DOE/PC/91059--T13

BIOLOGICAL UPGRADING OF COAL LIQUIDS

Final Report

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Contract No. DE-AC22-91PC91059

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ACKNOWLEDGEMENT

Financial support for this work was provided by the U.S. Department of Energy, Pittsburgh Energy Technology Center, on contract DE-AC22-91PC91059.

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ABSTRACT

A large number of bacterial enrichments have been developed for their ability to utilize nitrogen and sulfur in coal liquids and the model compound naphtha. These bacteria include the original aerobic bacteria isolated from natural sources which utilize heteroatom compounds in the presence of rich media, aerobic nitrogen-utilizing bacteria and denitrifying bacteria. The most promising isolates include Mix M, a mixture of aerobic bacteria; ERI5, a pyridine-utilizing isolate; ERI6, an aniline-utilizing isolate and a sewage sludge isolate. Culture optimization experiments have led to these bacteria being able to remove up to 40 percent of the sulfur and nitrogen in naphtha and coal liquids in batch culture.

Continuous culture experiments showed that the coal liquid is too toxic to the bacteria to be fed without dilution or extraction. Thus either semi-batch operation must be employed with continuous gas sparging into a batch of liquid, or acid extracted coal liquid must be employed in continuous reactor studies with continuous liquid flow. Isolate EN-1, a chemical waste isolate, removed 27 percent of the sulfur and 19 percent of the nitrogen in fed batch experiments. Isolate ERI5 removed 28 percent of the nitrogen in coal liquid in 10 days in fed batch culture. The sewage sludge isolate removed 22.5 percent of the sulfur and 6.5 percent of the nitrogen from extracted coal liquid in continuous culture, and Mix M removed 17.5 percent of the nitrogen from medium containing extracted coal liquid.

An economic evaluation has been prepared for the removal of nitrogen heteroatom compounds from Wilsonville coal liquid using acid extraction followed by fermentation. Similar technology can be developed for sulfur removal. The evaluation indicates that the nitrogen heteroatom compounds can be removed for \$0.09/lb of coal liquid treated.

1.0 INTRODUCTION

The United States currently imports about half of its liquid fuel requirements. Yet, the nation has massive and diverse reserves of coal, which represent our largest fossil energy resource, equivalent to 750 billion barrels of oil (Speeks and Klusman, 1982). Consequently, there have been intensive research efforts over the last two decades directed at processes to convert coal into liquid fuels.

The objective of coal liquefaction is to produce a synthetic crude that would give a good yield of high-octane gasoline. By comparison with petroleum, coal is low in hydrogen (0.8 H:C compared to 1.8); high in sulfur, nitrogen and oxygen; and high in mineral content. Therefore the coal liquefaction process must adjust the hydrogen content, reduce the heteroatom content, and remove the mineral matter.

Coal may be liquefied by pyrolysis or hydroliquefaction. The liquefaction process is thought to occur in a step-wise fashion (Hessley *et al.*, 1986; Yoshida *et al.*, 1976). Initially, there is a rapid thermal cleavage of the most reactive bonds to produce large organic fragments (mol. wt. about 1200), classified as preasphaltenes (soluble in pyridine, insoluble in benzene or hexane). These compounds contain a large amount of oxygen in the form of phenolic groups. Some oils (mol. wt. about 250, soluble in hexane) are also rapidly formed in the initial stages, possibly resulting from noncovalently bonded species trapped in the pore structure.

During the second stage, more resistant bridges are cleaved into smaller fragments, classified as asphaltenes (soluble in benzene, insoluble in hexane). These compounds have molecular weights in the range of 500. The final stage of conversion of asphaltenes into oils is a much slower process, requiring cleavage of aromatic rings, bridging structures, etc. The oils are the desirable components for a fuel and high yields of oils require more severe conditions (higher temperature and pressure, and longer residence times).

The coal-derived liquids are about 70 percent aromatic compounds, which is several times higher than petroleum. The sulfur content of coal syncrude is 0.3-3 percent, oxygen is 1-10 percent and nitrogen is 0.5-1.5 percent (Hessley *et al.*, 1986). By comparison, petroleum crude contains about 1.2 percent sulfur, little or no oxygen and 0.2 percent nitrogen content. To obtain a clean burning fuel, it is necessary to reduce both the aromaticity and the sulfur and nitrogen. These reductions are generally accomplished with a severe catalytic hydrogenation to convert the asphaltenes into oils and remove the bulk of the heteroatoms. The hydrogenation catalysts are effective in removing sulfur, but nitrogen, which is poisonous to refinery cracking catalysts, is much more difficult to remove.

Many processes are under development for the liquefaction of coal. These processes have yet to prove economical in the U.S. because of the high cost of hydrogen and the high capital and energy requirements for upgrading the liquids. A simple and economical process for upgrading coal liquids would enable commercialization of this technology and the establishment of a stable energy policy.

Microorganisms, which employ biocatalysts, may be used to upgrade coal liquids. Microbial processes offer some very specific advantages for coal syncrude upgrading. Perhaps, most importantly for this application, microorganisms can obtain hydrogen for biological reactions from water. Microorganisms are also quite specific in converting only certain substrates, resulting in higher yields and simpler separation technology. Biological processes function at ordinary temperatures and pressures, which usually results in substantial energy and capital savings. The products and by-products of biological reactions are non-toxic and compatible with the environment. Biocatalysts are

automatically and continuously regenerated by slow growth of the microorganisms. These advantages are offset, somewhat, by slower reaction rates for microbial processes.

As explained in detail later, microorganisms are known to remove heteroatoms from many compounds found in nature, and these same compounds are also found in coal. Preliminary experiments in our laboratories have demonstrated that certain cultures can reduce nitrogen, sulfur and oxygen in coal and shale oil liquids by 20 to 100 percent in a simple one-step incubation. In the process of removing heteroatoms, these microorganisms obtain energy, nutrients or carbon for growth and metabolism. In many of these reactions, the heterocyclic rings are also degraded. These compounds include phenol, dibenzofuran, pyridine, quinoline, aniline, indole, and dibenzothiophene, which are all common forms of oxygen, nitrogen and sulfur in coal. After biological reaction, the heteroatoms are in the form of H_2O , CO_2 , NH_3 or sulfate, all of which may be readily removed or recovered, possibly for resale. Therefore, it would be possible to develop a simple and economical biological step for removing the heteroatoms from coal liquids and, at the same time, reducing the aromaticity. Hydrogen would not have to be added separately to this process.

The objective of this three-year study is to survey cultures of known microorganisms for S, N, and O removal and aromaticity reduction. Isolates from natural sources will also be sought for these reactions. The performance of these cultures will be optimized and evaluated individually and as a mixed culture. The best cultures will be selected for performance evaluation in continuous submerged culture experiments in the CSTR. Reactor design and product removal processes will be projected and the economics of this technology compared with conventional methods.

1.1 Purpose

The purpose of this report is to present results from research aimed at demonstrating the technical and economic feasibility of biologically removing heteroatom compounds from coal liquids. A number of aerobic and anaerobic isolates have been obtained which are capable of removing nitrogen and sulfur compounds as well as reducing the aromaticity. These cultures have been optimized for heteroatom removal and then used in batch, semi-batch and continuous culture experiments. Finally, process economics are developed for the most promising and mature technology.

2.0 BIOLOGICAL REMOVAL OF NITROGEN, OXYGEN AND SULFUR FROM COAL LIQUIDS

In coal liquids, the heteroatoms (nitrogen, oxygen and sulfur) are found primarily in the asphaltene and preasphaltene fractions. These compounds increase the viscosity of the liquid and cause serious problems in storage. The nitrogen and sulfur are catalyst poisons in the subsequent refining steps and cause high oxide emissions from combustion. Nitrogen, which occurs in the organic fraction of coal, originated from plant, animal, or microbe protein and exists as anilines, pyridines, quinolines, indoles and carbazoles (Hessley *et al.*, 1986).

Oxygen is known to occur in several different forms in coal, including phenolic hydroxyl, carboxylic acid, ether linkages, carbonyl, and heterocyclic compounds, such as furan and benzofurans. The sulfur in coal is both organic and inorganic. Inorganic sulfur is present primarily as iron pyrite. The organic sulfur represents 30-70 percent of the total sulfur and is present (Deno *et al.*, 1978) as derivatives of thiophene, aromatic and aliphatic sulfides, thiols and thiophenols (Attar and Corcoran, 1977).

The various biological reactions with each of these compounds are summarized in the following paragraphs. No work has been reported on the biodegradation of nitrogen and oxygen containing aromatics in coal. However, many bacteria are known to actively degrade nitrogen and oxygen heterocyclic rings in nature. Intensive investigations have been made to investigate the biodegradation of inorganic and organic sulfur compounds in coal. Technology for the removal of inorganic sulfur is nearing commercialization and promising results are being obtained for organic sulfur removal. These various cultures will be surveyed and examined for removal of N, O and S from coal liquids.

2.1 Biological Removal of Nitrogen Heteroatoms

It is extremely difficult to remove nitrogen heteroatoms by catalytic hydrogenation. While somewhat speculative, the nitrogen heteroatom compounds in coal liquids are believed to include pyridine, anilines, quinolines, isoquinolines, indoles and carbazoles. Many of these compounds are known to be degraded by bacteria in nature and these biological reactions are summarized below.

2.1.1 Pyridine Biodegradation

The availability of pyridine as a substrate for microbial growth has been reported in many early studies (Moore, 1949; Ensign and Rittenberg, 1963; Stafford and Callely, 1970). The biological degradation of the nitrogen in pyridine to NH_3 was implied in very early studies (Funchess, 1917) and confirmed by Moore (1949), Stafford and Callely (1970) and Houghton and Cain (1972), who obtained nearly quantitative recovery of ring N into NH_3 . The fate of the carbon skeleton was not identified in these studies.

Watson and Cain (1975) isolated two bacteria, a *Bacillus sp.* and a *Nocardia sp.*, from soil enrichment with 0.1 percent pyridine. These species grew rapidly on pyridine as sole carbon, nitrogen and energy sources. The ring cleavage of pyridine consumed 2.5-3.0 mol O_2 /mol of pyridine and produced acetate, succinate, formate and ammonia. Acetate, succinate and formate were further oxidized to CO_2 . Based on oxygen consumption and carbon and nitrogen deposition, the complete oxidation of pyridine was as follows:

A similar result was obtained in recent studies of Shukla and Kaul (1986) who used a *Nocardia sp.* for degradation of pyridine and its derivatives. In this study, the bacterium could utilize pyridine, pyridine N-oxide (PNO), and 2-hydroxypyridine as sole sources of carbon, nitrogen and energy and produced acetate, succinate, formate and NH_3 . The product acids were further degraded to CO_2 .

Taylor and Amador (1988) isolated several bacteria from marine sediments which could aerobically grow on pyridine dicarboxylate. Intermediate products were ammonia, carbonyl and carboxylic acids, and 2,3-dihydroxypicolinic acid. All those intermediates were further consumed for bacterial growth:

Nocardia rhodochrous LL100-21 utilized 2-, 3-, or 4-pyridine carbonitrile and the corresponding pyridine carboxamide as a source of nitrogen for growth (Vaughan *et al.*, 1988). Ammonia and nicotinic acid were detected as final products:

2.1.2 Quinoline Biodegradation

Quinoline, isoquinoline and their derivatives have been found as contaminants in groundwater near creosote wood preservation and fossil fuel processing facilities. Several *Pseudomonas sp.*, that are able to utilize quinoline aerobically as a sole source of carbon and nitrogen, have been isolated from sewage, garden soil, and creosote-contaminated soil (Bennett *et al.*, 1985; Al-Najjar *et al.*, 1976; Shukla 1986). Studies indicate that those bacteria completely degraded quinoline by initial attack of the benzene ring and then of the pyridine ring. Hydroxyquinoline was found as an intermediate which accumulated in the culture fluid and later disappeared.

Brockman *et al.* (1989) isolated two gram-negative, motile bacteria from deep subsurface sediments which could aerobically grow on quinoline and many quinoline derivatives, including 2-hydroxyquinoline, quinaldic acid, and kyrenic acid, as their sole carbon and nitrogen source. This bacterium rapidly mineralized quinoline at concentrations as low as 0.002 $\mu\text{g/ml}$. Studies with C14-labeled quinoline indicated that CO_2 was the final product. Under anaerobic conditions, those isolates transformed quinoline to many unidentified soluble products and mineralization of quinoline was not observed.

Aislabie *et al.* (1989) enriched a mixed culture from oil- and creosote- contaminated soils and this culture could use isoquiniline as the sole source of carbon and nitrogen. From this culture, an isoquinoline-degrading *Acinetobacter* strain was isolated and degradation of isoquinoline was accompanied by the accumulation of a red cell-associated pigment and of 1-hydroxyisoquinoline, which was further degraded to unknown intermediary ring-cleavage products and carbon dioxide.

2.1.3 Aniline Biodegradation

Anilines are used in the manufacture of pesticides, dyes, plastics, and pharmaceuticals. Walker and Harris (1969) isolated a *Pseudomonas sp.* from soil which grew with aniline as its sole carbon source. This bacterium oxidized aniline by an induced enzyme system and liberated ammonia. Recently a *Pseudomonas sp.* (strain KI) has been found to be able to grow rapidly on aniline with a doubling time of 2 h (Konopka *et al.*, 1989). Catechol 2,3-oxygenase activity was induced by aniline and catechol could be degraded as well as aniline, but this species could not use halogenated anilines, which indicates that the aromatic ring was cleaved via the meta pathway.

A *Moraxella* sp. (strain G) was able to utilize aniline, and many aniline derivatives including fluoro-, chloro-anilines as sole source of carbon and nitrogen (Zeyer, *et al.*, 1985). Aniline and aniline derivatives were first attacked by aniline oxygenase and yielded the corresponding catechol. Catechol was then degraded by 1,2-dioxygenase into muconic acids which was mineralized via the ortho-cleavage pathway. Ammonia and CO₂ were the final products.

Acinetobacter calcoaceticus (strain DON26) from river water assimilated aniline effectively at micromolar concentrations and could grow rapidly at concentrations of aniline greater than 16 mM (Wyndham, 1986). Catechol and ammonia were transiently accumulated in the early stages and subsequently rapidly consumed by the bacterium for its growth.

2.1.4 Indole Biodegradation

Indole was found to be anaerobically degraded to methane, NH₃ and CO₂ by a mixed methanogenic culture (Wang *et al.*, 1984). Methane gas was produced from indole almost immediately after the initiation of the experiment and methane production averaged 23.2 ml/g of indole carbon. Similar results were obtained in the studies of Berry *et al.* (1987) and Madsen *et al.* (1988), in which oxindole was detected as a temporary intermediate and was completely converted into methane. Based on stoichiometric amounts of products, the anaerobic degradation of indole was described as:

Fujioka and Wada (1968) reported that a soil microorganism could transform indole to anthranilic acid and CO₂, with 2,3-dihydroxy-indole and isatoic acid as intermediate metabolites. Later, Claus and Kutzner (1983) found that *Alcaligenes* (strain In3) could grow aerobically on indole and cellular respiratory activity was high in the presence of both 3-hydroxyindole and isatin. They reasoned that indoxyl and isatin must be intermediate metabolites during indole metabolism. Ensley *et al.* (1983) also reported that *Pseudomonas putida* and a recombinant strain of *Escherichia coli* transformed indole to indoxyl. Based on these studies, the aerobic degradation of indole was suggested as the following:

2.1.5 Significance

Pyridines, quinolines, analines and indoles are readily degraded by many bacterial species, such as *Bacillus*, *Nocardia*, *Pseudomonas*, *Acinetobacter*, *Maraxella*, *Alcaligenes*, etc. Ring structures are broken and ammonia is the final nitrogen compound. Many intermediate organic compounds are formed which may be further degraded to CO₂ or methane.

2.2 Biological Removal of Oxygen Heteroatoms

Oxygen occurs in coal liquids in several forms, including phenolics and heterocyclic compounds, such as furan, benzofuran or dibenzofuran. These compounds also occur in nature and can be degraded biologically.

2.2.1 Biodegradation of Phenols

Many microorganisms can grow on phenol as sole carbon and energy sources and contain an active phenol-degrading capacity. The most common catabolic pathway for biodegradation of phenol involves conversion of phenol to catechols by a monooxygenase or a dioxygenase, (Gibson, 1968; Stanier and Ornston, 1973; Johnson and Stanier, 1971; Haggblom, 1990). The resultant catechols are catalyzed by nonspecific 1,2-dioxygenases (ortho-pathway) to form muconic acids. Muconic acids are degraded to yield B-ketoadipic acids. Further metabolism of B-ketoadipic acids yields succinate and acetate. Catechol can also be catalyzed by 2,3-dioxygenases (meta-pathway) to form hydroxy muconic semialdehyde which is further metabolized to produce acetate, formate and pyruvate. Succinate, acetate, formate and pyruvate could be used as the carbon source for the synthesis of bacterial cell mass or further degraded to CO₂ under aerobic conditions.

Feist and Hegeman (1969) reported that *Pseudomonas putida* strains could grow on phenol as the sole carbon and energy source with a generation time of 100 min. Catechol was detected as an intermediate. The enzyme studies indicated that these strains cleaved aromatic rings via either the meta- or ortho-pathway.

Recently Herrmann *et al.* (1987) reported that *Pseudomonas putida* (strain H) is able to utilize phenol as sole carbon and energy source, and the bacterial plasmid pPGH1 was found to be involved in phenol degradation via the meta-cleavage pathway. Masque *et al.* (1987) reported that a *Pseudomonas sp.* (strain QT31), isolated from activated sludges of a wastewater treatment plant of an oil refinery, could degrade phenol at the rate of 28 mg/l phenol/h. The studies showed that *Strain QT31* could grow at much higher concentrations of phenol than other species and could grow on a variety of phenolic derivatives, such as chlorophenol and m-cresol.

An obligate thermophilic strain of *Bacillus stearothermophilus* (strain PH24), isolated from industrial sediment, grew readily at 55°C on phenol or cresol as carbon sources (Buswell, 1975). Cell extracts converted phenol to catechol with a concomitant uptake of 1 mol of oxygen per mol of substrate. Catechol was further dissimilated by cell extract via the meta-cleavage route. Gaal and Neujahr (1979) reported that *Trichosporon cutaneum* was grown with phenol as the carbon source, and B-ketoadipate was formed in phenol degradation. Metabolism of phenol begins with o-hydroxylation and followed by ortho-ring fission.

Johnson and Stanier (1971) reported that *Alcaligenes eutrophus* degraded phenol and p-cresol via a catechol meta-cleavage pathway and this report was further confirmed by the study of Hughes and Bayly (1983). The enzyme, 2,3-oxygenase, and subsequent enzymes were identified from this bacterium and mutant studies indicated that the structural gene encoding these enzymes existed in the same operon.

Recently, studies of the degradation of phenol by immobilized cells of *Fusarium flocciferum* were conducted (Anselmo *et al.*, 1985). This bacterium was entrapped in agar, K-carrageenan, alginate or polyurethane and incubated with phenol at a variety of concentrations. Results showed that entrapped cells in polyurethane were able to degrade phenol up to 4 g/l with no loss of their activity under repeated use for more than two months.

Anaerobic degradation of phenol was recently investigated with an obligate syntrophic culture which was selected in mineral medium with phenol as the only carbon and energy source (Knoll and Winter, 1989). The consortium consisted of a short and long rod-shaped bacteria and of low numbers of *Desulfovibrio* cells, and grew only in syntrophy with methanogens, such as *Methanospirillum hungatei*. Under N₂/CO₂, phenol was degraded via benzoate to acetate, CH₄ and CO₂.

2.2.2 Biodegradation of Furans

Biodegradation of dibenzofurans has received attention because its halogenated derivatives have been produced commercially and exhibit high levels of toxicity. Engesser *et al.* (1989) isolated two dibenzofuran-degrading bacteria, *Brevibacterium* (strains DPO 1361 and DPO 220) which could utilize dibenzofuran and fluorene as the sole source of carbon and energy. Based on their studies, dibenzofuran was initially degraded by dioxygenase, followed by ring cleavage and subsequent rearomatization by dehydration with 2,2',3-trihydroxybiphenyl as the immediate product.

Fortnagel *et al.* (1989, 1990), isolated a *Pseudomonas* sp. (strain HH69) and a mixed culture (HH27) from soil enrichment with dibenzofuran as sole sources of carbon and energy. Both the pure strain and the mixed culture grew aerobically on dibenzofuran, biphenyl and a number of isomeric hydroxybenzoates. Degradation of dibenzofuran proceeded via salicylic acid, which was branched into the gentisic acid and the catechol pathway. Both salicylic acid and gentisic acid accumulated in the culture and were slowly metabolized after growth ceased. These results indicated that a novel type of dioxygenase is responsible for the attack on the biarylether structure of the dibenzofuran molecule. A meta-fission mechanism for cleavage of the the dihydroxylated aromatic nucleus of 2,2'3-trihydroxybiphenyl is suggested as the next enzymatic step in the degradative pathway.

2.2.3 Significance.

Phenols and furans are degraded by many bacterial species, such as *Pseudomonas*, *Bacillus*, *Alcaligenes*, *Tricosparon*, *Fusarium*, etc. The ring structures are degraded into various

intermediates, which may be subsequently converted to CO₂, H₂O or CH₄. Many of these bacteria also remove nitrogen heteroatoms. Therefore, simultaneous removal of nitrogen and oxygen heteroatoms might be achieved by the same culture.

2.3 Biological Removal of Sulfur Heteroatoms

Over the last decade, extensive research has been conducted to define cultures that remove sulfur from solid coal. Much of this work has been done with the model compound, dibenzothiophene (DBT), which is typical of many sulfur compounds found in the degradation products of coal liquefaction (Hessley *et al.*, 1986). Therefore, this work should be readily extended to the biological removal of sulfur heteroatoms from coal liquids. Present work on the removal of inorganic sulfur is also summarized below.

2.3.1 Organic Sulfur Removal

Dibenzothiophene represents a model compound of the organic sulfur integrated in the coal matrix and is also found to represent most of sulfur-containing heteroatoms in coal-derived liquids. It has been reported that many bacteria can utilize DBT as carbon and energy for growth and convert organic sulfur to sulfate.

Yamada *et al.* (1968) isolated six strains of *Pseudomonas* from various soils. These strains could convert 40 percent of DBT (4.6 g/L) into water-soluble organic acids under aerobic conditions. Later studies (Kodama *et al.*, 1970; Kodama *et al.*, 1973) identified these water-soluble products as DBT-5-dioxide, 3-hydroxy-2-formyl benzothiophene which were not further degraded by these strains. Similar results were obtained in the studies of DBT degradation with *Beijerinckia* species (Laborde and Gibson, 1977) and *Pseudomonas putida* (Mormile and Atlas, 1988). However, recent research (Mormile and Atlas, 1988) indicated that a mixed culture enriched from oil-exposed soil could mineralize DBT-5-dioxide and 3-hydroxy-2-formyl benzothiophene to CO₂, but release of sulfur or sulfate was not detected.

Kargi and Robinson (1982) reported that *Sulfolobus acidocaldarius* could remove part of the organic sulfur from coal in addition to pyritic sulfur. Later studies (Kargi and Robinson, 1984) showed that this bacterium could remove 65 percent of the sulfur in DBT (500 mg/l) and release sulfate as a product. The removal rate decreased only at high concentrations of DBT (> 1000 mg/l).

Recently a *Brevibacterium* species (strain DO), was isolated to grow on DBT as sole source of carbon, sulfur and energy (Afferden *et al.*, 1990). During dibenzothiophene utilization, sulfur was released in stoichiometrical amounts and was further oxidized to sulfate. Three intermediates were isolated and identified as dibenzothiophene-5-oxide, dibenzothiophene-5-dioxide and benzoate, and these intermediates were rapidly mineralized by the growing culture. No water soluble or organic metabolites were detected in the stationary phase of growth. The proposed degradation pathway was as follows:

Recently, scientists at the Institute of Gas Technology (IGT, Chicago, IL) have isolated a mixed culture (IGTS7) and two pure cultures (*Rhodococcus rhodochrous*, IGTS8, and *Bacillus sphaericus*, IGTS9) that specifically cleave carbon-organic sulfur bonds (Bioprocessing Technology, 1991). Analysis of coal samples treated with IGTS7 showed a decrease in organic sulfur of 91 percent, in 212 days.

2.3.2 Pyritic Sulfur Removal

Several genera of bacteria, including *Thiobacillus*, *Sulfolobus*, and *Pseudomonas*, are capable of oxidizing pyrite in finely ground coal. These bacteria catalyze the oxidation of iron pyrite to ferric ions and sulfate:

and

Thiobacillus ferrooxidans has been reported as a major species for removal of pyrite from coal (Andrews and Maczuga, 1982; Hoffmann *et al.*, 1981; Kargi and Weissman, 1984). This bacterium can selectively attach to pyrite in coal and remove 68-90 percent of the pyritic sulfur from a variety of coals, but the process usually requires long retention times (Myerson and Kline, 1983; Townsley *et al.*, 1987). Andrews and Maczuga (1982) reported that *T. ferrooxidans* and *T. thiooxidans* could remove more than 90 percent of the pyritic sulfur from coal in about two weeks. They also found that coal particle size had a significant effect on desulfurization. If the particle size is larger than 60 x 100 mesh, the ultimate removal was low (less than 50 percent). Further experiments indicated that the exposed pyrite area is the main rate-determining criterion.

The leaching of pyritic sulfur from coal employing *T. ferrooxidans* was studied in a continuous stirred tank reactor at a variety of dilution rates (0.02-0.11 h⁻¹) and coal surface areas (0.25-1.0 m²/ml) (Myerson and Kline, 1984). The bacterial leaching rate (2-24 $\mu\text{iron/hr/cm}^2$) was found to increase with increasing coal surface area concentration and increasing dilution rate.

The thermophilic and facultative autotrophic organism, *Sulfolobus acidocaldarius*, has been reported to be able to completely remove inorganic sulfur (more than 90 percent) at a temperature of 70°C and pH 1.5, with a residence time was 10 days (Kargi and Robinson, 1985). The microbial desulfurization rate was improved nearly tenfold by adjusting the N/P and N/Mg ratios in the nutrient medium. A solids density of 20 percent, particle size of 48 μM , and an initial cell density of 10¹² cells/g pyrite in coal were found to be optimal. Recently this bacterium was also found to be able to attach to coal particles in a 20 percent coal slurry (Chen and Skidmore, 1987).

Organisms of the genus *Pseudomonas* are widely distributed in the environment and are found in high-sulfur content coals. Preliminary studies (Rai and Reynier, 1988) indicated that *P. aeruginosa* and *P. putida* could function in desulfurization of bituminous coals and lignite. Further studies showed that *P. putida* was much more effective than *P. aeruginosa* in removing the sulfur compounds

present in the coals. *P. putida* reduced the pyritic sulfur by 75 percent from Texas lignites in 5-7 days for particle sizes varying from 74 μM to 295 μM .

2.3.3 Significance

It is well established that organic and pyritic sulfur can be removed from coal by bacteria such as *Pseudomonas*, *Sulfolobus*, *Brevibacterium*, *Thiobacillus*, etc. The sulfur is oxidized to sulfate.

The microorganisms that remove N, O and S compounds utilize these compounds as sole carbon and nutrient sources. The metabolism of these heteroatom compounds also provides energy for the growth of the organisms. Many of the bacteria, in particular the *Pseudomonas* and *Bacillus* species, utilize N, O and S compounds. It is quite possible that a single bacteria, or possibly a co- culture, can be used to remove S, N and O from coal liquids. These experiments must be conducted and culture performance optimized in order to demonstrate feasibility.

3.0. EXPERIMENTAL METHODS

3.1 Liquefied Coal

Five gallons of each of the following liquefied coals were received: (1) Illinois No. 6 Coal, boiling point range 80 to 240°C; (2) Illinois No. 6 Coal, boiling point range 240 to 400°C; (3) Sub-bituminous Coal, boiling point range 80 to 240°C; and (4) Sub-bituminous Coal, boiling point range 240 to 400°C. The samples were stored under inert gas at 5°C in a refrigerated room.

3.2 Model Compound Selection

Model compounds have been selected for study to help assess the relative effectiveness of each of the cultures for nitrogen, sulfur and oxygen compound removal. These compounds, listed in Table 3.1, were chosen because they are similar to compounds known to be present in coal liquids.

Table 3.1 Model Compounds for Heteroatom Removal

<u>Group I: Nitrogen Compounds</u>	<u>Group II: Oxygen Compounds</u>	<u>Group III: Sulfur Compounds</u>
quinoline	phenol	ferrous sulfide
pyridine	furan	benzothiophene
aniline	benzofuran	dibenzothiophene

3.3 Screening and Isolation Protocol

An outline of the culture screening protocol is shown in Figure 3.1. Pure cultures were obtained from culture collections and cultures were isolated from a variety of natural sources including the coal liquids themselves. After isolation and culture activation, the organisms were selected for use in CSTR studies based upon their ability to degrade the model compounds and more importantly, heteroatom compounds in coal liquids.

As is noted in Figure 3.1, a model compound matrix was used to partially evaluate the suitability of a given culture. Refer to Table 3.1 for identification of the compounds. If a given culture is able to degrade all of the given model compounds in a group (all of the nitrogen compounds, for example) it was then used in co-culturing experiments with other similarly suitable compounds in an attempt to degrade all nine of the model compounds. If a given culture was unable to degrade all of the compounds in a group it was rejected as a viable culture.

A similar set of experiments was performed concurrently with coal liquids. Experiments were performed to test whether a culture removed a significant fraction of one heteroatom species (nitrogen compounds, for example). These successful cultures were combined with others to form a co-culture. The objective of these studies was to develop a culture or group of cultures that will degrade a significant fraction of all the heteroatom compounds in coal liquids. Once a culture (or co-culture) has been selected for combined heteroatom removal, growth and conversion conditions will be optimized prior to use in the CSTR.

3.4 Continuous Culture Isolation and Optimization

A continuous culture isolation and optimization technique is being evaluated for its ability to obtain enrichments capable of aerobically or anaerobically removing heteroatom compounds from coal liquids. Extracts were prepared from chemical waste contaminated samples and hot springs sludges and incubated at 35°C. A schematic of the continuous apparatus is shown in Figure 3.2. A sodium succinate (10g/L) medium supplemented with vitamins and salts was fed continuously to the culture. Air was supplied to the culture growing aerobically, and N₂ was sparged through the anaerobic culture to help maintain anaerobic conditions. Coal liquid E was added semi-continuously to maintain a concentration of approximately 1 percent in each reactor. The aqueous phase was monitored periodically for pH and optical density, and the organic phase (coal liquid) was collected periodically for elemental analysis.

Culture Screening and Isolation

Figure 3.1 Culture Screening and Isolation Protocol

Figure 3.2 Schematic of Continuous Culture Isolation/Optimization System

3.5 The Use of Denitrifying Bacteria

Denitrifying bacteria are being developed as additional isolates capable of removing sulfur from coal liquids. These bacteria have several advantages over the other isolates including anaerobic growth, the utilization of a sulfur-free medium and rapid growth.

Anaerobic growth is desired in order to minimize hydrocarbon degradation in the coal liquids which may be significant under aerobic conditions. It also has the advantage of not requiring as much gas flow through the system with concomitant removal of readily volatilized components in the naphtha or coal liquids. Denitrifying bacteria will couple the complete oxidation of an added carbon and energy source to CO_2 with the reduction of nitrate to N_2 (both innocuous products), under anaerobic conditions. Anaerobiosis need not be strict. The added carbon and energy source may be selected from a number of inexpensive chemicals. The use of a sulfur-free medium will require any microbial growth and nutritional sulfur to be at the expense of the sulfur-containing materials in the coal liquids. Finally, denitrifiers typically grow with doubling times in the 1.5-4 hr range, allowing a rapid development of the culture and its sulfur removing biochemical activity.

Denitrifiers have been isolated which use naphtha as their sole source of sulfur at 37°C . The isolation medium is shown in Table 3.2. The carbon and energy sources so far used are acetate, succinate and glucose. These are mixed cultures which have been subcultured four times, thus the sulfur limitation in the medium is well established. A temperature of 30°C should typically be used, since it allows a wider variety of microbes to develop.

Table 3.2. Sulfur-Free Denitrifier Medium

Ingredient	g (ml)/liter
KH_2PO_4	0.24
K_2HPO_4	0.24
NH_4Cl	0.19
NaCl	0.48
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	0.068
NaNO_3	5.0
$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$	0.08
PFN Trace Metals ¹	3 ml

1. Uses chloride salts of cations of Fe, Zn, Mn, Co, Cu and Ni and contains Mo, Se and B as anions.

The pH of the medium is adjusted to 6.6 by the addition of solid Na_2CO_3 .

3.6 Analytical Methods

A CHNS-O model EA1108 elemental analyzer was obtained for monitoring overall nitrogen, sulfur and oxygen in heteroatom compounds during the biodegradation study. The EA1108 Elemental Analyzer is an instrument designed for the micro and macro determination of total C, H, N, S, and O present in a wide range of organic and inorganic samples which includes oils, coal, soils, fertilizers, etc. The sample can be in solid, liquid or gaseous forms.

Other analytical methods were developed to quantify the degradation of model compounds and possible specific coal liquid fractions. In addition, methods were developed to analyze for developed product gases such as nitrogen, and liquid phase products such as ammonia and sulfate.

Liquid column chromatographic methods were used to separate and concentrate the heteroatom-rich fractions for subsequent use in culture isolation experiments. In this manner, isolates will be obtained which are capable of growing on heteroatom compounds without utilizing other fractions of the coal liquid as growth substrates. The asphaltene fractions have been separated from two, Wilsonville coal liquids (V-178, Run 257; V-178; G-4S; T-4; N-41). V-198 coal liquid is dark brown in color, has low viscosity and has an asphaltene content of about 0.25 percent by weight. At room temperature, V-178 appears to be a highly viscous liquid. At 35°C, the viscosity of V-178 is greatly reduced, appearing as a dark brown liquid. The asphaltene content of V-178 is about 4.7 percent by weight.

The asphaltene fraction in both coal liquids was separated by dissolving the coal liquid in pentane. The precipitates were separated as asphaltenes by filtration or by allowing them to adhere on the bottom of the flasks prior to separating the liquid phase. The deasphaltene fractions were distilled to recover the pentane for recycle.

An elemental analysis was performed on each coal liquid fraction: The original coal liquid, the deasphaltene fraction and the asphaltene fraction. The results are shown in Table 3.3. As is noted, the sulfur and nitrogen heteroatom compounds were successfully concentrated in the asphaltene fraction for coal liquid V-198, and the nitrogen heteroatom compounds were successfully concentrated in the asphaltene fraction for coal liquid V-178.

Table 3.3 Elemental Analysis of Coal Liquids and Fractions

Sample	Elemental Analysis (Wt. Percent)		
	N	C	S
Coal Liquid, V-178, G-45, T-4, N-41	0.2542	89.2390	0.0000
Deasphaltene Fraction	0.2257	89.5452	0.0000
Asphaltene Fraction	2.5024	82.6522	0.2332
Coal Liquid, V-178, Run 257	0.9601	86.3868	0.0452
Deasphaltene Fraction	0.7837	87.0957	0.0345
Asphaltene Fraction	2.1001	81.7430	0.0361

4.0 RESULTS AND DISCUSSION

Four pure cultures that have the capacity to degrade either O or N heteroatoms were procured from the ATCC. *Rhodococcus rhodochrous* ATCC 33278 can degrade acetonitrile, propionitrile, and 2,3- or 4-cyanopyridine; *Pseudomonas putida* ATCC 17484 can utilize naphthalene; and *Pseudomonas putida* ATCC 33015 can decompose aniline. *Pseudomonas putida* ATCC 11172 can degrade phenol. In addition, eight cultures have been identified that can remove sulfur compounds from coal liquids. These include *Sulfolobus acidocaldarius* ATCC 33909, *Pseudomonas putida*, ATCC 12633, *Pseudomonas aeruginosa* ATCC 27853, *Thiobacillus thiooxidans* ATCC 8085 and ATCC 19377, and *Thiobacillus ferrooxidans* ATCC 13598, ATCC 13661, and ATCC 19859. Numerous cultures have been isolated from natural sources, including soils, chemical waste areas and the coal liquids themselves.

4.1 Model Compound Degradation (Batch Culture)

Examples of model compound degradation are shown in Figures 4.1 and 4.2, as well as in Tables 4.1-4.9. The results of a preliminary phenol degradation study using the isolate ERI4 is shown in Figure 4.1. As is shown, the phenol concentration fell from 0.26 g/L to 0 in about 24 hours. At the same time, the cell concentration rose from the inoculum level of 0.03 g/L to 0.26 g/L, concurrent with the utilization of phenol. Growth stopped after the phenol was depleted from the medium, indicating that phenol was used as a growth substrate.

Similar results are shown in Figure 4.2 for pyridine degradation by the isolate ERI5. The pyridine concentration fell from 0.61 g/L to 0.1 g/L in 24 hrs and fell to 0 in 48 hrs. Again, growth paralleled pyridine utilization, increasing from 0.04 g/L initially to 0.32 g/L in 48 hrs.

Rhodococcus rhodochrous, ATCC 33278, has been shown to rapidly grow on phenol (1.0 g/L) or pyridine (0.5 g/L) as the sole sources of carbon and energy. The results of batch experiments with *R. rhodochrous* are shown in Table 4.1. The medium in these studies contained only salts, vitamins and ammonia in addition to the heteroatom compound. As is seen in the table, both phenol and pyridine were utilized completely in 24 hours. In addition, the cell concentration increased significantly. In fact, the increase in cell density using 1.0 g/L phenol was nearly double the increase with 0.5 g/L pyridine. Thus, phenol and pyridine are nearly equal growth substrates.

Table 4.1. Phenol and Pyridine Utilization
by *R. rhodochrous* (ATCC33278)

Synthetic Heteroatom	Time (hr)	Synthetic Heteroatom	
		Concentration (g/L)	Cell Mass (g/L)
Phenol	0	0.9276	0.018
Phenol	24	0.0012	0.500
Pyridine	0	0.4267	0.025
Pyridine	24	0.0021	0.317

Figure 4.1 Phenol Degradation by Isolate ERI4

Figure 4.2 Pyridine Degradation by Isolate ERI5

As is shown in Table 4.2, *Pseudomonas putida*, ATCC 17484, was able to completely utilize pyridine (0.5 g/L) in 24 hours. Again, the medium contained vitamins, salts, ammonia and pyridine. The cell density rose in a manner similar to *R. rhodochrous* growing on phenol or pyridine. *P. putida*, ATCC 17484, was also shown to be able to utilize quinoline (0.1 g/L) as a sole carbon and energy source with rapid growth (data not shown). Experiments with the third nitrogen heteroatom

compound, aniline, have been initiated. So far, no growth on aniline has been observed. Experiments with other carbon sources while simultaneously attempting to degrade aniline will be undertaken next. *P. putida*, ATCC 17484, appears to be an excellent candidate for nitrogen heteroatom removal.

Table 4.2. Pyridine Utilization by *P. putida*, ATCC 17484

Time (hr)	Pyridine Concentration (g/L)	Cell Mass (g/L)
0	0.4263	0.041
24	0.0016	0.341

Table 4.3 shows phenol utilization by *Pseudomonas putida*, ATCC 11172. This *P. putida* species degraded phenol completely in 48 hours, while simultaneously utilizing phenol as a sole carbon and energy source. In comparing phenol utilization by *P. putida*, ATCC 11172, and *R. rhodochrous*, ATCC 33278, it is seen that *R. rhodochrous* appears to be the superior organism. However, additional heteroatom compounds, as well as coal liquids, will also be tried with this bacterium.

Table 4.3 Phenol Utilization by *P. putida*, ATCC 11172

Time (hr)	Phenol Concentration (g/L)	Cell Mass (g/L)
0	0.9372	0.033
24	0.4263	0.261
48	0.0053	0.474

Sulfur heteroatom degradation experiments have also been carried out with pure cultures, chosen for their ability to utilize inorganic and organic sulfur compounds. The organisms *Pseudomonas aeruginosa*, ATCC 27853; *Pseudomonas putida*, ATCC 12633; and *Sulfolobus acidocaldarius*, ATCC 33909 were utilized in media essentially devoid of all other sulfur species. The model compounds used were FeS, benzothiophene (BT) and DBT. Controls were run with cultures and without model compounds, and without cultures but with the model compounds.

Tables 4.4 and 4.5 show the results of sulfur heteroatom removal studies with *P. aeruginosa*, ATCC 27853, and *P. putida*, ATCC 12633. Table 4.6 shows removal results with *S. acidocaldarius*, ATCC 33909. The results shown in Table 4.5 are a repeat of the experiments of Table 4.4. As is noted in Table 4.4, significant increases in sulfate concentrations were noted only for *P. aeruginosa* grown on FeS. Marginal production of SO₄ (greater than the control concentrations) was noted for *P. aeruginosa* grown on DBT and *P. putida* grown on FeS. Table 4.5 shows that both bacteria utilized FeS in producing SO₄, with spotty and marginal utilization of the other compounds. Table 4.6 shows that additional time is required for *S. acidocaldarius* to utilize the sulfur compounds.

**Table 4.4. Degradation of FeS, BT and DBT by *P. aeruginosa* as Measured by Sulfate Production
(Initial Studies)**

Bacteria	Initial Concentration Substrate	Sulfates Produced (mg/L)						
		0 (hrs)	26.5 (hrs)	47.0 (hrs)	72.0 (hrs)	96.0 (hrs)	140.5 (hrs)	164.5 (hrs)
<i>Pseudomonas aeruginosa</i> , ATCC 27853	FeS (1g/L)	32	76	107	126	112	117	98
	BT (400 mg/L)	16	22	22	23	29	31	27
	DBT (400 mg/L)	16	27	27	27	32	44	47
	None	12	14	15	15	21	32	18
<i>Pseudomonas putida</i> , ATCC 12633	FeS (1g/L)	43	30	33	37	52	41	41
	BT (400 mg/L)	20	23	20	21	29	18	16
	DBT (400 mg/L)	17	20	23	23	28	32	18
	None	12	13	14	14	20	19	19
None	FeS (1g/L)	33	32	31	45	54	41	30
None	BT (400 mg/L)	17	15	18	22	25	26	30
None	DBT (400 mg/L)	17	19	20	22	24	27	24

Table 4.5. Degradation of FeS, BT and DBT by *P. aeruginosa* as Measured by Sulfate Production (Repeat of Initial Studies)

Bacteria	Initial Concentration Substrate	Sulfates Produced (mg/L)							
		0 (hrs)	24.5 (hrs)	47.5 (hrs)	72.5 (hrs)	97.5 (hrs)	120.5 (hrs)	144.5 (hrs)	165.5 (hrs)
<i>Pseudomonas aeruginosa</i> , ATCC 27853	FeS (1g/L)	76	80	91	83	82	102	57	123
	BT (400 mg/L)	25	41	41	44	49	46	23	78
	DBT (400 mg/L)	40	33	39	51	64	59	22	26
	None	32	24	24	21	21	27	22	29
<i>Pseudomonas putida</i> , ATCC 12633	FeS (1g/L)	65	142	152	91	86	105	116	134
	BT (400mg/L)	28	32	25	32	30	39	19	-
	DBT (400 mg/L)	35	26	80	36	48	40	34	66
	None	(bottle broken)							
None	FeS (1g/L)	49	44	44	42	48	41	48	48
None	BT (400mg/L)	22	22	23	19	26	21	24	32
None	DBT (400mg/L)	9	16	15	10	17	11	22	24

Table 4.6. Degradation of FeS, BT and DBT by *S. acidocaldarius* as Measured by Sulfate Production

Bacteria	Initial Concentration Substrate	Sulfate Produced (mg/L)		
		0 (hrs)	79.5 (hrs)	110.5 (hrs)
<i>Sulfolobus acidocaldarius</i> , ATCC 33909	FeS (1 g/L)	39.5	84.7	118.1
	BT (400 mg/L)	29.1	46.1	47.6
	DBT (400 mg/L)	26.2	46.3	32.5
	None	30.2	37.2	62.8
None	FeS (1 g/L)	27.2	84.4	144.1
None	BT (400 mg/L)	16.7	31.3	31.3
None	DBT (400 mg/L)	17.7	24.2	35.3

A summary of the model compound growth studies is shown in Table 4.7 below. *Rhodococcus rhodochrous* was found to be able to grow on several nitrogen, oxygen and sulfur compounds including pyridine, aniline, phenol, furan and dibenzothiophene. By contrast, *Pseudomonas putida*, 17484, and *P. putida*, 33015, failed to demonstrate growth on any of the model compounds. Each of the model compounds tested were utilized as growth substrates by one or more of the cultures except for benzofuran, although only one culture utilized quinoline effectively as a growth substrate.

Table 4.7. Growth of Cultures/Isolates on Model Compounds

<u>Culture/Isolate</u>	<u>Nitrogen Compounds</u>			<u>Oxygen Compounds</u>			<u>Sulfur Compound</u>
	<u>Pyridine</u>	<u>Quinoline</u>	<u>Aniline</u>	<u>Phenol</u>	<u>Furan</u>	<u>Benzofuran</u>	<u>Dibenzothiophene</u>
<i>R. rhodochrous</i> , 33278	+	-	+	+	+	-	+
<i>P. putida</i> , 12633	+	?	+	+	+	-	-
<i>P. putida</i> , 17484	-	-	-	-	-	-	-
<i>P. putida</i> , 33015	-	-	-	-	-	-	-
<i>P. aeruginosa</i> , 27853	-	+	-	-	-	-	-
<i>S. acidocaldarius</i> , 33909	+	-	-	-	-	-	-
ERI4	+	-	-	+	-	-	-
ERI5	+	-	+	+	-	-	-
ERI6	+	-	+	-	-	-	-
ERI7	+	-	-	+	-	-	+

+ growth/degradation observed
 ? possible growth, some cloudiness observed
 - no growth/degradation observed

4.2 Utilization of Denitrifying Bacteria for Sulfur Removal

The first step in obtaining enrichments of denitrifying bacteria for sulfur removal was to inoculate a consortia of bacteria on various carbon sources in the presence of either naphtha or coal liquid as the sulfur source. A mixture of sludge, soil and petroleum waste was inoculated into a mixture of carbon sources and either naphtha or coal liquid as the source of sulfur. Both heavy straight run naphtha and full range cracked naphtha were used along with Wilsonville coal liquid E.

Table 4.8 shows the enrichment history on the naphtha model compounds. In this and all other tables, H denotes full range cracked naphtha and L denotes heavy straight run naphtha. In following Table 4.8, the consortium was first inoculated into a bottle containing 5 percent full range cracked naphtha and 1 g/L each of nine potential carbon sources. A relatively high optical density

(representing cell growth) was obtained. One to two drops of the resulting culture enrichment were then inoculated into bottles containing 5 percent naphtha and 5 g/L of one of the carbon sources. This procedure ensured that increases in optical density would be due to growth on the carbon source and naphtha instead of inoculum carryover. The procedure was repeated for eight transfers using the best carbon sources and either full range cracked or heavy straight run naphtha. Lactate and perhaps glycerol appeared to be suitable carbon sources for the rapid growth of the denitrifying bacteria. A similar procedure was designed to utilize heavy straight run naphtha (Table 4.9) or water extracted naphtha (Table 4.10) as the source of sulfur.

Table 4.11 shows the results of a similar experiment using coal liquid E as the sulfur source. Very good growth occurred when using glycerol as the carbon source through four transfers. However, the resulting sulfur uptake from the coal liquid was not appreciable in 312 hr, when using acetate as the carbon source (see Table 4.12).

In the next set of experiments, full range cracked naphtha was treated with an enrichment of denitrifying bacteria as a sole sulfur source. Whenever the culture grew to a relatively high optical density, the naphtha was extracted and added to fresh medium inoculated with the same culture. Continued growth indicated that the sulfur in naphtha had to be used as the sulfur source. It was expected that less growth would occur after the first few transfers, since the more tightly bound sulfur in the naphtha would become limiting. The results from the experiment are shown in Table 4.13.

From Table 4.13, it appears that after the sixth transfer, the culture started to grow better. This indicates that the culture adapted to naphtha and was capable of utilizing additional sulfur from the naphtha. Precipitation of FeS may be indicated by the appearance of finely disseminated black flakes in the vessel.

Table 4.8. Enrichment of Denitrifying Bacteria on Various Carbon Sources Using Naphtha as the Sulfur Source

Transfer	Amount of Inoculum	Bottle	Carbon Source	Sulfur Source	OD 600 nm
First 7/9	5 mL of mixture of sludge, soil and petroleum waste	A	Acetate, Pyruvate, Glucose, Fructose, Methanol, Lactate, Succinate, Ethanol, Glycerol (1 g/L each)	Naphtha (H, 5%)	0.576
Second 7/12	1-2 drops in 50 mL	Z1	Acetate (5 g/L)	Naphtha (H, 5%)	0.245
		Z3	Pyruvate (5 g/L)		0.359
		Z5	Glucose (5 g/L)		0.724
		Z7	Fructose (5 g/L)		0.661
		Z9	Methanol (5 g/L)		0.045
		Z11	Lactate (5 g/L)		0.385
		Z13	Succinate (5 g/L)		0.511
		Z15	Ethanol (5 g/L)		0.551
		Z17	Glycerol (5 g/L)		0.643
Third 7/16	1-2 drops in 50 mL	Z19	Pyruvate (5 g/L)	Naphtha (H, 5%)	0.428
		Z21	Glucose (5 g/L)		0.678
		Z23	Fructose (5 g/L)		0.605
		Z25	Lactate (5 g/L)		1.040
		Z27	Succinate (5 g/L)		0.716
		Z29	Glycerol (5 g/L)		0.442
		Z31	Acetate (5 g/L)		0.010
		Z33	Ethanol (5 g/L)		0.007
Fourth 7/19	1-2 drops in 50 mL	Z35	None	Naphtha (L, 5%)	0.011
		Z37	Pyruvate (5 g/L)		0.115
		Z39	Glucose (5 g/L)		0.178
		Z41	Fructose (5 g/L)		0.010
		Z43	Lactate (5 g/L)		0.292
		Z45	Succinate (5 g/L)		0.008
		Z47	Glycerol (5 g/L)		0.012

Fourth 7/19	1-2 drops in 50 mL	Da1	None	Naphtha (H, 5%)	0.012
		Da2	Pyruvate (5 g/L)		0.376
		Da3	Glucose (5 g/L)		0.308
		Da4	Fructose (5 g/L)		0.462
		Da5	Lactate (5 g/L)		0.471
		Da6	Succinate (5 g/L)		0.625
		Da7	Glycerol (5 g/L)		0.389
Fifth 7/20	1-2 drops in 50 mL	L1	Glucose (5 g/L)	None	0.113
		L2	Succinate (5 g/L)		0.123
		L3	Glycerol (5 g/L)		0.095
		L4	Lactate (5 g/L)		0.099
		L5	Acetate (5 g/L)		0.065
		L6	Fructose (5 g/L)		0.095
		L7	Pyruvate (5 g/L)		0.331
Fifth 7/21	1-2 drops in 50 mL	L8	Glucose (5 g/L)	None- Millipore Water	0.289
		L9	Succinate (5 g/L)		0.101
		L10	Glycerol (5 g/L)		0.078
		L11	Lactate (5 g/L)		0.059
		L12	Acetate (5 g/L)		0.037
		L13	Fructose (5 g/L)		0.324
		L14	Pyruvate (5 g/L)		0.137
Sixth 7/23	1-2 drops in 50 mL	C1	Lactate (5 g/L)	None	0.223
		C2	Lactate (5 g/L)	Naphtha (H, 5%)	0.504
		C3	Lactate (5 g/L)	Naphtha (L, 5%)	0.085
		C4	Glycerol (5 g/L)	None	0.221
		C5	Glycerol (5 g/L)	Naphtha (H, 5%)	0.286
		C6	Glycerol (5 g/L)	Naphtha (L, 5%)	0.189

Seventh 7/26	1-2 drops in 50 mL	L15	Lactate (5 g/L)	None	0.066
		L16	Lactate (5 g/L)		0.041
		L17	Lactate (5 g/L)		0.067
		L18	Lactate (5 g/L)	Naphtha (H, 5%)	0.410
		L19	Lactate (5 g/L)		0.560
		L20	Lactate (5 g/L)		0.510
		L21	Lactate (5 g/L)		-
	No Bacteria	L22	Lactate (5 g/L)		-
		L23	Lactate (5 g/L)		-
Seventh 7/28	1 mL of culture from C3 and C6	Da8	Lactate (5 g/L)	Naphtha (L, 5%)	-
		Da9	Glycerol (5 g/L)		-
Eighth 7/30	0.5 mL in 300 mL	Z(1)	Lactate (5 g/L)	Naphtha (H, 2%)	0.190
	None	Z(2)	Lactate (5 g/L)		0.090
	0.5 mL in 300 mL	Z(3)	Lactate (5 g/L)	None	0.027
	0.5 mL in 300 mL	Z(4)	Lactate (5 g/L)	Extracted Naphtha (H, 2%)	0.153
	None	Z(5)	Lactate (5 g/L)		0.085
	0.5 mL in 300 mL	Z(6)	Lactate (5 g/L)	None	0.038
Eighth 8/2	1-2 drops in 50 mL	DC1	Lactate (5 g/L)	Water extracted from naphtha (H, 5%)	0.460
		DC2	Lactate (5 g/L)		0.433
		DC3	Lactate (5 g/L)	None	0.019
		DC4	Lactate (5 g/L)		0.012
Eighth 8/3	1 mL from C3 and C6 in 50 mL	LB1			
		LB2			
		LB3			
		LB4			
		LB5			
		LB6			
		LB7			
		LB8	Lactate (5 g/L)		0.027

**Table 4.9. Enrichment of Denitrifying Bacteria on Various Carbon Sources
Using Heavy Straight Run Naphtha as the Sulfur Source**

Transfer	Amount of Inoculum	Bottle	Carbon Source	Sulfur Source	Growth 600 nm
First 7/23	2 mL of mixture of sludge, soil and petroleum waste samples	Mix 3	Acetate, Pyruvate, Glucose, Fructose, Methanol, Lactate, Succinate, Ethanol, Glycerol-1 g/L each	Naptha (L, 5%)	0.437
Second 7/26	1-2 drops from Mix3 in 50 mL	ZJ1	Acetate (5 g/L)		
		ZJ2	Pyruvate (5 g/L)		
		ZJ3	Glucose (5 g/L)		
		ZJ4	Fructose (5 g/L)		
		ZJ5	Methanol (5 g/L)		
		ZJ6	Ethanol (5 g/L)		
		ZJ7	Lactate (5 g/L)		
		ZJ8	Succinate (5 g/L)		
		ZJ9	Glycerol (5 g/L)		
First 8/3	1 mL of sludge	LA1	Pyruvate, Glucose Fructose, Succinate Glycerol, Acetate, Lactate 1 g/L each	Naphtha (L, 5%)	
	1 mL of pet. waste	LA2			
	1 mL of sludge 37°C	LA3			
	1 mL of pet. waste 37°C	LA4			

**Table 4.10. Enrichment of Denitrifying Bacteria on Various Carbon Sources
Using Water Extracted Naphtha as the Sulfur Source**

Transfer	Amount of Inoculum	Bottle	Carbon Source	Sulfur Source	Growth 600 nm*
First 7/30	Mixed sludge	Z(7)	Acetate, Pyruvate, Glucose, Fructose, Lactate, Succinate, Glycerol-1 g/L each	Extracted Naphtha (H, 5%) through water	0.456
Second 8/4	0.5 mL from Z(7) in 50 mL	Z(8)	Pyruvate (5 g/L)		
		Z(9)	Pyruvate (5 g/L)		
		Z(10)	Acetate (5 g/L)		
		Z(11)	Acetate (5 g/L)		
		Z(12)	Glycerol (5 g/L)		
		Z(13)	Glycerol (5 g/L)		
		Z(14)	Lactate (5 g/L)		
		Z(15)	Lactate (5 g/L)		

Table 4.11 Enrichment of Denitrifying Bacteria on Various Carbon Sources Using Coal Liquid E as the Sulfur Source

Transfer	Amount of Inoculum	Bottle	Carbon Source	Sulfur Source	Growth
First 7/9	5 mL of mixture of sludge, soil and petroleum waste samples	B	Acetate, Pyruvate, Glucose, Fructose, Methanol, Lactate, Succinate, Ethanol, Glycerol-1 g/L each	Coal Liquid E (5%)	++
Second 7/12	1-2 drops in 50 mL	Z2	Acetate (5 g/L)		-
		Z4	Pyruvate (5 g/L)		++
		Z6	Glucose (5 g/L)		++
		Z8	Fructose (5 g/L)		++
		Z10	Methanol (5 g/L)		-
		Z12	Lactate (5 g/L)		-
		Z14	Succinate (5 g/L)		-
		Z16	Ethanol (5 g/L)		-
		Z18	Glycerol (5 g/L)		++
Third 7/16	1-2 drops in 50 mL	Z20	Pyruvate (5 g/L)		++
		Z22	Glucose (5 g/L)		-
		Z24	Fructose (5 g/L)		++
		Z26	Glycerol (5 g/L)		
Fourth 7/19	1-2 drops in 50 mL	Z28	Glycerol (5 g/L)		++
		Z30	Glycerol (5 g/L)		++
Fourth 7/23	1-2 drops in 50 mL	Z32	Pyruvate (5 g/L)		
		Z34	Fructose (5 g/L)		
Fifth, 7/23	1-2 drops in 50 mL	Z36	Glycerol (5 g/L)		
Fourth 7/27	1-2 drops in 50 mL	Z38	Pyruvate (5 g/L)		+
		Z40	Fructose (5 g/L)		
Fifth, 7/27	1-2 drops in 50 mL	Z42	Glycerol (5 g/L)		
First 8/4	Mixture of sludge and pet. waste samples	D	Glucose, Glycerol, Succinate, Pyruvate, Acetate, Lactate, Fructose-1 g/L each		

++ very good growth
+ good growth
- no growth

Table 4.12. Sulfur Removal from Coal Liquid F (2%) by Denitrifying Enrichments Using Acetate as the Carbon Source

Time (h)	A (control)		B (DT4)		C (DT8)		D (DT3)	
	S%	pH	S%	pH	S%	pH	S%	pH
0	0.0528	8.05	0.0522	8.16	0.0559	8.24	0.0505	8.05
24	0.0516	8.19	0.0503	8.28	0.0541	8.50	0.0540	8.19
48	0.0550	8.34	0.0512	8.49	0.0523	8.54	0.0517	8.34
72	0.0523	8.62	0.0516	8.53	0.0466	8.59	0.0499	8.62
96	0.0538	8.61	0.0500	8.58	0.0494	8.64	0.0505	8.61
120	0.0531	8.31	0.0501	8.71	0.0486	8.79	0.0518	8.31
144		8.34	0.0510	8.54	0.0540	8.67		
168	0.0498	8.50	0.0506	8.67	0.0513	8.63	0.0524	8.34
192	0.0506	8.51	0.0509	8.69	0.0540	8.63	0.0531	8.50
216	0.0506	8.61	0.0500	8.69	0.0558	8.71	0.0517	8.51
264	0.0503	8.57	0.0526	8.58	0.0515	8.41	0.0506	8.61
288	0.0529	8.34	0.0532	8.50	0.0539	7.63	0.0519	8.57
312	0.0518	8.41	0.0481	8.52	0.0596	7.62	0.0525	8.34

Table 4.13. Bacterial Utilization of Sulfur from Full Range Cracked Naphtha by Denitrifying Bacteria

Inoculation	Carbon	Sulfur	OD (600nm)
First (8/15)	Lactate (5g/l)	Naphtha (H, 10%)	0.967
Second (8/20)	Lactate (5g/l)	1st-treated naphtha	0.786
Third (8/23)	Lactate (5g/l)	2nd-treated naphtha	0.465
Fourth (8/27)	Lactate (5g/l)	3rd-treated naphtha	0.367
Fifth (9/1)	Lactate (5g/l)	4th-treated naphtha	0.286
Sixth (9/8)	Lactate (5g/l)	5th-treated naphtha	0.612
Seventh (9/17)	Lactate (5g/l)	6th-treated naphtha	0.705
Eighth (9/26)	Lactate (5g/l)	7th-treated naphtha	0.721

Table 4.14. Growth of Denitrifying Bacteria with Heavy Straight Run Naphtha as the Sole Sulfur Source.

Inoculation	Carbon Source	Sulfur Source	OD (600nm)
First (7/23)	Mixture of sugars	Naphtha (5%)	0.437
Second (7/26)	Fructose (5g/L)	Naphtha (5%)	0.556
Third (8/9)	Pyruvate (5g/L)	Naphtha (5%)	1.125
Fourth (8/26)	Pyruvate (5g/L)	Naphtha (5%)	0.893
Fifth (9/1)	Pyruvate (5g/L)	Naphtha (5%)	

The naphtha was subjected to elemental analysis of sulfur. However, the analysis showed no significant drop in the sulfur content. This result could be due to (1) low sulfur content in bacterial mass; (2) the consumption of both sulfur and hydrocarbons in naphtha; (3) errors due to elemental analysis.

Several vendors have been contacted regarding the use of elemental analysis for sulfur analysis. Each of the vendors indicated that this technique is unsuitable for sulfur concentrations of 1000 ppm or below, the typical levels in coal liquids and naphtha. Other methods of analysis are being considered which can yield meaningful data at these sulfur concentrations.

In another set of experiments, a mixture of denitrifying bacteria was enriched with heavy straight run naphtha. This enrichment grew at a rapid rate with the naphtha as the sole sulfur source (see Table 4.14).

A GC-MS analysis of the two naphtha sources was prepared by an independent laboratory. In comparing the two sources, it was found that the full range cracked naphtha contains a variety of identifiable sulfur compounds including thiophenes and benzothiophenes. Heavy straight run naphtha, on the other hand, contained almost no identifiable sulfur compounds (see appendix).

Mixed denitrifying bacteria which can grow at the expense of high boiling point naphtha were grown with 2 percent naphtha and the cells were collected at roughly 7 day time intervals. The cells were dried and sent to Huffman Labs in Colorado for a LECO sulfur analysis. The results are shown in Table 4.15 and Figure 4.3. A total of 32.4 mg of sulfur were initially present in the naphtha.

Table 4.15. LECO Sulfur Analysis of Cells from High Boiling Point Naphtha

Time, days	Cell Dry Weight, mg	Sulfur, mg	% Released	Cumulative % Sulfur Released
0	0	0	0	0
7	353	2.15	6.7	6.7
14	443	1.68	5.2	11.9
21	822	6.33	19.5	31.4
31	433	2.51	7.7	39.1
40	no growth			

As is noted in the table and figure, a total of 12.67 mg of sulfur were converted into cellular material, or 39.1 percent of the sulfur from the naphtha.

Figure 4.3 Removal of Sulfur from High Boiling Naptha
Mixed Denitrifying Bacteria

4.3 Utilization of Sulfate Reducing Bacteria for Nitrogen Removal

Similar procedures are being utilized for nitrogen removal from naphtha and coal liquids by employing sulfate reducing bacteria. These bacteria grow slower than denitrifying bacteria, but also incorporate a larger percentage of the heteroatom compound into cell mass. Tables 4.16 and 4.17 present results of the enrichment histories of the sulfate reducing bacteria on various carbon sources and naphtha or coal liquid E, respectively. As is noted in Table 4.16, pyruvate, fructose and glucose appear to be promising carbon sources for nitrogen uptake from naphtha. Experimental results with coal liquid E will be apparent in a few weeks.

The heavy straight run naphtha has also been treated with sulfate-reducing bacteria to test bacterial growth after several transfers. No bacterial growth was detected with either naphtha or coal liquids as the nitrogen source.

Sulfate-reducing bacteria were employed in an attempt to remove heteroatom nitrogen from coal liquids. These bacteria were supplied sulfur, but depend upon the coal liquid as their sole source of nitrogen. Experiments have begun using various carbon sources for these sludge isolates. As is noted in Tables 4.18 and 4.19, no growth occurred under anaerobic or aerobic conditions.

Table 4.16. Enrichment of Sulfate-Reducing Bacteria on Various Carbon Sources Using Naphtha as the Nitrogen Source

Transfer	Amount of Inoculum	Bottle	Carbon Source	Nitrogen Source	Growth 600 nm
First 7/13	2 mL of mixture of sludge, soil and pet. waste samples	Z19	Acetate, Pyruvate, Glucose, Fructose, Lactate, Succinate, Glycerol-1 g/L each	Naphtha (H, 5%)	0.076
Second 7/19	1-2 drops in 50 mL	D1	Glucose (5 g/L)	Naphtha (H, 5%)	0.170
		D2	Glycerol (5 g/L)		0.008
Third 7/23	1-2 drops in 50 mL	Db1	Acetate (5 g/L)	Naphtha (H, 5%)	0.043
		Db2	Fructose (5 g/L)		0.148
		Db3	Glucose (5 g/L)		0.156
		Db4	Glycerol (5 g/L)		0.115
		Db5	Lactate (5 g/L)		0.036
		Db6	Pyruvate (5 g/L)		0.120
		Db7	Succinate (5 g/L)		0.032
Fourth 7/27	1-2 drops in 50 mL	L24	Fructose (5 g/L)	Naphtha (H, 5%)	
		L25	Fructose (5 g/L)	None	
		L26	Glucose (5 g/L)	Naphtha (H, 5%)	
		L27	Glucose (5 g/L)	None	
		L28	Glycerol (5 g/L)	Naphtha (H, 5%)	
		L29	Glycerol (5 g/L)	None	
		L30	Pyruvate (5 g/L)	Naphtha (H, 5%)	
Fourth 8/4	0.5 mL in 50 mL	L31	Pyruvate (5 g/L)	None	
		Db8	Fructose (5 g/L)	Naphtha (H, 2%)	
		Db9	Glucose (5 g/L)		
		Db10	Glycerol (5 g/L)	0.2 mL filtered Vitamin B	
		Db11	Pyruvate (5 g/L)		

H - full range cracked naphtha

Table 4.17. Enrichment of Sulfate-Reducing Bacteria on Various Carbon Sources Using Coal Liquid E as the Nitrogen Source

Transfer	Amount of Inoculum	Bottle	Carbon Source	Nitrogen Source	Growth 600 nm
First 7/13	2 mL of mixture of sludge, soil and pet. waste samples	Z20	Acetate, Pyruvate, Glucose, Fructose, Lactate, Succinate, Glycerol-1 g/L each	Coal Liquid E 5%	0.042
Second 7/23	1-2 drops in 50 mL	ZC1	Pyruvate (5 g/L)	Coal Liquid E 5%	
		ZC2	Glucose (5 g/L)		
		ZC3	Succinate (5 g/L)		
		ZC4	Acetate (5 g/L)		
		ZC5	Glycerol (5 g/L)		
		ZC6	Lactate (5 g/L)		
		ZC7	Fructose (5 g/L)		

Table 4.18. The Use of Sulfate Reducing Bacteria to Remove Nitrogen from Coal Liquids (Anaerobic, HCl Pretreatment)

Bottle No.	Source	Carbon	Growth
CB1	Sludge	Lactate (5g/l)	None
CB2	Sludge	Pyruvate (5g/l)	None
CB3	Sludge	Succinate (5g/l)	None
CB4	Sludge	Glucose (5g/l)	None

Table 4.19. The Use of Sulfate Reducing Bacteria to Remove Nitrogen from Coal Liquids (Aerobic, HCl Pretreatment)

Bottle No.	Source	Carbon	Growth
CB5	Sludge	Glucose (5g/l)	None
CB6	Sludge	Fructose (5g/l)	None
CB7	Murphy's	Glucose (5g/l)	None
CB8	Murphy's	Fructose (5g/l)	None
CB9	Murphy's	Succinate (5g/l)	None

4.4 Nitrogen-Utilizing Aerobes

Three nitrogen-utilizing enrichments were grown aerobically in a nitrogen-free mineral medium with vaporized coal liquid E as the sole nitrogen source and glucose (5 g/L) as the energy source. Many nitrogen-containing heteroatoms such as carbazole, quinoline, benzoquinoline and indole are vaporized at 30°C. Under the microscope, one of the enrichments contains mostly yeast-like microorganisms and the other two enrichments contain rods. Each of the enrichments grew rapidly in the second and third transfer. In the fourth transfer, a small amount of coal liquid E was added to each culture. However, the direct addition of coal liquid E into the culture caused a decline in growth. These cultures were inoculated onto agar plates (1.5% agar in nitrogen-free mineral medium, 5 g/L glucose and coal liquid E as the nitrogen source). Two isolates were picked from the plates and grown with coal liquid E as the nitrogen source and glucose as the energy source. These isolates were not stable. Sometimes they grew on coal liquid and sometimes they failed to grow with coal liquid as the nitrogen source. However, one enrichment was obtained which can grow on the coal liquid.

A number of enrichments were also grown aerobically in nitrogen-free mineral medium with carbazole, quinoline or benzoquinoline as the nitrogen sources. The experiment was performed as follows:

<u>Enrichment</u>	<u>Carbon Source</u>	<u>Nitrogen Source</u>
A	glucose (5 g/L)	carbazole (1 g/L)
B	carbazole (1 g/L)	carbazole (1 g/L)
C	quinoline (1 g/L)	quinoline (1 g/L)
D	glucose (5 g/L)	benzoquinoline (1 g/L)

These enrichments will be monitored with time.

The enrichments were also streaked onto agar plates (1.5% agar in nitrogen-free mineral medium, 5g/L glucose). Coal liquid E or carbazole was added in the lid (plate was placed upside down). The vaporized coal liquid or carbazole was used as the nitrogen source for culture growth. Two indole-utilizing isolates were picked from the agar plates. One is a short rod and the other is a cocci. These cultures are continuing to be transferred with glucose as the energy source and indole as the sole nitrogen source.

An enrichment which can utilize coal liquid E as its sole source of nitrogen has been obtained. The culture survives well in medium containing 0.4 percent coal liquid. Acclimation will continue.

Additional enrichments are being developed on other carbon and nitrogen sources as follows:

<u>Enrichment</u>	<u>Carbon and Energy Source</u>	<u>Nitrogen Source</u>
E	lactate	benzoquinoline
F	succinate	benzoquinoline
G	succinate	carbazole
H	glucose	carbazole
I	lactate	carbazole
J	acetate	carbazole
K	lactate	naphtha (L)

These enrichments were positive for growth after about 1 week of incubation at 30°C. Successive transfers are currently underway.

Most of the cultures with glucose as the carbon source showed the presence of spore forming bacteria as well as small Gram negative rods. The cultures also have the smell of butyric acid, showing that the oxygen was also used up and the glucose was then being fermented. Enrichments with succinate and lactate, which cannot be fermented, yield only non-spore forming bacteria.

4.5 Coal Liquid Degradation in Batch Culture

A summary of all of the culture screening and isolation studies is shown in Table 4.20. Of particular interest is the performance of concentrated cultures of Isolates ERI5 and ERI6. Concentrated ERI5 was able to remove 29 percent of the nitrogen and 21 percent of the sulfur from coal liquid E. Concentrated ERI6 removed 76 percent of the nitrogen and 43 percent of the sulfur. These experiments point out the benefits of concentrating the cultures to maximize biocatalytic activity.

Table 4.20. Nitrogen and Sulfur Reduction by Various Bacterial Cultures in Coal Liquids

<u>Culture/Isolate</u>	<u>Coal Liquid</u>	<u>% Reduction</u>	
		<u>Nitrogen</u>	<u>Sulfur</u>
<i>S. acidocaldarius</i> , 33909	C,D	17.0	0
Isolate ERI4	C,D	26.0	0
Concentrated Mixed culture (<i>P. aeruginosa</i> ; <i>P. putida</i> , 12633; EN-1; EN-2)	E	8.0	0
<i>P. aeruginosa</i> , 27853	E	6.1	0
Enrichment EN-1	E	35.3	14.0
Enrichment EN-1	F	8.4	0
Concentrated EN-1 Enrichment	E	0	31.4
Concentrated EN-1 Enrichment	F	0	16.5
Isolate ERI4	C,D	7.0	23.0
Isolate ERI5	C,D	10.0	0
Enrichment EN-1	F	0	24.0
Concentrated Isolate ERI5	E	29.1	21.4
Concentrated Isolate ERI6	E	76.0	42.9

Coal Liquid C contains 0.46% N, 86.3% C, 0.04% S.
 Coal Liquid D contains 0.45% N, 86.2% C, 0.05% S
 Coal Liquid E contains 0.36%N, 85.15%C, 0.04%S
 Coal Liquid F contains 0.23%N, 82.25%C, 0.03%S

4.6 Degradation of Naphtha as a Model Compound in the CSTR

Naphtha was chosen as a model compound for CSTR sulfur removal studies because of its high sulfur content (1000 ppm) and its similarity to coal liquids as a liquid fuel. Naphtha was inoculated with enrichment EN-1 in the CSTR. The medium used was basal medium supplemented with glucose, trypticase and yeast extract. Air was continuously sparged into the reactor containing 5 percent naphtha in the inoculated medium. A low-temperature condenser was installed in the air outlet to prevent air entrained naphtha from leaving the system. Samples were removed from both the aqueous and organic phases with time and analyzed for elemental sulfur (organic phase) and sulfate (aqueous phase).

Plots of SO_4^{2-} production in the aqueous phase and elemental sulfur concentration in the organic phase are shown in Figures 4.4 and 4.5. As is noted in Figure 4.4, the sulfate concentration increased from 50 to 550 ppm throughout the course of the 350 hr experiment. More importantly, the sulfur content of the organic phase, shown in Figure 4.5, decreased from 1031 ppm to about 700 ppm in just over 50 hr, before holding constant at this level throughout the duration of the experiment.

Figure 4.4 Sulfate Production in the Aqueous Phase During Naphtha Desulfurization by EN-1 in the CSTR

Figure 4.5 Elemental Sulfur Concentration in Naphtha During Desulfurization by EN-1 in the CSTR

Figures 4.6 and 4.7 show the elemental sulfur content of the two model compounds, full range cracked naphtha and heavy straight run naphtha, as a function of time of inoculation, again using enrichment EN-1. The initial O.D. in the full-range naphtha study was 0.1 and the initial O.D. in the heavy straight run naphtha was concentrated to 2.1. As noted in Figure 4.6, the sulfur content of the full range naphtha was not altered by incubation with Enrichment EN-1 during 250 hr of incubation. The sulfur content of the heavy straight run naphtha was decreased by about 20 percent in 60 hr (see Figure 4.7). These results demonstrate the importance of high cell concentration during biological heteroatom removal, but may also signal that only some of the sulfur compounds in naphtha are being removed during biological treatment.

Figure 4.6 Sulfur Removal from Full Range Naphtha Using Enrichment EN-1 in the CSTR

Figure 4.7 Sulfur Removal from Heavy Straight Run Naphtha Using Enrichment EN-1 in the CSTR

4.7 Coal Liquid Degradation in Continuous Culture

4.7.1 The Use of Modified Gas Wash Bottles

Modified gas wash bottles were also used as stirred tank reactors for heteroatom removal from coal liquids. Since the coal liquids are less volatile than naphtha, a cold condenser was not needed. Air was continuously sparged through the liquid containing a concentrated inoculum (6-8X) of Enrichment EN-1 in mineral medium. The pH was maintained at 6.6 and the temperature was held constant at 37°C. Samples were removed from the reactors after approximately 2 weeks of incubation and analyzed for elemental nitrogen and sulfur content. As is noted in Table 4.21, Enrichment EN-1 concentrated 8 times resulted in a nitrogen reduction of 13 percent and a sulfur reduction of 6 percent. In a related experiment, Isolate EN-1 concentrated 6 times resulted in a nitrogen reduction of 19 percent and a sulfur reduction of 27 percent. These results are better than previous batch results with Isolate EN-1, indicating that continuous air introduction enhances heteroatom removal.

Table 4.21. Nitrogen and Sulfur Removal from Coal Liquid F in the CSTR Using Concentrated Enrichment EN-1

<u>Concentration</u>	<u>% Reduction</u>	
	<u>Nitrogen</u>	<u>Sulfur</u>
8X	13.3	5.8
6X	18.8	27.3

Additional experiments were performed with cultures ERI5, ERI6 and EN-1 were used in these studies. The procedures used in these experiments were as reported previously. Concentrated cultures (OD = 1.7-2.4) and a 2 percent coal liquid concentration were employed in the experiments. The medium for cultures ERI5 (a pyridine-growing isolate) and ERI6 (an aniline-growing isolate) was a mineral medium without ammonia. The medium for Isolate EN-1 was a basal medium containing trypticase, yeast extract and glucose. All experiments were performed with agitation at 300 rpm.

Tables 4.22 - 4.24 show results of the heteroatom removal experiments using the three cultures. As is noted in Table 4.22, culture ERI5 removed 28 percent of the nitrogen present in coal liquid F in 10 days. Sulfur removal was not observed. Culture ERI6 reduced the nitrogen content by 20.5 percent in 17 days (see Table 4.23). Finally, Isolate EN-1 was shown to be ineffective in removing nitrogen or sulfur heteroatom compounds from coal liquid.

Table 4.22. Nitrogen and Sulfur Removal from Coal Liquid F by Culture ERI5 in Gas Wash Bottles with Continuous Air Flow

<u>Time (days)</u>	<u>Sample</u>	<u>N (%)</u>	<u>S (%)</u>
5	Control	0.3549	0.0536
	ERI5	0.3550	0.0677
10	Control	0.3794	0.0610
	ERI5	0.2730	0.0666
17	Control	0.3908	0.0629
	ERI5	0.3268	0.0734

**Table 4.23. Nitrogen and Sulfur Removal from Coal Liquid F
by Culture ERI6 in Gas Wash Bottles with Continuous Air Flow**

<u>Time (days)</u>	<u>Sample</u>	<u>N (%)</u>	<u>S (%)</u>
5	Control	0.3549	0.0569
	ERI6	0.4194	0.0821
10	Control	0.3794	0.0610
	ERI6	0.3754	0.0668
17	Control	0.3908	0.0629
	ERI6	0.3106	0.0648

**Table 4.24 Nitrogen and Sulfur Removal from Coal Liquid F
by Isolate EN-1 in Gas Wash Bottles with Continuous Air Flow**

<u>Time (days)</u>	<u>Sample</u>	<u>N (%)</u>	<u>S (%)</u>
0	Control	0.3383	0.0536
	EN-1	0.3070	0.0500
5	Control	0.3549	0.0569
	EN-1	0.3579	0.0580
10	Control	0.3794	0.0610
	EN-1	0.3546	0.0660
17	Control	0.3908	0.0629
	EN-1	0.3723	0.0570

Three additional experiments were performed in gas wash bottle reactors with continuous gas flow. The wash bottles used in previous experiments have been replaced by larger volume reactors in order to permit more frequent sampling. The procedures used in the experiments were as reported previously. The cultures used were Isolate ERI6 (an aniline-growing isolate), Isolate EN-1 and an anaerobic isolate previously subjected to a continuous feed of coal liquids over a period of one month (see May, 1993 monthly report). The coal liquid was sample F which contains 4500 ppm nitrogen and 500 ppm sulfur. The medium in each experiment was a basal medium supplemented with trypticase, yeast extract and glucose. All experiments were performed at 37°C and an initial pH of 6.6 with continuous gas bubbling. Air was used as the gas phase for isolates ERI6 and EN-1, and N₂ was used as the gas phase for the anaerobic isolate. The initial optical densities were 0.5, 3.0 and 0.02 for the ERI6, EN-1 and anaerobic enrichments, respectively.

Figures 4.8 and 4.9 show sulfur and nitrogen utilization results, respectively, for the three isolates in comparison to a control without culture. As is noted in Figure 4.8, Isolate EN-1 was the most promising in removing sulfur from the coal liquid. The sulfur content decreased about 10 percent during the course of the experiment, and contained, on the average, about 17 percent less sulfur than the control. Isolate ERI6 contained about 10 percent less sulfur than the control, and the

anaerobic isolate showed little change from the control. As is noted in Figure 4.9, each of the isolates had only 3-5 percent less nitrogen than the controls. Also, the experiments with cultures actually showed an increase in nitrogen during the latter stages of the experiment, perhaps due to cell death.

4.7.2 Enrichment of Sewage Sludge Culture

Figures 4.10 and 4.11 show experimental results from culture enrichment of a sewage sludge culture using aerobic conditions, and Figures 4.12 and 4.13 show experimental results from anaerobic conditions. As is noted in Figure 4.10, the optical density under aerobic conditions fell with operating time, possibly indicating that culture selection for the coal liquid compounds was taking place. The pH was rather high due to death of the organisms. Figure 4.11 shows the elemental analysis of the coal liquid with time of inoculation under aerobic conditions. Both nitrogen and sulfur utilization were significant in comparison to control levels. The average sulfur utilization was 6.5 percent and the maximum was 15.8 percent. The average nitrogen utilization was 22.5 percent and the maximum was 46.3 percent.

Figure 4.8 Sulfur Removal from Coal Liquid F in Gas Wash Bottles with Continuous Gas Flow

Figure 4.9 Nitrogen Removal from Coal Liquid F in Gas Wash Bottles with Continuous Gas Flow

Figure 4.10 Aqueous Phase Optical Density and pH Measurements in the Aerobic Continuous Culture System

Figure 4.11 Elemental Analyses of Coal Liquid E Subjected to Continuous Aerobic Conditions

Figure 4.12 Aqueous Phase Optical Density and pH Measurements in the Anaerobic Continuous Culture System

Figure 4.13 Elemental Analyses of Coal Liquid E Subjected to Continuous Anaerobic Conditions

Figures 4.12 and 4.13 show similar results under anaerobic conditions. Anaerobically, the average sulfur utilization was 4.2 percent, while the maximum was 19.8 percent. The average nitrogen utilization was 25 percent and the maximum was 70.3 percent. It is obvious that this technique is very good in isolating and optimizing aerobic and anaerobic cultures for heteroatom removal.

A CSTR was assembled from glassware to study the biological removal of volatile and water soluble nitrogen-containing compounds from coal liquids using the sewage sludge culture. A schematic of the apparatus is shown in Figure 4.14. The reactor has a sampling port to permit routine sampling. Agitation is provided by use of a magnetic stirrer. A pH probe is submerged into the reactor to monitor pH, and, when necessary, the pH is adjusted manually. A thermolamp is installed in the system to control the temperature of the coal liquid prior to feeding. Oxygen is continuously fed into the coal liquid holding vessel, from which coal liquid vapor is carried along with the oxygen into the reactor. Fresh medium is mixed with reacted coal and medium, from the reactor, and finally separated from the coal liquid prior to feeding the medium to the reactor. Exhaust gas passes through an absorber where unconsumed coal liquid vapor can be absorbed by water or dilute acid. The apparatus is also designed so that unconsumed or unabsorbed coal liquid vapor can be recycled into the nutrient holding vessel or pass through an ice condenser into a receiver. Both the absorber and receiver have sampling ports. The operating conditions in the reactor are as follows:

Reactor Working Volume: 1750 mL
 Reactor Temperature: 29°C
 Oxygen Flow Rate: 44 mL/min
 Medium Flow Rate: 0.4 mL/min
 Heating Temperature (to vaporize coal liquid): 55°C
 pH: 7.0±0.05
 Agitation: brisk
 Medium Composition (in 1000 mL):

salt C (w/o NH ₄ ⁺)	8 ml	
PFN		3 ml
glucose	4 g	

Inoculation: About 10 g of sludge collected from Springdale, AR was extracted with 100 mL of deionized water. The water extracts were then inoculated into the CSTR reactor.

Maximum growth to date: 0.52 g/L (O.D. = 1.86)

Results from an experiment with the sludge mixture are shown in Table 4.25. The data show that about 50 percent of the nitrogen-containing components have been extracted (.44 reduced to <.26% N in coal liquids). It is known that the medium at pH 7, 4 or 3 supports the growth of a good population of bacteria (about 10⁸ bacteria/mL). These microbes appear to mainly belong to the *Pseudomonas* genus upon microscopic examination.

Figure 4.14 Apparatus for Removing Volatile and Water Soluble Nitrogen Containing Compounds from Coal Liquid

Table 4.25. Results from Sludge Isolation Experiments, pH 3

Sample, description	Date Collected	%N
Coal E (<12>)	-----	.44
Coal E, from extractor	5/26	.29
Coal E, from extractor	5/28	.26
Coal E, from extractor	5/30	.23
Waste permeate	5/27	.09
Medium, aqueous	5/27	.08
Aqueous, reactor	5/27	.08
Water gas trap	5/27	.08
Waste permeate	5/28	.08
Medium, aqueous	5/28	.08
Aqueous, reactor	5/28	.08
Water gas trap	5/28	.08
Waste permeate	5/30	.11
Medium, aqueous	5/30	.08
Aqueous, reactor	5/30	.08
Water gas trap	5/30	.08

4.7.3 Heteroatom Removal in Continuous Flow Reactors

Three cultures were selected for study in flow type CSTRs for the removal of S-, N- and O-containing elements from coal liquids. A schematic of the experimental apparatus is shown in Figure 4.15. The apparatus includes a reactor (400 ml), a fresh nutrient container and a waste holding vessel. Fresh coal liquid is directly fed along with the required nutrients into the reactor. Reactor agitation is controlled by a magnetic stirrer. A pump controls the fresh nutrient and coal liquid flow rate. Fermented broth with treated coal liquid overflows into a waste holding vessel. A sample port mounted on the reactor allows periodic sampling of the reactor contents for sulfur, oxygen and hydrogen.

The CSTR was used to test three cultures for their ability to remove heteroatom compounds from coal liquids in continuous culture:

- Isolate Mix M, an aerobic bacterial mixture which grows in rich medium
- ERI5, a pyridine degrading bacterium, which removes nitrogen compounds from coal liquids
- ERI6, an aniline degrading microorganism, which removes amines from coal liquids

The bacterial mixture Mix M was inoculated into the CSTR containing rich medium. In previous results, this bacterial mix was shown to remove 30 percent of the nitrogen from the model compound naphtha. The conditions were as follows:

Figure 4.15 Schematic of CSTR System for Treating Coal Liquids

Liquid volume: 400 mL
 Coal liquid addition: 2 mL every other day (1 mL/d)
 Liquid flow rate: 4 mL/hour
 O₂ flow rate: continuous flow
 Temperature: 30°C
 Agitation rate: 200 rpm

Prior to addition, 20 mL of coal liquid E was extracted with 80 mL of 1N HCl for 24 hr as a pretreatment. The reactor was run over a period of several months. Growth was adequate, indicating that the coal liquid in this concentration was not toxic. The elemental analysis data of Table 4.26 were obtained at the conclusion of the experiment. As is noted, the nitrogen concentration dropped by 17.5 percent with bacterial treatment.

Table 4.26. Elemental Analysis of Coal Liquid E After Treatment with Mix M

	N(%)	C(%)	S(%)
Coal Liquid E	.44	85	.05
Medium Extracted Coal Liquid	.40	85	.05
Bacteria Treated Coal Liquid	.33	85	.05
1N HCl Extract*	.23	.70	0

* 20 mL of coal liquid extracted with 80 mL of 1N HCl for 24 hr

ERI5, a pyridine-utilizing bacterium previously isolated from chemical waste samples, was inoculated into a CSTR with pyridine as the sole carbon and nitrogen source. The CSTR conditions were as follows:

reactor volume: 400 mL
 liquid flow rate: 30-60 mL/day
 O₂ flow rate: continuous
 temperature: 30°C
 agitation rate: 200 rpm

The culture grew rapidly on pyridine, reaching an optical density of 1.0 within three days before gradually dropping to 0.65. Once the culture was stable, coal liquid E-extracted medium was used to replace the pyridine medium.

The coal liquid E-extracted medium was prepared by mixing 1.0N HCl with coal liquid E (20%, v/v) for 24 hours, followed by the removal of the coal liquid. Elemental analysis indicated that the coal liquid E-extracted solution contained 231 ppm nitrogen and 698 ppm carbon. The coal liquid E-extracted solution (500 ml) was added to mineral medium (500 ml). The solution pH was adjusted to 6.5 with NaOH.

Figure 4.16 shows optical density readings from the CSTR with time. The medium was shifted to coal liquid E-extracted medium after 18 days of operation. Immediately after the medium shift, the culture OD significantly increased up to 1.35. Under the microscope, a lot of small motile rods were observed. Ten days after the medium shift, the cell optical density had dropped to 0.55. With time, the bacterial OD dropped further, indicating that the coal liquid E-extracted medium hardly supported bacterial growth.

Figure 4.16 Bacterial Growth of Isolate ERI5 on Coal Liquid E-Extracted Medium

After 20 days of operation the cell OD had decreased to 0.17. The liquid dilution rate was slowed from 0.15 day^{-1} to 0.075 day^{-1} . After the change in liquid dilution rate, the cell OD started to increase and was finally stable at 0.35. Microscopic examination showed a fair number of microorganisms, with small rods the predominant species.

It is evident that the coal liquid E-extracted medium could only support minimal bacterial growth. Possible reasons for this include:

1. Low concentration of carbon substrates
2. Slow utilization of carbon-containing substrates

Based on the experimental results, the addition of alternative carbon sources such as glucose and phenol could be a key in improving bacterial growth.

Consistent with previous work, ERI5 was cultured in the CSTR with coal liquid E-extracted medium. Glucose and phenol were added as an extra energy source to stimulate bacterial growth. Bacterial optical density was again used as the indication of cell growth. The same CSTR conditions as described above were utilized.

Glucose (2 g/l) was added to the culture as a carbon and energy source after 68 days of operation, and continuously added for 10 days. As is noted in Figure 4.17, little change in bacterial optical density was observed. Under the microscope, there was no significant change in cell numbers. Phenol (0.5 g/L) was then added on the 78th day. No change in cell density was detected with this

addition either. Based on the results, there are two possible explanations for the behavior: (1) ERI5 may not use glucose or phenol as energy and carbon sources, or (2) the available nitrogen in the coal liquid E-extracted medium is limiting.

Isolate ERI6, an aniline utilizing bacterium previously isolated from chemical waste, was also utilized in removing nitrogen from medium containing waste-extracted Wilsonville coal liquid (coal liquid E) in the CSTR. The isolate was inoculated into a CSTR with aniline as the sole carbon and nitrogen sources. After the culture had grown and was stable, the aniline medium was replaced with a coal liquid E-extracted medium. The addition of glucose was also tested to see if an extra energy source would stimulate bacterial growth. The medium containing water extracted coal liquid E was prepared as before and the CSTR conditions were as outlined for Isolate ERI5.

As is noted in Figure 4.18, ERI6 grew rapidly with aniline as its sole carbon and nitrogen source. The cell optical density reached 1.0 within five days of incubation. The culture O.D. remained stable at 0.95, and under a microscope the culture looked healthy and very motile. When coal liquid E extracted medium replaced the aniline medium after nine days, the culture optical density gradually decreased. By the 26th day, the culture O.D. was 0.35 and remained stable at this level. Glucose (2 g/L) was added on the 28th day with little effect observed. The culture pH was relatively constant (6.5-7.0) during the fermentation, indicating little acid production. Since ERI6 was known to be able to use glucose for growth in the presence of ammonia, it is likely that the coal liquid E-extracted medium contains a very limited amount of nitrogen available for ERI6 growth.

In order to clarify the total nitrogen contents in the coal liquid E-extracted medium, a total nitrogen analysis was conducted in a series of coal liquid E-extracted solutions with the elemental analyzer. Table 4.27 shows the results from this study. Based on the results, 24 percent of the nitrogen is extracted from coal liquid E if the coal liquid to water ratio is 1:5 in 1.0N HCl solution. The nitrogen in coal liquid E-extracted water is 457 ppm and thus there is 228 ppm N in the medium. If all nitrogen in the medium is available for bacterial growth, 1628 mg/L cell mass should be produced. However, there is only 110 mg/L of cell mass in the reactor and this big discrepancy is best explained by assuming that most of the extracted nitrogen in the medium is unavailable to bacterial utilization.

Table 4.27. Elemental Nitrogen Analysis of Coal Liquid and Extract Water

No.	Analysis of	Coal Liquid/Water	HCl Conc (N)	N (ppm)
1	Extracted Water	1:5	1	457
2	Extracted Coal Liquid E	1:5	1	5718
3	Extracted Water	1:2	1	629
4	Extracted Coal Liquid E	1:2	1	5597
5	Extracted Water	1:10	1	830
6	Extracted Coal Liquid E	1:10	1	5883
7	Extracted Water	1:5	2	607
8	Extracted Coal Liquid E	1:5	2	5598
9	Extracted Water	1:5	0.5	618
10	Extracted Coal Liquid E	1:5	0.5	5479
11	Water			384
12	Coal Liquid E			7482

Figure 4.17 ERI5 Growth in Coal Liquid E-Extracted Medium With Glucose and Phenol Additions

Figure 4.18 Growth of ERI6 in the CSTR with Coal Liquid E-Extracted Medium

5.0 ECONOMIC PROJECTIONS

A preliminary economic evaluation has been prepared for the biological removal of nitrogen heteroatom compounds from coal liquids. The process is based on acid extraction of the coal liquid to remove nitrogen compounds prior to fermentation by Isolates ERI5 or ERI6. This process was chosen from among the various technologies developed at BRI because of its reliability and maturity. Sulfur removal, if desired, utilizes similar technology.

5.1 Process Description

A process for the biological removal of the heteroatom compounds is shown in Figure 5.1. Liquefied coal is first extracted using a 2:1 by volume ratio of 1 N HCl to remove 25 percent of the total nitrogen contained in the coal liquid. The extracted aqueous solution is then neutralized with sodium carbonate to pH 6.5 prior to fermentation. A liquid retention time of 11 days is used in the fermenter. Air is continuously sparged through the culture to ensure aerobic conditions for culture growth. After fermentation, the cells and water are separated in a hollow fiber system. The water is reused in preparing the 1 N HCl solution for coal liquid extraction.

5.2 Design Basis and Principles

The design is based on handling 100 tons of coal liquid per day. The coal liquid is assumed to have a density of 0.8 and the same physical properties of Wilsonville coal liquid E.

The 1 N HCl preparation tank was sized by assuming a 10 min liquid retention time. A 500 gallon jacketed, agitated, and closed (50 psi) stainless steel tank was selected. The heat of dilution in preparing 1 N HCl from 38 percent HCl was estimated at 177,700 Btu/hr. The cooling water flow rate is 4270 gph, and obtains a 5°C increase in temperature prior to discharge. The cooling water pump size is 3 hp, the size of the pump required for transferring HCl solution to the extractor is 3 hp and the coal liquid pump is 1 hp.

The extraction column was sized based on the assumption of a 30 min total liquid retention time. The column diameter was selected so that the height of the column did not exceed 100 ft. The size of the column is 2 ft in diameter and 90 ft in height. The tray spacing is set at 36 in.

The neutralization tank has a 15 min liquid retention time. A 750 gal, 50 psi, agitated, stainless steel tank was used. The heat of reaction was estimated as 1.87 million Btu/hr. The cooling-water requirement is 45,000 gph and the cooling water pump size is about 29 hp. Nutrients were also added to the aqueous solution in this neutralization tank. The solution was pumped through a heat exchanger to be heated to 38°C. The temperature increase was 13°C, the overall heat transfer coefficient is 100 Btu/ft² °F, and the temperature difference is 122.9 °F. The surface area is 50 ft². The 3 hp pump should be sufficient to send the aqueous solution through the heat exchanger and to the reactor.

The fermenter was based on an 11 day liquid retention time. The culture volume is 80 percent of the total reactor volume. The agitation power was set at 0.1 hp/m³. The maximum reactor size was set at 500,000 gal. The system thus requires two 500,000 gal stainless steel reactors, with each reactor requiring a 200 hp agitator. A blower system supplies air at a retention time of 1 hr and a pressure increase of 10 psi. The blower requires 75 hp per reactor. A hollow fiber unit is used to recover 90 percent of the water from culture bleed to be reused in HCl preparation. The hollow fiber filter has a permeate flow rate of 41.3 gpm. A pressure of 60 psi is required to operate the hollow fiber filter. Thus, the culture bleed pump prior to the hollow fiber is 3 hp. The permeate recycle pump was estimated at 1 hp, and a 1 hp water make-up pump is required to pump 25,000 L/day.

5.3 Process Evaluation

The equipment list and delivered prices are shown in Table 5.1. The prices are based on the information from Garrett (1989), except for the market price for the hollow fiber unit. The fixed capital investment calculation was based on the method of Chilton, and a factor of 3 on the delivered equipment cost was used. The total fixed capital investment was \$3.2 million. A summary of utility usage is shown in Table 5.2. Electricity for the fermenters and compressed air was the major required source of energy.

The economic evaluation for treating 100 tons/day of liquified coal is shown in Table 5.3. The chemical costs are based on data from the September, 1994 Chemical Marketing Report. The utility cost estimates were \$0.05/kwhr for electricity, \$4.00/MM Btu for steam, and \$0.05/M gal for cooling water. The treatment cost was \$6,042,000 annually or \$0.09 per lb of liquified coal. The major cost is from the raw materials which are about 79 percent of the total cost. On a per lb basis, \$0.07 of the cost was from raw materials and utilities. This means that an increase in the size of the plant will not reduce the production costs.

Figure 5.1 Schematic of Coal Liquid Treatment Process

Table 5.1 Equipment List for Heteroatom Removal

Item, Number of Units	Capacity/Unit	Delivered Cost, \$
1 N HCl Preparation Tank, 1	500 gal	26,000
Cooling Water Pump, 1	3 hp	2,000
Permeate Recycle Pump, 1	1 hp	2,000
Water Make Up Pump, 1	1 hp	2,000
38% HCl Pump, 1	1 hp	2,000
1 N HCl Feed Pump, 1	3 hp	4,000
Coal Liquid Feed Pump, 1	1 hp	2,000
Extraction Column, 1	2 ft X 90 ft	92,000
Trays, 30	2 ft	21,000
Coal Liquid Product Pump, 1	1 hp	2,000
Extracted HCl Pump, 1	3 hp	4,000
Neutralization Tank, 1	750 gal	32,000
Cooling Water Pump, 1	29 hp	6,000
Medium Pump, 1	3 hp	4,000
Heat Exchanger, spiral plate, 1	50 ft ²	9,000
Fermenters, 2	500,000 gal	500,000
Agitators, 2	200 hp	276,000
Hollow Fiber Filter, 1	42 gpm/25 ft ²	30,000
Culture Bleed Pump, 1	3 hp	4,000
10 psi Blower, 2	75 hp/1114 sfm	34,000
Total Delivered Equipment Cost		1,054,000
Fixed Capital Investment		3,162,000

Table 5.2 Summary of Utility Requirements

Section	Electricity, kwhr	Steam, MM Btu/hr	Cooling Water, M gal/hr
Extraction	10.44	0.0	4.27
Neutralization	23.86	0.615	45.00
Fermentation	412.37	0.0	0.0
Total	446.67	0.615	49.27

Table 5.3 Economic Evaluation

Item	Annual Cost, \$/yr
Operating Costs	
Raw Material	
38% HCl, 24 tons/day, \$75/ton	630,000
Na ₂ CO ₃ , 29 tons/day, \$381/ton	3,888,000
Process Water, 6,600 gal/day, \$0.2/M gal	500
Medium, \$30/hr	252,000
Total Raw Material Costs	4,771,000
Utilities	
Electricity, \$0.05/kwhr	188,000
Steam, \$4.00/MM Btu	21,000
Cooling Water, \$0.05/M gal	21,000
Total Utilities Cost	230,000
Labor, 4.2 Shift, 2 people per shift, \$30,000	252,000
Supervision & Overhead = Labor	252,000
Maintenance, 5% FCI	158,000
Insurance, 2% FCI	63,000
Total Operating Costs	5,726,000
Depreciation, 10% FCI	316,000
Total Cost	6,042,000
Product, 100 tons/day	35,000 tons/yr
Product Cost	\$0.09/lb

6.0 CONCLUSIONS

Several pure cultures and isolates have been obtained which are capable of removing sulfur and nitrogen from model compounds and coal liquids. The conditions for heteroatom removal by these cultures have been determined and optimized. Enrichments ERI5 and EN-1 are particularly promising for this application, with the ability to remove nearly 40 percent of the nitrogen and 30 percent of the sulfur in batch culture. Denitrifying bacteria are also promising since they utilize the sulfur in naphtha and coal liquids as their sole source of sulfur under anaerobic conditions. Continuous culture experiments showed that the coal liquid is too toxic to the bacteria to be fed without dilution or extraction. Thus either semi-batch operation must be employed with continuous gas sparging into a batch of liquid, or acid extracted coal liquid must be employed in continuous reactor studies with continuous liquid flow. Isolate EN-1, a chemical waste isolate, removed 27 percent of the sulfur and 19 percent of the nitrogen in fed batch experiments. Isolate ERI5 removed 28 percent of the nitrogen in coal liquid in 10 days in fed batch culture. The sewage sludge isolate removed 22.5 percent of the sulfur and 6.5 percent of the nitrogen from extracted coal liquid in continuous culture, and Mix M removed 17.5 percent of the nitrogen from medium containing extracted coal liquid.

An economic evaluation has been prepared for the removal of nitrogen heteroatom compounds from Wilsonville coal liquid using acid extraction followed by fermentation. Similar technology can be developed for sulfur removal. The evaluation indicates that the nitrogen heteroatom compounds can be removed for \$0.09/lb of coal liquid treated.

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APPENDIX

GC-MS Analysis of Full Range Cracked Naphtha and Heavy Straight Run Naphtha

CORE LABORATORIES

LABORATORY TESTS RESULTS

06/24/93

NUMBER: 932627

CUSTOMER: TEXACO INC

ATTN: JAMES MUEHA

NT I.D.:
SAMPLED: / /
SAMPLED:
DESCRIPTION: 291-92-0627-000

Full range FC naphtha

LABORATORY I.D.: 932627-0001

DATE RECEIVED: 06/21/93

TIME RECEIVED: 12:01

REMARKS:

Total S = 0.1499 wt%

ANALYZED BY: CHELLINGHOUSE DATE ANALYZED: 06/24/93 TIME ANALYZED: 11:14 METHOD: GC/SEC DILUTION:

DESCRIPTION	FINAL TEST RESULT	UNITS OF MEASURE	DETECTION LIMIT	TECHNICIAN INITIALS
ropan Sulfide	<1	ppm wt sulfur	1	
bonyl Sulfide	2	ppm wt sulfur	1	
fur dioxide	<1	ppm wt sulfur	1	
bon Disulfide	<1	ppm wt sulfur	1	
hyl Mercaptan	<1	ppm wt sulfur	1	
yl Mercaptan	1	ppm wt sulfur	1	
propyl Mercaptan	1	ppm wt sulfur	1	
ropyl Mercaptan	1	ppm wt sulfur	1	
t-Butyl mercaptan	<1	ppm wt sulfur	1	
-Butyl mercaptan	<1	ppm wt sulfur	1	
butyl Mercaptan	<1	ppm wt sulfur	1	
utyl Mercaptan	1	ppm wt sulfur	1	
hyl Sulfide	<1	ppm wt sulfur	1	
yl Methyl Sulfide	<1	ppm wt sulfur	1	
yl Sulfide	<1	ppm wt sulfur	1	
hyl Disulfide	<1	ppm wt sulfur	1	
yl Methyl Disulfide	1	ppm wt sulfur	1	
yl Disulfide	1	ppm wt sulfur	1	
lophene	37	ppm wt sulfur	1	
tra-Hydro Thiophene	16	ppm wt sulfur	1	
Methyl Thiophene	75	ppm wt sulfur	1	
Methyl Thiophene	101	ppm wt sulfur	1	
Ethyl Thiophene	39	ppm wt sulfur	1	
Ethyl Thiophene	63	ppm wt sulfur	1	
nzothiophene	273	ppm wt sulfur	1	
thyl benzothiophenes	359	ppm wt sulfur	1	
methyl benzothiophenes	82	ppm wt sulfur	1	
identified sulfur compounds	510	ppm wt sulfur	0	

Low b.p.

P O BOX 34262
HOUSTON, TX 77234-4262
(713) 943-9776

A
BWS

CORE LABORATORIES

LABORATORY TESTS RESULTS

06/24/93

LABORATORY ID: 932827

CUSTOMER: TEXACO, INC.

ATTN: JAMES HUDRA

LABORATORY I.D.:
SAMPLED: / /
SAMPLED:
DESCRIPTION: 291-91-0617-000

Heavy SR Naptha

LABORATORY I.D.: 932827-0002
DATE RECEIVED: 06/21/93
TIME RECEIVED: 12:01
REMARKS:

Total S = 0.1084 wt %

ANALYSIS BY: Chemiluminescence DATE ANALYZED: 06/24/93 TIME ANALYZED: 11:14 METHOD: DC/SCD DILUTION:

DESCRIPTION	FINAL TEST RESULT	UNITS OF MEASURE	DETECTION LIMIT	TECHNICIAN INITIALS
Open Sulfide	2	ppm wt sulfur	1	
Thiophene Sulfide	2	ppm wt sulfur	1	
Sulfur Dioxide	<1	ppm wt sulfur	1	
Carbon Disulfide	<1	ppm wt sulfur	1	
Ethyl Mercaptan	<1	ppm wt sulfur	1	
Propyl Mercaptan	<1	ppm wt sulfur	1	
Isopropyl Mercaptan	<1	ppm wt sulfur	1	
tert-Butyl mercaptan	<1	ppm wt sulfur	1	
sec-Butyl mercaptan	<1	ppm wt sulfur	1	
Isobutyl mercaptan	<1	ppm wt sulfur	1	
Isopentyl mercaptan	<1	ppm wt sulfur	1	
Isopentyl Sulfide	<1	ppm wt sulfur	1	
Isopentyl Methyl Sulfide	<1	ppm wt sulfur	1	
Isopentyl Sulfide	<1	ppm wt sulfur	1	
Isopentyl Disulfide	<1	ppm wt sulfur	1	
Isopentyl Methyl Disulfide	<1	ppm wt sulfur	1	
Isopentyl Disulfide	4	ppm wt sulfur	1	
Isopentene	<1	ppm wt sulfur	1	
Isopentyl Hydro Thiophene	<1	ppm wt sulfur	1	
Isopentyl Thiophene	<1	ppm wt sulfur	1	
Isopentyl Thiophene	<1	ppm wt sulfur	1	
Isopentyl Thiophene	<1	ppm wt sulfur	1	
Isopentyl Thiophene	<1	ppm wt sulfur	1	
Isopentyl Thiophene	2	ppm wt sulfur	1	
Identified Sulfur Compounds	310	ppm wt sulfur	0	

High b.p.

P O BOX 34282
HOUSTON, TX 77234-4282
(713) 943-9776

A. Aug

unidentifiable
= 318