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Final Report

**Microbial Recovery of Metals from Spent Coal
Liquefaction Catalysts**

Contract No. DEAC2289PC89881

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I. Introduction

A. Problem

This project was initiated on October 1, 1989, for the purpose of recovering metals from spent coal liquefaction catalysts. Two catalyst types were the subject of the contract. The first was a Ni-Mo catalyst supported on alumina (Shell 324), the catalyst used in a pilot scale coal liquefaction facility at Wilsonville, Alabama. This plant was run and operated by Southern Clean Fuels. A large sample of spent catalyst from this facility was obtained. The second material was an unsupported ammonium molybdate catalyst used in a pilot process by the Department of Energy at the Pittsburgh Energy Technology Center. This material was obtained in late February 1990 but has not been pursued since the Mo content of this particular sample was too low for the current studies and the studies at the Pittsburgh Energy Technology Center have been discontinued. The object of the contract was to treat these spent catalysts with microorganisms, especially *Thiobacillus ferrooxidans*, but also other *Thiobacillus* spp. and possibly *Sulfolobus* and other potential microorganisms, to leach and remove the metals (Ni and Mo) from the spent catalysts into a form which could be readily recovered by conventional techniques.

1. Coal liquefaction

Current direct coal liquefaction technologies suffer from some severe practical problems (1-5, 7). One of the main problems is the requirement for relatively expensive catalysts which have short lives because they are prone to poisoning especially by mineral components in the coal (6). This leads to heavy catalyst loading levels. At Wilsonville the loading was approximately 1 lb of catalyst per ton of coal liquefied. With lower rank coals, this loading was bound to increase. This has prompted much catalyst research by the Department of Energy and also led to the funding of this work to remove and recover for reuse the metals used in direct liquefaction technologies. Commercialization of coal liquefaction using Shell 324 or a similar catalyst would rapidly deplete the world's supply of Mo and possibly produce unacceptable solid wastes containing Ni. The direct liquefaction requires the use of a high hydrogen-containing solvent to transfer hydrogens to the coal structure during liquefaction. Thus the use of Shell 324, an excellent hydrogen transferring catalyst, is easily justified. This coal cracking leads to relatively high quality coal liquids which can then be refined for further added value and use.

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2. Catalysts - Wilsonville

The pilot plant at Wilsonville, Alabama has run direct liquefaction processes for several years. This plant has studied the catalyst, the main research emphasis of this contract, in detail. The production records and parameters are well understood. We obtained spent catalyst (Shell 324) from a "typical" run (our actual catalyst sample was a combination from runs 255 and 258) for our studies and this catalyst will be further investigated in future contracts. The specifics of this run are not available to us at this time. Although many other catalysts are being currently pursued, Shell 324 or the related Amocat (Amoco Corp.) are still the benchmark for direct liquefaction catalysts. The chemical composition of Shell 324 is shown in Table 1.

Table 1

Metal Content of Catalyst Samples

Catalyst Sample	% Mo	%Ni
Shell 324	12.7	3.5
Spent Shell 324	8.1	2.2
Presulfided Shell 324	9.3	2.6
10 Mesh THF-Extracted Spent Shell 324	9.1	2.7
Coal Liquids, not filtered	7 ppm	31 ppm

3. Metals

Various metals have been studied as components of coal liquefaction catalysts. The most commonly used metal is Molybdenum, often in conjunction with Cobalt and Nickel. In addition, Cobalt (Co) and Tungsten (W) (as CoWMo or CoMo formulations) are sometimes also incorporated into direct liquefaction processes. In earlier work Cr was sometimes used. However, Cr is an environmentally sensitive metal and its use has decreased in many of its earlier applications including direct coal liquefaction.

4. PETC Approach

The Pittsburgh Energy Technology Center (PETC), United States Department of Energy, as part of its own in-house research efforts pursued the use of an unsupported Mo-containing catalyst

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(Dr. B. Blaustein). It was hoped that low catalyst loading without a support would facilitate the development of a "throw away" catalyst, thus obviating waste disposal problems and new catalyst costs. Although this work at PETC has been suspended, other laboratories are currently pursuing this concept of direct coal liquefaction catalysis. For a review see the work of Bockrath et al. (12).

B. Biohydrometallurgy - history; applications

1. Acid mine drainage

Some acidophilic bacteria are capable of the oxidation of mineral sulfides with the concomitant solubilization of metals and the formation of copious amounts of acid. This natural process can lead to the production of acidic drainage from natural sources of metal sulfides. Coal mines are one such example where the pyrite and other iron-containing minerals found in coal is converted by these acidophilic bacteria, exemplified by *T. ferrooxidans*, into oxidized iron and sulfuric acid. The acid is then responsible for further solubilization of the pyrite and other sulfide minerals. The result is a powerful impact on local environments such as streams and lakes because of both acidification and the increase in soluble heavy metals. Studies on controlling this process have been ongoing for many years, but the effects of exposing metal sulfides to an aerobic, aqueous environment containing an ubiquitous microorganism such as *T. ferrooxidans* are not easily controlled and remedied.

2. Cu, U, others

Microbiological leaching of metals from ores is currently practiced in both underground and heap leaching situations for U and Cu. Gold, Ni and Co may also be retrieved through the use of bioleaching processes (8). For further details on these processes I refer the reader to the recent book, *Microbial Mineral Recovery* (8). In summary, many metals can be either released into solution by microorganisms or may be deleteriously released as a result of the metabolic action of *T. ferrooxidans* (a case in point is the release of Mo from molybdenite in Mo mines in New Mexico and Colorado).

C. *T. ferrooxidans*

1. Use in metal recovery

Thiobacillus ferrooxidans has been used extensively in laboratory scale leaching processes. Under these conditions, it is possible to control the physical and chemical environment to such a degree that process optimization can be accomplished.

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However, practical processes cannot employ pure cultures and other acidophilic microorganisms contaminate practical processes, including heterotrophic, acidophilic bacteria and other Thiobacilli. In most cases, however, these contaminating microorganisms do not seriously affect the success of the process. In this project, we assumed that mixed cultures would be the only ones of practical use, and no attempt was made to keep cultures pure except in those studies where careful control of the cultures was necessary. The environmental dynamics of mixed acidophiles means that *T. ferrooxidans* necessarily will be the dominant microorganism. However, other microorganisms may play significant roles in some situations. However, scale up of pure processes to mixed processes with *T. ferrooxidans* usually are successful with only minor modifications. This philosophy extended to the work with denitrifying bacteria, where mixed cultures were chosen solely by virtue of their ability to grow with specific carbon and energy sources and cultures which could be maintained in a process environment without significant microbiological controls.

2. Metal tolerances

Much work has been done with *T. ferrooxidans* which demonstrates significant tolerance for many metals normally toxic for most microbes (including Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Hg, Pb and Bi). However, *T. ferrooxidans* has unusually low tolerance for both Mo in the form of molybdate and W in the form of tungstate (also As and Sb, although tolerant strains are readily obtained). Nothing is known about why *T. ferrooxidans* exhibits these intolerances. Speculation centers around the interference of these ions with either sulfate metabolism or phosphate metabolism. As far as is known, however, there is no known difference between the phosphate and sulfate metabolism of *T. ferrooxidans* and that of any other microorganism. For example, *Escherichia coli*, an organism not known for its metal tolerances, is capable of growth in 100 mM molybdate and tungstate. In contrast, *T. ferrooxidans*, an organism well known for its metal tolerances, is completely inhibited by 100 μ M molybdate and 10 μ M tungstate (See Tables 11-15 below).

II. Materials and Approaches

A. Catalysts

1. Wilsonville - Shell 324

The catalyst used in these studies was obtained from the Wilsonville pilot plant (runs 255 and 258). Its chemical characterization is presented in Table 6 below.

2. PETC - unsupported

Unsupported ammonium molybdate spent catalyst was obtained from PETC in early 1990, but its Mo level was too low to be of interest and the study of this unsupported material was discontinued.

B. Microorganisms

1. *T. ferrooxidans* and other *Thiobacilli*

a. Strains

Cultures of *T. ferrooxidans* and *T. denitrificans* and related organisms were obtained from INEL, the American Type Culture Collection, and isolated from natural sources.

b. Culture conditions

Cultures of *T. ferrooxidans* and *T. denitrificans* and related organisms were obtained from INEL, the American Type Culture Collection, and isolated from natural sources. The INEL medium (Table 2) for the cultivation of *T. ferrooxidans* was used with an initial pH of 2.0; this pH was later changed to 2.5 when better and more reproducible results were obtained at that pH. The medium was further modified (NOAL Broth) to remove the Al present in the INEL Broth. This was necessary since we wished to determine Al solubilized from the alumina catalyst support during leaching. *Thiobacillus denitrificans* was cultured in the ATCC medium of choice (usually medium 450 or 132 (Tables 3 and 4)). Heterotrophic denitrifiers were cultured in medium 337 of Hirsch and Conti (see Table 5 below) supplemented with 10 g/l NH_4NO_3 or KNO_3 . Denitrifying bacteria were isolated from various soil samples and from water samples collected from Alaskan oil fields. These organisms were isolated in mixed culture and maintained as mixed cultures for use in these experiments. Depending on the carbon source they may be very different phylogenetically. However, for the carbon sources so far tested most of the microorganisms belong to the genus *Pseudomonas*.

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Table 2

INEL Broth

Ingredient	g/l
$(\text{NH}_4)_2\text{SO}_4$	.15
KCl	.15
K_2HPO_4	.15
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	3.36
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	1.28
$\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$	2.25
$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	.12
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	7.46

Adjust pH to 2.5 with H_2SO_4

NOAL Broth

This medium has the same composition as INEL Broth with the $\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$ eliminated from the formulation. Growth of *T. ferrooxidans* was unaffected by this change.

Table 3

Medium 132 (for *T. denitrificans*)

Chemical Component	g/l
Na_2HPO_4	1.2
KH_2PO_4	1.8
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	.4
NH_4Cl	.5
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	.03
$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	.02
FeCl_3	.02
NaHCO_3	1.0
KNO_3	5.0
$\text{Na}_2\text{S}_2\text{O}_3$	10.0
SL4 Trace Metals	10 ml

Table 4

Medium 450 (for *T. denitrificans*)

Chemical Component	g/l
$\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$	5.0
NH_4Cl	1.0
KNO_3	5.0
KH_2PO_4	2.0
NaHCO_3	2.0
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.8
SL4 Trace Metals	10 ml

Table 5

Medium 337, Sulfate Free

Chemical Component	g/l
KH_2PO_4	1.4
Na_2HPO_4	2.1
$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$	.2
NH_4NO_3	10.0
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	.01
SL-4 Trace Metals (see Table 6)	20 ml

Table 6

SL-4 Trace Metals, Sulfate Free

Chemical Component	mg/l
EDTA, di Na salt	500
$\text{FeCl}_2 \cdot 5\text{H}_2\text{O}$	100
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	25
$\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$	10
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	20
$\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$	20
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	30
Na_2WO_4	5
Na_2SeO_3	5
H_3BO_3	25

The salt solution and the trace metal solution have been reformulated to remove all salts containing S and replace them with chloride salts. This forces growing organisms to scavenge S from the catalyst. We know from other experiments that these denitrifying bacteria are capable of utilizing sulfide as a

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source of nutritional sulfur and that the "insoluble" sulfides such as FeS may serve as a nutritional S source.

2. *Sulfolobus*

Sulfolobus was obtained from two sources, from INEL and Brierley.

3. Other microorganisms

Other microorganisms used in the leaching/metal release experiments were isolates from natural sources. Geo-Microbial Technologies has soil samples from all over the world and these served as sources for microbes capable of denitrifying with the spent catalyst as their sole source of sulfur. Specifically samples from Bolivia and New Guinea were employed. However, we found that exotic soil samples were not necessary and that the strains we isolated were readily obtained from local water or soil samples.

a. Strains

Denitrifying strains were only crudely characterized due to the necessity, mentioned above, for impure, mixed cultures. Subcultures were stored simply as "mixed denitrifiers" or as denitrifiers with the capability to use specific carbon and energy sources to drive denitrification (i.e. "citrate utilizing denitrifiers"). These cultures remained remarkable constant. Crude examination of the mixture revealed that most of the organisms, regardless of carbon and energy source, probably belonged to the genus *Pseudomonas*.

b. Culture conditions

Denitrifying bacteria were cultured in selective media consisting of a mineral salts medium, with no added S and NH_4NO_3 as both nitrogen source and substrate for denitrification. The catalyst, which contains significant sulfur in the form of sulfide, served as a nutritional source of sulfide. The catalyst therefore exerted a significant selective pressure for both denitrification, anaerobic growth, Ni and Mo tolerance, as well as carbon and energy source selectivity.

C. Analytical Procedures

1. Chemical

Early in the project we used wet chemical methods for the analysis of Mo and Ni. These methods appeared to be reproducible, but gave artificially high numbers and were replaced early by atomic absorption.

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2. Atomic absorption

Atomic absorption was used for the determination of metals. A Perkin-Elmer model 703 atomic absorption spectrometer was used. At various times, Ni, Mo, W, V, Co, Fe and Al were analyzed. This method was reproducible and highly accurate with good sensitivity for all of these metals. It is not possible to distinguish between Fe^{++} and Fe^{+++} with atomic absorption. Traditional techniques for this differentiation suffer from interferences, specifically by Ni^{++} and therefore could not be used.

3. External analyses

At various times we used external laboratories for analyses, especially for the analysis of whole catalyst samples. Based on the recommendation of Dr. B. Blaustein, we had Huffman Laboratories in Colorado perform catalyst analyses using a wet chemical ashing technology. They also performed an ICP method of total chemical composition. We have discovered a technique recently which will allow us to perform these analyses in house without significant additional equipment. This technology is proprietary to Inorganic Ventures, New Jersey.

4. Xanes/Xafs

Dr. Gerald Huffman, Fossil Fuel Consortium, University of Kentucky, performed a Xanes/Xafs, x-ray analysis on the leached material to determine the state of the Mo. This analysis required the use of the accelerator at Argonne National Laboratory. Dr. Huffman, the Fossil Fuel Consortium and Argonne National Laboratory are gratefully acknowledged for their contributions.

5. Ion Exchange

We leached about 100 g of THF-washed spent catalyst and collected the supernatant solution containing both Ni and Mo. This solution was filtered through a $0.22\mu\text{M}$ filter to remove the bacteria and other small particulate matter. This solution was then passed over a Chelex 100 column in the Na^+ form and the column washed with 10 ml deionized water. The washings were combined with the unabsorbed material and analyzed for Ni and Mo. The column was then washed with 1 N HCl to elute the bound metals and the eluate analyzed. The liquids with initially unabsorbed ions to which the washings were added were then treated to determine if the Mo was in an anionic state. This solution was passed through a Dowex 1X-200 anion exchange resin column in the chloride form. After loading the column it was washed with deionized water and the washings added to the unabsorbed liquid

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materials. The column was then eluted with 2 M KNO_3 and the eluate analyzed for Ni and Mo.

6. Microbial Growth

Growth of *T. ferrooxidans* was analyzed by direct microscopic counts using a Petroff-Hauser counting chamber. Growth of organisms other than *Thiobacilli* were measured by conventional technologies such as light scattering and plate counts. These organisms posed no serious problems in measuring growth such as those found with *T. ferrooxidans*.

III. Experimental Results

A. Catalyst Characterization

1. Metal Content

The metal content of catalyst samples was determined by a wet ashing methods by Huffman Laboratories. The results are shown in Table 1 above.

We believe that the relatively high metal content of the oil was due to fines from the catalyst, since there is no reason to expect to find Mo in coal. A filtered sample was analyzed at a later date and no appreciable Mo was found and <5 ppm Ni was present. The Ni may be naturally present in coal as it is in many crude oils.

2. Non-Microbial Leaching

a. Rates of Leaching

Experiments were performed to determine the rate of release of Ni and Mo from various catalyst samples, employing different preliminary washes. We obtained 4 catalyst samples for these analyses. The first is Shell 324 as obtained from the manufacturer. This can be viewed simplistically, from a biohydrometallurgical point of view, as a high quality Ni and Mo containing ore. The second sample was obtained from Wilsonville and was a presulfided Shell 324. Before use, Shell 324 is treated with H_2S under pressure and heat to reduce the Ni and Mo to their sulfides, the form in which they are active in the liquefaction process. This material is stored under oil to prevent chemical oxidation. This can be viewed as a first stage in the complication of Shell 324. The third stage of this series is spent Shell 324 which was exhaustively extracted with tetrahydrofuran (THF) in a Soxhlet extractor. This can be viewed as an ore contaminated with mineral components and non THF-

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soluble carbon-containing materials such as coke. The final sample is spent Shell 324 as it comes from the reactor pilot plant. This material has low activity and is contaminated with both minerals and coal process liquids. It is obviously the most complex of our samples. These samples were subjected to extraction by either INEL broth or 0.5 M H_2SO_4 . Washes were obtained at various times and the amount of Ni and Mo released were analyzed. The results are shown in Table 7. These data are reduced to initial rates (Table 9) to compare the efficacy of acid and INEL broth as prewashes for the catalyst samples. The results are shown graphically in Figures 1-10. Various conclusions can be drawn from these figures. First, new Shell 324 shows little effect on selectivity of Ni or Mo and that these metals are rapidly released with a high percentage yield (figures 1 and 5). The presence of oil in the Presulfided catalyst samples clearly shows that the release of Mo is greatly reduced compared with Ni (Figures 2 and 6). Why this is the case is not clear. This selectivity is also exhibited with spent Shell 324 and the THF-extracted spent catalyst (Figures 3, 4 and 7). An opposite effect is shown with the INEL washing of spent Shell 324. Although the ultimate outcome is the same, the kinetics are quite different. Clearly sulfuric acid is able to solubilize much material from catalyst samples which are free of contaminating coal liquids (Figures 9 and 10).

Table 7
Extractions of Catalysts

Catalyst Material, Wash	Time, hrs	Cumulative Released	
		% Ni ⁺⁺	% Mo
Oily Wilsonville, INEL pH 2.5	24	0.27	0.6
	48	0.40	1.2
	72	0.56	1.4
	96	0.72	1.4
	168	1.0	1.4
	192	1.2	1.4
Oily Wilsonville, H_2SO_4 0.5 M	24	13	1.0
	48	20	1.8
	72	22	2.9
	96	24	4.1
	168	28	5.4
	192	28	5.8

10 Mesh THF-Washed, INEL pH 2.5	24	18	4.4
	48	32	4.8
	72	40	5.0
	96	46	5.2
	168	52	5.5
	192	53	5.6
10 Mesh THF-Washed, H ₂ SO ₄ 0.5 M	24	67	1.6
	48	78	4.0
	72	85	6.6
	96	87	9.3
	168	90	12
	192	90	14
Presulfided Shell, INEL 324 Ph 2.5	24	1.2	0.09
	48	26	1.4
	72	31	2.2
	96	32	2.6
	168	33	3.3
	192	34	3.6
Presulfided Shell, H ₂ SO ₄ 324 0.5 M	24	26	3.4
	48	31	6.6
	72	32	9.6
	96	33	12
	168	36	15
	192	37	16
Shell 324, INEL pH 2.5	24	35	5.5
	48	48	25
	72	53	34
	96	55	38
	168	57	41
	192	57	43
Shell 324, H ₂ SO ₄ 0.5 M	24	66	69
	48	80	93
	72	83	102
	96	85	105
	168	85	107
	192	88	107

There are some obvious trends in this data. The first is that washing Shell 324 catalyst with 0.5 M H₂SO₄ removes almost all of the Ni and Mo and dissolves about 92% of the catalyst. Once the catalyst has been sulfided, washing even with 0.5 M H₂SO₄ does not readily release all of the metals. The oil, obviously, also plays an important role in this protection since the presulfided catalyst must be stored under oil to prevent oxidation. Spent Wilsonville catalyst contaminated with product

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oil shows the same protective phenomenon due the residual liquefaction oil. There is also a probable role played by the mineral deposition on the spent catalyst from Wilsonville since washing THF-extracted spent Wilsonville catalyst with 0.5 M H_2SO_4 only removes about 10% of the Mo as compared to 100% of the Mo with Shell 324 raw catalyst. There is also another observation which bears on this problem. These extractions were performed on a rotary shaker at 250 rpm, which certainly could lead to the physical diminishing of the catalyst particle size due to abrasion. If the extractions were done under gentler conditions, perhaps less of the catalyst support would have been lost. The separations were done by decanting, and fines were therefore lost at each step. See Table 8 for the data concerning the losses during extractions due to either dissolution or fines.

Table 8

Sample	Treatment	Original, g*	Final, g	% Lost
Spent, with Coal Liquids	INEL	27.64	22.01	20
Spent, with Coal Liquids	H_2SO_4	26.91	13.68	49
Spent, <10 mesh THF-extracted	INEL	21.34	18.19	15
Spent, <10 mesh THF-extracted	H_2SO_4	20.17	8.48	58
Presulfided	INEL	28.33	17.65	38
Presulfided	H_2SO_4	27.92	6.71	76
Shell 324	INEL	20.16	16.80	17
Shell 324	H_2SO_4	24.61	2.06	92

* - Amount of catalyst including associated oil or coal liquids, if present.

Table 9

Rates of Release of Ni and Mo from Chemically Leached Catalyst Samples

Sample, Leaching Solution ^{1,2}	Initial Rates, %/hr	
	Ni	Mo
New Shell 324		
Leached with Sulfuric Acid	2.8	2.9
Leached with INEL Broth	.96	.46
THF-Extracted Spent		
Leached with Sulfuric Acid	2.8	.13
Leached with INEL Broth	.63	.21
Presulfided Shell 324		
Leached with Sulfuric Acid	1.1	.13
Leached with INEL Broth	.44	.03
Spent Shell 324		
Leached with Sulfuric Acid	.40	.08
Leached with INEL Broth	.001	.03

1. 0.5 M H₂SO₄

2. For the composition of INEL broth, see the Methods section.

Sulfuric acid is the best prewash in all cases, although with spent catalyst it does not give much of an advantage over broth washing. Clearly organic and mineral materials which poison the catalyst prevent acid from releasing the catalytic metals. However, as we show below, microorganisms (both *T. ferrooxidans* and denitrifying bacteria) are quite capable of releasing those remaining metal values through metabolic activity over time.

b. Leaching Efficiencies and Mass Balance

It is clear from the above data that chemical leaching may be effective with some catalysts. This is especially true of the new Shell 324, where the metals are in the oxide form and where the catalyst pores have not been clogged with coke like materials or minerals. In the case of the spent Shell 324, with or without the presence of the coal liquids, acidic solvents do not perform as well by themselves. A mass balance was obtained and is shown

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in Table 8.

3. Solvent Extractions

The spent catalyst from Wilsonville was treated initially by extensively washing the material with tetrahydrofuran in a Soxhlet extractor and evaporating the THF from the residual catalyst and the oil/THF-containing liquid. The dried catalyst was then crushed in a ball mill, producing fractions from 10 to smaller than 100 mesh. Minus 100 mesh material was initially used to assay metal release. This has since been replaced by 10 mesh or uncrushed material since microbial leaching appears to proceed reasonably well without crushing when utilizing *T. ferrooxidans*. However, the effect of mesh size on the ability of denitrifying bacteria to release metals from the spent catalyst is greater and some crushing is necessary. We looked for solvent efficacy for the removal of residual coal liquids. Table 10 shows the solvents examined.

Table 10

Solvents for Catalyst Extraction

Methanol
Hexane
Toluene
Ethyl acetate
Acetone
Water
Chloroform
Carbon tetrachloride
Methylene chloride
Acetic acid
Formic acid
Tetrahydrofuran
Pyridine
Isopropanol
Acetonitrile
p-Dioxane
t-Butanol

A visual examination of the liquid material obtained from catalyst showed that several solvents were more efficacious in removing contaminating organic materials from the catalyst. For example, hexane extracted a clear yellow oil from the catalyst. At the opposite end of the spectrum, pyridine extracted a thick black oil containing high levels of solids and clearly removed the most material of the solvents on a weight basis. These observations were reflected in the ability of the denitrifying

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bacteria to release metals from the catalysts extracted with different solvents (see also Table 23). Water was a poor solvent. Acetic acid not only was good at extraction of coal liquids, but also "bleached" some of the catalyst particles. It is possible that the bleached particles were those with the shortest residence time in the reactor.

4. Coal Liquids - Effect on Growth of Microorganisms

The process coal liquids contain a large variety of hydrocarbon-containing materials. Many of these components are quite toxic to higher organisms (9). In addition, *T. ferrooxidans* is well known for its inability to tolerate significant quantities of organic material in its growth medium. We found that the tolerance of *T. ferrooxidans* for organic material was quite high if the cultures were not pure, as they were in our experiments. However, the coal liquids were still quite inhibitory to *T. ferrooxidans* and its acidophilic consortium even under these conditions. If the coal liquids were extensively extracted with dilute acid, which would remove N, S and O containing material with some polarity, the growth tolerances could be increased by a factor of two, but they were still too inhibitory to grow *T. ferrooxidans* readily in the presence of oil saturated catalyst. However, the denitrifying bacteria were completely tolerant of as much as 2% THF-extracted coal liquids added to their growth medium. They were unable, however, to use the coal liquids as sole source of carbon and energy. It is clear that any process in which the coal liquids may remain would be inhibitory to *T. ferrooxidans* but not the denitrifying bacteria.

5. Crushing

A ball mill and a sieve shaker was used to crush and separate particle sizes of the solvent-extracted catalysts (THF). In later studies we used a Retsch grinder to produce >100 mesh catalyst fractions. It was not possible to crush the catalyst with the coal liquids present. A very hard and insoluble asphalt like material was produced which solidified and it was impossible to work with this material or extract the coal liquids after crushing.

B. *T. ferrooxidans* - Metal Tolerance Studies

1. Metal Tolerances

Preliminary to the actual leaching of the catalyst samples with the microorganisms, it was necessary to determine their tolerances for the metals. Initially, it was determined that Ni and Mo would be tested, since they are integral components of the

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catalyst. As can be seen below, there was no problems with the tolerance of *T. ferrooxidans* for Ni, but, as reported elsewhere, this organism's tolerance for Mo in the form of molybdate was not good. We thought that we might increase this Mo intolerance through the use of increased resistance to V, Cr or W (vanadate, chromate and tungstate) and therefore these metals were also tested. Later we found no intolerance of the denitrifying bacteria for Mo or Ni, and, since there was no apparent problem, these were not pursued in detail.

a. Nickel

Table 11

Tolerance of *T. ferrooxidans* for Nickel

Growth

Nickel Concentrations

Strain	0 μ M	50 μ M	100 μ M	500 μ M	1mM	50mM	100mM	120mM	150mM
DT-3	+	+	+	+	+	+	+	+	-
DT-5	+	+	+	+	+	+	+	+	-
DT-6	+	+	+	+	+	+	±	-	-
DT-7	+	+	+	+	+	+	±	-	-
D-26	+	+	+	+	+	±	-	-	-
A-4-1	+	+	+	+	+	+	-	-	-
A-4-2	+	+	+	+	+	+	±	-	-
F221	+	+	+	+	+	+	+	±	-

These strains exhibited high overall tolerance for Ni⁺⁺, as expected. These tolerance levels are high enough that a process for the recovery of metals from spent catalyst would not be significantly impacted by Ni intolerance.

b. Molybdenum

Table 12

Molybdate Tolerances of *T. ferrooxidans* Strains

Growth

Molybdate Concentrations

Strain	0 μ M	2 μ M	8 μ M	16 μ M	50 μ M	62.5 μ M	75 μ M	87 μ M	100 μ M
DT-3	+	+	+	+	+	+	+	±	-
DT-5	+	+	+	+	+	+	+	+	-
DT-6	+	+	+	+	+	+	±	-	-
DT-7	+	+	+	+	+	+	-	-	-
D-26	+	+	+	+	+	+	±	-	-
A-4-1	+	+	+	+	+	+	+	-	-
A-4-2	+	+	+	+	+	+	±	-	-
F221	+	+	+	+	+	+	+	+	-

The tolerances of these strains for molybdate (Mo) are about as would be expected from reports in the literature (10). We expected to increase these tolerances, both through mutagenic procedures and natural strain isolations through repeated growth in the presence of released Mo. We also performed studies where we tested a combination of Ni and Mo. 50 mM NiCl₂ was added to different concentrations of molybdate. The molybdate tolerances for all of the strains shown in the above table remained essentially the same. Therefore there was no negative synergistic reaction between the presence of both Ni and Mo. The Ni also did not spare Mo intolerance.

c. Chromium

Table 13

Chromate Tolerances of *T. ferrooxidans* Strains

Growth

Chromate Concentrations

Strain	0 μ M	5 μ M	10 μ M	50 μ M	100 μ M	500 μ M
DT-3	+	+	+	+	++	++
DT-5	+	+	+	+	++	++
F221	+	+	+	++	++	++

* - Although there was visible oxidation of ferrous iron in
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these cultures before growth was initiated, significant growth with the production of typical cell densities still occurred in these cultures. Of course the Chromate concentration was lowered because of the chemical oxidation, but, obviously the Cr still remained in some form in the culture vessel. Chromium chemistry at low pH values is quite complex and the possible forms and complexes are numerous.

After discovering the Mo tolerance for the tested strains was lower than we could use in a practical process, we tested Cr, in the form of chromate, to see if we could use chromate resistance as a marker for molybdate tolerance. Unfortunately, chromate, at the low pH of growth for *T. ferrooxidans*, forms some dichromate (11) and there is chemical oxidation of the ferrous iron energy source for the microorganisms. However, we did confirm good growth of *T. ferrooxidans* with chromate up to 500 μ M. After growth in these cultures, no higher molybdate tolerance was noted. Because of the great disparity in tolerances, chromate obviously does not in the same manner as molybdate at the molecular level.

d. Vanadium

The same reasoning for the above work with Cr led us to test V as metavanadate. Vanadate also becomes an oxidizing agent at low pH and exists in different forms, but it is not nearly as reactive or as strong an oxidizing agent as chromate (11).

Table 14

Vanadate Tolerances of *T. ferrooxidans* Strains

Strain	Growth					
	Vanadate Concentrations					
	0 μ M	5 μ M	50 μ M	100 μ M	500 μ M	1mM
DT-3	+	+	+	+	+	-
DT-5	+	+	+	+	+	-
F221	+	+	+	+	+	±

The results from this study revealed high vanadate tolerance and the *T. ferrooxidans* recovered from these flasks with this high vanadate tolerance still did not show increased molybdate tolerance.

e. Tungsten

Tungstate and molybdate are very similar chemically (11). We expected to find similar tolerances for these two elements. The major difference between W and Mo is in their natural occurrences. Mo is found in several mineralogical types, with molybdenite (a form of MoS_2) being the ore of commerce. Tungsten is only found naturally as tungstate derivatives (such as Scheelite). However, when Mo ores are subjected to aerobic aqueous environments, solubilized Mo rapidly oxidizes to various forms of Mo^{+6} and molybdate anions. Still, a susceptibility to tungstate could be used as a marker for molybdate intolerance. Therefore tungstate was tested. The results are shown below.

Table 15

Tungstate Tolerances of *T. ferrooxidans* Strains

Strain	Growth					
	Tungstate Concentrations					
	0 μM	5 μM	10 μM	50 μM	100 μM	500 μM
DT-3	+	+	***	-	-	-
DT-5	+	+	***	-	-	-
F221	+	+	+	-	-	-

** - These cultures took extremely long to grow, 21 days vs 5-7 days for the other cultures which were positive for growth.

It is clear from these data that tungstate is considerably more inhibitory than molybdate. Cultures grown in 10 μM tungstate continued to be highly inhibited for growth with tungstate and showed no increase in molybdate tolerance.

Because of the importance of metal tolerances to the entire project, Table 16 below summarizes the ability of *T. ferrooxidans* to tolerate appropriate metals. It should be noted that, since the denitrifying bacteria showed little Ni or molybdate inhibition except at high levels, they were not tested for these other metal ions. Since many of the denitrifiers appear to be Pseudomonads, they should be relatively resistant to the above metal ions with the possible exception of chromate. Also, the denitrifiers grow at neutral pH and therefore both vanadate and chromate would not form highly oxidizing or chemically complex species (11) and any inhibitory effects would probably be through a completely different mechanism.

Table 16

Average Maximum Tolerance of *T. ferrooxidans* Strains
for Molybdenum, Nickel and Related Metals

Metal	Average Maximum Tolerance, μM^*
Ni, as Ni^{++}	8900
Mo, as molybdate	78
Cr, as chromate	~1000**
V, as vanadate	670
W, as tungstate	10

* - average of 3 to 8 strains

** - estimate due to precipitation.

2. Natural Isolates

After obtaining natural isolates as described below, we tested them for Ni and molybdate tolerances. Although molybdate tolerances were higher by as much as three times (see Table 17), the total tolerance was not sufficiently high enough for practical purposes. There was no significant difference in Ni tolerances with these strains and the laboratory strains discussed earlier.

3. Mutation Studies

It is clear from our previous work and work in the first year that the development of a highly molybdate resistant strain of *T. ferrooxidans* would have been very difficult, time consuming and possibly not successful enough to warrant the use of these organisms in the leaching of metals from Mo or W containing catalysts. In addition, *T. denitrificans* and *T. thiooxidans* as well as *Sulfolobus* sp. are not proficient leachers of the spent Shell 324 catalyst. It may still be possible to build a technology around *T. ferrooxidans* if the leached metals can be efficiently and economically removed from the leachate as they are produced. We were not successful in producing molybdate tolerant strains with significantly increased tolerance levels. The best we were able to produce through the use of mutagens (ethylmethane sulfonate, nitrosoguanidine and nitrous acid) was a tolerance level of about 300 μM molybdate. This is not that much higher than natural isolates from a molybdenite mine which we sampled and the results of which are presented in another section

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of this report and we were never able to improve on this level after extensive efforts with either laboratory strains or natural isolates.

a. Natural strains

Through Dr. Robert Kleinmann of the U.S. Bureau of Mines, we were able to identify both a molybdenum mine and a tungsten mine which served as sources of naturally-occurring molybdate tolerant strains of *T. ferrooxidans*. The owners/operators of both facilities agreed to cooperate in both collection of samples and access to the sites. Site access proved to be a major stumbling block, especially with molybdenum mines.

Samples were obtained in late November 1990 from three sources. First, a molybdenum mine in Questa, New Mexico was sampled. This mine is quite large and has been mining molybdenite (a form of MoS_2 , which is the chemical form of the Mo in the spent catalyst) for about 70 years. The site is quite well contained now, but samples were obtained from several sites around the property and at the base of the tailings piles. Since this area also contained some thermal springs, we also sampled them for potential thermophilic strains of *T. ferrooxidans* and other *Thiobacilli*. The third site was the Curtis Tungsten mine in Upland, California. The form of tungsten in this mine is Scheelite (WO_3 , which has very limited water solubility), which in solution would form tungstate. Samples were obtained from streams in the area of this mine and the tailings pond. The results from these studies are presented in Table 17. As can be seen, natural isolates of *T. ferrooxidans* from Mo rich areas do indeed exist which are more resistant to molybdate than previously tested laboratory isolates. However, the increase in the molybdate tolerance is not great enough to be practically useful and reflect the environment. Water and soil samples collected from the molybdenum and tungsten mines were extracted with INEL broth (soil samples) and analyzed by atomic absorption spectrometry for Mo and W. Both were below detectible levels (20 ppm W or 1 ppm Mo, on a solid or liquid basis, depending on the particular sample). The best increase is from the typical approximate 100 μM of natural strains isolated from non-molybdenum containing sites to the 250 μM concentration of the molybdenum mine tailings isolate. While this isolate may prove useful, it still is not resistant enough to molybdate to make a significant impact on potential processes to recover Mo from spent coal liquefaction catalysts.

Table 17

T. ferrooxidans Isolates and their Molybdate Sensitivities

Isolate	Source	Maximum MoO_4^- Tolerance, μM
Q-Mo1	Base of Tailings Pile	250
Q-Mo2	River Across from Tailings	200
Q-Mo4	Outside Plant Gate	150
Q-Mo7	Upstream from Plant Gate	100
J-Mo1	Soda Dam, Jemez Springs	175
J-Mo3	Soda Dam, Fe precipitated material	150
C-W3	W millings	200
DT-5	INEL	85

b. ATCC cultures

We obtained all of the available strains of *T. ferrooxidans* through the American Type Culture Collection. These strains did not exhibit any significantly different tolerances for W or Mo than the natural strains or those obtained from INEL.

4. Coal Liquids and Organic Compound Tolerance

As mentioned above, *T. ferrooxidans* tolerated significant levels of organic materials when the cultures were not pure, but showed inhibition in pure culture. However, the process coal liquids showed significant inhibition for *T. ferrooxidans* and the associated microorganisms found with them. This inhibition was measured by the lack of iron oxidation and also by the total number of microorganisms present.

C. Leachate Characterization

We leached about 100 g of the THF-washed spent catalyst and collected the supernatant solution containing both Ni and Mo. This solution was filtered through a $0.22\mu\text{M}$ filter to remove the bacteria and other small particulate matter. This solution was then passed over a Chelex 100 column in the Na^+ form and the column washed with 10ml deionized water. The washings were combined with the unabsorbed material and analyzed for Ni and Mo. The column was then washed with 1N HCl to elute the bound metals and the eluate analyzed. The results are shown in Table 18. The initial unabsorbed liquids to which the washings were added were then treated to determine if the Mo was in an anionic state. This solution was passed through a Dowex 1X-200 anion exchange resin column in the chloride form. After loading the column it was washed with deionized water and the washings added to the unabsorbed liquid materials. The column was then eluted with 2M KNO_3 and the eluate analyzed for Ni and Mo. These results are

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also shown in Table 18.

It is quite clear from these data that the Ni is in a cationic form, i.e. most likely Ni^{++} since typically only that form occurs in ordinary, ambient chemistry, the other oxidation states are very rare and the Ni in Shell 324 is in predominantly in the +2 oxidation state. The data also unambiguously tell us that the Mo is in an anionic form. One would expect this because Mo in an aerobic aqueous solution usually is in some ionic form related to molybdate. However, since the form of the Mo in the catalyst is MoS_2 (i.e. the Mo is Mo^{+4}), the oxidation state of the leached Mo may still be in doubt. Clearly the leached Mo has formed some type of anion, and the penchant of Mo to form polyanions of mixed oxidation states and complexes, may leave its actual oxidation state open to question. To try to determine the actual oxidation state of Mo in these leachates we sent a sample of dried leachate to Dr. G.P. Huffman, Director of the Consortium for Fossil Fuel Liquefaction Science at the University of Kentucky who determined the valence of the Mo in the leachate. While this is important we should not lose sight of the fact that the leached Mo was readily recovered with the Dowex resin and the knowledge of its actual oxidation state may not be absolutely required for success of the project.

Table 18

Ion Exchange Properties of Leached Metals

Sample	Total mg Ni	Total mg Mo
Leachate	1280	2300
Material not absorbed to Chelex + column washings	63	2410
Chelex eluate	1120	<10
Material not absorbed to Dowex + column washings	35	120
Dowex eluate	<5	2050

The total leachate was examined by XAFS spectroscopy analysis for the oxidation state of the Mo. The following is a report on the sample. It clearly shows that the Mo is in the +6 (probably molybdate) form.

"We obtained a Mo L₁ edge XANES (edge energy = 2520 eV) spectra for a coal liquefaction catalyst leachate sample and an ammonium molybdate standard compound in fluorescence geometry.

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Top figures (see Figure 59) compares these two spectra. From the position of the peaks and their relative heights, we can conclude that the Mo exists in +6 state in the leachate sample.

To further confirm these findings, we also obtained a sulfur K edge (edge energy = 2472 eV) spectra for the leachate sample. The position of the main peak in this spectra at around 10 eV confirms that the sulfur is in the sulfate (+6) state and that there is no evidence of any sulfide phases.

A minor peak at around -3 eV in Mo XANES could be due to lower Mo oxidation state. However, from the sulfur XANES it appears that is most probably due to a background signal from sulfur edge. Since we do not have any Mo XANES for standard compounds with +5 and +5.5 oxidation states, we have not shown their spectra for comparison. If such phases exist, based on this XANES analysis, their concentration would be less than 10%."

D. Leaching Using *T. ferrooxidans*

The first indications that microorganisms could be useful in the release and recovery of metals from spent Shell 324 were found in the data shown in Table 19 below. In this experiment, we first washed THF-extracted catalyst with INEL broth until the washings were reaching a low plateau of both Ni and Mo release. At that point we then added medium and microorganisms. It is clear from these data that the microorganisms played an active role and increased metal release by approximately 20 fold. Thus, even though these organisms are sensitive to molybdate, these organisms were still able to solubilize both Ni and Mo from the catalyst matrix.

Table 19

Microbial Leaching of INEL-Washed THF-Extracted Catalyst

Sample	μM Ni Released	μM Mo Released
H ₂ O	0	0
INEL Broth	14	9
1st Wash - 24 hr	2147	782
2nd Wash - 96 hr	1295	219
3rd Wash - 24 hr	358	146
4th Wash - 24 hr	273	156
5th Wash - 48 hr	85	167
Cultures: *		
F221	256	2272
DT-3	273	2637
DT-5	324	2616
Control (uninoculated)	70	138

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* Cultures were grown in INEL broth supplemented with 1% INEL washed, THF-extracted minus 100 Mesh catalyst. The cultures were incubated for 12 days prior to analysis.

The graphical impact of these data is shown in Figure 12. The release by the microorganisms is very pronounced. This is the first data that unambiguously showed that *T. ferrooxidans* could leach metals from a catalyst matrix.

Each catalyst sample with its prewashes are described below. In addition, we have summarized these findings in a mass balance shown in Table 20. As can be seen, with the appropriate amount of time, 90% metal recovery from the catalyst is readily achieved.

A continuation of the microbial leaching experiments showed that essentially all of the Ni and most of the Mo can be released through the combined effects of chemical, physical and microbiological processes utilizing *T. ferrooxidans* in all tested catalyst samples. A summary of this data is presented in Table below.

Table 20

Percentages of Leached Metals

Catalyst Sample	% Mo Leached	% Ni Leached
Oily Wilsonville INEL Pre Wash	84	95
Oily Wilsonville H ₂ SO ₄ Pre Wash	92	91
10 Mesh-THF Extracted INEL Pre Wash	141	95
10 Mesh-THF Extracted H ₂ SO ₄	122	100
Presulfided Shell 324 INEL Pre Wash	116	74
Presulfided Shell 324 H ₂ SO ₄	84	85
Shell 324 INEL Pre Wash	111	88
Shell 324 H ₂ SO ₄	77	99

1. Shell 324

After the above data were collected we began to test *T. ferrooxidans* and its ability to leach metals from each of the four catalyst samples described earlier. The catalysts were first washed extensively with either INEL broth or 0.5M H₂SO₄ as described for Table 19. These washed catalyst samples were then treated with *T. ferrooxidans* and the results of these leaching experiments are shown in Figures 25-8. It is clear that the sulfuric acid wash is as good or better in releasing metals from new Shell 324. However, when washed with INEL broth, *T. ferrooxidans* released Mo and Ni at much higher levels and with greater rates.

2. Presulfided Shell 324

The presulfided catalyst was treated as a control on the effect of oil (substitute for coal liquids) on the ability of *T. ferrooxidans* to leach the catalyst. In each case of prewash, the microorganisms were effective in releasing both Ni and Mo from the catalyst matrix (Figures 21-4).

3. Spent Catalyst with Coal Liquids

Spent catalyst as obtained from the Wilsonville pilot plant contains about 18% coal liquids. An ideal process to release and recover metals from this material would bypass a solvent extraction. Therefore we washed this material with sulfuric acid and INEL broth, as above, and subjected them to *T. ferrooxidans*. The results of these studies are shown in Figures 13-6. Again, as with the presulfided catalyst, *T. ferrooxidans* proved to be active at the release of both Ni and Mo from this catalyst. However, the kinetics of metal release differed from Mo to Ni, with the Ni being more readily release. This catalyst material is the most complex of those studied because it not only has contaminating coal liquids, but also is poisoned by the presence of minerals deposited during the liquefaction process.

4. Solvent Extracted Spent Catalyst

The case of THF-extracted spent catalyst is simpler due to the removal of the coal liquids. However, the mineral deposits are still present. These data are shown in Figures 17-20. The kinetics of metal release are similar to those of the presulfided catalyst in that there is a delay in the Mo release and its rate of release is slower than that of Ni. However, good release was found all cases studied. This sample most closely represents the type of material which would be used in an actual process.

5. Soxhlet Reactors

Since the leaching of the catalyst involved both large volumes of liquid and long time periods, Soxhlet reactors were set to microbiologically leach Ni and Mo from both oily Wilsonville spent catalyst and Tetrahydrofuran extracted oily Wilsonville spent catalyst (10 mesh). Significant Mo and Ni were recovered from the THF extracted material and the oily Wilsonville spent catalyst. This simple reactor design had many advantages and one major disadvantage. The reactor tends to clog the material which is supporting the catalyst in the reactor. A periodic mix overcomes this negative aspect. There was a fairly high level of non microbial release of Mo and Ni in the THF-extracted material, but little non-specific release in the oily Wilsonville spent catalyst. After about 120 days we determined

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that the THF-extracted material has been leached as far as *T. ferrooxidans* is capable. The final data are presented in Table 21 below. These data show that we have successfully removed the Ni from the THF washed material and most of the Mo. The oily spent catalyst also released significant quantities of both of these metals, although some still remained. We achieved approximately a ten fold decrease in the volume necessary to treat a gram of catalyst over our previous shake flask process (275ml/g for the soxhlet reactor vs 3275ml/g for the shake flask studies employing the THF extracted material). It is quite clear that we could still significantly reduce this volume with further work.

These reactors were dismantled after we determined that little more Ni and Mo were being released. The remaining catalyst was sent out for Ni and Mo analysis. Those analyses allowed us to calculate the balances reported in Table 4. 5.4 liters of liquid have been used so far in the THF-extracted reactor (#2) for 300 g of material or 18ml/g catalyst. 5.8 liters of liquid have been used so far in oily material reactor (#1) for 300 g of material (252 g not including the oil) or 23ml/g catalyst.

We can conclude from this data that, except for oily catalyst samples (Oily Wilsonville and Presulfided), the Ni in the catalyst samples is essentially completely recovered. Also, only in the oily Wilsonville catalyst samples is the recovery of Mo slow. We can only presume that the disparate recovery numbers are due to poor initial values.

Table 21

Reactor Metal Release Studies

Reactor #	Catalyst Sample	% Ni Released	% Mo Released
1	Oily Wilsonville	78.45	76.36
2	THF-Extracted	97.27	93.56

E. *Sulfolobus* and other Thiobacilli

Further microbial leaching experiments were performed. These employed both *T. thiooxidans* and *Sulfolobus*. When these cultures were kept pure, they were very poor at leaching Mo and Ni from THF-washed spent catalyst (both showed <5% increase over uninoculated controls). If the cultures were not kept sterile, then better leaching occurred, but the cultures then contained a majority of the microorganisms as *T. ferrooxidans* and presumably the leaching was due to the presence of *T. ferrooxidans* as a

contaminant. The outcome of these experiments shows that the only potential acidophilic organism with good leaching characteristics is *T. ferrooxidans*.

F. Denitrifying Bacteria

In work unrelated to this contract, we noticed that denitrifying bacteria which are regularly found in oil fields are capable of utilizing FeS as a source of S and can solubilize the Fe and use this chemical as both a source of nutritional Fe and S. Since the spent coal liquefaction catalyst contains both Ni and Mo sulfides as well as Fe sulfides, this ability might be used to recover both Mo and Ni from the spent catalyst.

1. Advantages/Disadvantages

In addition these organisms would have many more advantages not available with the typical leaching microorganisms exemplified by *T. ferrooxidans*. These include:

1. High resistance to molybdate
2. Good Ni tolerance.
3. These organisms are anaerobic, making simple leaching piles easy to operate without the constraints imposed by the necessity of supplying oxygen.
4. The organisms are apparently very resistant to the toxic effects of the coal liquids.
5. The products of their metabolism, CO₂, N₂ and water are environmentally innocuous.

Initial experiments showed very good growth in the presence of the spent catalyst and the presence of the coal liquids. This is greatly different from the effects noticed for *T. ferrooxidans* earlier. In addition very good growth occurred in the presence of THF-washed catalyst (10 mesh).

In 60 days over 90% of the Mo was released in 2 cultures, utilizing a volume only 8% required by *T. ferrooxidans* for a similar release of metals performed in the first year. There also appears to be a great potential for decreasing the volume of these leachings. In addition there appears to be some selectivity for Mo, the targeted metal of this contract, depending on the organic growth substrate. These experiments were performed with 10 mesh THF-washed catalyst and finer material may very well release more of the metal.

2. Reaction with Coal Liquids

Microorganisms grown under denitrifying conditions were exposed to spent catalyst containing coal liquids and to added

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coal liquids up to 2% (v/v). These microorganisms grew just as rapidly under these conditions as they did when the coal liquids were not present. Clearly, the coal liquids are not toxic or inhibitory for denitrifiers as they are for *T. ferrooxidans*. However, denitrifiers do not leach metals from the spent catalyst very well in the presence of coal liquids. Therefore, solvent pretreatment of the spent catalyst is necessary for denitrifiers to successfully release metals from the spent catalyst.

3. Carbon Sources

Initially we thought that the ability of denitrifiers to release metals from the catalyst might be related to the fact that citrate and succinate are relatively good chelating agents. We selected carbon and energy sources based on the criteria that they are not fermentable and that they are relatively inexpensive. We also wanted a diverse group of microorganisms present, so we chose carbon and energy sources which are typically widely used by microorganisms. Therefore we chose the following compounds for further study: adipic acid, citric acid, EDTA (not a growth substrate, used as a control), lactic acid/EDTA mixture, lactic acid, malonic acid, oxalic acid and succinic acid. The results of these studies are shown in Figures 29-36 and summarized in Table 23. It should be emphasized that the growth medium for these studies contained no S and the organisms were obliged to scavenge nutritional S from the catalyst.

Table 22

Release of Mo and Ni from THF-Washed Spent
Coal Liquefaction Catalyst by Denitrifying Bacteria

Catalyst Sample	C Source	Initial		Released		% Released	
		g Mo	mg Ni	mg Mo	mg Ni	Mo	Ni
THF-Washed	Succinate	1.00	207	545	13	88	71
THF-Washed	Lactate	1.04	215	702	29	88	77
THF-Washed	Lactate-EDTA	1.27	262	791	200	92	89
THF-Washed	Adipate	.95	197	639	18	88	66
THF-Washed	Malonate	.99	204	789	78	97	76
THF-Washed	Citrate	.98	202	789	96	96	78
THF-Washed	Oxalate*	.98	203	518	83	68	51
THF-Washed	EDTA	1.00	205	502	126	56	62
THF-Washed	none	1.01	205	422	11	62	55

* - There was very poor growth under denitrifying conditions with oxalate as a sole source of carbon and energy.

In 26 days up to 81% of the Mo was released, utilizing a
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volume only 8% of that required by *T. ferrooxidans* for a similar release of metals. As can be seen the presence of the chelator appears to facilitate the release of Ni from the spent catalyst, but does not seem to affect the release of Mo. All of the carbon and energy sources were chosen because of the ability of the carbon compound to serve two functions: 1) to act as carbon and energy sources for the denitrifying bacteria and 2) to act as chelators of divalent cationic metal ions. In addition there appears to be some selectivity for Mo, the targeted metal of this contract, depending on the organic growth substrate. We still do not know the extent of this specificity. It may be possible to selectively release either of these two metals, although our previous studies have shown that the metals are readily recovered, specifically, from a mixed solution. We also do not know the effect of mesh size on metal release. These experiments were performed with 10 mesh THF-washed catalyst and finer material may very well release more of the metal. In the remaining time on the contract we will pursue these experiments to see if we can make the economics and feasibility of the process attractive enough for further work. Notice the dramatic reduction in time frames compared with previous reports.

4. Particle Size

We began the rather laborious task of crushing each of the solvent-extracted catalyst samples for the determination of the effect of crushing on leaching rate and per cent metal recovered. We searched for an alternative method which could save time. We found that a Retsch mill or other electric powered device might allow us to more efficiently process these samples. However, this device produces material of only one particle size. The crushing of these catalyst samples using a ball mill takes approximately 1 week to crush each sample. Therefore a mill was used to produce a fine powder and these samples were tested using denitrifying bacteria for their ability to release Ni and Mo from these samples. The data are shown in Figures 37-52 as well as comparisons for rates of Ni and Mo released in relation to catalyst solvent pretreatment. These data were obtained with citrate as a carbon and energy source for the microorganisms. It is quite clear that crushing the catalyst allows for a more rapid release of these metals from the catalyst samples, regardless of the organic solvent used to pretreat the catalyst. However, the effects of the different solvents are still evident, although not as striking, after the catalysts are crushed (see previous reports). There also still appears to be a greater ease in removing Mo over Ni, a case particularly prevalent if ethyl acetate, methanol or xylene is used as the pretreatment solvent. In any case, it appears that a variety of solvents will be useful and that an optimized system could yield >90% release and recovery of both Ni and Mo in as little as 10 days of treatment.

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5. Extraction Solvent Effects

As reported earlier, the use of a solvent helps in the microbiological release of metals from the spent catalyst. Depending on the solvent, varying efficiencies of release of bot Ni and Mo are obtained. These are summarized below in Table 23. Table 10 shows the solvents tested. Previous work had centered primarily on tetrahydrofuran, but it appears from the data that other solvents may be superior to tetrahydrofuran. Figures 53-9 summarize these findings.

Table 23

Quality of Solvents for Catalyst Pretreatment

Solvent	Relative Efficiency (100 = best)		
	Ni	Mo	Ni+Mo
Tetrahydrofuran, 100 mesh	97	99	98
Pyridine	29	63	46
Dioxane	32	44	38
Acetic Acid	28	45	37
Toluene	24	42	33
iso-Propanol	25	37	31
Formic Acid	29	31	30
Methanol	15	38	27
Tetrahydrofuran	14	39	27
Acetone	15	38	27
Chloroform	15	37	26
Ethyl Acetate	13	38	26
Methylene Chloride	12	38	25
Hexane	14	33	24
Xylenes	11	36	24
Carbon Tetrachloride	22	21	22
Water	24	11	18

6. Carbon Source Effects

The primary carbon source effect noticed for the denitrifying bacteria is the apparent and reproducible selective release of Mo over Ni in some cases. This is exemplified by the cases of succinate, malonate and citrate, all of which are metal chelators. Other than this selectivity, there appears to be no good ground at this point for superiority of one carbon and energy source over another.

IV. Conclusions

The project goals were simple. GMT was to leach coal liquefaction catalyst with *T. ferrooxidans* and other organisms to release and recover metals from these catalysts. In addition, a good mass balance was desired. We were successful in all of these goals. Basically our conclusions are listed below:

1. *T. ferrooxidans* is capable of leaching both Ni and Mo from spent Shell 324. However, the inhibitory effects of molybdate on *T. ferrooxidans* make the organism relatively unappealing for practical purposes. In order to completely leach Ni and Mo from spent catalyst efficiently, solvent extraction, large volumes (>2000ml/g catalyst) and long times (4 months) are required.

2. Leached metals can be readily recovered from the leachate by conventional techniques such as ion exchange. Characterization of the leachate revealed that the Ni was in the form of Ni^{++} and that the Mo was in an anionic form (from XANES/XAFS data), presumable as molybdate or some complex polyanion.

3. Heterotrophic denitrifying bacteria are capable of solubilizing metal sulfides and are good at releasing metals from the spent catalyst which has been extracted with various organic solvents. There is some unexpected selectivity of Mo over Ni based on the carbon and energy source. Pyridine appears to be the best solvent for extraction of the coal liquids. Another advantage is the anaerobiosis of the system and the volumes required for complete metal release (about 20ml/g). Times are also far superior to those employing *T. ferrooxidans* with >90% recovery occurring in less than 20 days compared to 4 months with *T. ferrooxidans*.

4. Future work will concentrate on identifying the best solvents, carbon and energy sources and physical conditions for efficient leaching. In addition, optimization of times and volumes required for treatment will be performed.

V. Technology Transfer

Several activities have been instigated to facilitate technology transfer. These are summarized below.

Publications.

A paper was presented at the American Society for Microbiology Annual Meetings in New Orleans in May 1992. *Microbiological Recovery and Removal of Molybdenum and Nickel*

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from Spent Coal Liquefaction Catalysts. Donald O. Hitzman, Penny L. Sperl and George T. Sperl. Bacteriol Proc. p. 346.

A paper was also presented at the Second International Symposium on the Biological Processing of Coal, San Diego, Ca. 1991.

Patent.

A patent was filed on May 22, 1992: Inventors: George T. Sperl and Donald O. Hitzman. ~~Method for Treatment of Catalysts Using Denitrifying Bacteria.~~ Serial No. 07/887,011

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Figure 1

Leaching of Ni and Mo from Shell 324 with Sulfuric Acid

Chemical Leaching of New Shell 324 Leached with Sulfuric Acid

Figure 2

**Leaching of Ni and Mo from Presulfided Shell 324 with Sulfuric
Acid**

Chemical Leaching of Presulfided Shell 324
Leached with Sulfuric Acid

Figure 3

**Leaching of Ni and Mo from THF-Extracted Spent Shell 324 with
Sulfuric Acid**

Chemical Leaching of THF-Extracted Spent Shell 324 Leached with Sulfuric Acid

Figure 4

Leaching of Ni and Mo from Spent Shell 324 with Sulfuric Acid

Chemical Leaching of Spent Shell 324 Leached with Sulfuric Acid

Figure 5

Leaching of Ni and Mo from Shell 324 with INEL Broth

Chemical Leaching of New Shell 324 Leached with INEL Broth

Figure 6

Leaching of Ni and Mo from Presulfided Shell 324 with INEL Broth

Chemical Leaching of Presulfided Shell 324 Leached with INEL Broth

Figure 7

**Leaching of Ni and Mo from THF-Extracted Spent Shell 324 with
INEL Broth**

Chemical Leaching of THF-Extracted Spent Shell 324 Leached with INEL Broth

Figure 8

Leaching of Ni and Mo from Spent Shell 324 with INEL Broth

Chemical Leaching of Spent Shell 324 Leached with INEL Broth

Figure 9

**A Comparison of the leaching of Ni from Various Catalyst Samples
with Sulfuric Acid and INEL Broth**

Chemical Leaching of Ni from Catalyst Samples

Figure 10

**A Comparison of the Leaching of Mo from Various Catalyst Samples
with Sulfuric Acid and INEL Broth**

Figure 11

**XANES/XAFS Fluorescence Spectra of Leachate Solids from THF-
Extracted Spent Shell 324**

Figure 12

Leaching with *T. ferrooxidans* of Ni and Mo from INEL-Washed,
THF-Extracted Spent Shell 324

Leaching of Additional Metal from Washed Catalyst

Figure 13

Leaching of Ni with *T. ferrooxidans* of INEL-Washed Spent Shell
324

Microbial Leaching of Ni from Spent Shell 324 INEL Broth Wash

Figure 14

Leaching of Mo with *T. ferrooxidans* of INEL-Washed Spent Shell
324

Microbial Leaching of Mo from Spent Shell 324 INEL Broth Wash

Figure 15

**Leaching of Ni by *T. ferrooxidans* of Sulfuric Acid Washed,
Spent Shell 324**

Microbial Leaching of Ni from Spent Shell 324 Sulfuric Acid Wash

Figure 16

**Leaching of Mo by *T. ferrooxidans* from Sulfuric Acid Washed
Spent Shell 324**

Microbial Leaching of Mo from Spent Shell 324 Sulfuric Acid Wash

Figure 17

**Leaching of Ni by *T. ferrooxidans* of INEL-Washed, THF-Extracted
Spent Shell 324**

Microbial Leaching of Ni from THF-Extracted Spent Shell 324
INEL Broth Wash

Figure 18

**Leaching of Mo by *T. ferrooxidans* from INEL-Extracted,
THF-Extracted Spent Shell 324**

Microbial Leaching of Mo from THF-Extracted Spent Shell 324
INEL Broth Wash

Figure 19

Leaching of Ni by *T. ferrooxidans* from Sulfuric Acid Washed,
THF-Extracted Spent Shell 324

Microbial Leaching of Ni from THF-Extracted Spent Shell 324
Sulfuric Acid Wash

Figure 20

**Leaching of Mo by *T. ferrooxidans* from Sulfuric Acid Washed,
THF-Extracted Spent Shell 324**

Microbial Leaching of Mo from THF-Extracted Spent Shell 324 Sulfuric Acid Wash

Figure 21

Leaching of Ni by *T. ferrooxidans* from INEL-Washed, Presulfided
Shell 324

Microbial Leaching of Ni from Presulfided Shell 324 INEL Broth Wash

Figure 22

**Leaching of Mo by *T. ferrooxidans* from INEL-Washed, Presulfided
Shell 324**

Microbial Leaching of Mo from Presulfided Shell 324 INEL Broth Wash

Figure 23

Leaching of Ni by *T. ferrooxidans* from Sulfuric Acid Washed,
Presulfided Shell 324

Microbial Leaching of Ni from Presulfided Shell 324 Sulfuric Acid Wash

Figure 24

**Leaching of Mo by *T. ferrooxidans* from Sulfuric Acid Washed,
Presulfided Shell 324**

Microbial Leaching of Mo from Presulfided Shell 324 Sulfuric Acid Wash

Figure 25

Leaching of Ni by *T. ferrooxidans* from INEL-Washed, New Shell 324

Microbial Leaching of Ni from Shell 324 INEL Broth Wash

Figure 26

Leaching of Mo by *T. ferrooxidans* from INEL-Washed, New Shell 324

Microbial Leaching of Mo from Shell 324 INEL Broth Wash

Figure 27

**Leaching of Ni by *T. ferrooxidans* from Sulfuric Acid Washed,
New Shell 324**

Microbial Leaching of Ni from Shell 324 Sulfuric Acid Wash

Figure 28

**Leaching of Mo by *T. ferrooxidans* from Sulfuric Acid Washed,
New Shell 324**

Microbial Leaching of Mo from Shell 324
Sulfuric Acid Wash

Figure 29

**Leaching of Ni and Mo from Whole THF-Extracted Spent Shell 324
with Denitrifying Bacteria with Adipate as the Carbon and Energy
Source**

Catalyst Leaching by Denitrifying Bacteria

Carbon & Energy Source: Adipate

Figure 30

**Leaching of Ni and Mo from Whole THF-Extracted Spent Shell 324
with Denitrifying Bacteria with Citrate as the Carbon and Energy
Source**

Catalyst Leaching by Denitrifying Bacteria

Carbon & Energy Source: Citrate

Figure 31

**Leaching of Ni and Mo from Whole THF-Extracted Spent Shell 324
with Denitrifying Bacteria with EDTA as the Carbon and Energy
Source**

Catalyst Leaching by Denitrifying Bacteria Carbon & Energy Source: EDTA

Figure 32

**Leaching of Ni and Mo from Whole THF-Extracted Spent Shell 324
with Denitrifying Bacteria with Lactate/EDTA as the Carbon and
Energy Source**

Catalyst Leaching by Denitrifying Bacteria Carbon & Energy Source: Lactate/EDTA

Figure 33

**Leaching of Ni and Mo from Whole THF-Extracted Spent Shell 324
with Denitrifying Bacteria with Lactate as the Carbon and Energy
Source**

Catalyst Leaching by Denitrifying Bacteria Carbon & Energy Source: Lactate

Figure 34

**Leaching of Ni and Mo from Whole THF-Extracted Spent Shell 324
with Denitrifying Bacteria with Malonate as the Carbon and Energy
Source**

Catalyst Leaching by Denitrifying Bacteria Carbon & Energy Source: Malonate

Figure 35

**Leaching of Ni and Mo from Whole THF-Extracted Spent Shell 324
with Denitrifying Bacteria with Oxalate as the Carbon and Energy
Source**

Catalyst Leaching by Denitrifying Bacteria

Carbon & Energy Source: Oxalate

Figure 36

**Leaching of Ni and Mo from Whole THF-Extracted Spent Shell 324
with Denitrifying Bacteria with Succinate as the Carbon and
Energy Source**

Catalyst Leaching by Denitrifying Bacteria

Carbon & Energy Source: Succinate

Figure 37

**Leaching of Ni and Mo from Crushed (>100 mesh) and Whole Acetone-
Extracted Spent Shell 324 using Denitrifying Bacteria with
Citrate as the Carbon and Energy Source**

Leaching of Crushed vs Whole Catalyst Acetone-Washed

Figure 38

Leaching of Ni and Mo from Crushed (>100 mesh) and Whole Acetic Acid-Extracted Spent Shell 324 using Denitrifying Bacteria with Citrate as the Carbon and Energy Source

Leaching of Crushed vs Whole Catalyst Acetic acid—Washed

Figure 39

**Leaching of Ni and Mo from Crushed (>100 mesh) and Whole
Acetonitrile-Extracted Spent Shell 324 using Denitrifying
Bacteria with Citrate as the Carbon and Energy Source**

Leaching of Crushed vs Whole Catalyst Acetonitrile-Washed

Figure 40

**Leaching of Ni and Mo from Crushed (>100 mesh) and Whole
Chloroform-Extracted Spent Shell 324 using Denitrifying Bacteria
with Citrate as the Carbon and Energy Source**

Leaching of Crushed vs Whole Catalyst Chloroform-Washed

Figure 41

**Leaching of Ni and Mo from Crushed (>100 mesh) and Whole Dioxane-
Extracted Spent Shell 324 using Denitrifying Bacteria with
Citrate as the Carbon and Energy Source**

Leaching of Crushed vs Whole Catalyst Dioxane-Washed

Figure 42

Leaching of Ni and Mo from Crushed (>100 mesh) and Whole Formic Acid-Extracted Spent Shell 324 using Denitrifying Bacteria with Citrate as the Carbon and Energy Source

Leaching of Crushed vs Whole Catalyst Formic acid-Washed

Figure 43

**Leaching of Ni and Mo from Crushed (>100 mesh) and Whole
Methanol-Extracted Spent Shell 324 using Denitrifying Bacteria
with Citrate as the Carbon and Energy Source**

Leaching of Crushed vs Whole Catalyst Methanol-Washed

Figure 44

**Leaching of Ni and Mo from Crushed (>100 mesh) and Whole Xylenes-
Extracted Spent Shell 324 using Denitrifying Bacteria with
Citrate as the Carbon and Energy Source**

Leaching of Crushed vs Whole Catalyst Xylenes—Washed

Figure 45

**Leaching of Ni and Mo from Crushed (>100 mesh) and Whole Ethyl
Acetate-Extracted Spent Shell 324 using Denitrifying Bacteria
with Citrate as the Carbon and Energy Source**

Leaching of Crushed vs Whole Catalyst Ethyl Acetate—Washed

Figure 46

**Leaching of Ni and Mo from Crushed (>100 mesh) and Whole Hexane-
Extracted Spent Shell 324 using Denitrifying Bacteria with
Citrate as the Carbon and Energy Source**

Leaching of Crushed vs Whole Catalyst Hexane-Washed

Figure 47

**Leaching of Ni and Mo from Crushed (>100 mesh) and Whole
Isopropanol-Extracted Spent Shell 324 using Denitrifying Bacteria
with Citrate as the Carbon and Energy Source**

Leaching of Crushed vs Whole Catalyst Isopropanol-Washed

Figure 48

**Leaching of Ni and Mo from Crushed (>100 mesh) and Whole
Methylene Chloride-Extracted Spent Shell 324 using Denitrifying
Bacteria with Citrate as the Carbon and Energy Source**

Leaching of Crushed vs Whole Catalyst Methylene Chloride—Washed

Figure 49

Leaching of Ni and Mo from Crushed (>100 mesh) and Whole
Pyridine-Extracted Spent Shell 324 using Denitrifying Bacteria
with Citrate as the Carbon and Energy Source

Leaching of Crushed vs Whole Catalyst Pyridine-Washed

Figure 50

**Leaching of Ni and Mo from Crushed (>100 mesh) and Whole
Tetrahydrofuran-Extracted Spent Shell 324 using Denitrifying
Bacteria with Citrate as the Carbon and Energy Source**

Leaching of Crushed vs Whole Catalyst Tetrahydrofuran—Washed

Figure 51

**Leaching of Ni and Mo from Crushed (>100 mesh) and Whole Toluene-
Extracted Spent Shell 324 using Denitrifying Bacteria with
Citrate as the Carbon and Energy Source**

Leaching of Crushed vs Whole Catalyst
Toluene-Washed

Figure 52

**Leaching of Ni and Mo from Crushed (>100 mesh) and Whole Water-
Extracted Spent Shell 324 using Denitrifying Bacteria with
Citrate as the Carbon and Energy Source**

Leaching of Crushed vs Whole Catalyst Water-Washed

Figure 53

Mo Release from Crushed and Whole Spent Catalyst by Denitrifying Bacteria with Citrate as a Carbon and Energy Source. Catalysts were washed with the following solvents: Methanol, Formic Acid, Dioxane, Chloroform, Xylenes

Mo Release from Spent Catalyst Crushed vs Whole, Solvent-Washed

Figure 54

**Mo Release from Crushed and Whole Spent Catalyst by Denitrifying
Bacteria with Citrate as a Carbon and Energy Source. Catalysts
were washed with the following solvents: Toluene,
Tetrahydrofuran, Pyridine, Methylene Chloride**

Mo Release from Spent Catalyst Crushed vs Whole, Solvent-Washed

Figure 55

Mo Release from Crushed and Whole Spent Catalyst by Denitrifying Bacteria with Citrate as a Carbon and Energy Source. Catalysts were washed with the following solvents: Isopropanol, Hexane, Ethyl Acetate, Carbon Tetrachloride

Mo Release from Spent Catalyst Crushed vs Whole, Solvent-Washed

Figure 56

Mo Release from Crushed and Whole Spent Catalyst by Denitrifying Bacteria with Citrate as a Carbon and Energy Source. Catalysts were washed with the following solvents: Water, Acetone, Acetic Acid

Mo Release from Spent Catalyst Crushed vs Whole, Solvent-Washed

Figure 57

Ni Release from Crushed and Whole Spent Catalyst by Denitrifying Bacteria with Citrate as a Carbon and Energy Source. Catalysts were washed with the following solvents: Xylenes, Methanol, Formic Acid, Dioxane

Ni Release from Spent Catalyst Crushed vs Whole, Solvent-Washed

Figure 58

Ni Release from Crushed and Whole Spent Catalyst by Denitrifying Bacteria with Citrate as a Carbon and Energy Source. Catalysts were washed with the following solvents: Toluene, Tetrahydrofuran, Pyridine, Methylene Chloride

Ni Release from Spent Catalyst Crushed vs Whole, Solvent-Washed

Figure 59

Ni Release from Crushed and Whole Spent Catalyst by Denitrifying Bacteria with Citrate as a Carbon and Energy Source. Catalysts were washed with the following solvents: Isopropanol, Hexane, Ethyl Acetate, Carbon Tetrachloride

Ni Release from Spent Catalyst Crushed vs Whole, Solvent-Washed