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Coal Bioprocessing Research at the Institute of Gas Technology

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ABSTRACT

Coal bioprocessing research at the Institute of Gas Technology (IGT) has included solubilization, gasification, desulfurization, denitrogenation, production of specialty chemicals, and the remediation of organic and inorganic wastes associated with coal utilization. Currently, research is focused on desulfurization and remediation. Desulfurization research concerns the development of processes to remove organic sulfur or to convert a portion of pyritic sulfur to sulfuric acid rapidly, thereby serving as a pretreatment to aid the thermochemical conversion of coal to coke and liquid products. The removal of as much as 91% organic sulfur from coal has been achieved, and biodesulfurization of coal has been confirmed by seven analytical techniques performed in six different laboratories. Recent studies have involved the use of molecular genetics to develop strains of bacteria with higher levels of desulfurization activity, and the development of methods for the preparation, storage, and utilization of biodesulfurization catalysts. Remediation studies include the development of *in situ* and on-site technologies to treat soil contaminated with coal tar, the leaching of metals from fly ash, and the treatment of waste water resulting from fly ash leaching or from acidic mine drainage (AMD). IGT currently has two projects in EPA's SITE program concerned with the remediation of coal tar-contaminated soil, and other related technologies are being developed. Efficient laboratory-scale processes for the removal of metals from fly ash and from soil so that the solids pass EPA's TCLP test, and the subsequent treatment of the leachates or AMD to meet all regulatory requirements have been developed. Data obtained in these projects are presented in particular, and a general discussion of the application of biotechnology to coal is offered.

Index Entries: Coal bioprocessing; desulfurization of coal; Institute of Gas Technology; gasification of coal; SITE program; bioremediation.

INTRODUCTION

Coal accounts for well over half of all electric power generation in the US, and steady increases in coal utilization worldwide are forecast for the next decade and beyond (*1*). Nonetheless, coal utilization, particularly in the US is facing strong challenges, and the health of the US coal industry is by no means assured (*2*). The recently released National Energy Policy contains almost no mention of coal, even though coal is America's most abundant fossil fuel (*1*). The chief problems associated with coal are environmental issues, such as particulate emissions, acid rain resulting in part from sulfur and nitrogen oxides in combustion gases, air toxins, such as heavy metals, carbon dioxide emissions contributing to the greenhouse effect, acidic metal-laden drainage from pyritic mine tailings, and similar problems. There are also problems associated with coal because of its nature as a solid in contrast to other energy sources that are liquids and gases. The physical removal of coal from geological deposits by strip-mining or subsurface mining is required in contrast to the less costly and less invasive/environmentally sensitive pumping technologies that are appropriate for harvesting and distributing liquid and gaseous fuels. The coal industry, therefore, must address the issues of land reclamation and subsidence where these issues are comparatively nonexistent in the gas and petroleum industries. For these reasons, there is a need for new technologies to recover and process coal.

Bioprocesses have the potential to address efficiently and effectively many of the environmental issues associated with coal and to convert coal to alternative products. Coal bioprocessing includes such topics as pyrite removal, organic sulfur removal, ash/metals removal, solubilization, gasification, acid pretreatment for thermal gasification, chemical production, metal recovery/ash stabilization, treatment of acidic mine drainage, remediation of coal tar-contaminated soil, and elucidation of the chemical structure of coal. These topics have been investigated in numerous laboratories in the US and worldwide (*2*). The purpose of this article is not to review the entire field of coal bioprocessing research, but rather to summarize coal bioprocessing research at the Institute of Gas Technology (IGT) with a focus on current research topics.

SELECTED RESULTS OF COAL BIOPROCESSING RESEARCH

IGT has been involved to varying degrees with virtually every aspect of coal bioprocessing research; however, current research focuses on the removal of organic sulfur from coal, remediation of coal tar-contaminated soil, and stabilization/metal recovery of fly ash. Previous work at IGT documented the ability of various species of fungi and bacteria to solubilize coal, particularly oxidized and low-rank coals (3,4). The mechanisms of coal solubilization were shown by IGT and numerous other researchers to be the production of alkalinity, and the removal of divalent cations chiefly by the production of the metal chelating agent, oxalic acid. There is also an enzymatic component to the solubilization of coal by some microorganisms (5,6). Coal biogasification research at IGT examined the direct conversion of coal to methane, the gasification of biologically or chemically solubilized coal products, and a two-step conversion in which microorganisms were grown aerobically with coal as the sole source of carbon. Subsequently, the produced biomass and soluble products were anaerobically digested and converted to methane. No convincing evidence for the direct conversion of coal to methane was obtained, but soluble products and coal-derived biomass readily responded to biogasification. However, coal solubilization could not achieve quantitative conversion, particularly of higher-rank coals, and only minor percentages of coal could be converted to methane. Consequently, research concerning coal solubilization and coal biogasification has been terminated at IGT.

The microbiological removal of nitrogen from coal and the bioconversion of coal to specialty chemicals was briefly examined at IGT. In-depth research on either topic has not yet been performed, nor are these topics being currently explored. An existing microbial culture(s) capable of selectively cleaving carbon–nitrogen bonds has not been identified, but an appropriate culture could presumably be developed. The direct conversion of coal to specialty chemicals is hampered by the lack of a clearly defined product(s) that is desired and the likelihood of low yields. The microbiologically mediated production of chemicals, such as ethanol and methane from coal-derived synthesis gas, is a more promising approach that has even been developed by other researchers to a degree approaching commercialization (7). Although IGT is not currently engaged in coal biogasification research or the direct conversion of coal to specialty chemicals, the bioconversion of methane to specialty chemicals, particularly stereospecific chemicals, is a topic of current research (8). However, since that project is not directly related to coal bioprocessing, it will not be discussed further here.

Coal Desulfurization

The precombustion removal of organic sulfur from coal is a key topic of current coal bioprocessing research at IGT. Effective technologies are in place to remove sulfur after or during combustion, but no technology for the removal of sulfur prior to combustion is currently available that can meet the Clean Air Act standards. Sulfur exists in coal in two basic forms: organic and inorganic. There are physical, chemical, and microbiological technologies that can effectively remove inorganic sulfur from coal prior to combustion; however, the removal of organic sulfur remains problematic.

If sulfur could be effectively removed from coal prior to combustion:

1. The market for coal would expand because desulfurized coal would not require the use of flue gas scrubbers or other desulfurization equipment that is currently impractical and too expensive for intermediate- to small-volume users of coal;
2. Coal users would have no sulfur wastes to dispose of; and
3. Regulatory agencies could monitor the removal and correct disposal of sulfur at a few coal processing sites, eliminating the need to monitor sulfur emissions or correct disposal of sulfur wastes from the many users of coal.

Biological processes occur under very mild reaction conditions compared to chemical reactions, so it is hoped that if a suitable means of desulfurizing fuels using biological systems can be found, then economically favorable precombustion fuel desulfurization processes will result. *Rhodococcus rhodochrous* IGTS8 appears to be an appropriate microbial culture (*9–14*).

R. rhodochrous IGTS8 has the ability to cleave selectively carbon–sulfur bonds in thiophenes, sulfides, disulfides, mercaptans, sulfoxides, sulfones, and more importantly in coal and in petroleum (*11*). The stability of the desulfurization trait of IGTS8 and the validity of claims that IGTS8 can selectively cleave carbon–sulfur bonds has been confirmed by numerous independent investigators at the University of North Dakota, University of Mississippi, Notre Dame University, Purdue University, University of Georgia, Massachusetts Institute of Technology, Canterbury University (UK), University of Houston, Illinois Institute of Technology, Petrolite Corporation, and Energy BioSystems Corporation (*12–14*). The selective removal of organic sulfur from biotreated coal has been confirmed using several analytical techniques: ASTM sulfur forms analyses, examination of elemental composition/ratios, the electron microbeam technique (*15*), controlled atmosphere programmed temperature oxidation (CAPTO) (*16*), X-ray photoelectron spectroscopy (XPS) (*17*), laser desorption/mass spectroscopy (*18*), and X-ray absorption near edge spectroscopy (XANES) (*19*). These coal analyses were performed by several independent laboratories including IGT, DOE-PETC, Iowa State University, ViroLac Industries, University of Illinois, University of Kentucky, and Huffman Laboratories, Inc. The removal of as much as 91% of the organic sulfur from solid coal by *R. rhodochrous* IGTS8 has been observed (*4,9,20*), and organic sulfur reductions in other biotreated coal samples of 80% have been confirmed by DOE-PETC using XPS analysis (*20*).

Recent research has focused on the biodesulfurization of mild coal gasification liquid products. The mild coal gasification process developed at IGT converts coal to fuel gas (5–19%), oils and tars (13–35%), and char/coke (54–76%) (*21*). The liquid products resulting from mild coal gasification do not compare favorably with petroleum because of relatively high sulfur, aromaticity, and viscosity. Biodesulfurization can potentially be used to upgrade mild coal gasification liquids by addressing all three

Table 1
Characterization of Biodesulfurized Samples of Mild Gasification Liquid

Biodesulfurization catalyst		Time of catalyst storage at $-70°C$, d before testing	Carbon	Hydrogen	Sulfur	BTU/lb	Carbon/sulfur	% Desulfurization[a]
Inactive control	rep 1	0	80.37	8.69	1.17	15,255	68.69	29.1
	rep 2	0	80.65	8.63	1.20		67.21	27.3
No. 1	rep 1	7	73.06	10.63	0.21	15,561	347.90	87.3
	rep 2	7	72.92	10.54	0.23		317.04	86.1
No. 2	rep 1	7	72.09	11.21	0.21	15,564	343.28	87.3
	rep 2	7	71.55	11.03	0.19		376.58	88.5
No. 3	rep 1	30	71.52	10.84	0.28	15,562	255.43	83.1
	rep 2	30	71.54	10.89	0.29		246.68	82.4
No. 4	rep 1	108			1.05			36.4
	rep 2	108			0.83			49.7
No. 5	rep 1	185			0.52			68.5
	rep 2	185			0.58			64.8
Untreated 360–440°F oil			76.79	8.47	1.65	15,105	46.54	

[a] The percent biodesulfurization was calculated by comparing the sulfur values of biotreated samples with the untreated 360–440°F oil. Missing values in the table indicate that insufficient sample was available.

Table 2
Biodesulfurization of Unfractionated Mild Coal Gasification Liquids Using Freeze-Dried Cells: High Oil Loading (1:1 Oil-to-Water Ratio)

Sample	Carbon	Sulfur	Nitrogen	Biodesulfurization
Biotreated 1	77.86	2.14	0.95	25.3%
Biotreated 2	76.25	2.27	0.76	20.8%
Biotreated 3	74.54	2.03	0.90	29.1%
Biotreated 4	76.49	1.90	1.00	33.6%
Biotreated 5	76.27	1.93	0.93	32.6%
Biotreated 6	76.31	2.36	0.84	17.6%
Biotreated 7	76.53	2.24	0.94	21.8%
Biotreated 8	76.85	1.75	0.91	39.0%
Untreated	79.36	2.85	0.82	—
Untreated	78.97	2.88	0.95	—

issues. As shown in Table 1, as much as 88% removal of sulfur from a middle distillate fraction of mild coal gasification liquids has been demonstrated using biocatalyst preparations that had been stored at –70°C for various times prior to being incubated for 16 h at room temperature with oil-to-water ratios of 1:20. More importantly, as much as 39% removal of sulfur from unfractionated mild coal gasification liquids has been demonstrated using freeze-dried cells incubated for 16 h at room temperature and equal ratios of oil to water as shown in Table 2. The importance of these results is that the test conditions used are highly relevant to the development of a practical biodesulfurization process. Freeze-dried biocatalysts can be produced in one location, stored indefinitely, then shipped (at reduced weight, volume, and cost) to another location for use in a coal biodesulfurization process. Moreover, the use of equal volumes of oil and water is the highest oil loading successfully tested so far, which means that smaller reactor sizes than previously thought can be used in a coal biodesulfurization process. Experiments with model compounds have demonstrated successful biodesulfurization with oil-to-water ratios of 9:1.

R. rhodochrous IGTS8 does exactly what is needed for effective biodesulfurization: sulfur is removed, but carbon remains intact. The development of biodesulfurization technology at IGT has resulted in several patents that have been licensed by Energy BioSystems Corporation, whose sole focus is to commercialize biodesulfurization technology. The key item in need of improvement in order to ensure the commercial success of biodesulfurization technology is to improve the level of desulfurization activity in IGTS8 cultures. Genetic engineering is being used to develop cultures with enhanced levels of desulfurization activity.

There are three genes comprising the desulfurization (dsz) operon (*12*). The products of these dsz genes combine to form an enzyme complex associated with the external surface, cell wall/membrane of *R. rhodochrous*

IGTS8 cells. The product of one of the dsz genes is responsible for the conversion of dibenzothiophene (DBT) to dibenzothiophene sulfone, whereas the products of the other two dsz genes are responsible for the conversion of dibenzothiophene sulfone to 2-dihydroxybiphenyl and sulfite. Current efforts to develop cultures capable of expressing dsz genes at higher levels have focused on the isolation of strong *Rhodococcus* promoters to replace the relatively weak native promoter of the dsz genes. Toward that end several promoter probe vectors have been constructed/utilized.

Four promotor probe vectors have been constructed, and one commercially purchased vector has also been utilized for studying promoters in IGTS8. These vectors are called pRCM1, pRCAT1, pRCAT2, and pRCAT3, and the commercial vector pKK232 (Pharmacia, NJ). Each of these vectors contains structural genes encoding chloramphenicol resistance but lacks a promoter to express these resistance genes. One or more unique restriction sites are present on each vector just proximal to the resistance genes, allowing the insertion of DNA fragments that can be assessed for the presence of promoters by selection for those derivatives that express chloramphenicol resistance. Except for pRCM1, which has the Cm gene from pRF29 whose mechanism of resistance is related to permeability rather than acetylation of chloramphenicol, the promoter probe vectors can be used to measure the relative strength of the promoter inserts through the chloramphenicol acetyl transferase (CAT) assay, which is a convenient, well-documented assay. Numerous Cm-resistant colonies have been isolated using these vectors. These derivatives have been found to contain inserts of *R. rhodochrous* IGTS8 chromosome ranging in size from about 100 to 2000 bp and providing levels of chloramphenicol resistance ranging from 25- to 200 μg/L.

A strategy for the isolation of strong inducible promoters is currently being studied through a technique called subtractive RNA hybridization. Subtractive RNA hybridization allows a specific gene to be identified through a multistep procedure. Genomic DNA, or a genomic library of cloned DNA fragments, is immobilized on nitrocellulose by Southern blotting. Unlabeled total RNA of uninduced cells is used as a component of the prehybridization solution to bind to sites that are not specific for the gene of interest, then the radiolabeled total RNA isolated from induced cells is used as a hybridization probe to detect the gene of interest. A flowchart depicting subtractive RNA hybridization is shown in Fig. 1.

Bioremediation of PAH Contamination

Research concerning the remediation of environmental problems associated with coal utilization includes the remediation of coal tar-contaminated soil, the removal of leachable metals from fly ash and from soil, and the treatment of acidic mine drainage and other metal-laden waste water. Until recent decades, much of the US had no access to geological

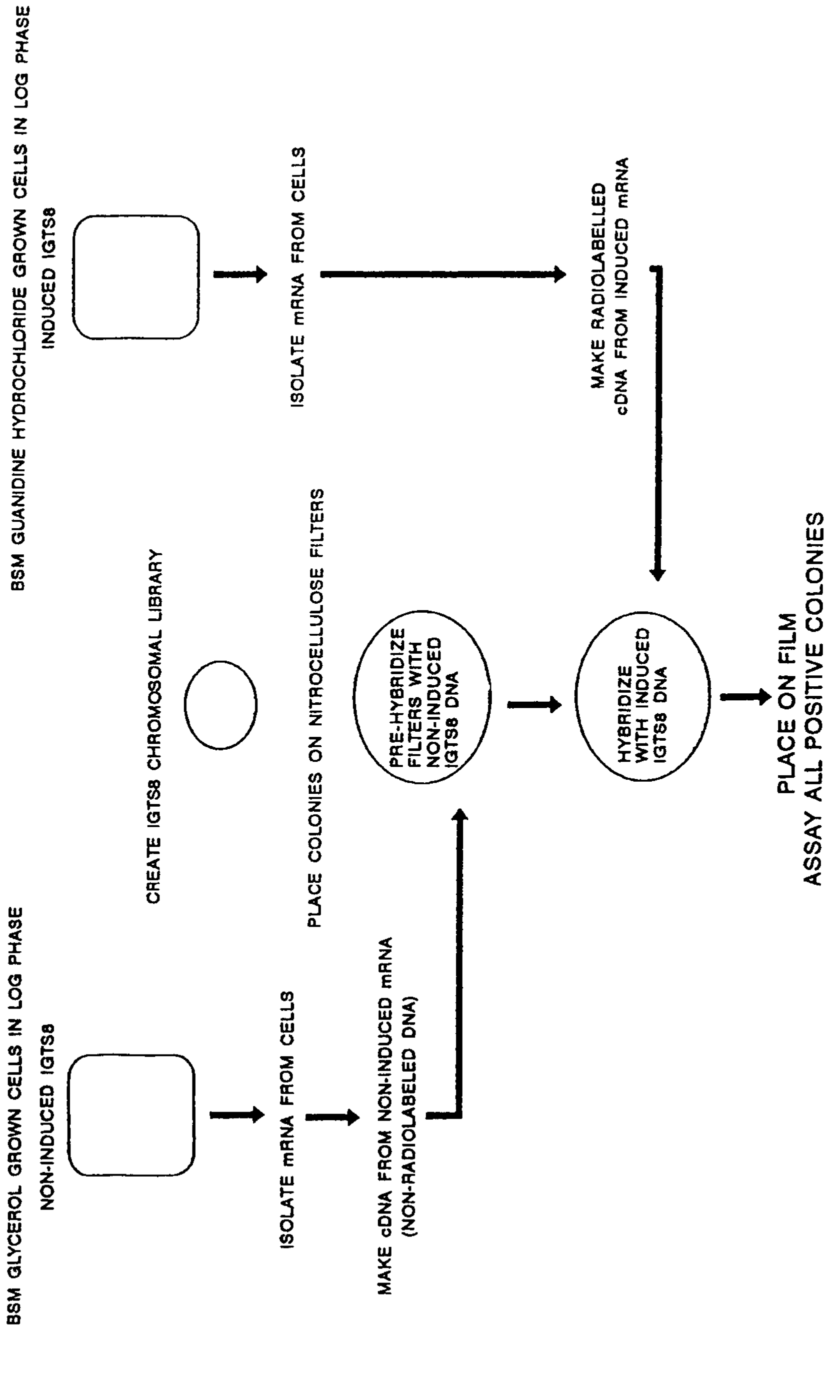

Fig. 1. Subtractive RNA hybridization flowchart.

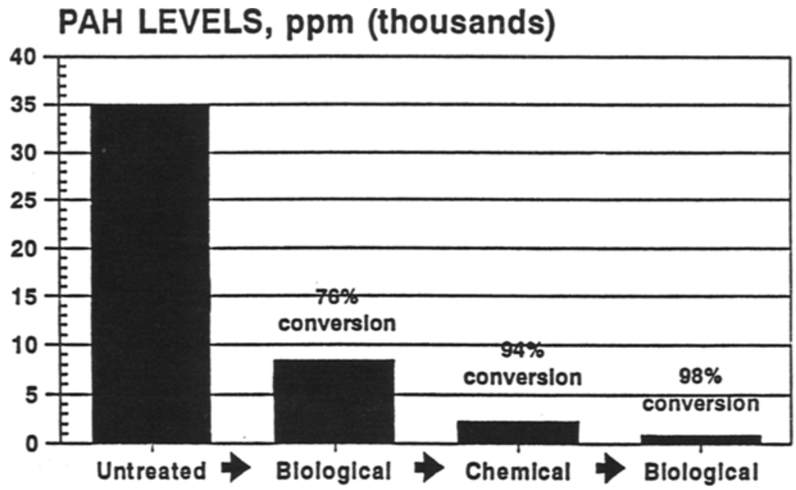

Fig. 2. CBT process: Sequential biological-chemical-biological treatment of PAH-contaminated soil.

deposits of natural gas, so that coal or other available substances were thermochemically converted to natural gas. A generally unwanted by-product of this conversion process was coal tar, which was usually dumped or landfilled on-site such that today several thousand such sites are known. Coal tar contains polyaromatic hydrocarbons (PAHs) that are potentially carcinogenic, as well as other organic contaminants, and some coal tar-contaminated sites are on the EPA's Superfund priority list of sites requiring remediation. IGT has developed several integrated biological/chemical/physical techniques to treat coal tar-contaminated soil: chemical biological treatment (CBT) (22), fluid extraction biodegradation (FEBD) (23), and several other approaches for enhanced *in situ* bioremediation. Both the CBT and the FEBD processes are being demonstrated in EPA's Strategic Innovative Technology Evaluation (SITE) program. The CBT process involves the combined use of chemical oxidation of organic pollutants by the Fenton's reaction (hydrogen peroxide and ferric ion catalyzed free radical reactions) and biodegradation. High-mol-wt PAHs are often recalcitrant to biodegradation, especially when sorbed to the organic matrix of soil; however, the Fenton's reagent preferentially attacks higher mol-wt compounds transforming them to products that are much more amenable to biodegradation. Field demonstrations of the CBT process employed as a land-farming treatment or as a soil slurry treatment have been completed and/or are in progress. Typical results obtained in CBT tests are illustrated in Fig. 2.

The FEBD process employs environmentally benign supercritical fluids (typically carbon dioxide) to extract PAHs and other pollutants from contaminated soil. Subsequently, the extracted pollutants are biodegraded

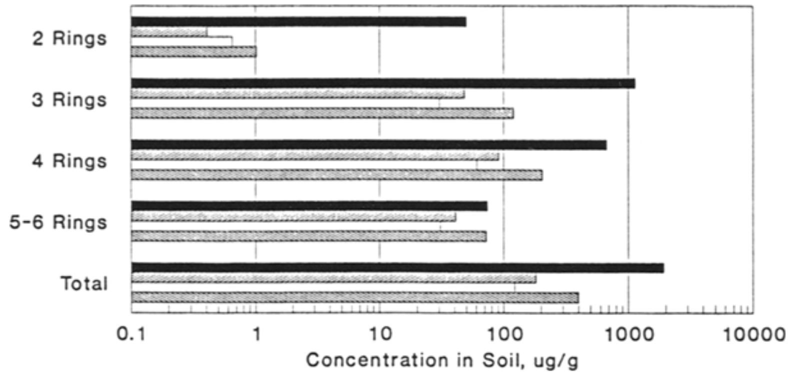

Fig. 3. FEBD: The effect of temperature on the extraction of PAH contaminants from soil. Concentration in soil, μg/g. ■ contaminated soil, ▨ T = 91°F, □ T = 113°F, and ▧ T = 171°F. Pressure = 2000 psig. CO_2/contaminants = 6700 lb/lb.

with or without the aid of chemical oxidation. The FEBD process can rapidly cleanse soil of pollutants, and it is relatively easy to verify that the entirety of soil treated by the FEBD process meets the desired/regulated clean-up goals as compared with some other techniques of soil remediation, since the FEBD process is a batch treatment that uniformly extracts the contaminants in a given batch of soil or sediment, and extraction cycles can be repeated as often as needed to ensure that regulatory compliance is achieved. Typical results obtained with the FEBD process for the extraction of PAHs from soil using supercritical carbon dioxide are shown in Fig. 3.

Removal of Metals

Another area of research at IGT relevant to coal bioprocessing is the treatment of solids and liquids containing heavy metals. Fly ash resulting from the combustion of coal can result in the leaching of significant quantities of metals when the fly ash is contacted by distilled water as shown in Table 3. The characteristics of fly ash regarding their propensity to leach metals when contacted by water can vary, but the results reported in Table 3, which were obtained using a fly ash sample supplied by a coal-utilizing utility company, illustrate that the leaching of metals from fly ash can be a source of environmental problems. IGT has developed solutions that can be used to promote the leaching of metals from fly ash or from soil so that leachable metals can be removed in a controlled fashion and the treated solids pass EPA's toxicity characteristic leaching procedure (TCLP) test (EPA Method 1311). Additionally, bioreactors containing sulfate-reducing bacteria (SRB) have been used at IGT to remove heavy metals subsequently from leachate solutions derived from fly ash or soil, or to treat metal-laden acidic mine drainage solutions. Figure 4 illustrates the

Table 3
Significant Quantities of Heavy Metals Can Be Leached from Fly Ash by Water

Element	Metal concentration in the leachate, ppm
Al	68
B	55
Ca	595
Cd	2.1
Cu	1.7
Fe	9.3
Mg	28.5
Mn	3.8
Ni	1.7
S	7750
Zn	17.5

Volume:weight ratio of liquid vs solid is 20:1; leaching time is 24 h.

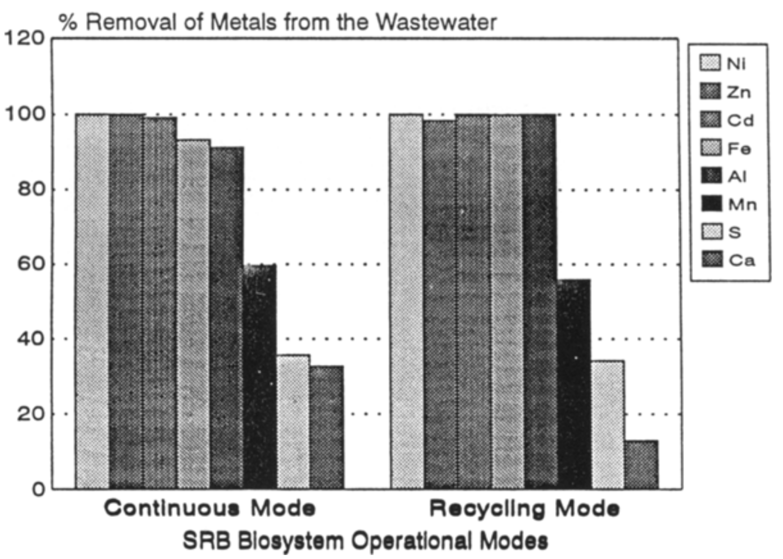

Fig. 4. Comparison of waste-water treatment efficiencies of two different operational modes of the SRB biosystem.

metal removal efficiency of the SRB biosystem. SRBs are particularly useful for metals removal, because they produce hydrogen sulfide, resulting in the precipitation of metal sulfides, produce alkalinity, can alter the valence state of certain metals, such as chromium and uranium, can biosorb metal ions, filter particulates, and can even biodegrade some organic pollutants.

SUMMARY AND CONCLUSIONS

The coal industry currently faces numerous challenges primarily because of environmental problems associated with the use of coal, and somewhat because of land reclamation and subsidence issues associated with the mining of coal. Coal is North America's most abundant fossil fuel, and the continued use of coal is essential for the foreseeable future if the US is to maintain and increase its industrial productivity, standard of living, and energy self-sufficiency. Nevertheless, the switching of utilities from coal to natural gas, petroleum, or nuclear energy has become common. Innovative technologies are needed. Coal bioprocessing can provide numerous alternatives, and economics will decide which, if any, of the coal bioprocessing technologies will be used commercially. Since coal is a relatively inexpensive commodity that is utilized in immense quantities, applications of any technology to coal face rigorous challenges. Biotechnology is not generally perceived to be appropriate for the production of low-value, high-volume products such as coal. Rather, the production of high-value, low-volume products, such as pharmaceuticals, is often thought to be the more appropriate niche for biotechnology. However, municipal waste-water treatment plants, agriculture, the brewing industry, acrylamide production, and Nutrasweet® production are just a few examples of bioprocesses that are operated on a large scale. Energy BioSystems Corporation is in business solely to commercialize biodesulfurization (organic sulfur removal) technology for applications to fossil fuels, and Engineering Resources, Inc. expects that it will soon have a commercial process for the conversion of fossil fuel-derived gases to ethanol. Coal bioprocessing has enormous potential, and integrated biological/chemical/physical processes for coal are the wave of the future. Although most new technologies for coal seek to or are urged to conform as closely as possible to traditional practices within the coal industry, the best means of addressing problems associated with the use of coal, and the best means of maintaining and increasing the use of coal in the long term may require transformations within the coal industry.

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