 0.1% yeast extract, and the produced colonies split green pigment, this characteristic is Pseudomonad species. As mentioned before, Pseudomonad species is described to be able to degrade DBT. The species of each isolation was determined by microscopic observation. This is only the first step in DBT degradation. All these organisms should be further characterized.

Table 6. Characteristics of Isolated Organisms on Agar Plates

	A-1	A-2	Ar-1	S-D1	S-d'	T3-2
Medium I + 0.1% DBT	++++	+++	+++	++++	+++	++++
Medium I + 0.1% DBT + 0.1%Dimethyl-mamide	+++++ +++	++++	+++++	+++++ +	+++++	+++++
Medium I + 0.1%DBT+0.1%YE	+++++ +++++ ++	+++++ ++	+++++ +++++	+++++ +++++	+++++ ++++	+++++ ++++
Medium I + 0.1% Demethyl-mamide	+++++	+++	+++	++++	+++	+++++
Medium I + 0.1% DBT in Di-methylformamide	+++++ +	+++++	+++++ ++	+++++ +	+++++	++++
Medium I + 0.1% Y.E.	+++++ +++++	+++++ ++	+++++ ++++	+++++ ++++	+++++ +++++	++++ ++
Medium I + 0.1%DBT in CH_2Cl_2	++	+				+
Medium I	+		+			+

notice: more "+" means that organisms grow well.

Test and Improvement of Analytical Methods

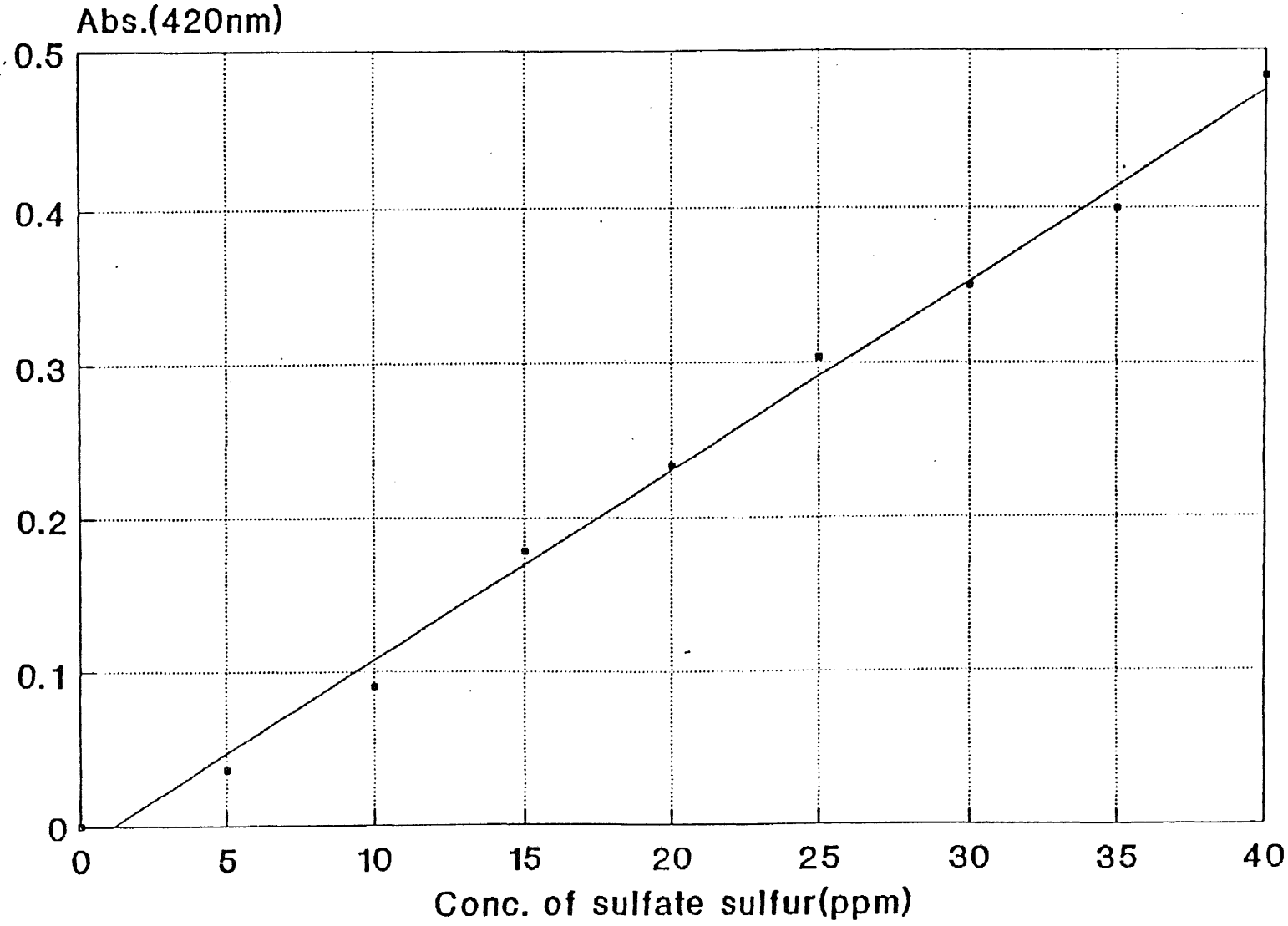
In order to determine the sulfur content in various fractions associated with coal desulfurization, standard analytical methods were evaluated. The turbidimetric method (Method 9038) was evaluated for determination of sulfate sulfur. A UV-Vsible spectrophotometer was employed for this analysis. The concentration of standard sulfate sulfur solution (Na_2SO_4) and the absorption at 420 nm was determined as indicated in Table 7.

Table 7. The Absorption of Standard Sulfate Sulfur Solution by Turbidimetric Method

number	blank	1#	2#	3#	4#	5#	6#	7#	8#
SO4(mg/L)	0	5	10	15	20	25	30	35	40
Condition reagent (ml)	2	2	2	2	2	2	2	2	2
Water dilute to (ml)	100	100	100	100	100	100	100	100	100
BaCl2 (234.3mg/100ml)	50mg in 25ml	50mg in 25ml	50mg in 25ml	50mg in 25ml	50mg in 25ml	50mg in 25ml	50mg in 25ml	50mg in 25ml	50mg in 25ml
Absorption (420nm)	0	0.036	0.090	0.178	0.233	0.303	0.350	0.399	0.483

The standard solution calibration curve for sulfate sulfur by turbidimetric method can be seen in Figure 4. and 5. The linearity of the absorption with the concentration, Figure 5, is not very good. The problem with this method is the need to measure barium sulfate turbidity at 30-sec intervals and it is difficult to get reproducible readings in 30-seconds because the gowth rate of $BaCl_2$ crystals significantly affects the absorption.

Figure 4. Calibration Curve
(Turbidimetric Method)



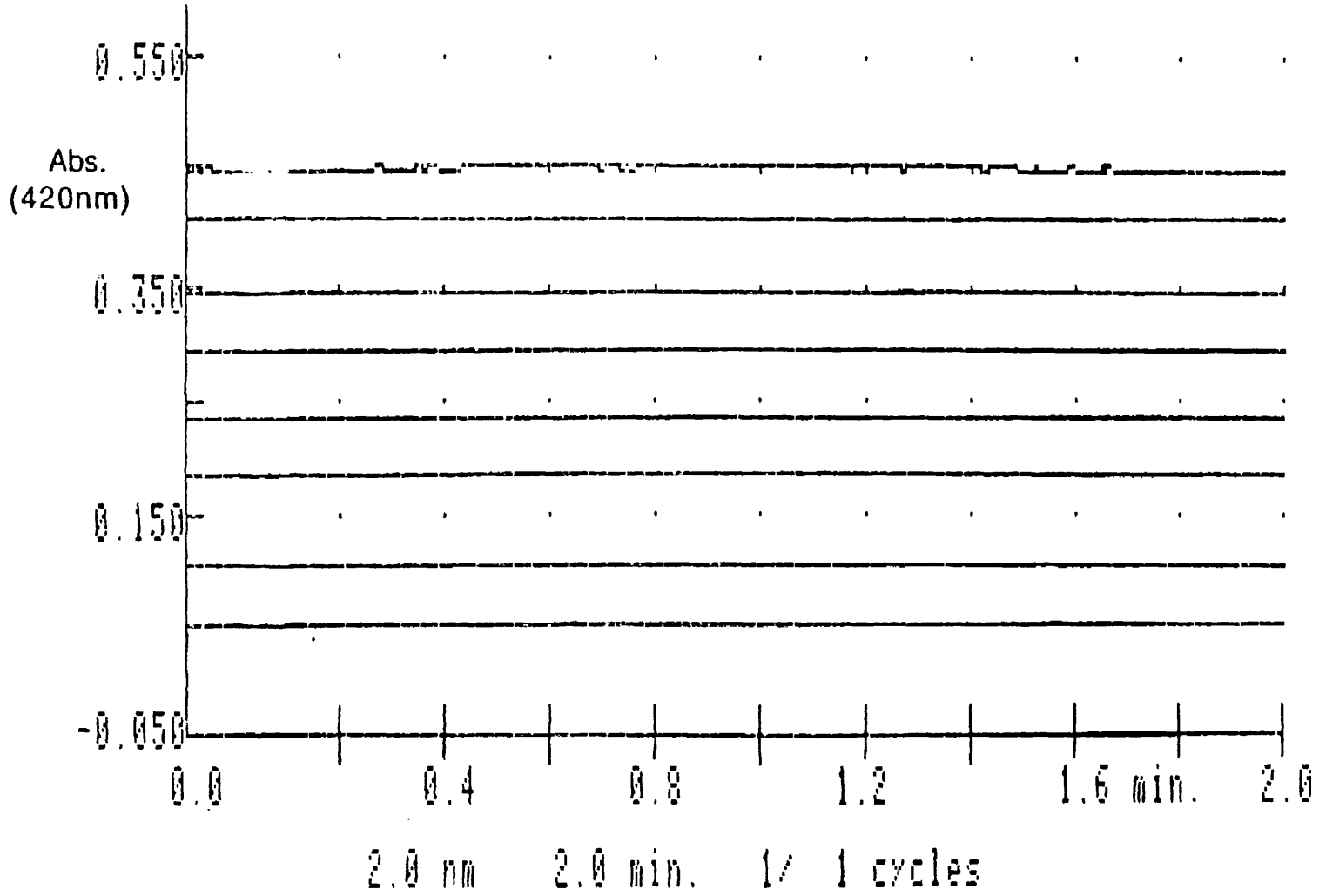


Figure 5. Spectrum of Sulfate Sulfur Standard (Turbidimetric Method)

Since the turbidimetric method does not give good linear results, ASTM D 2492-84 and D 3177-84 were evaluated for sulfur analysis of coal samples. ASTM D 2492-84 is used to determine the three forms of sulfur in coal: sulfate sulfur, pyritic sulfur and organic sulfur. ASTM D 3177-84 is used to determine total sulfur with Eschka and Bomb Washing Methods. In both ASTM methods, the sulfur is dissolved in hot water and is determined by precipitation methods. Because the precipitation methods take a longtime for filtration, ashing and weighing small amounts of samples, large errors are introduced. Consequently, ion-chromatography (IC) was evaluated as a method to determine sulfate sulfur, organic sulfur and total sulfur after extracting these from coal samples.

The models of the Dionex and Waters IC systems that were evaluated are listed in Table 5. The Waters Ion chromatographic configuration is depicted in Figure 6. The eluents for different IC and columns are summarized in Appendix 3.

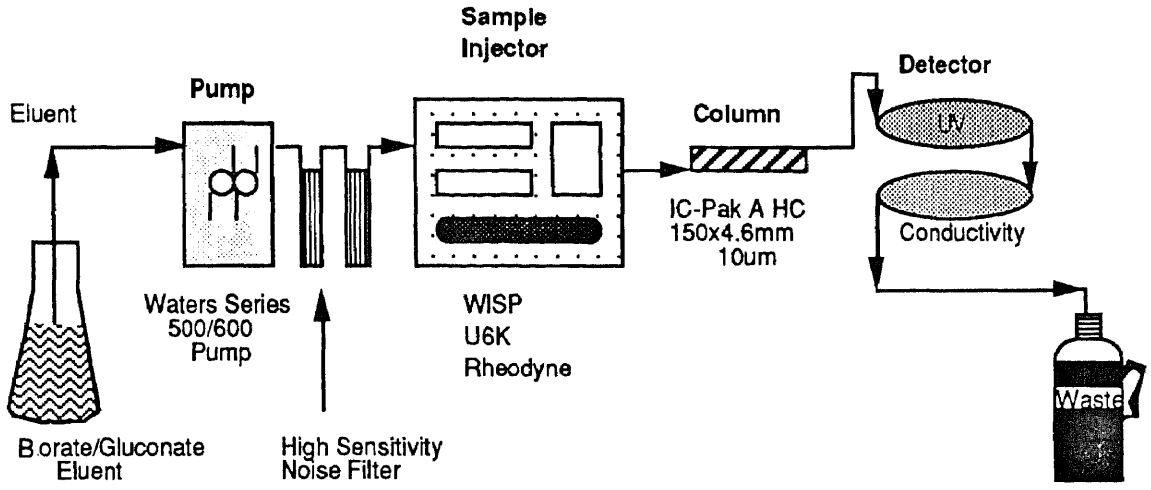


Figure 6. Waters' Ion Chromatography Instrumentation

The concentration of standard sulfate sulfur solution and chromatographic areas are summarized in Table 8. The calibration curve is plotted in Figure 7. IC is available between 0 to 200ppm of sulfate sulfur.

Table 8. The Standard Sulfate Sulfur Solution of IC Method

SO4(ppm)	0	4.950	14.851	24.752	34.653	44.554
Na2SO4(ml) (175.4mg/L)	0	4.17	12.52	20.87	29.22	37.57
Areas(mVs)	0	1042.041	4257.185	6674.588	9438.004	11743.994

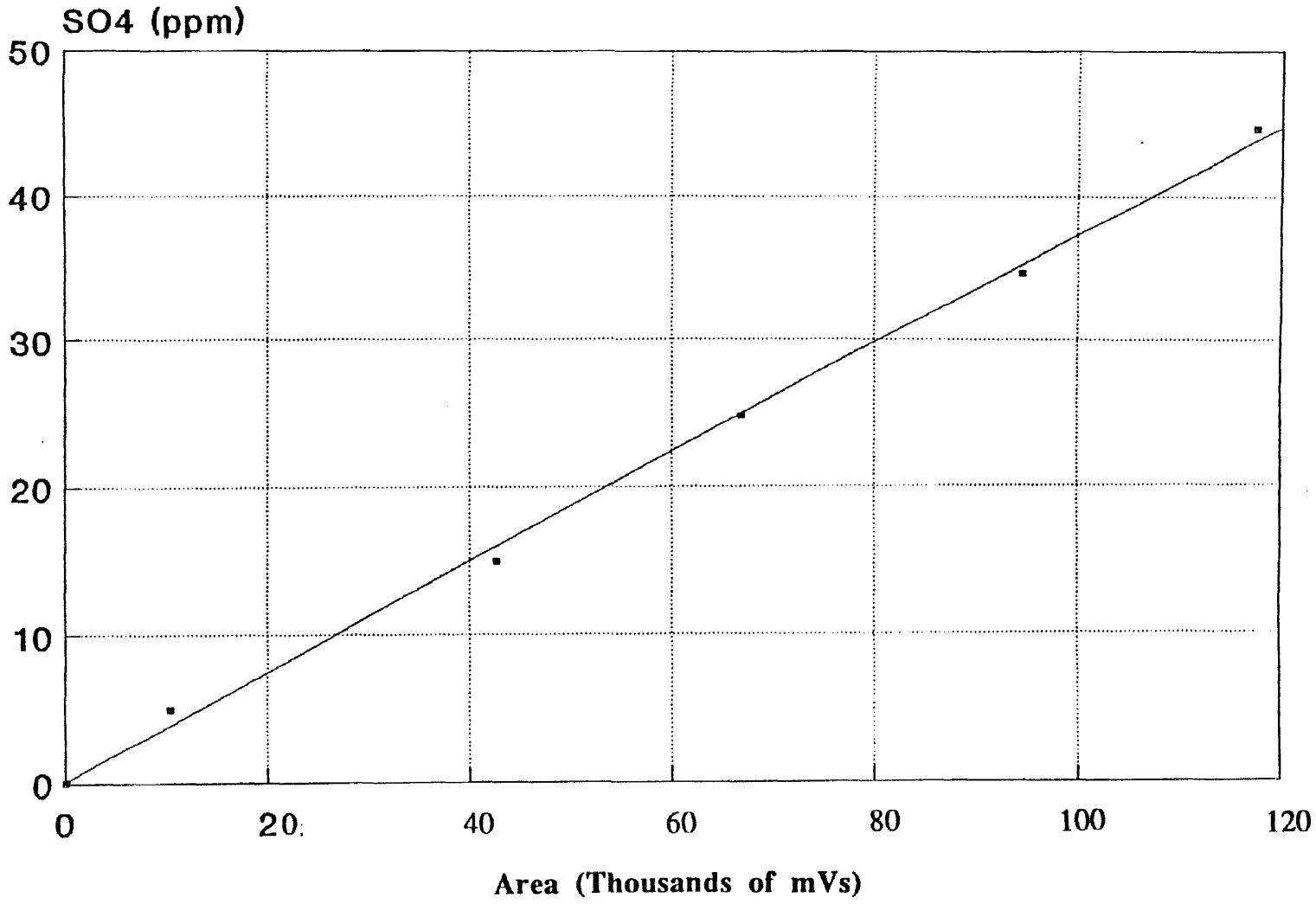
Inorganic sulfur salt, Na_2SO_4, and organic sulfur compound, DBT, were used to verify the ion chromatographic method following extraction according to the ASTM method. Table 9. shows the results of inorganic sulfur and organic sulfur measurements by ion chromatography in Eschka mixture method.

Table 9. Sulfur Compounds Determined by IC

samples / parameters	DBT-1	DBT-2	Na2SO4(98%)
Weight (g)	0.1144	0.1149	0.2161
Areas (mVs)	53050.958	52891.572	343316.00
Determined total sulfur	19.08 mg	19.02mg	42.66mg
Calculated total sulfur	19.90 mg	19.99mg	47.79mg
Differents between determined and calculated results	4.12%	4.85%	10.73%

Figure 7

Calibration Curve
(Sulfate sulfur standard for IC-method)



The results showed that the differences between determined and calculated results of sulfur compounds are between 4.5% to 10.73%, which is well within the accuracy of the ASTM methods.

Total sulfur in different kind of coal samples, Juliana, Geo-chemical and Brooks Run from PSE&G, were determined using the Eschka method in conjuction with ion chromatography. Coal samples were dried overnight at 105°C. Table 10. presents the results from the coal samples, which were calculated from IC-calibration equation.

Table 10. Total Sulfur in Coal Samples (Juliana, Geo-chemical and Brooks Run Coal)

sample / parameters	Juliana		Geo-chemical		Brooks Run	
weight (g)	0.5015	0.5049	0.5950	0.5054	0.5052	0.5012
mean areas (mVs)	42541	45375	52683	42449	41427	41170
SO4 (%)	2.480	2.676	2.474	2.455	2.380	2.380
sulfur (%)	0.828	0.893	0.826	0.819	0.794	0.794
mean of total sulfur	0.860%		0.823%		0.794%	
total sulfur given by PSE&G(wt%)	0.903%		0.976%		0.838%	
Deviation	4.8%		15.7%		5.3%	

The total sulfur in coal IBC-108 (8031001 and 8031202) were determined by ion chromatography using Eschka mixture and bomb calorimeter ashing method. Coal samples were dried overnight at 105°C. Table 11. presents the measurement of coal moisture in samples IBC-108-80 1001 and 8031202. Tables 12. and 13.

summarizes the results of total sulfur in both coal samples determined by the different ashing methods.

Table 11. Test of Moisture of Coal IBC-108

	IBC-108-8031001	IBC-108-8031202
Empty beaker	22.8901 g	23.1676 g
Sample+beaker	37.7540 g	35.1605 g
Weitht after heating overnight at 105°C	31.4500 g	29.8732
moisture (% wet coal)	42.4	44.1

Table 12. Total Sulfur of Coal IBC-108 Determined by IC (Eschka Mixture Method)

coal / parameters #	blank	IBC-108-8031001		IBC-108-8031202	
		1#	2#	1#	2#
E.mixture(g)	4.0	4.0	4.0	4.0	4.0
Coal sample(g)	0.0	1.0015	1.0036	1.0032	1.0219
Deviation water	250	250	250	250	250
pH	>11	>11	>11	>11	>11
Injection(ul)	100	100	100	100	100
Areas(mVs)	4185.39	50246.35	49794.71	44207.54	49267.57
Total sulfur(%)	-	2.33	2.31	2.03	2.24
Average	-	2.32%		2.14%	
Total sulfur given by Illinois	-	2.63%		2.63%	
Deviation	-	11.8%		18.6%	

Table 13 Total Sulfur of Coal IBC-108 Determined by IC (Oxygen-bomb Calorimeter)

coal / parameters	Blank	IBC-108-8031001		IBC-108-8031202	
		1#	2#	1#	2#
Coal sample(g)	0	1.0124	1.0015	1.0029	0.5001
deionized water	250	250	250	250	250
pH	-7.0	-7.0	-7.0	-7.0	-7.0
Injection(ul)	100	100	100	100	100
Aresas(mVs)	3883.32	57295.13	50246.35	45728.42	27037.76
Total sulfur(%)	-	2.36	2.07	2.10	2.32
Average(%)	-	2.22%		2.21%	
Total sulfur given by Illinois	-	2.63%		2.63%	
Deviation	-	15.6%		17.0%	

Results showed that there are some differences between the present measurements and the values given by Illinois State Geological Survey . It might be caused by the different moisture in the sample or an analytical error. The moistures given by Illinois State Geological Survey is 45%, but the sample used in experiment is

about 42.4% and 44.1%. And also, in reference to the Eschka total sulfur determination, the presence of Ba^{+2} or large concentration of Ca^{+2} can cause precipitation of the alkaline earth sulfate on the unextracted residue, leading to low values for determined sulfate content. The sulfate sulfur and pyritic sulfur have not been determined because they are only 0.01% and 0.37% in coal IBC-108.

As noted above, Oxygen-bomb method like Eschka mixture method ultimately measure the sulfate content of an aqueous extract of the burned coal sample, the presence of Ba^{+2} or very high concentration of Ca^{+2}, could result in retention of insoluble $BaSO_4$ or slightly soluble $CaSO_4$ on the residue and thus provide a low total sulfur value.

A comparison of the results from the Eschka mixture and bomb calorimeter shows that both methods give almost the same results. Because the bomb calorimeter method requires each sample to be ashed separetely while the Eschka mixture method can ash all the sample simultaneously, the Eschka mixture can save analytical time.

For the coal IBC-101, the sulfate sulfur, organic sulfur and total sulfur were determined in Eschka mixture method by ion chromatography, and the pyritic sulfur was determined by Atomic Absorption. The atomic absorption instrumental parameters are summarized in Table 14. The iron standard solution for atomic absorption calibration is given in Table 15. Figure 8. is the calibration curve for iron.

Table14. Instrumental Parameters of Atomic Absorption

Light Source:	Hollow Cathode	
Lamp NO:	62810162810-02	
Lamp Current:	Normal and D2 Operation:	8.0 ma
	Smith-Itieftje Operation: (Bky)	3.0 ma
	(Signal) Match to Bky intensity	
Wavelength:	248.3 nm	
Bandpass	0.3 nm	
Flame Description:	Air-Acetylene	
	Oxidizding: Fuel Lean, Blue	

Table 15. Iron Standard Solution of Atomic Absorption

Iron solution(ppm)	0	20	30	40
Lanthanum(ml)	10	10	10	10
Absorption(248.3nm)	0	0.035	0.065	0.124

The determined results of sulfate sulfur, pyritic sulfur, organic sulfur and total sulfur are summarized in Table 16.

Figure 8. Callbration Curve

(Iron standard solution for A-A)

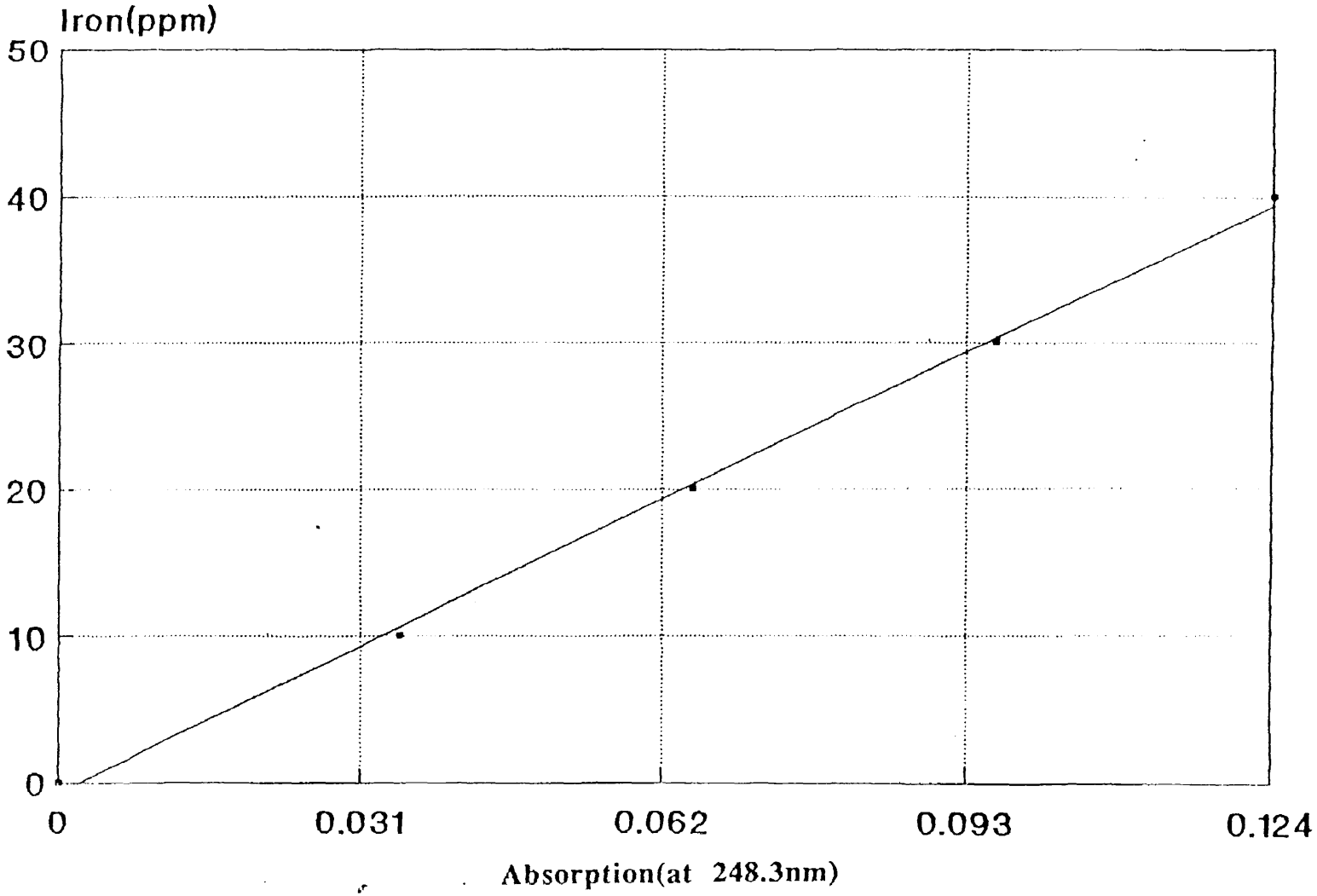


Table16. The Different Kinds of Sulfur in IBC-101 Determined by Ion Chromatography and Atomic Absorption

sulfur / parameters	Blank	Sulfate sulfur		Pyritic sulfur		Organic sulfur		Total sulfur	
		1#	2#	3#	4#	5#	6#	7#	8#
Coal sample(g)	0	1.0035	1.0076	filter residue from 1#	filter residue from 2#	filter residue from 3#	filter residue from 4#	0.5014	1.0019
E.mixture(g)	4.0	4.0	4.0	-	-	4.0	4.0	4.0	4.0
pH	>11	>11	>11	<2	<2	>11	>11	>11	>11
M.Q.water(ml)	250	250	250	50	50	250	250	250	250
Injection(ul)	100	100	100	-	-	100	100	100	100
Areas(mVs)/ Absorption	5547.5	6155.2	7095.4	2x0.069	2x0.071	57888.5	55197.6	44881.9	83381.7
Total sulfur(%)	-	0.03	0.08	1.36	1.31	2.89	2.73	4.35	4.31
Average(%)	-	0.055%		1.335%		2.81%		4.33%	
Number given by Illinois	-	0.05%		1.27%		3.00%		4.32%	
Differences between determined and number	-	10.0%		5.11%		6.33%		0.23%	
Total	-	0.055% + 1.335% + 2.81% = 4.20%							

The analysis for pyritic sulfur is actually the analysis for pyritic iron, since it is the HNO_3 soluble iron which is determined. In the analysis, the sample has been extracted with diluted HCl for the sulfate analysis prior to HNO_3 digestion, and is thus free of any iron other than pyritic. But an oxidized coal might contain jarositic sulfate($Fe_3(SO_4)_2(OH)_5.2H_2O$), which would have escaped HCl extraction but which would have dissolved in HNO_3. This could cause an apparent high pyritic iron value resulting in an apparent high pyritic sulfur value and an apparent low organic sulfur, like the results showed in Table 16.

Data from the analysis of inorganic and organic sulfur compounds and coal samples obtained from many analytical tests using ion chromatography and atomic absorption are presented above. The average deviation between the analyzed data and the measured data by PSE&G are about 0.23% to 15%. Because the ASTM precipitate method may vary +/-10%, the analytical results develeped here are considered to agree closely with the ASTM results. Consequently, ion chromatography used to analyze sulfate sulfur following the extraction by ASTM method can save much time and requires less effort than the precipitate method. Figure 10. illustrates an ion chromatogram for total sulfur analysis.

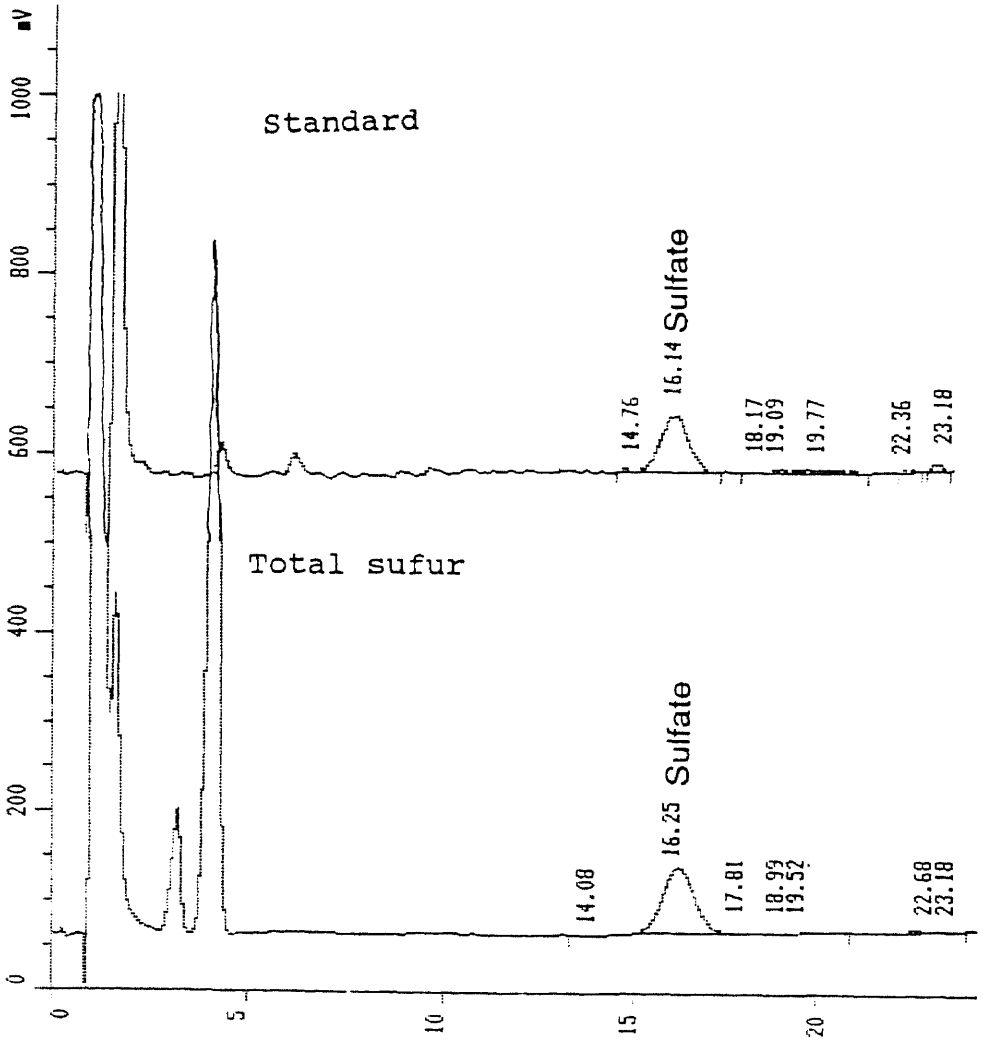


Figure 9. Ion Chromatogram(total sulfur analysis)

Tests of Coal Desulfurization Using Activated Sludge And Individual Cultures

The two sources of microorganisms used for removal of sulfur from coal in the experiments are activated sludge from aerobic sewage treatment plants, and pure cultures isolated from different sludge/wastes. Initial experiment were done in shaker flasks/ incubators. These experiments were followed using different laboratory-scale bioreactors. For the determination of the total sulfur left in coal, the coal sample was centrifuged and washed three time using millin quality (M.Q.) water. The same amount of biomass from activated sludge was determined as a blank.

Sulfur removal data obtained from shaker flask experiments from Juliana, Geo-chemical and Brooks Run coal are presented in Tables 17. and 18. Approximately 30% of total sulfur was removal from Juliana coal, 49% from Geo-chemical coal and 31% from Brooks Run coal.

Table 17. Activated Sludge Cultures with Coal in Flasks

parameters \ coal	Juliana	Geo-chemical	Brook Run
A. Sludge(ml)	100	100	100
Coal sample(g)	10.1091	10.0586	10.0403
Total sulfur left	0.67%	0.51%	0.58%
Total sulfur measured by PSE&G(wt%)	0.903%	0.976%	0.838%
Total sulfur removal	25.80%	47.75%	30.79%

- activated sludge was obtained from Livegston sewage treatment pant
- culture condition: 30°C, 200 rpm shaker for 49 days

Table 18.Biomass/Medium III Cultures with Coal in Flask

parameters \ coal	Juliana	Geo-chemical	Brooks Run
Biomass/Medium	100 ml	100 ml	100 ml
Coal sample(w/w%)	6.7%	6.7%	6.7%
Total sulfur left	0.59%	0.48%	0.57%
Total sulfur given by PSE&G(wt%)	0.903	0.976	0.838
Total sulfur removal (%)	34.66%	50.82%	31.98%

- activated sludge was obtained from Livingston sewage treatment plant
- biomass was obtained from 100ml activated sludge by centrifuged at 3,000 rpm for 10 mins
- 0.1% vitamin was add into medium III
- culture condition: 30°C, 200 rpm shaking for 35 days

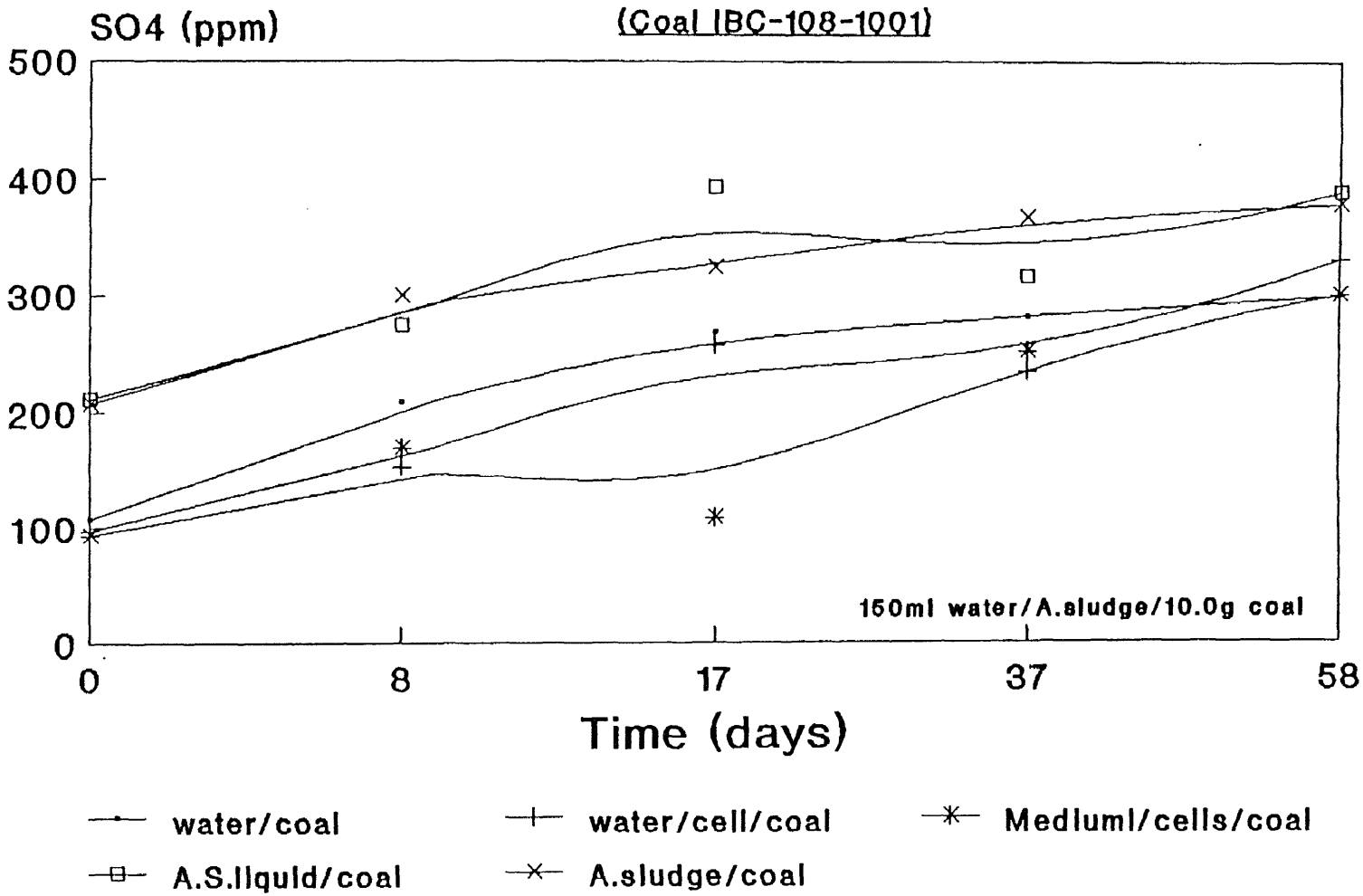
In the experiments with shaker flasks, coal IBC-108(8031001 and 8031202) were added into different cultures. Water plus biomass from activated sludge, Medium I plus biomass from activated sludge, activated sludge liquid without biomass, activated sludge, as well as pure cultures, A-1, S-D1, T3-2, Ar-1 and mixed culture of those are used, and water is as blank. The activated sludge was obtained from the Parsippany sewage treatment plant. Although the results presented in Table 19, Table 20 , and Figure 10, Figure 11, have not shown very different results between blank and activated sludge culture, there is about 55% total sulfur removal from coal. Table 21 and Figure 12. show unexpectedly high results in pure culture flasks. The total sulfur removal is 62.74% in A-1 specie culture, 62.13% in S-D1 culture, 63.31% in T3-2 culture, 52.31% in Ar-1 culture and 71.86% in the mixed culture. In comparison with a blank flask of 14.45% sulfur removal, these results indicate that species A-1, S-D1, T3-2 and

Table 19. **Different Coal Cultures in Flasks**

(Coal IBC-108:sulfate 0.01%,pyrite 0.37%,org.sulfur 2.25%,total sulfur 2.63%)

culture / days	150ml water 10.0g coal		150ml water + cells 10.0g coal		150ml media + cells 10.0g coal		150ml liquid of A.sludge 10.0g coal		150ml A.sludge 10.0g coal	
	pH	SO4 (ppm)	pH	SO4 (ppm)	pH	SO4 (ppm)	pH	SO4 (ppm)	pH	SO4 (ppm)
0	7.50	106.91	7.62	97.02	7.50	93.05	7.50	210.49	7.50	206.41
8	5.36	207.25	5.66	152.32	6.03	167.80	5.96	273.69	5.67	298.83
17	3.56	267.34	4.77	256.03	5.34	107.49	4.81	392.39	4.80	323.22
37	3.29	280.60	4.34	234.19	3.90	251.10	3.56	316.46	4.36	367.35
58	3.01	300.10	2.98	331.50	3.82	301.10	4.19	389.05	3.30	378.98
Total sulfur left	1.06%		0.80%		1.22%		1.38%		1.17%	
Sulfur removal (%)	59.70%		69.58%		53.61%		47.53%		55.51%	

Figure 10. The Change Of Sulfate Sulfur Concentration In Activated Sludge Coal Cultures Of Flasks



(Coal IBC-108-1202:sulfate 0.01%,pyrite 0.37%, org.sulfur 2.25%,total sulfur 2.62%)

culture / days	150ml water 5.0g coal		150ml water + cells 5.0g coal		150ml media + cells 5.0g coal		150ml liquid of A.sludge 5.0g coal		150ml A.sludge 5.0g coal	
	pH	SO4 (ppm)	pH	SO4 (ppm)	pH	SO4 (ppm)	pH	SO4 (ppm)	pH	SO4 (ppm)
0	5.42	60.80	7.01	52.18	7.31	33.40	7.24	108.72	7.81	87.03
8	3.19	90.54	4.53	104.22	5.37	85.49	5.46	161.94	4.94	144.88
17	3.24	118.20	4.32	112.93	4.91	102.34	5.09	128.45	4.01	163.23
37	3.12	219.52	3.14	294.10	3.61	179.72	4.49	230.90	4.32	314.10
58	3.24	281.80	2.96	430.00	2.71	259.20	3.64	373.40	3.62	527.6
Total skulfur left	1.07%		0.81%		1.16%		0.97%		1.16%	
Sulfur removal (%)	59.32%		69.20%		55.69		63.12%		55.69%	

Figure 11. The Change of Sulfate Sulfur Concentration of Activated Sludge Coal Cultures in Flasks

(Coal IBC-108-1202)

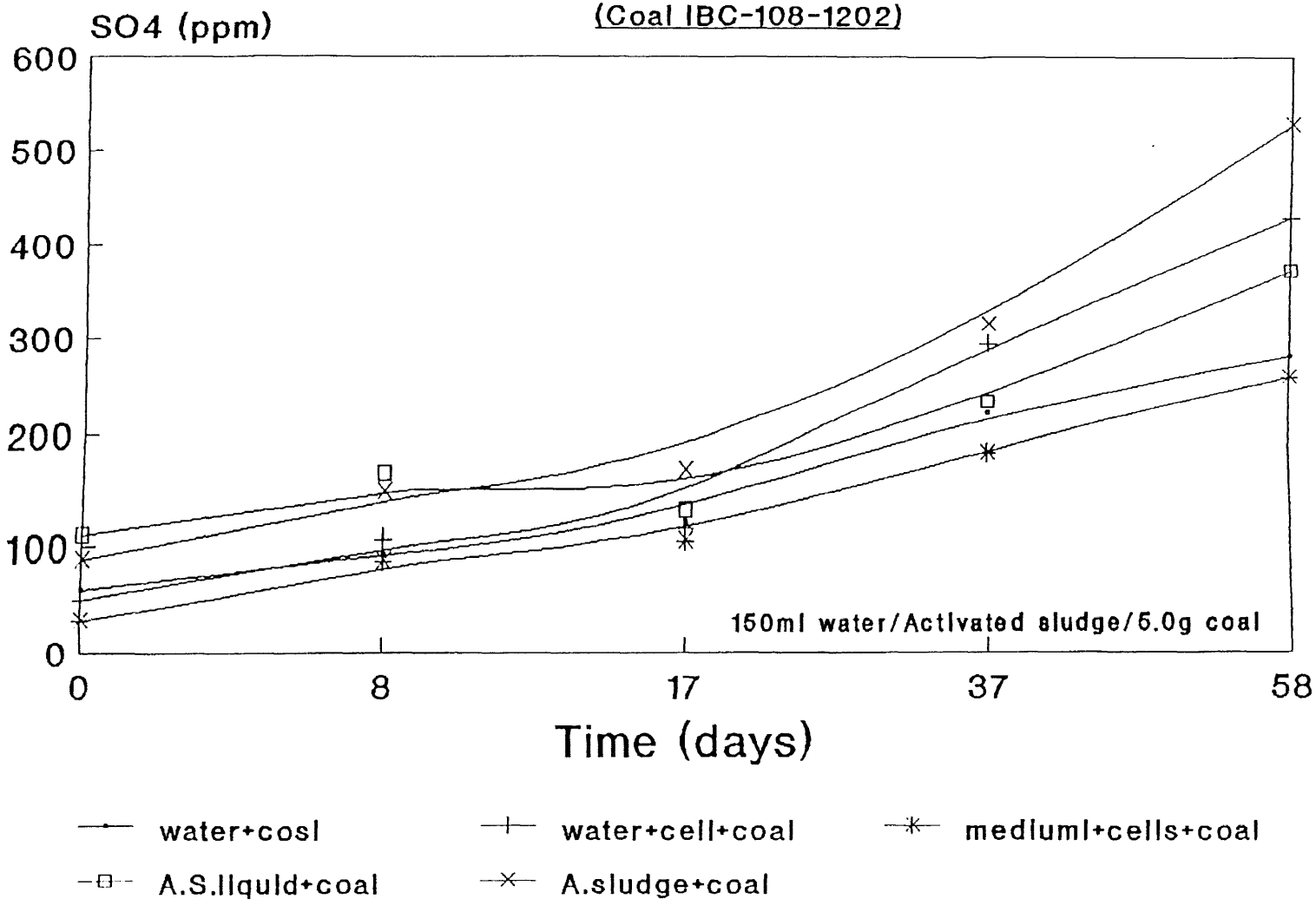
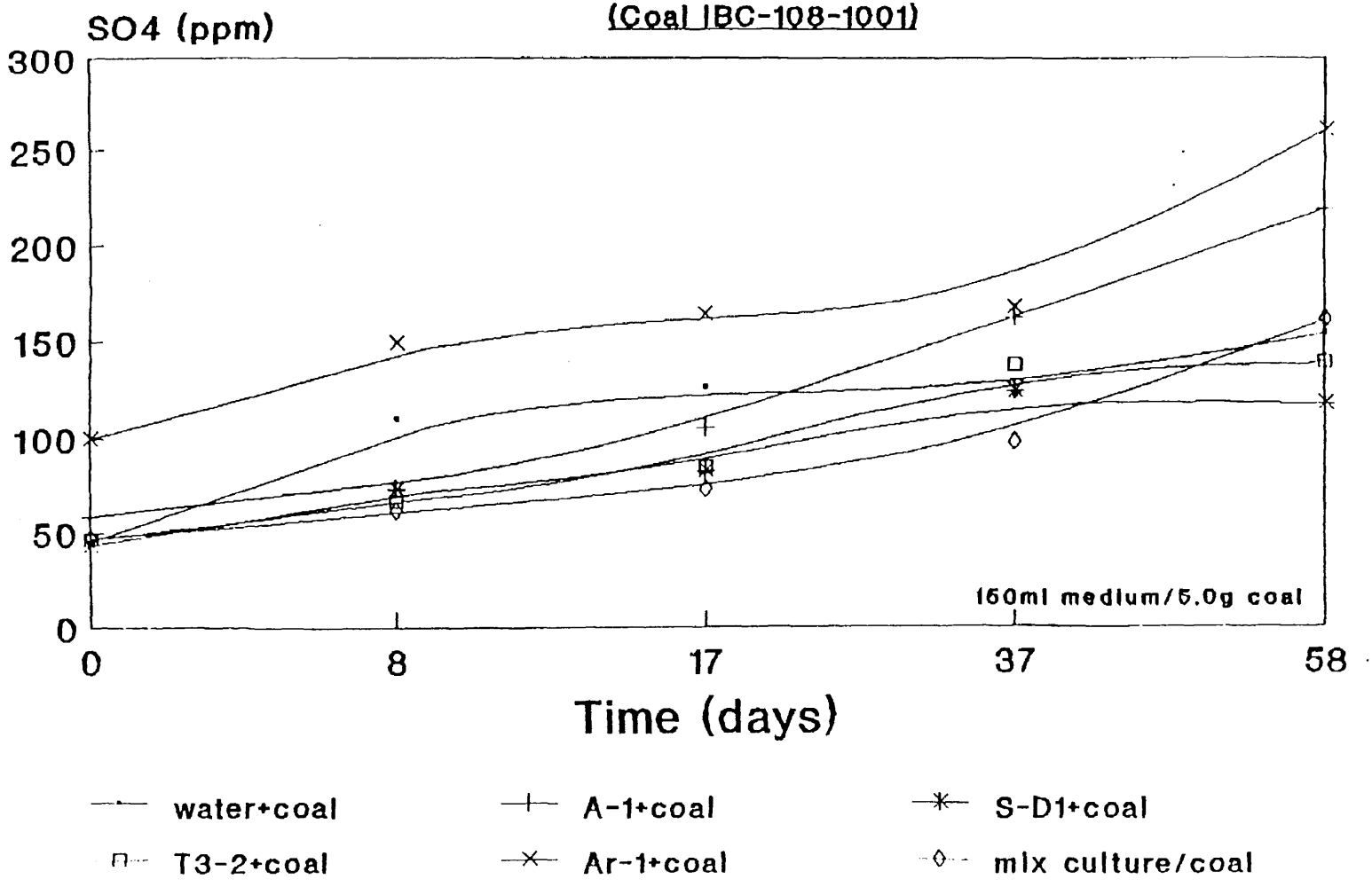


Table 21. Microorganisms Medium Coal Cultur in Flasks

(Coal IBC-108:sulfate 0.01%,pyrite 0.37%,org.sulfur 2.25%, total sulfure 2.63%)

Culture / Days	150ml water 5.0g coal		150ml medium A-1 5.0g coal		150ml medium S-D1 5.0g coal		150ml medium T3-2 5.0g coal		150ml medium Ar-1 5.0g coal		150ml medium mix culture 5.0g coal	
	pH	SO4 (ppm)	pH	SO4 (ppm)	pH	SO4 (ppm)	pH	SO4 (ppm)	pH	SO4 (ppm)	pH	SO4 (ppm)
0	7.40	45.20	7.40	58.09	7.40	43.22	7.40	46.18	7.40	99.56	7.40	46.53
8	6.43	110.32	7.01	73.25	6.87	72.90	6.97	66.02	6.49	148.77	6.96	61.28
17	5.07	126.47	6.83	105.48	6.75	82.20	6.86	84.37	6.47	163.99	6.92	72.69
37	3.62	122.49	6.61	162.32	6.59	124.34	6.66	137.71	5.13	168.08	6.69	97.93
58	3.40	219.20	6.67	220.20	6.57	119.10	6.56	140.16	6.26	261.36	6.52	162.60
Total sulfur left	2.25%		0.98%		0.47%		0.97%		1.25%		0.74%	
Sulfur removal (%)	14.45%		62.74%		62.13%		63.31%		52.31%		71.66%	

Figure 12. The Change of Sulfate Sulfur Concentration of Medium Coal Culture in Flasks



Ar-1 have the ability of removing sulfur from coal. Because coal IBC-108 contains only 0.01% sulfate sulfur and 0.37% pyritic sulfur, 85.93% is organic sulfur, the above results of total sulfur removal are to be shown at least 85.93% organic sulfur.

The pH change of cultures with coal IBC-108 in different shaker flasks have been shown in Figures 13, 14, and 15. The pH of cultures with 10.0g IBC-108-1001 coal decreased with culture time(Figure 13). From the beginning to 17 days, the pH deceased fast, pH changed from 7.5 to about 4.0. The pH change for the blank culture, water and coal sample, showed faster pH change than activated sludge cultures. The pH for cultures with 5.0g coal changed from 7.0 to 4.5 during first 8 days, in blank, the pH has changed to 3.19. After 8 days, the change of pH become very slow. At the end of shaking for 58 days, the pH changed to about 3.2. Unlike the biomass culture, the pH of media coal cultures did not change very much(Figure 15). The pH of pure culture and mix culture change from pH 7.40 to about 6.60 during 58 days period, but the pH of the bank culture, water and 5.0g coal, changed from pH 7.40 to pH 3.40.

In the experiment, we found that the aqueous sample of medium with biomass and activated sludge flasks in 8 days has some soluble-coal on the top of aqueous sample after centrifuged and the soluble-coal disappeared from late samples. This phenomenon shows that the biomass from activated sludge can transform coals to soluble products and it will disappear with pH decreasing. The results of this experiment suggest that biomass has ability to catalyze a surface attack on coal during solubilization and may catalyze sulfur removal from the surface of finely-ground coal particles. The coal-solubilizing and sulfur-oxidizing organisms in activated sludge biomass may be particularly active at the interface between organic and inorganic regions within coal. The coal-solubilizing ability decreased with decreasing pH.

Figure 13. The pH Change of Activated Sludge Coal Cultures

(Coal IBC-108-1001)

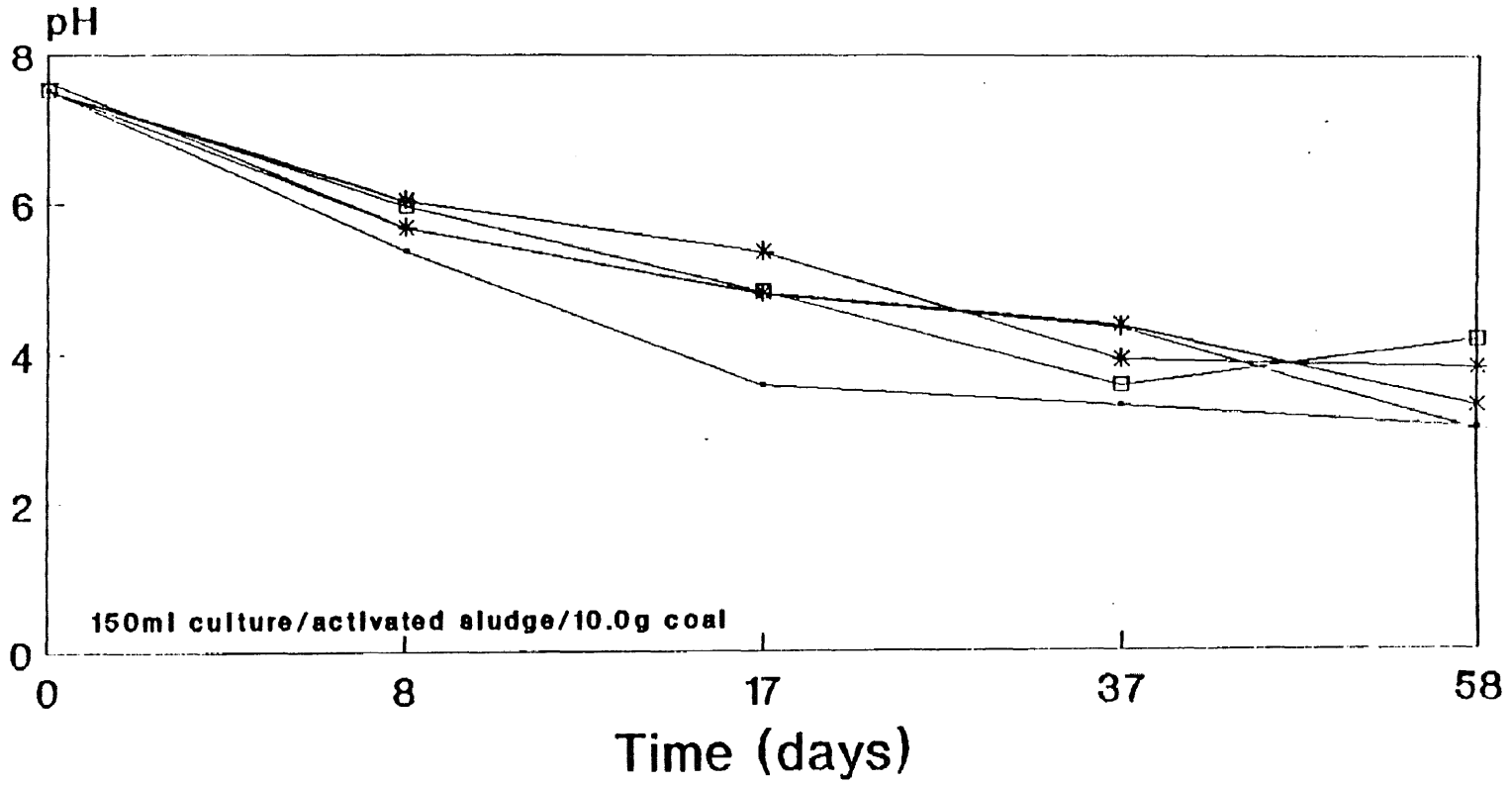


Figure 14. The pH Change of Activated Sludge Coal Cultures

(Coal IBC-108-1202)

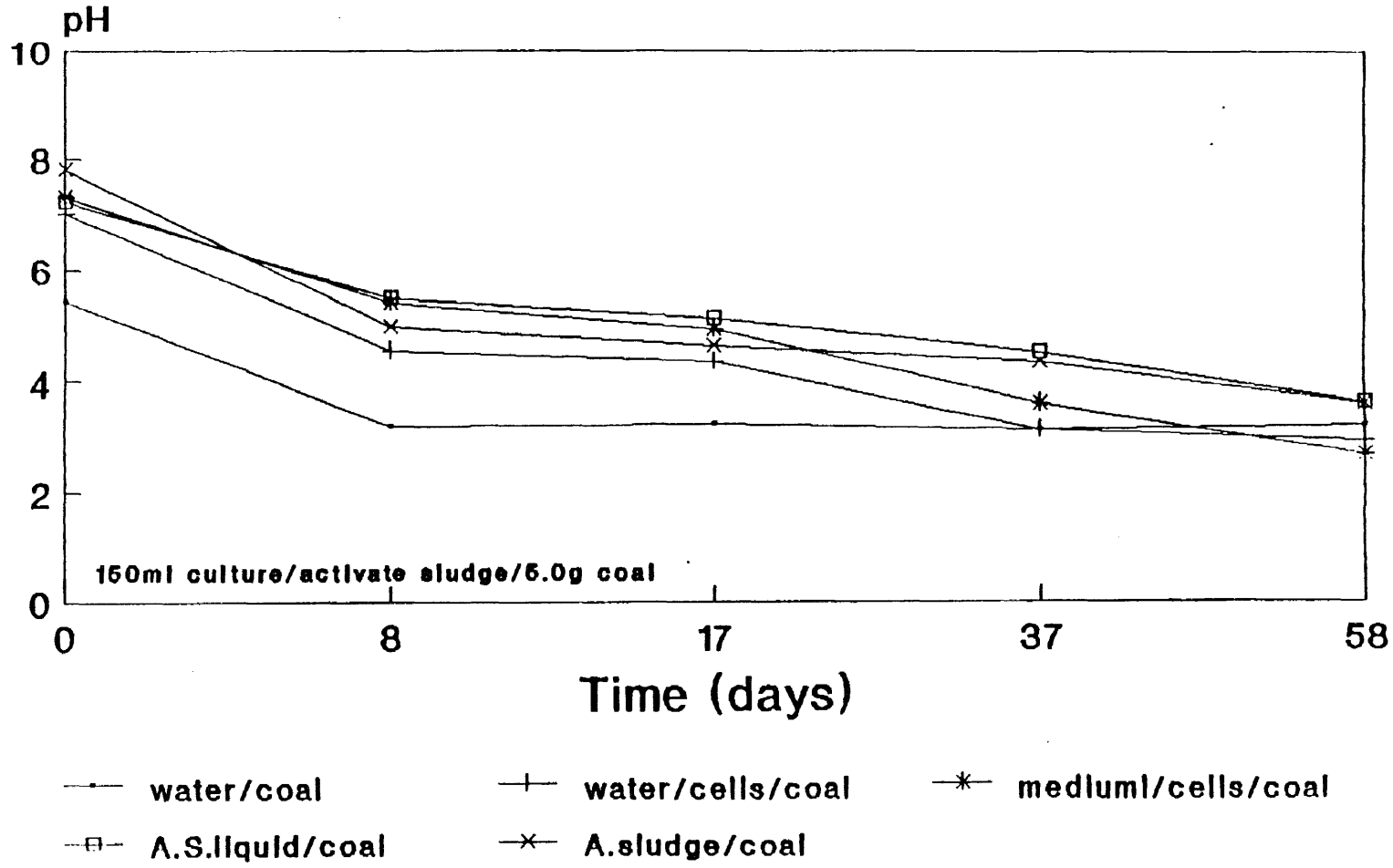


Figure 15. The pH Change of Medium Coal Cultures

(Coal IBC-108-1001)

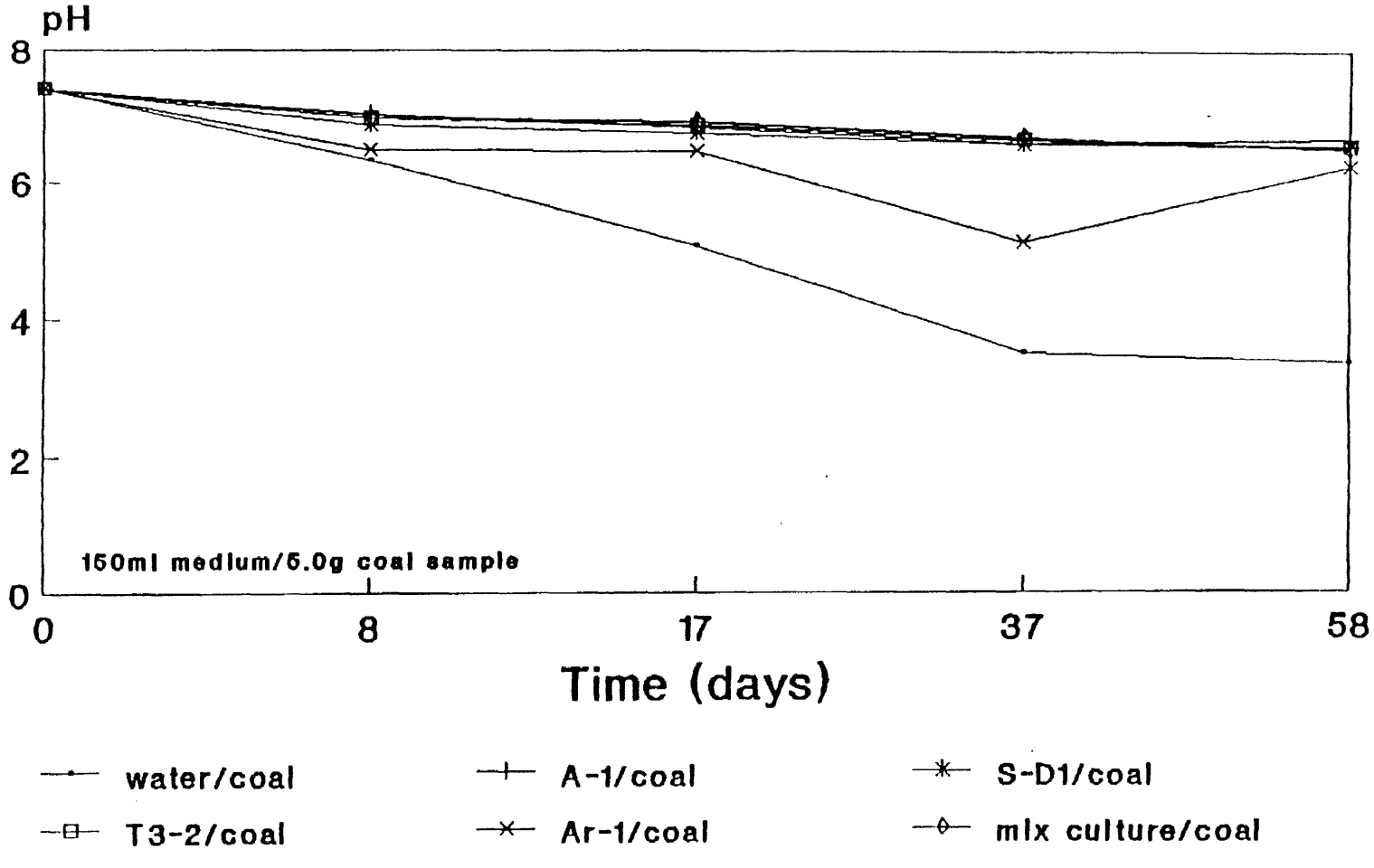


Figure 16. shows ion chromatograms for 150ml activated sludge and 10.0g IBC-108 coal culture in flask at beginning and end culturing. Figure 18. is the ion chromatogram for 150ml activated sludge and 5.0g IBC-108 coal culture in flask at beginning and end shaking.

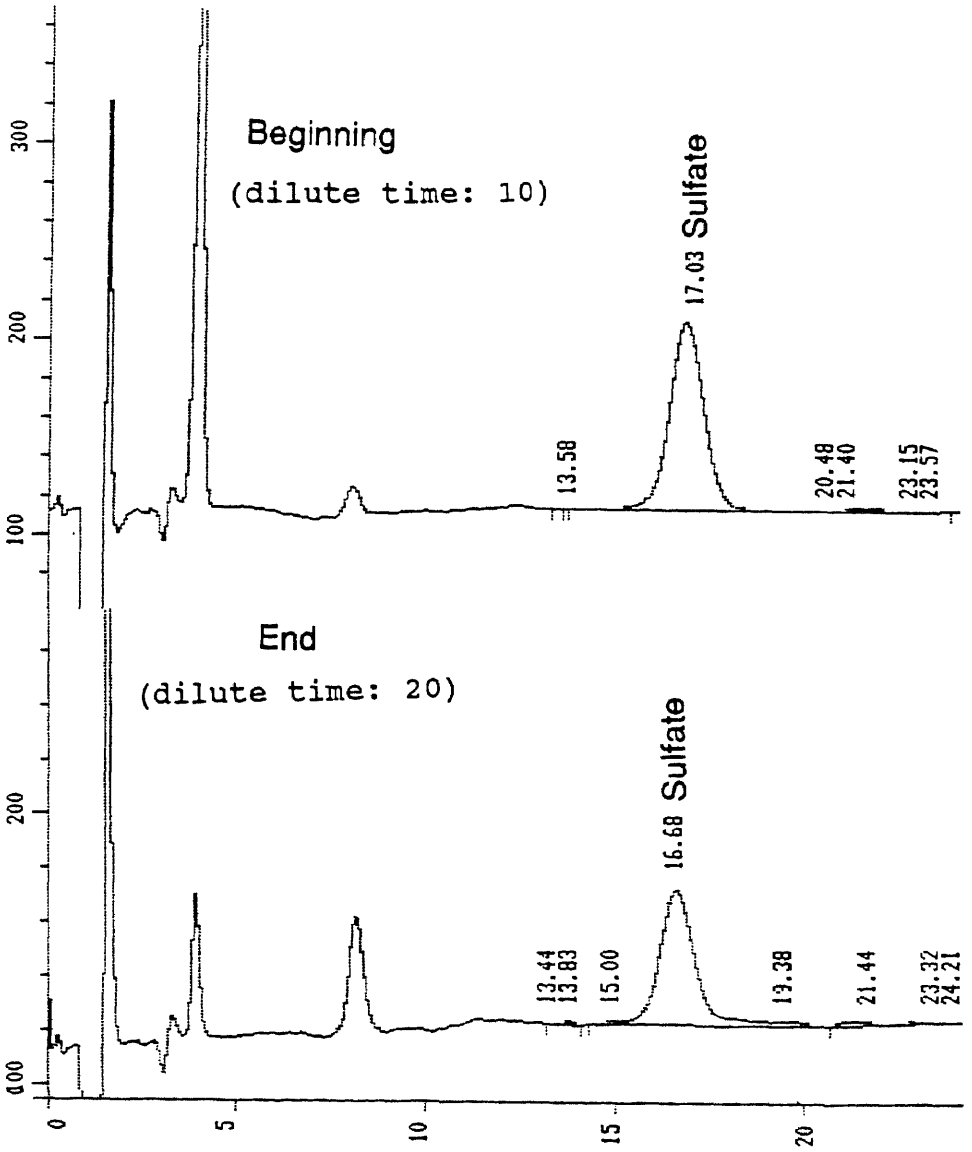


Figure 16. Ion Chromatogram (150ml A.Sludge and 10.0g Coal in Flask)

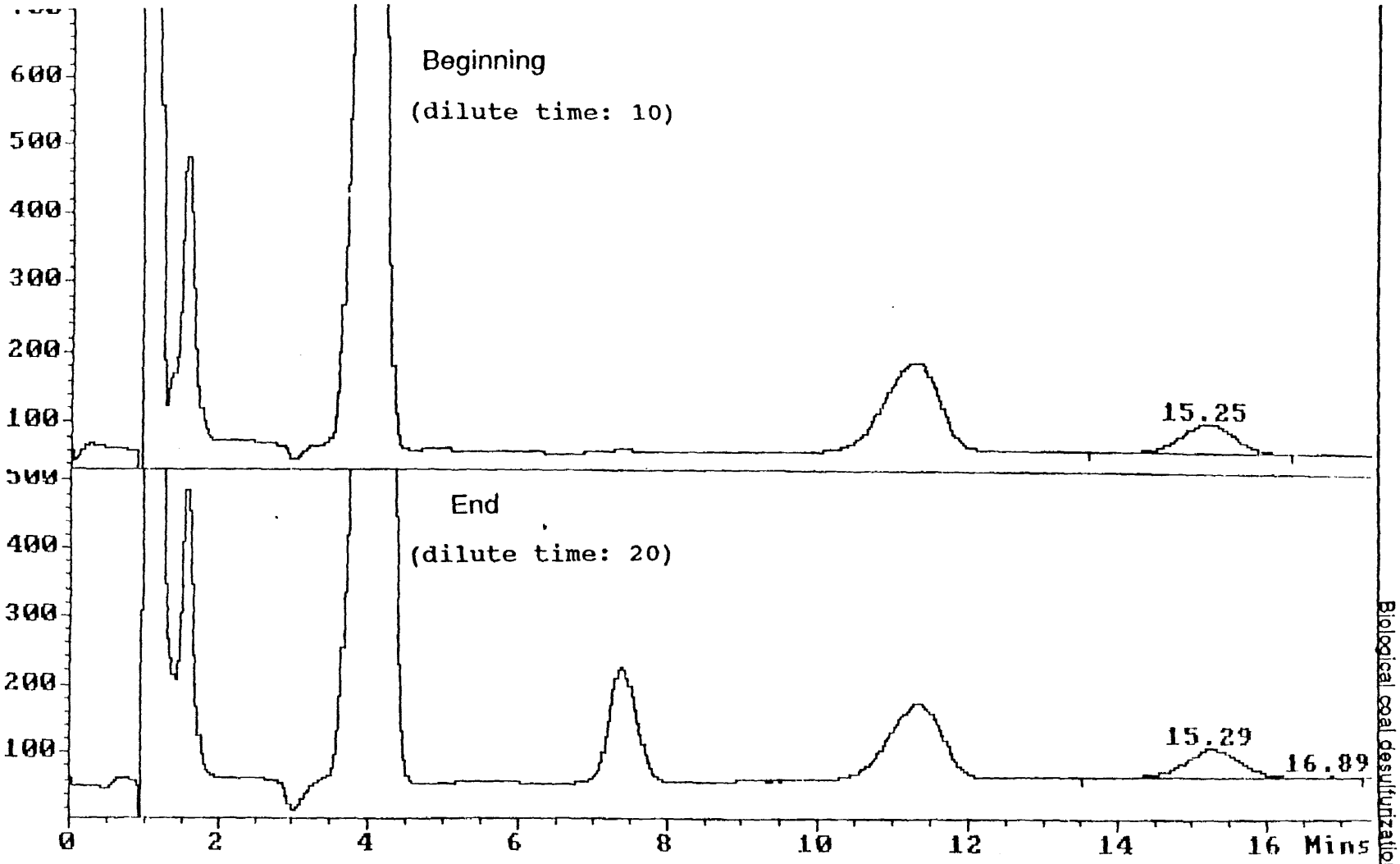


Figure 17. Ion Chromatogram
(150ml A. Sludge and 5.0g Coal in Flask)

Coal IBC-101(sulfate: 0.5%, pyrite: 1.27%, organic sulfur: 3.00% and total sulfur:4.32%) is also used in flasks/incubators experiments. The same as cultures with coal IBC-108, the cultures with coal IBC-101 of water, water plus biomass, medium I plus biomass, activated sludge without biomass, activated sludge and pure cultures, A-1, S-D, S-D, T3-2, Ar-1, as well as with mixed culture of those, were used for desulfurization test of IBC-101 in shake flasks. The activated sludge was obtained from the Parsippany sewage treatment plant. Table 22 is the results of activated sludge coal cultures and Table 23 is the results of microorganisms coal cultures from flasks. The results showed that there is 28.7% sulfur removal from activated sludge flask, but only 8.1% is removed from the blank flask. There is about 25 to 29% sulfur removal from pure cultures, however the blank is only 8.8%. Compared with the results of sulfur removal from coal IBC-108, the results of sulfur removal from coal IBC-101 are less than from coal IBC-108, because coal IBC-108 is acid-pretreatment coal, but coal IBC-101 is not. The different results of both coal IBC-108 and IBC-101 indicate that the acid-pretreatment of coal has great effects on biological coal desulfurization.

Figure 18 and 19 show the change of sulfate sulfur concentration of activated sludge and medium coal cultures in flasks. The concentration of sulfate sulfur increase with culture time. The pH change of cultures with coal IBC-101 in different shaker flasks are shown on Figure 20 and 21. The pH of activated sludge coal cultures did not change very much in first 14 days. After 14 days, the pH of blank, water plus biomass and medium plus biomass cultures decreased with culture time, from about 5.8 to 3.3, but the pH of activated sludge without biomass and activated sludge cultures didn't change very much, from 6.6 to 5.7. Unlike the activated sludge coal cultures, the pH of media coal cultures did not change very much(Figure 21).

Table 22. Different Coal Cultures in Flasks

(Coal IBC-101: Sulfate 0.05%, Pyrite 1.27%, Organic sulfur 3.00%, total sulfur 4.32%)

Culture / Days	150ml water 10.0g coal		150ml water + biomass 10.0g coal		150ml media + biomass 10.0g coal		150ml liquid from A.sludge 10.0g coal		150ml A. sludge 10.0g coal		150ml A.sludge enriched in 0.05% DBT for 30 days 10.0g coal	
	pH	SO4 (ppm)	pH	SO4 (ppm)	pH	SO4 (ppm)	pH	SO4 (ppm)	pH	SO4 (ppm)	pH	SO4 (ppm)
0	5.84	162.25	5.58	88.59	6.43	129.70	6.66	352.75	6.79	296.86	6.57	350.45
14	6.21	386.04	5.68	302.17	5.77	296.39	5.96	383.72	5.55	282.33	5.88	488.80
33	4.16	497.92	4.87	511.61	3.81	331.35	6.01	420.13	5.48	508.84	6.18	648.14
43	3.45	574.70	4.45	562.49	3.19	438.05	5.81	539.65	5.11	526.31	5.82	678.14
56	3.31	591.90	4.21	584.56	2.59	488.22	5.77	520.71	5.05	558.67	5.70	708.92
Total sulfur left	3.94%		3.15%		3.19%		3.29%		3.03%		3.97%	
Total sulfur removal	8.80%		27.08%		26.16%		23.84%		29.86%		20.37%	

Table 23. Microorganisms Coal Culture in Flasks Media

(Coal IBC-101: Sulfate 0.05%, Pyrite 1.27%, Organic sulfur 3.00%, total sulfur 4.32%)

Culture / Days	150ml water 5.0g coal		150ml media A-1 5.0g coal		150ml media S-01 5.0g coal		150ml media T3-2 5.0g coal		150ml media Ar-1 5.0g coal		150ml media mix culture 5.0g coal	
	pH	SO4 (ppm)	pH	SO4 (ppm)	pH	SO4 (ppm)	pH	SO4 (ppm)	pH	SO4 (ppm)	pH	SO4 (ppm)
0	6.56	150.84	6.59	140.76	6.58	158.14	6.61	173.85	6.58	159.76	6.59	167.96
14	6.66	105.24	6.58	162.30	6.65	206.29	6.73	316.39	6.58	188.03	6.68	246.93
33	6.33	276.53	6.40	269.02	6.39	262.55	6.50	263.27	6.42	272.43	6.46	282.35
43	6.02	329.26	6.06	351.75	6.03	298.65	6.15	289.58	6.20	354.26	6.30	290.80
56	6.02	350.88	6.01	366.88	6.04	346.78	6.15	303.00	6.08	332.42	6.13	313.19
Total sulfur left	3.97%		3.07%		3.38%		3.20%		3.08%		3.14%	
Total sulfur removal	8.10%		28.93%		21.76%		25.94%		28.70%		27.31%	

Figure 18. The Change Of Sulfate Sulfur Concentration of Activated Sludge Coal Cultures in Flasks

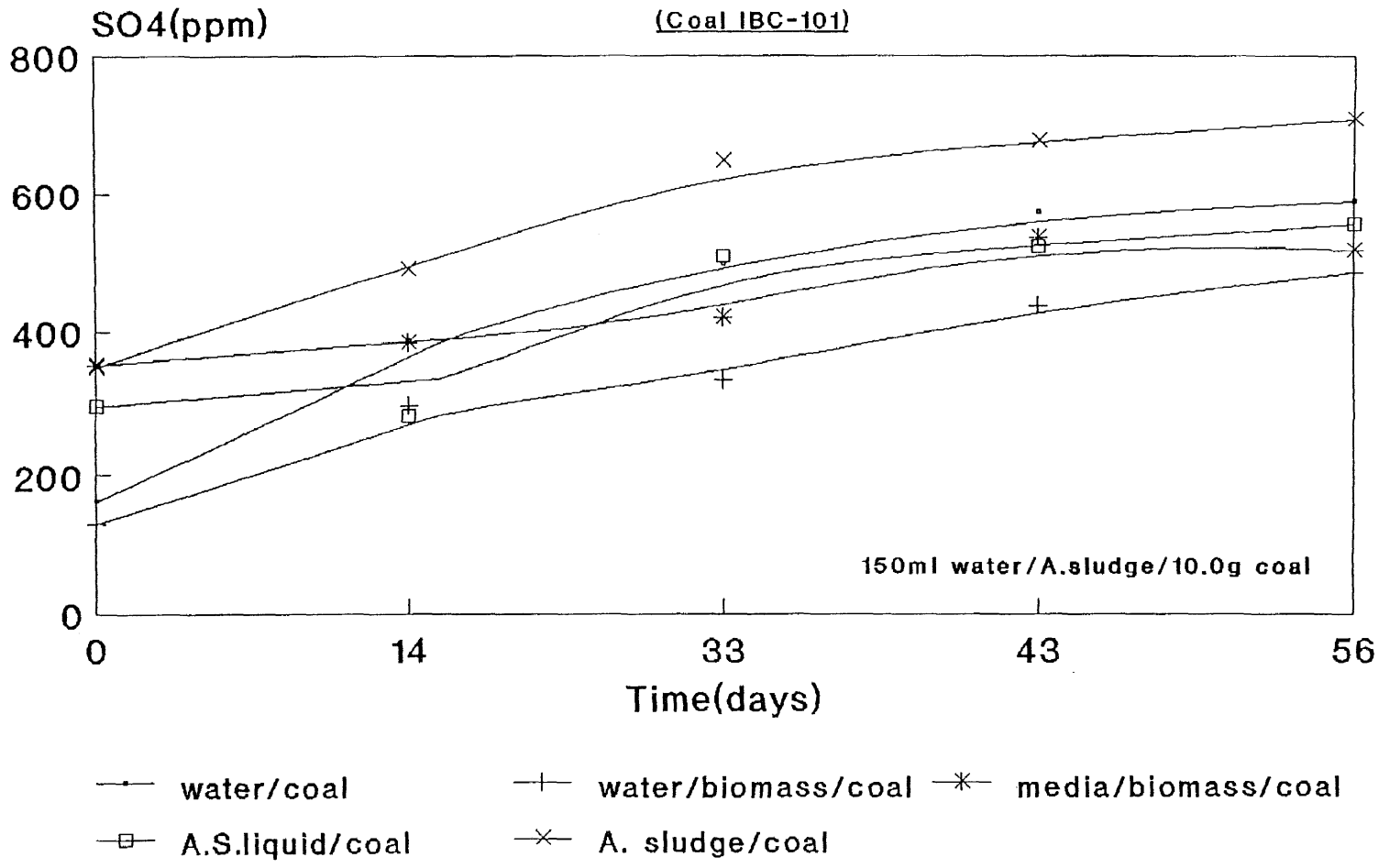


Figure 19. The Change Of Sulfate Sulfur Concentration of Medium Coal Culture in Flasks

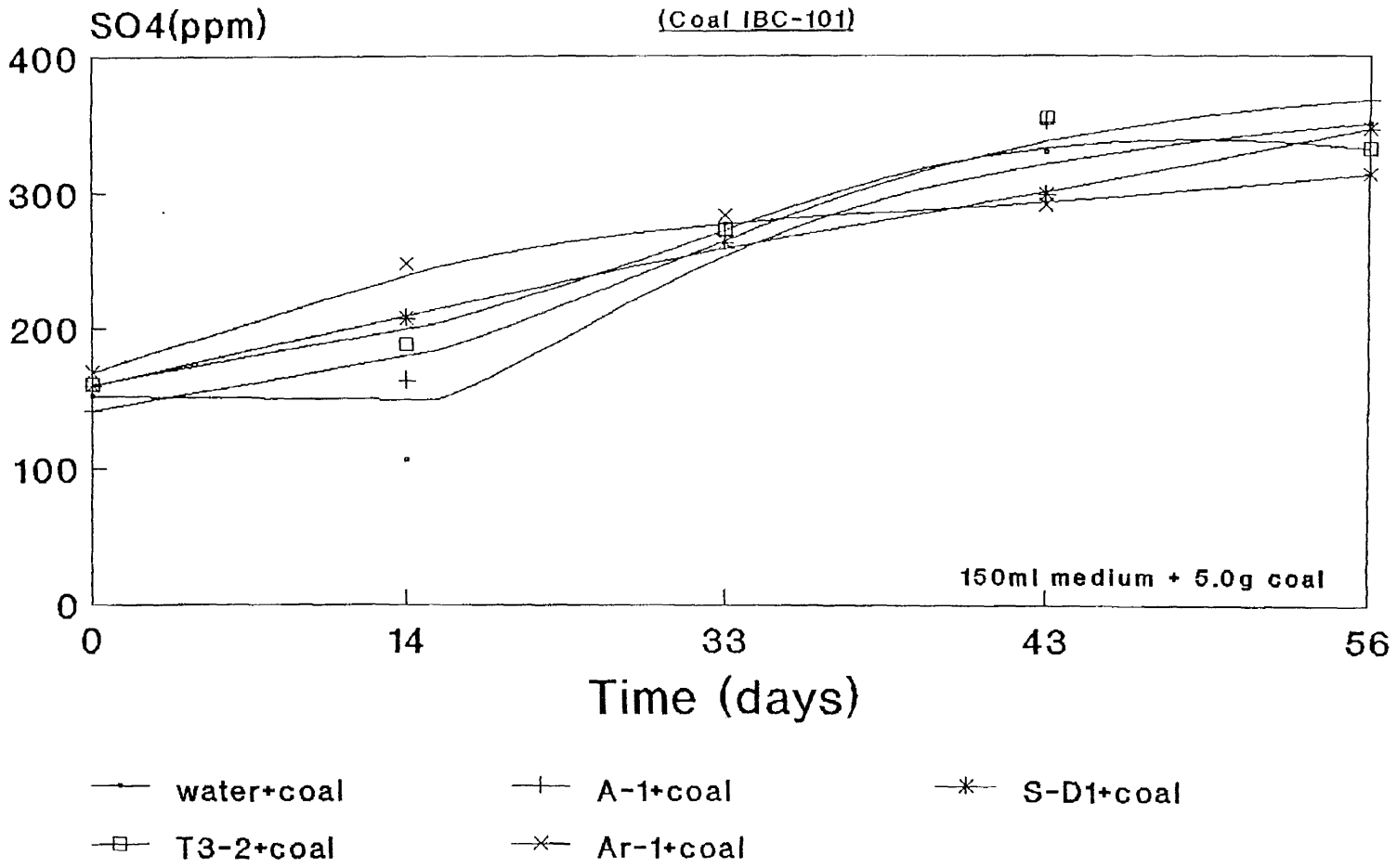


Figure 20 The pH Change Of Activated Sludge Coal Cultures

(Coal IBC-101)

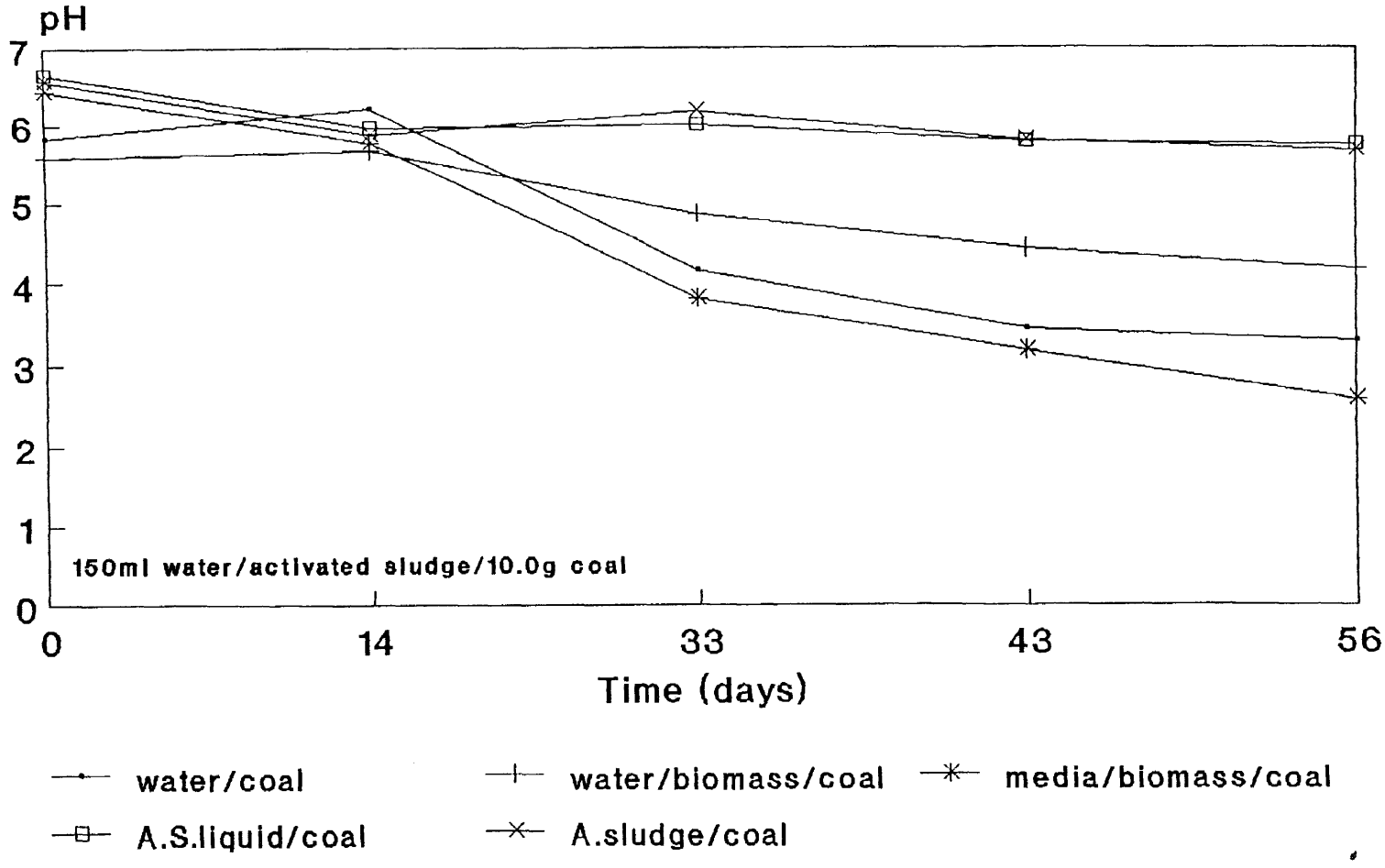
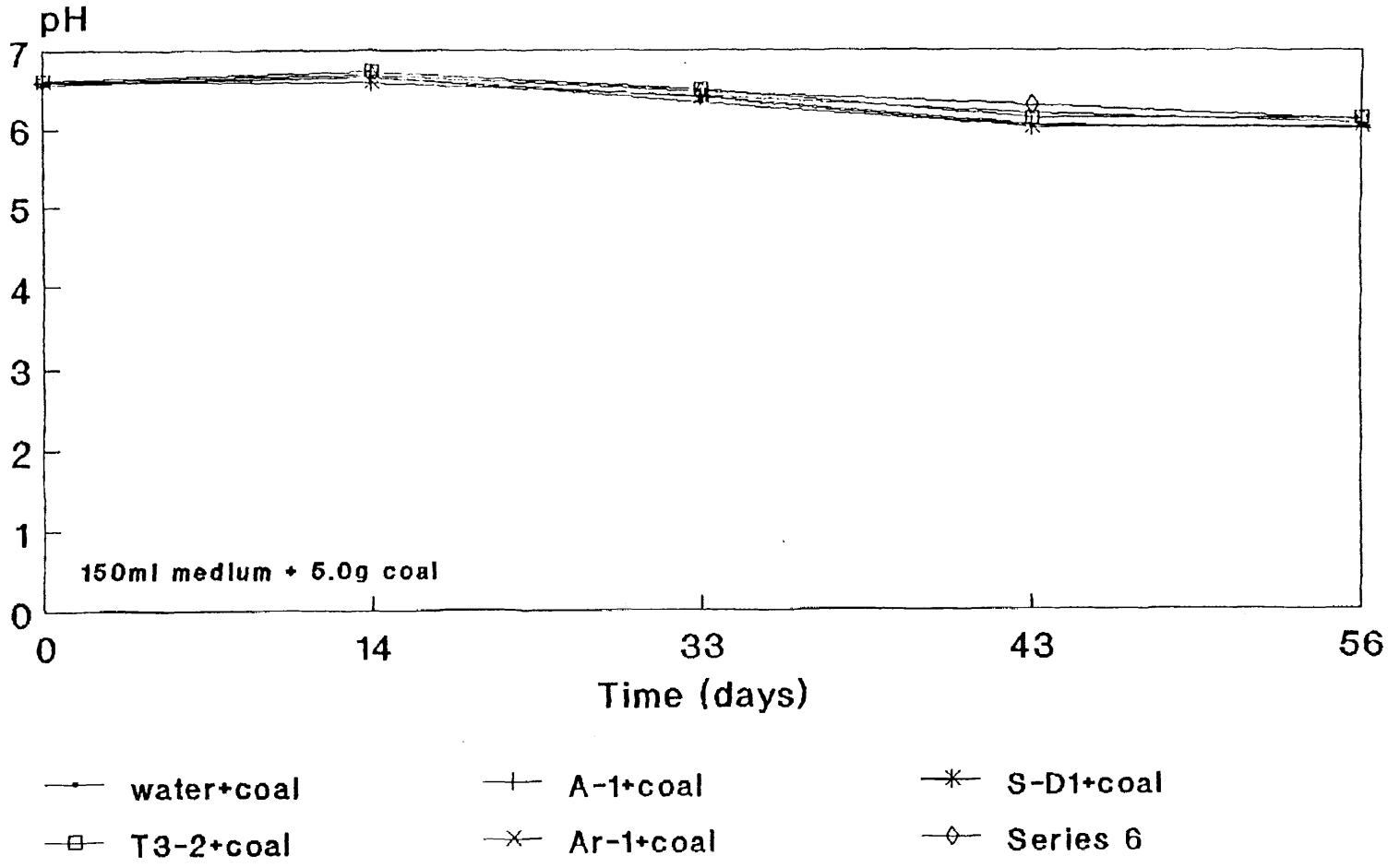


Figure 21 The pH Change Of Medium Coal Cultures

(Coal IBC-101)



Based on the results of the shaker flask, three different laboratory scale bioreactors are designed and used for biological sulfur removal from coal by activated sludge. One diagram of recycling packed bed leaching reactor is given in Figure 22. Coal sample were mixed with porcelain bead and packed in column. At the bottom, the glass wood was packed and used to support and filter coal sample. The diameter of column is 30 mm and length is 220 mm. The results of sulfur removal by this reactor have been shown in Table 24. The changes of pH and sulfate sulfur in aqueous have been showed in Figure 23. The pH change is slow, the concentration of sulfate sulfur increased slowly in first 12 days and after 22 days. During 12 to 22 days, the concentration increases a bit more quickly.

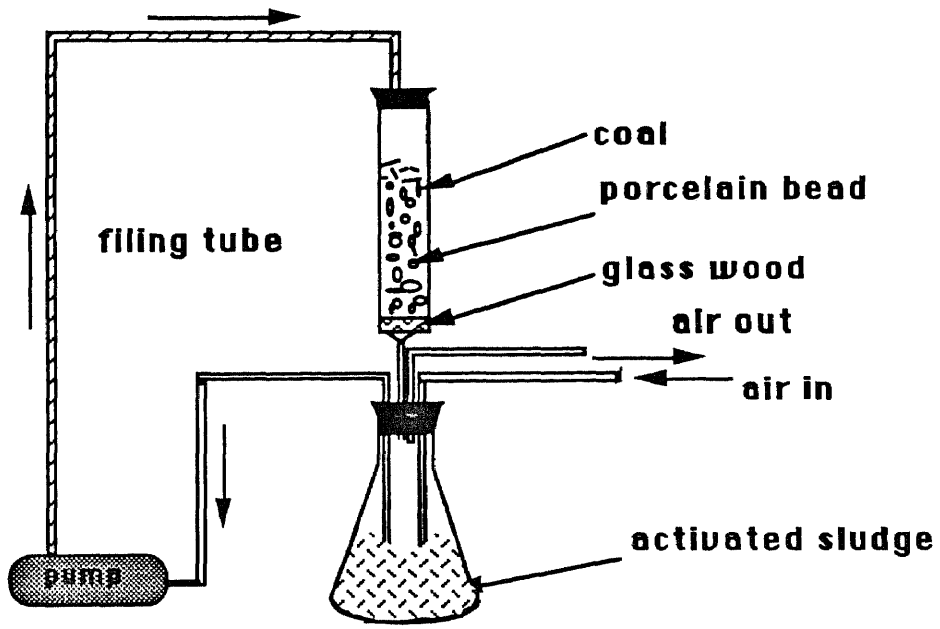


Figure 22. Schematic diagram of Laboratory Scale Activated Sludge Cycling Bioreactor Used for Biological Desulfurization of Coal

Figure 23. The Change of pH & Sulfate Sulfur Concentration In Recycling Leaching Reactor

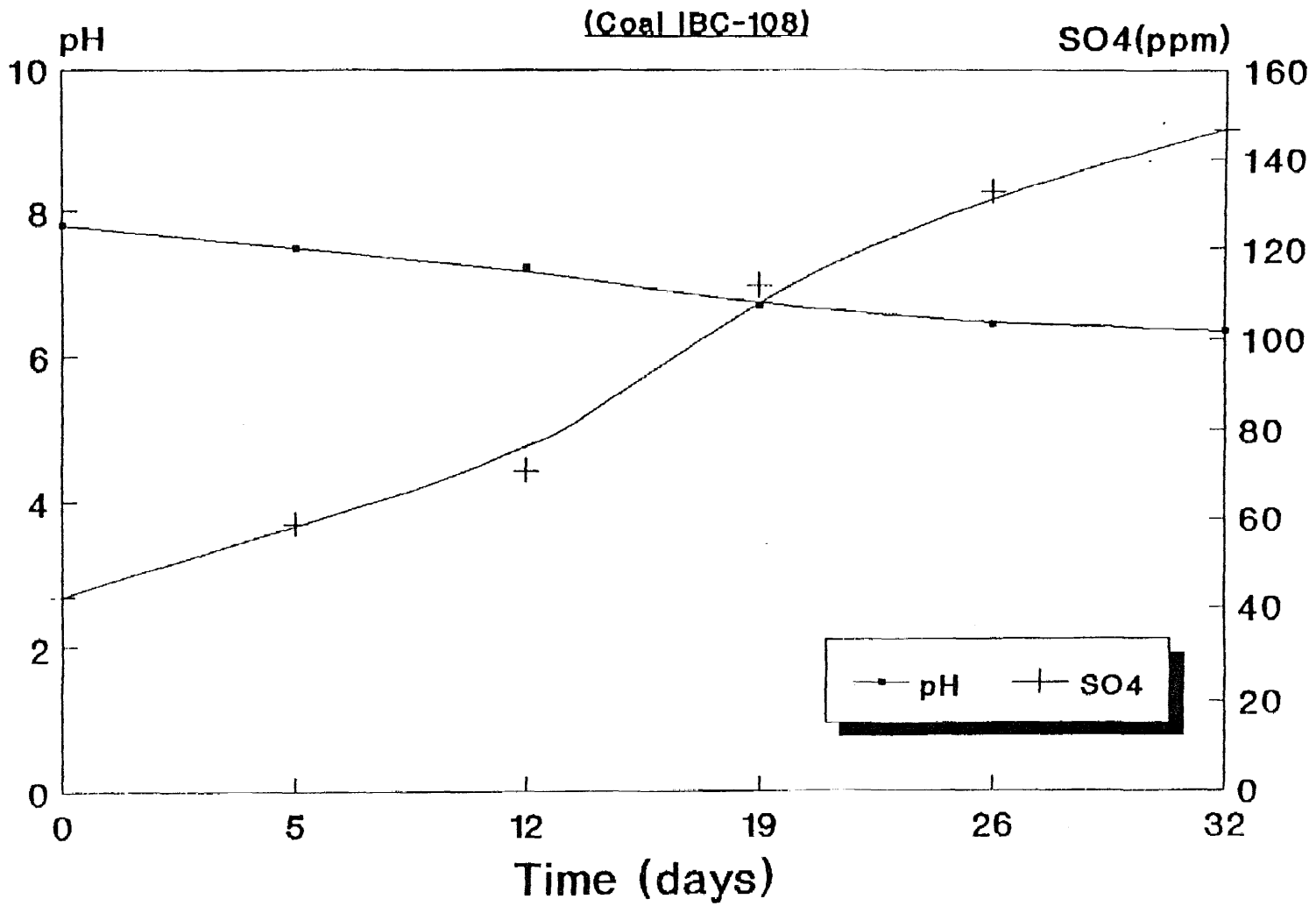


Figure 24 is a diagram of no-cycling bioreactor. The activated sludge flows into a column on top, passes through the column, and flows out from bottom by a pump. The size of this column is the same as that in the recycling-reactor. The degree of sulfur removal obtained under continuous activated sludge flow condition is illustrated in Table 24. The change of pH and sulfate sulfur in aqueous phase can be see in Figure 25. The results showed the pH remams almost constant. The concentration of sulfate sulfur changed little in the first 33 days. After 33 days, it increased by a factor of 2 to 2.5.

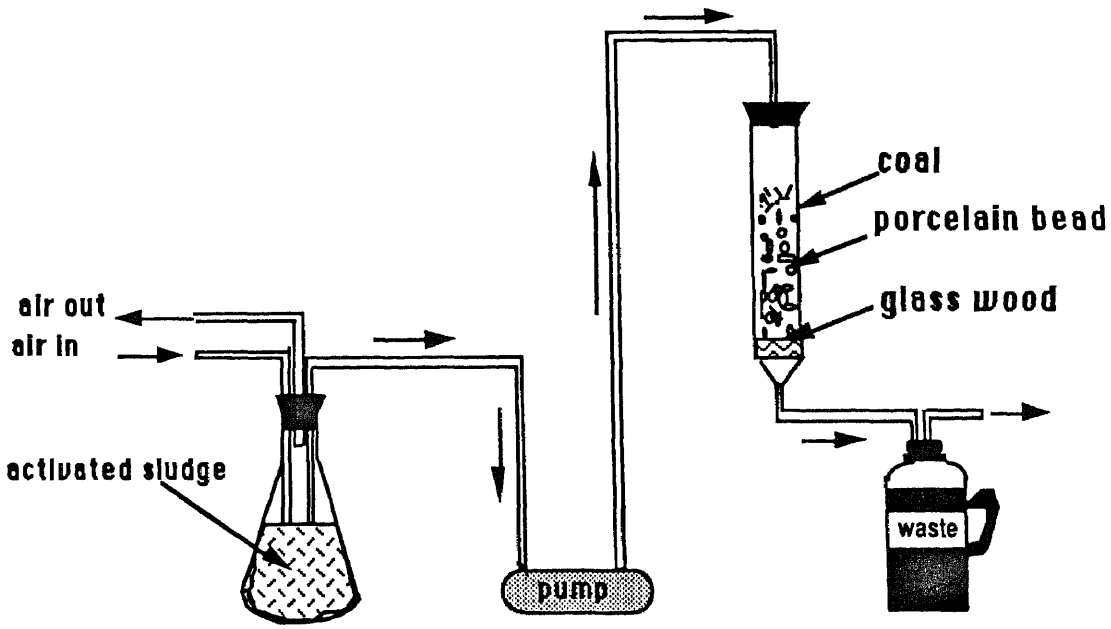
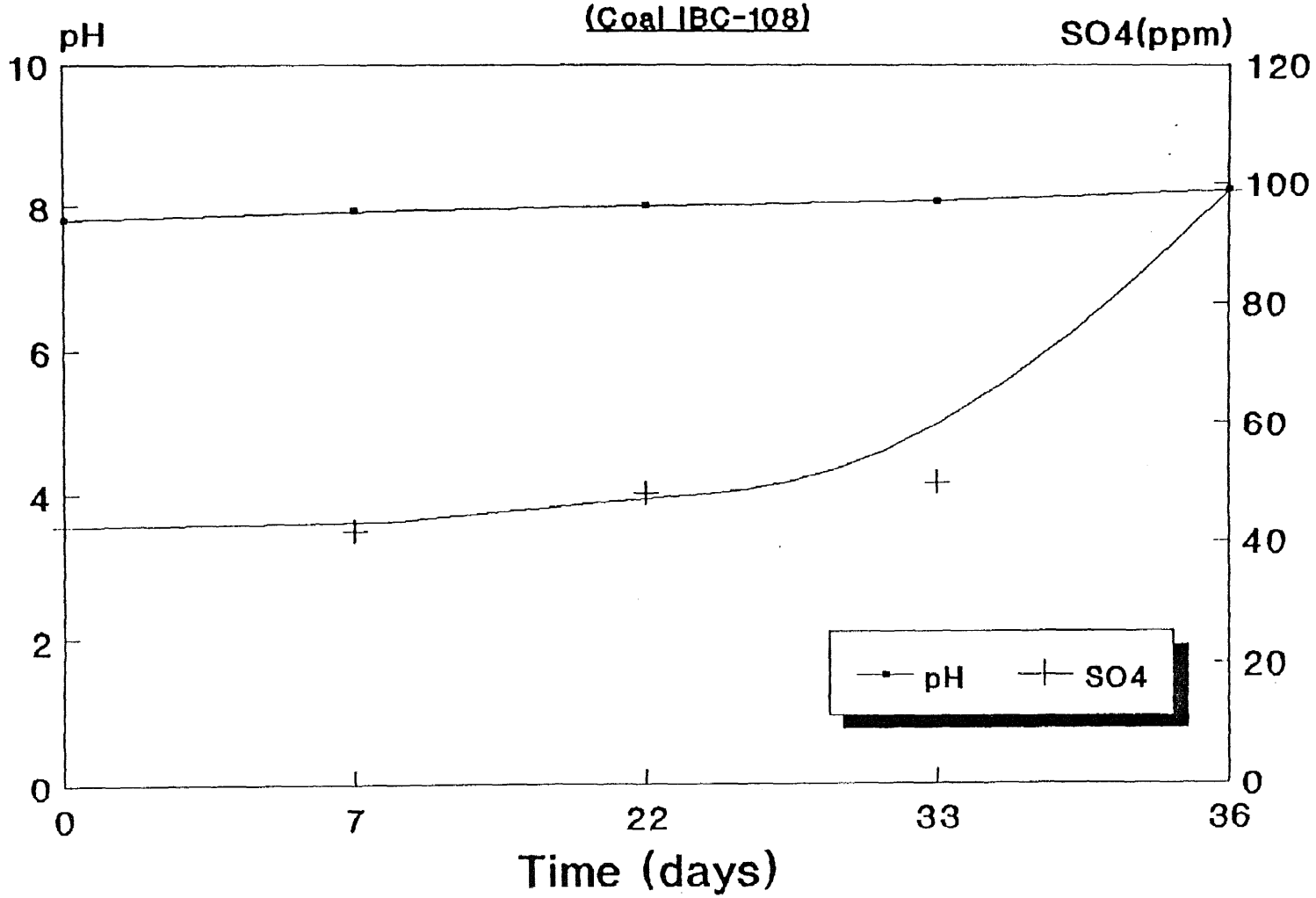


Figure24. Schematic Diagram of Laboratory Scale Activated Sludge N0-Cycling Bioreactor Used for Biological Desulfurization of Coal

Figure 25. The Change of pH & Sulfate Sulfur Concentration In No-Cycling Leaching Reactor (Coal IBC-108)



Unlike the packed column cycling or no-cycling reactor, Figure 26. is a diagram of an aerated continuous tank reactor. Activated sludge mixed with coal sample was continuously pumped into first tank, then following a series of aerated tanks was pumped into a sedimentary tank, in which, the coal and heavy materials sediment to the bottom. In the experiments, at the beginning, the flow rate is 6ml/min and the activated sludge with coal was recycling in first two weeks because the retention time is not enough for a bioreaction at high flow rate. After two weeks the flow rate changes to 0.4 ml/min. The results are summarized in Table 24. and in Figure 27. The Figure 27. showed the pH decreasing in all culture-times and sulfate sulfur increasing during all culture periods.

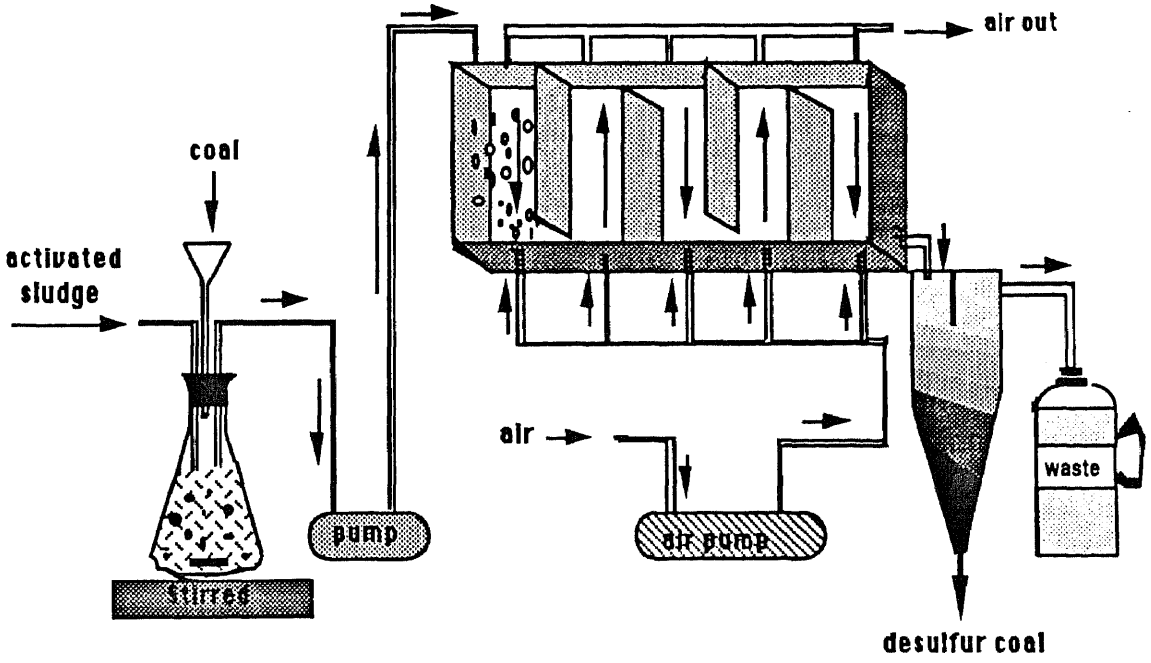
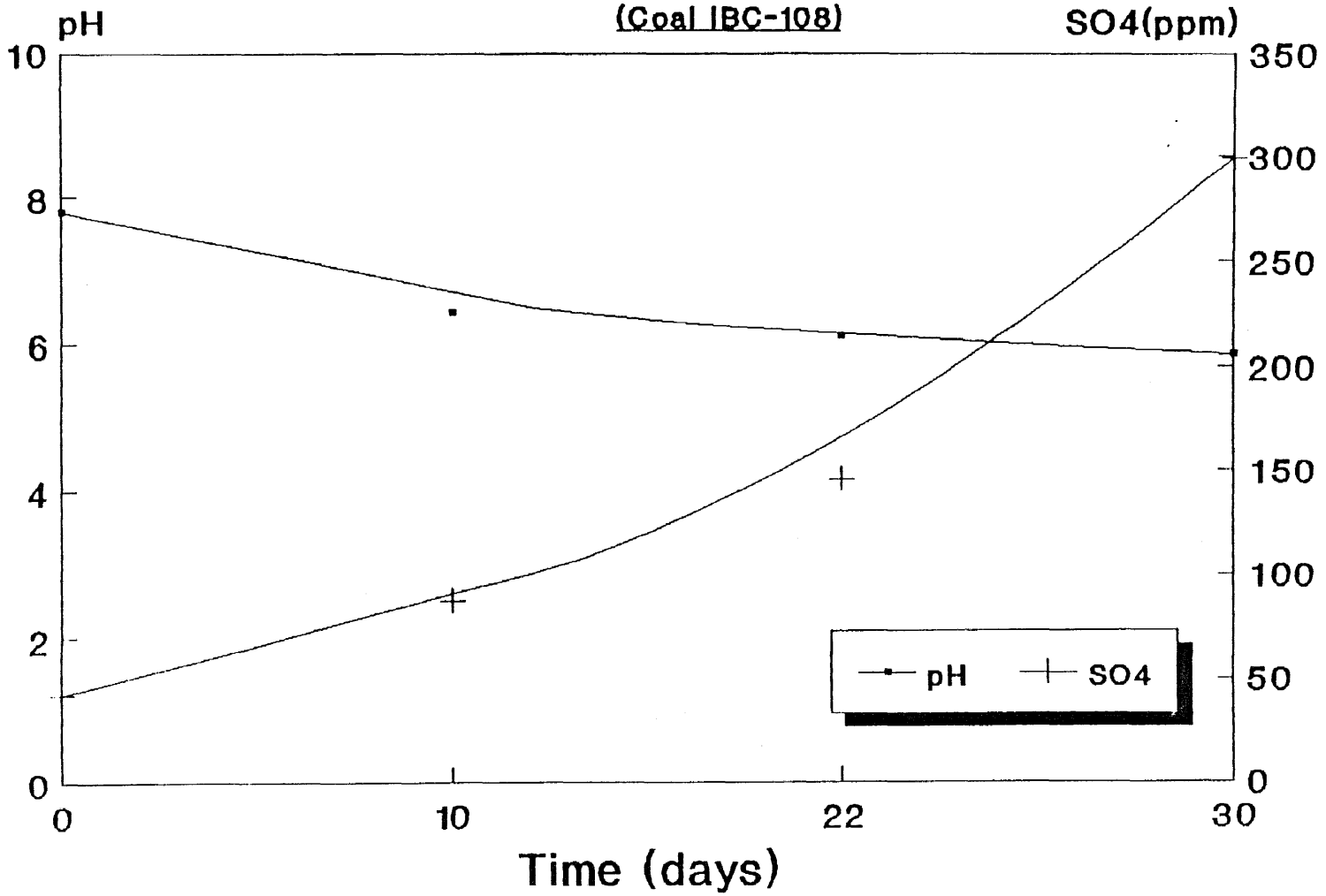


Figure 26. Schematic Diagram of Laboratory Scale Aerated Continuous Tank Reactor Used for Biological Desulfurization of Coal By Activated Sludge

Figure 27. The Change of pH & Sulfate Sulfur Concentration In Aerated Continuous Tank Reactor



The experimental results for the three reactors are shown in Table 24. About 32.33% total sulfur can be removed from coal IBC-108 by activated sludge recycling reactor, 49.81% total sulfur removed by no-cycling reactor and 17.49% removed by aerated continuous tank reactor. Comparing the pH change in flasks and reactors, when pH is between 5.5 to 7.5, sulfur was removed from coal. The lower the pH, the less sulfur was removed from coal. The pH has significant effects on microbial desulfurization.

Table 24. The Results of Sulfur Removal From Coal in laboratory Scale Bioreactors by Activated sludge

reactors / parameters	A.sludge recycling leaching reactor			A. sludge no-cycling leaching reactor			A. sludge aerated continous tank reactor		
Culture temperature	30 °C			30 °C			30 °C		
coal IBC-108	5.0g			5.0g			5.0g/100ml		
A.sludge	200ml			2ml/min			336ml		
Reactor volum	150ml			150ml			400ml		
	days	pH	SO4(ppm)	days	pH	SO4(ppm)	days	pH	SO4(ppm)
	0	7.80	42.58	0	7.80	42.58	0	7.80	42.48
	5	7.47	58.88	7	7.92	41.87	10	6.42	87.57
	12	7.21	70.92	22	8.01	48.16	22	6.09	145.14
	19	6.66	111.28	33	8.06	49.75	30	5.87	299.34
	26	6.44	132.21	36	8.24	98.72			
	32	6.36	146.60						
Total sulfur left	1.78%			1.32%			2.17%		
Total sulfur removal	32.33%			49.81%			17.49%		

Economic Aspects of Desulfurization by Activated Sludge

An industrial-scale commercial operation of microbial desulfurization has not yet been attempted. In general, the cost of microbial coal desulfurization is determined largely by culture-cost and large reactors.

Based on the investigation of Parsippany Sewage Treatment Plant and research results, a model of biodesulfurization coal by activated sludge has been established (Figure 28.). In Parsippany sewage treatment plant(see Appendix 5.), 45 million liters wastewater are treated every day. According to the results of experiment, if 100ml activated sludge can mix with 5.0g coal, 45 million liters activated sludge in Parsippany sewage treatment plant can desulfurize about 2,000 tons coal every day. The cost of biomass incinerated in plant should be considered. If all this biomass are used to coal desulfurization, it is a significant benefit for both the sewage treatment plant and desulfurization process. As the model showed, the coal particles also can deduct the load of filter in leaching process for sewage treatment process. Whether biomass and solid materials from activated sludge sediment in desulfurized coal dilute the heating value still needs to be determined.

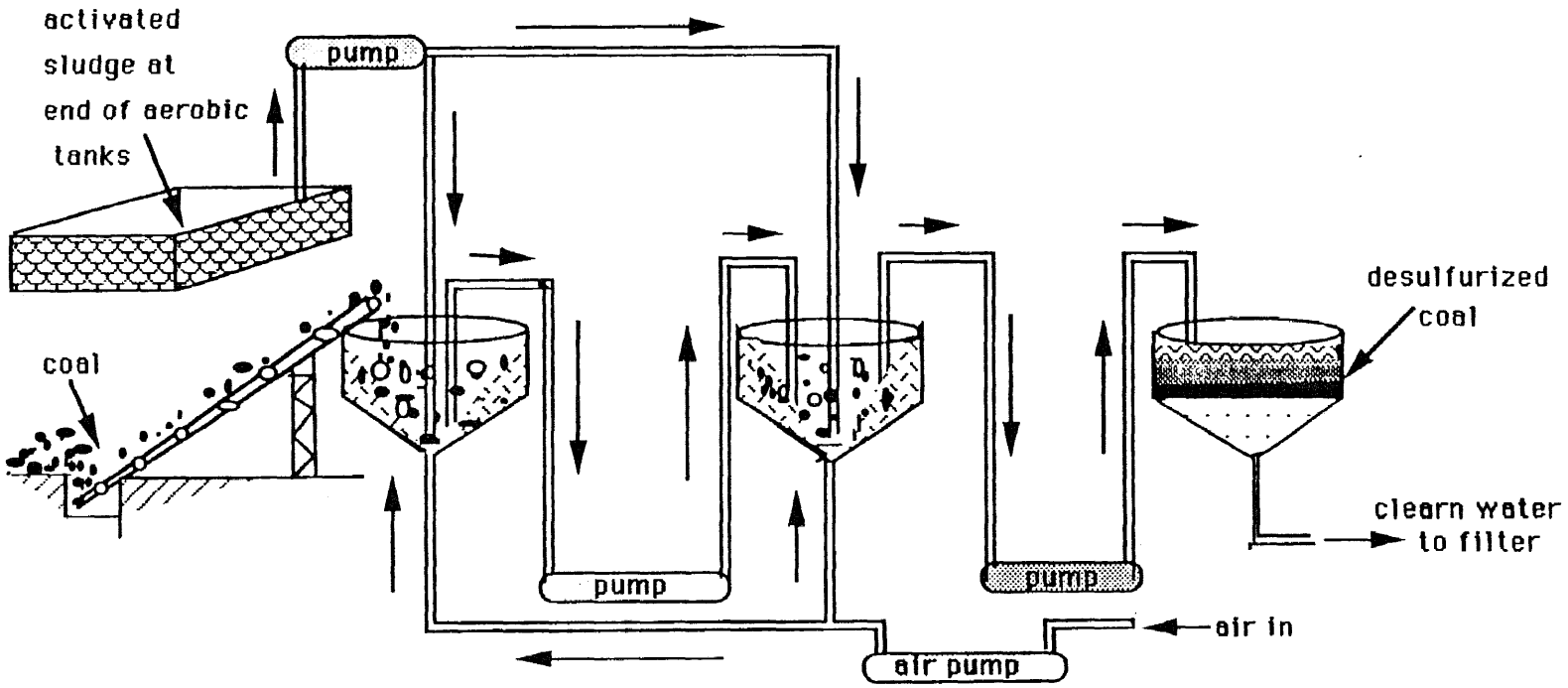


Figure 28. Schematic Model of Coal Desulfurization By Activated Sludge Biomass

IV. Conclusion

Several unidentified microorganisms capable of removing sulfur from coal were isolated using sulfur-limited media. The mixed culture of strains A-1, S-D1, T3-2 and Ar-1 showed a high ability for removing sulfur from coal.

The application of ion chromatography and atomic absorption spectrometry following ASTM extraction for sulfate, pyritic and total sulfur analysis show good results. These results agree with ASTM results and save analytical time, as well as reduce the effort required for the ASTM method.

The results from these experiments confirm that activated sludge can remove sulfur from coal and suggest that the biomass in activated sludge can solubilize coal as well. Activated sludge may catalyze removal from the surface of finely-ground coal particles. pH has significant effects on activated sludge desulfurization.

Tests of three different kind of reactors indicate the no-recycling activated sludge leaching process is possible for coal desulfurization.

Based on the experimental results and the investigation about sewage treatment plant suggested a mode, which can be used in industrial scale and benefit both sewage treatment and coal desulfurization processes.

References

1. R. Markuszewski, C. W. Fan, R. T. Greer and T. D. Wheelock, "Evaluation of Removal of Organic Sulfur from Coal", In. Symposium on New Approaches in Coal Chemistry, American Chemical Society, Pittsburgh, PA, USA (1980)

2. J. P. Bojudou, J. Bouleque, L. Malechanx, M. Nip, "Identification of Som Sulfur Species in a High Organic Sulfur Coal", Fuel, 66, pp.1558-1569 (1987)

3. Christian A. Fligwe, Microbial desulfurization of coal, Fuel, Vol. 67, April pp451-458, 1988

4. L. Wicholas Ornston and Albert Balovs, "Annual Review of Microbiology", Ann. Rev. Microbiol. 39, pp371-89, 1985

5. F. Kargi, Enzyme Microb. Technol., 4:13-19,1982

6. H. B. Gala, R. D. Srivastava, R. H. Rhee and R. E. Hucko, "An Overview of the Chemistry of the Molten-Caustic Leaching Process", Coal Preparation, 6, 102, 1988

7. G. Edward Ruby, "Fractionation of Stable Carbon Isotopes during Chemoautotrophic Growth of Sulfur-Oxidizing Bacteria", Applied and Environmental Microbiology, Aug. pp1940-1943, 1987

8. A. K. Parameswaran, "Sulfur Reduction by the Extremly Thermophilic Archaebaterium", Applied Environmental Microbiology, July pp1690-1693, 1987

9. G. I. Karavaiko, "Microbiological Processes for the Leaching of Metals from Ores, United Nations Environment Programme (USSR Commission for UNEP)", Centre for International Projects, L. E. Miner, Sei. Eng. 12,121,1980

10. S. K. Garg and S. Neelakanan, "Enhancement of Microbial Removal of Pyritic Sulfur from Coal Using Concentrated Cell Suspension of T. ferrooxidans and an External Carbon Dioxide Supp"; Biotechnology and Bioengineering, Vol. XXIV, pp.749-752, 1982

11. Fikret Kargi, "Kinetic Parameter Estimation in Microbial Desulfuriztion of Coal", Biotech. and Bioeng., Vol. 30, pp.1063-1066, 1987

12. Fikret Kargi, "A Dynamic Mathematical Model for Microbial Removal of Pyritic Sulfur from Coal", Biotech. and Bioeng., Vol.,XXVI, pp.604-612, 1984

13. P. Kline and A. S. Myerson, Biotechnol. Bioeng., 25, 1669, 1983

14. M. David Tillet, "The Removal of Pyritic Sulfur from Coal Empolying Thiobacillus Ferrooxidans in a Packed Column Reactor", Biotech. and Bioeng., Vol. XXIX, pp146-150, 1987

15. Michael Heyer, "Microbial Removal of Pyritic from Coal Using a Percolation Bioreactor", Biotechnolgy Letters, Vol. 9, N. 1, pp19-24, 1987

16. W. J. Ingleclew, Biochim. Biophys. Acta, 683:89-117, 1982

17. G. F. Andrews and J. Maczuga, Biotechnol. Bioeng. Symp. 12, pp337, 1982

18. F. Kargi and J. M. Robinson, Biotechnol. Bioeng, 24, 2115, 1982

19. F. Kargi and J. M. Robinson, Appl. Environ Microbiol., 44, 878, 1982

20. N. Wakao, M. Mishina, Sakurai. Y. Shiota, H. J. Gen. Appl. Microbiol. 29, pp.177-85, 1983

21. Yun Chea Chang and allan S. Myerson, "Growth Models of the Continuous Bacterial Leaching of Iron Pyrite by Thiobacillus ferrooxidans", Biotech. and Bioeng. Vol. XXIV, pp.889-902, 1982

22. F. Kargi. Enzyme Microb. Technol., 4, pp.13-19, 1982

23. F. Kargi, J. M. Robinson, Biotechnol. Bioeng, 26, pp.687-90, 1984

24. F. Kargi, T. D. Cervoni, Biotechnol. Lett. 5, pp.33-38, 1983

25. F. Kargi,J. M. Robinson, Biotechnol. Bioeng, 27, pp.41-49, 1985

26. J. D. Isbister, "Mutant Microorganism and its Use in Removing Organic Sulfur Compounds", U.S. Patent 4,562,156. 1985

27. J. D.Isbister, and E. A. Kobylinski, "Microbial desulfurization of Coal, in Processing and Utilization of High Sulfur Coals", Attia. Y., Ed, Elsevier, N.J., 1985

28. H. Tributsch and J. C. Bennett, "Semiconductor-electrochemical Aspects of Bacterial Leasching, part 2, survey of Rate-Controolling Sulfide Properties", J. Chem. Tech.,Biotechnol., 31, 627, 1981

29. J. John, Kilbane. "Biodesulfurization of Coal", Presented at the IGI symposium on Gas, Oil and Coal Biotechnology, New Orleans LA, Dec. 5-7, 1988

30. M. Van afferden, S.Schacht, M. Beyer, "Microbial Desulfurization of Dibenzothiophene", Am. Chen. Soc. 34(4), pp.561-572, 1988

31. Fikret Kargi, "Microbial Methods for Desulfurization of Coal", Biotechnol. Bioeng, november, 1986

32. H. John Shinn, "From Coal to Single-Stage and Two-Stage Products: a reactive model of coal stracture", Fuel, Vol, 63, Sep., 1984

33. Steven Krawiec, "Bacterial Desulfurization of Thiophenes: Screening Techniques and Som Speculations Regading the Biochemical and Genetic Bases", Society for Industrial Microbiology(Lehigh University)

34. D. L. Stoner, J. E. Wey, "Modification of Water-Soluble Coal-Derived Products by dibenzothiophene-Degrading Microorganisms", Applied. and Environ. Microbiology, Sep., pp2667-2676, 1990

35. D. J. Monticello, D. Bakker, and w. R. Finnertry, "Plasmic-Mediated Degradation of Dibenzothiophene by Pseudomonas Species", Appl. Environ. Microbiol., 49, 756, 1985

36. Fikret Kargi and Janes M. Robinson, "Microbial Oxidation of Dibenzothiophene by the Thermophilic Organism Sulfolobus Acidocaldarius", Biotechnology and Bioengineering, Vol., XXVI, pp.687-690, 1984

37. Proceedings: 1990 First International symposium of the biological Processing of Coal, EPRI, Sep., 1990

38. P. R. Dugan, "Microbial Desulfurization of Coal and its Increased Monetary Value", Biotechnol Bioeng. Symp., Sep.,No. 16, 185,1986

39. B. A. Laseke, H. J. Johnson and D. A. Oerger, "Evaluation of Flue Gas Desulfurization as an Acid Rain Control Strategy for Utility Coal-Fired Boilers in the State of Ohio" in Proceedings: Tenth Symposium on Flue Gas Desulfurization, EPRI CS-5176, May 1987,pp3-55.

40. J. P. Maree and Wilma F. Strydom., "Biological Sulfate Removal from Industrial Effluent in an Upflow Packed Bed Reactor", Wat. Res. 21,No. 2, pp. 141-146, 1981

41. Joann. C. Radway, "Microbially Mediated leaching of Low-Sulfur Coal in Experimental Coal Columns", Appled and Environmental Microbiology, May, pp.1056-1063, 1987

42. Henry Shaw and David Kafkewitz, Proposal: Biological Coal Desulfurization, 1989

43. ASTM, Designation: D 2492-84, Standard Test Method for Form of Sulfur in Coal

44. ASTM Designation: D 3177-84, Standard Test Methods for Total Sulfur in the Analysis Sample of Coal and Coke

Appendix 1. Biological Coal Desulfurization - Literature Survey

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Thiobacillus ferrooxidans: (2)(3)(4)(7)(10)(14)(18)(19)(21)

Sulfolobus acidocaldarius: (2)(3)(4)(22)(23)(24)(25)

Pyritic sulfur removal: (4)(10)(14)(15)(17)(31)

Organic sulfur removal: (1)(20)(22)(26)

Dibenzothephene(DBT): (4)(18)(26)(30)(34)(35)(36)

Coal structure: (27)(29)(32)(33)

Mechanism of biodesulfurization: (4)(27)

Analytical method: (37)(42)(43)

Other microorganisms of desulfurization: (2)(3)(4)(5)(7)(8)(16)(19)(33)(34)

Activated sludge: (41)

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Appendix 2. Analytical Procedure of Sulfate, Pyritic, and Total Sulfur Determination

Sulfate Sulfur:

Sulfate sulfur is determined by extracting the coal with dilute hydrochloric acid and deteminning the sulfur by ion chromatography.

Weight about 2g of coal sample and add 50ml HCl(2+3) in a small beaker while stirring. A few drops of enthenol to the coal facilitates the wetting process. Place on a moderately hot plate and boil gently for 1/2 hr. Carefully filter and wash the filter paper. Just the pH to 8.4 to 8.5 by KOH. The sulfate sulfur was measured by ion-chromatogrphy.

Pyritic Sulfur:

Transfer the filter paper and extracted residue from last step and slowly add 50ml of HNO_3(1+7) with stirring. Boil gently for 30 mins. Filter and wash the residue at six times with water. Add 2ml of 30% H_2O_2 and boil for 5mins to oxidize salts. The pyritic sulfur was determined by atomic absorption.

The residue can be used to determine the organic sulfur by the total sulfur analytical method, or organic sulfur is calculated by deducting the percentage sums of sulfate and pyritic sulfur from the total sulfur.

Total Sulfur:

Arccording to Eschka Method, coal sample and Eschka mixture are intimately mixed and ignited together, the sulfur is dissolved in hot water. Take about 0.5 to 1.0g coal total sample and mix with 3.0g Eschka mixture, and cover with 1.0g of Eschka mixture in porcelain crucible. Place the crucible in a cold-vented muffle and gradually raise the temperature to 800+/-25^{o}C in about 1 hr. Remove the crucible and empty the contents into a beaker and digest with 50ml of hot water for 1/2 hr, while stirring occasionally. Decant the solution through filter paper. After washing five times, just the filtrate to neutral pH 8.4 to 8.5 with KOH. Then analyze SO_4 by ion-chromatography.

Appendix 3. Eluents of Ion Chromatography

a. Eluent used for IC-Anion-Guard 1653, IC-Anion-PW

Gluconic acid potasium salt	0.3g
Sodium tetraborate	0.5g
Boric acid	0.5g
5.0g/L Glycerin(1ml/5g)	5.0ml
Acetonitrile	120ml
N-Butyl Alcohol	30ml
M.Q.water	1 liter

b. Eluent used for IC-Anion-Guad, Catolog 127-0056, Serial 16532 IC-Anion-PW Catolog 127-00 62, Serial 10917

(Bio-Gel IC-Anion-PW Column Eluent)

1). a). 300mg gluconic acid potassium salt($C_6H_{11}KO_7$)
b). 500mg sodium tetraborate ($NO_2B_4O_7.10H_2O$)
c). 500mg boric acid (H_3BO_3)
d). 5.0g glycrerine
2). Put the compounds in a 1 liter volumetric flask and dissolve in 500 ml of distilled deionized water
3). Mix together
a). 120ml acetonitrile
b). 30ml n-butyl alcohol
4). Add the organic mixture to the flask and bring to volume with distilled deionized water
5). Confirm a pH of approximate 8.4-8.5
6). Filter eluent
7). Degas 30mins

c. Eluent for IC-Pak A HC Column

(Lithium borate/gluconate concentrate)

a). Place approximately 500ml of Milli-Q water in a 1 liter volumetric flask. To this flask add:
- 7.2g of lithium hydroxid, monolydrate, 98%,stir until dissolved.
- 25.5g of boric acid, 98%, A.C.S. reagent, stir until dissolved
- 13.2ml of gluconic acid, 50% wt in water solution
- 94ml of glycerol, 99.5% A.C.S. gragent

b). Add Milli-Q water to the mark and mix thoroughly. Store in a polypropylene or polyethylene container (shelf life 6 months)

To prepare lithium borate/gluconate eluent

a). to a 1 liter volumetric flask add:
- 20ml of the lithium borate/gluconate concentrate
- 120ml acetonitrile, 99.9% HPLC grade

b). add Milli-Q water to the mark and mix thoroughly use the eluent the same day it is prepared

c). Filter and degas using a solvent clarification system with Millipore Durapore 0.22 um membrane, 47 mm filter

Appendix 4. Components of Vitamin Media

Biotin	0.002	g/L
Folic acid	0.002	g/L
Pyridokinehydrochloride	0.01	g/L
Riboflavin	0.005	g/L
Thiamin B_1	0.005	g/L
Nieotinic acid	0.005	g/L
Pantothenic acid	0.005	g/L
B12	0.0001	g/L
P-aminobenzoic acid	0.005	g/L
Thioctic acid	0.005	g/L

Appendix 5. Sewage Treatment Plant Schematic