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Microbial Recovery of Metals from Spent Coal Liquefaction Catalysts

Final and Quarterly Report

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Summary

Mo release from spent coal liquefaction catalysts has been shown to be dependent upon many parameters, but release is dominated by microbial growth. The microbial Mo release is a rapid process requiring less than one week for 90% of the releaseable Mo to be solubilized from whole washed (THF) catalyst. It could be expected that the rates would be even greater with crushed catalyst. Efforts were centered on optimizing the parameters that stimulate microbial growth and action and further efforts centered on catalyst pre-treatment prior to microbial bio-leaching. Recent experiments suggest that hydrogen peroxide promises to be an effective pre-treatment wash. Hydrogen peroxide was also found to be an effective and economical agent for metals solubilization per se and could promote solubilization without subjecting the catalyst to microbial growth.

Project Objective

The project objectives outlined in the previous reports involved defining conditions and cultures best suited to achieve the most effective metal release from spent coal liquefaction catalysts by microbial processes. Various microbiological and physiochemical parameters which may be important in microbial release of metals from spent coal liquefaction catalysts were investigated so that this technology could be optimized.

Introduction

The work initiated in the first and second quarters of 1994 was continued and expanded using solvent extraction systems defined by the multiple solvent tests used for washing the catalysts. Several of the solvents had desirable characteristics and would provide an extracted test catalyst which would respond well to the leaching ability of the cultures. Upon review of the data, tetrahydrofuran (THF) was selected as a representative system following consideration of attributes including cost, ease of handling, and extractive capabilities. In the present simplified, extractive system, the catalysts are washed with the solvent with simple agitation to prevent catalyst breakage. This approach of using unbroken catalyst was considered feasible since the controlled breakage and particle sizing would involve extensive grinding and sieving to obtain selective and reproducible samples having similar surface areas. It has been shown that the greater the surface area, the greater and more rapid will be the metals release. Thus, it can be expected and demonstrated that positive results with unbroken catalysts can be enhanced by increasing surface area. Studies on the effect of catalyst surface area were continued in conjunction with the progressive development of the optimized microbial metal releasing system. Since THF has proven to be a superior solvent system as judged by metal release, research concentrated on using THF as the solvent system which allowed work to proceed rapidly. Also, changing the oxidation state of the metals within the

catalyst matrix took on new importance as hydrogen peroxide shows promise as a pre-treatment solvent which also acts as a metals releasing agent. These studies include measurements of metal release at various catalyst/media ratios. The importance of the catalyst/media ratios assumed a greater importance since it is desired to reduce the media volume to the lowest level possible while still retaining good metals release. To maintain continuity and demonstrate the rational for the experiments performed in the last quarter, the results from last report are included herein followed by the results for this quarter starting with test number 44.

Methodology

The solvent washed catalysts were examined in a series of controlled studies of microbial growth curve data versus observed metal release using a carbon source selected from the previously tested multiple fermentative carbon sources. Previous work has shown that many carbon sources can be used to enhance the metals releasing capabilities of the denitrifying cultures. Thus, the selection of the carbon source would be governed by a series of parameters involving cost and availability considerations in addition to increased growth efficiency. The carbon source selected was citrate because it results in microbial growth enhancement and its ability as a chelating agent. The effect of metabolizable chelating sources in the growth media was studied and their impact on the observed molybdenum and nickel release determined. It has been found that chelators (such as citrate and EDTA) will cause initial rapid release of the metals. This chelating effect confers an added advantage to the biological releasing system and was more fully investigated.

Multiple microbial metal releasing experiments with a series of comparative growth systems were established. The basic growth medium (Table 1) was supplemented with various carbon sources and catalyst loadings as well as a trace metal solution (Table 2). All cultures were incubated at 30° C. The growth studies were conducted with THF solvent extracted catalyst and with various concentrations of media additions and include various tests of catalyst-media ratios. Metals released at shorter time periods (every two days) were determined and comparative responses related to microbial growth (turbidity), pH changes, and gas production.

TABLE 1
MEDIUM B

1.0g KH₂PO₄
1.8g Na₂HPO₄
10.0g NH₄NO₃
5.0g Na₃ Citrate
1.0ml SL-4 trace metals (See Table 2)
1L dH₂O

Used for growing bacteria in the presence of catalyst

NOTE: For lactate and isopropanol growth medium, substitute 5g Na Lactate or 5 ml isopropanol for Na Citrate and experiments in the last quarter employed medium B in which the citrate concentration and ammonium nitrate concentration were elevated to 1 and 1.5% respectively.

TABLE 2
SL-4 Trace Metals (500X)

Chemical Component	mg/l
EDTA, di Na salt	500
FeCl ₂ •5H ₂ O	100
MnCl ₂ •4H ₂ O	25
CuCl ₂ •2H ₂ O	10
CoCl ₂ •6H ₂ O	20
NiCl ₂ •6H ₂ O	20
Na ₂ MoO ₄ •2H ₂ O	30
Na ₂ WO ₄	5
Na ₂ SeO ₃	5
H ₃ BO ₃	25

Thermophilic pure cultures were obtained from the American Type Culture Collection (*Bacillus stearothermophilus* ATCC 12016) and the cultures were grown at 60°C for metal releasing studies. It was later noted that higher levels of catalyst were inhibitory for growth of this thermophile, therefore strain development for tolerance to catalyst was being undertaken. Strain tolerance has not been successfully developed yet. A culture of *Metallosphaera sedula* was obtained from Dr. Thomas Clark (DOE-PETC) in order to determine if this microorganism had the capability of anaerobic growth under denitrifying conditions. We have not been able to grow *Metallosphaera sedula* under denitrifying conditions and have had

difficulties reviving this culture under aerobic conditions. We had hoped to obtain the thermophilic denitrifying strains FV-A and ITI 382-H-90 from Dr. Jacob Kristjansson (Institute of Biology, University of Iceland) in order to test the metal releasing capabilities of these bacteria isolated from Icelandic hot springs and we obtained a sampling permit from the U.S. Park Service so that we could use enrichment cultures for thermophilic denitrifiers using Yellowstone National Park hot spring algal-bacterial mat material as a source of inocula. In addition, Dr. Pieter Visscher, USGS, Menlo Park, CA kindly consented to supplying us with a culture of his unique marine denitrifier (ASN-1) which degrades alkyl sulfides so that this organism can be tested for its ability to release metals from spent coal liquefaction catalysts.

All work will centered on development of a low cost process system having optimum metal (molybdenum) releasing capabilities for initiation of preliminary economic evaluation studies.

The effectiveness of the heterotrophic denitrifying bacteria to remove the nickel and molybdenum at high rates and for the system to operate efficiently at small volumes was further defined to develop a practical and cost effective processing system for coal liquefaction catalysts.

Data from several of the tests conducted since the last quarterly report are summarized below in the text and in some cases are reported in tabular form. Each test was designed to provide insights into several parameters that might be important for metals release and are numbered in chronological order. Tabular data will be presented for molybdenum and nickel release. Except where noted, tests employed 100 ml of medium contained in capped prescription bottles and solubilized metals were analyzed by atomic adsorption after media ultrafiltration to remove cell biomass and catalyst debris.

In this last quarter a new culture transfer/rejuvenation procedure was adopted wherein mixed denitrifying cultures were transferred every 3 to 4 days into fresh medium of a composition which duplicated the culture conditions which would probably be used in specific tests. This rejuvenation medium always contained 1.0 g of spent THF washed catalyst. This new procedure led to faster and greater levels of Mo release than has been seen in the past.

Test Results

It was found that initial and rapid metal release was due to the chelating power of the citrate medium followed by increased release due to microbial growth. Metal release by microbial growth was slower than the initial chelating effect and a preferential release of the molybdenum was evident. Tests involving the ability of cultures which have been refortified with rejuvenated nutrient supplementation to release more metals were undertaken. In addition, the effects of non chelating

carbon sources such as lactate to enhance microbial growth, but which have no chelating power, were compared to comparable growth media employing citrate.

Data from a representative, newly initiated experiment (only several days old) is provided in Table 3 below as an illustration of the efforts to define optimum economic operational parameters.

TABLE 3
Comparative Mo Release in Presence of Isopropanol
and THF Washed Catalysts
% Mo Released After 11 Days

Sample	Isopropanol Washed Shell 324	THF Washed Shell 324
1	18.5	24.6
2A	31.4	48.5
2B	27.2	47.2
3	36.4	62.6
4	33.7	56.8
5	24.1	34.0
6	28.7	40.0
7	27.2	39.7
8	29.0	36.7

Sample

- 1 3.0g cat in H₂O
- 2A 3.0g cat in citrate media
- 2B Same as 2A, but media rejuvenated at day 7
- 3 1.5g cat in citrate media
- 4 5.0g cat in citrate media
- 5 3.0g cat in isopropanol media
- 6 3.0g cat in lactate media
- 7 1.5g cat in thermophilic culture at 60°C
- 8 3.0g cat in thermophilic culture at 60°C

Test 1 Results

Test 1 was designed primarily to determine if the alteration of components of the medium employed (see Table 1), or changing the levels of catalyst treated, affected metals release. It is evident from the data presented in Table 4 that several variables affect the release of Mo from the catalyst (see Table 4 below). In general, the greatest percentage of Mo was released when the catalyst/media ratio was lowest and citrate levels were higher than the medium recipe outlined previously in Table 1. Varying the levels of nitrate in the medium had little effect for the release of Mo

in these cultures and overall best release for Mo and Ni from the catalyst occurred in the culture employing 1.0% citrate and 1.5% nitrate. Bottles were monitored for metals release for up to 24 days after inoculation. While higher percentages of Mo were released at 24 days, the increase in percentage Mo released was never more than 10% higher (data not shown) than after 10 days. Therefore, a 10 day incubation after inoculation was chosen as an appropriate sample time frame for examining incubation parameters.

TABLE 4
Test 1
% Mo Released After 10 Days Under Various Culture Conditions

Sample	Media	Grams Catalyst	% Citrate	% NH ₄ NO ₃	% Mo Released
1	Medium B	1.5	-	-	55
2	Medium B	3.0	-	-	46
3	Medium B	5.0	-	-	43
4	Medium B	10.0	-	-	39
5	Altered Medium B*	3.0	0.25	1.0	38
6	Altered Medium B	3.0	0.75	1.0	47
7	Altered Medium B	3.0	1.0	1.0	47
8	Altered Medium B	3.0	0.5	1.5	43
9	Altered Medium B	3.0	0.5	0.5	43
10	Altered Medium B	3.0	0.25	1.5	34
11	Altered Medium B	3.0	0.25	0.5	38
12	Altered Medium B	3.0	1.0	1.5	47

* Medium B altered by changing levels of catalyst, citrate and NH₃NO₃

Test 2 Results

One parameter investigated in Test 2 was particularly revealing because it showed that (see Table 5 below) more Mo is leached from the spent catalyst as a result of bacterial growth versus an uninoculated blank control. Also, more Mo was released when growth occurred in the presence of nitrate as opposed to the chloride ion suggesting that denitrifying growth promotes Mo release. In general, higher turbidity readings correlated with increased Mo release, whereas, it was surprising that bacterial growth conferred no added Ni release suggesting that Ni release is preferentially influenced chemically. Ni release was no greater for bottles exhibiting good growth and no increase in Ni release was observed after three days (data not shown). These data support the idea that Ni is preferentially chemically leached. These results confirm previous experiments that Mo release paralleled bacterial growth, and the majority of the Mo release occurred within 10 days.

TABLE 5
Test 2
Comparison of Metal Release and Microbial Growth
% Mo and Ni Released After 10 Days* and Turbidity

<u>Sample</u>	<u>Media</u>	<u>Aerobic/ Anaerobic</u>	<u>% Mo Release</u>	<u>% Ni Release</u>	<u>Turbidity</u>
1	Citrate B	Aerobic	47.9	13.1	0.72
2	Citrate B - Uninoculated	Aerobic	30.5	29.1	0.04
3	Lactate B	Aerobic	19.0	3.2	0.15
4	Lactate B - Uninoculated	Aerobic	30.0	11.6	0.02
5	Water Blank	Aerobic	28.3	11.6	0.01
6	Citrate B NH ₄ Cl (as N Source)	Aerobic	27.9	13.3	0.36
7	Citrate B NH ₄ Cl - Uninoculated	Aerobic	37.4	27.6	0.04
8	Citrate B	Anaerobic	46.0	14.5	0.36
9	Citrate B - Uninoculated	Anaerobic	28.0	27.5	0.04
10	Lactate B	Anaerobic	29.7	12.9	0.11
11	Lactate B - Uninoculated	Anaerobic	29.6	11.5	0.05
12	Water Blank	Anaerobic	30.1	11.6	0.05
13	Citrate B NH ₄ Cl (as N Source)	Anaerobic	30.7	13.8	0.24
14	Citrate B 0.25% Citrate	Anaerobic	39.6	13.5	0.44
15	Citrate B 0.25% Citrate Uninoculated	Anaerobic	37.4	11.9	0.04
16	Citrate B 0.75% Citrate	Anaerobic	38.8	27.3	0.78
17	Citrate B 0.75% Citrate Uninoculated	Anaerobic	29.1	31.3	0.06
18	Citrate B 1.0% Citrate	Anaerobic	29.4	32.4	0.50
19	Citrate B 1.0% Citrate Uninoculated	Anaerobic	19.6	29.1	0.04

* All bottles received 150 ml media or water and 1.5 grams catalyst

Test 3 and 4

Test 3 and 4 confirmed the findings in test 2 that all Ni release occurred by day three and in some cases uninoculated controls exhibited more Ni in solution than in bottles where good growth had occurred (data not shown). It is possible that more Ni was released from the spent catalyst in cases of good growth, but was not revealed in our analysis procedure because some of the solubilized Ni might have been incorporated into bacterial enzyme complexes. Experiments investigating this possibility indicate that some Ni is associated with the cell biomass or possible redeposition of Ni on the catalyst occurred.

Test 5

In this experiment, the thermophilic culture *B. stearothermophilus* (ATCC 12016) was grown at 60° C in a simple defined minimal salts medium and no Mo or Ni release above an uninoculated control could be demonstrated. This minimal salts medium contained MgSO₄ such that, during growth, the sulfate instead of the metal sulfides were used as a source of nutritional sulfur. It was noted previously that higher levels of the spent catalyst were toxic for growth of *B. stearothermophilus* in the absence of MgSO₄ and attempts are currently underway to develop catalyst tolerant strains. While a previous experiment did show thermophilic metals release (Table 3), that experiment was conducted in a rich undefined medium and was not compared to a parallel uninoculated control.

Test 6

The experiments in test 6 were designed to reconfirm the differences in the kinetics of metals release between Mo and Ni as well as to compare release between aerobically and anaerobically grown cultures (Table 6). Test 6 also showed the metal releasing capabilities of enrichment cultures developed in this laboratory. These enrichment cultures were started using soil from near our laboratory as a source of enrichment inoculum. The cultures were enriched using citrate B as the enrichment medium and reduced metal sulfides (Cu, Mo Fe and Ni sulfides) as the only source of nutritional sulfur. Earlier test were confirmed that crushed catalyst gave the greatest percentage Mo release. Also, while Mo release continued with time, levels of solubilized Ni actually decreased with time especially in aerobically grown cultures

TABLE 6
% Mo and Ni Released After 4 and 7 Days

<u>Sample</u>	<u>Inocula</u>	<u>Conditions</u>	<u>Mo</u> <u>3 Days</u>	<u>Mo</u> <u>7 Days</u>	<u>Ni</u> <u>3 Days</u>	<u>Ni</u> <u>7 Days</u>
1	Mixed Denitrifiers	Capped +	10.7	19.2	10.0	4.4
2	Uninoculated	Capped +	4.2	18.2	2.4	16.3
3	Mixed Denitrifiers	Capped + Crushed Catalyst	34.3	49.0	21.4	18.2
4	Mixed Denitrifiers	Anaerobic	28.2	33.6	9.3	7.3
5	Mixed Denitrifiers	Aerobic / Not Agitated	23.6	37.8	9.2	0.4
6	Enrichment Culture	Anaerobic	28.0	33.2	20.8	16.8
7	Enrichment Culture	Aerobic	27.8	40.3	20.3	5.9

* All bottles received 150 ml liquid, 1.5 grams catalyst.

+ Bottle closed but not air tight.

Test 7

Experiments conducted in test 7 investigated some further medium parameters that were thought might influence metals release (Table 7). The standard medium, citrate B (see Table 1) was modified to contain different levels of citrate, nitrate, or a medium component not normally present, EDTA. Again it can be seen that growth (versus uninoculated controls) generally promoted good Mo release, and the presence of EDTA or higher levels of citrate showed higher levels of solubilized Ni after ten days of incubation.

TABLE 7
Effect of Media Composition and Chelating Agents on Metal Release
% Mo and Ni Released After 10 Days

<u>Sample</u>	<u>Conditions</u> °	<u>Metal Release</u>	
		<u>% Mo</u>	<u>% Ni</u>
1	Citrate B ⁺ 0.1% EDTA Combined Denitrifiers *	32.3	32.7
2	0.1% EDTA only - NO Inoculum	5.1	35.9
3	1.0% Citrate, 1.5% NO ₃ Combined Denitrifiers	31.6	32.2
4	1.0% Citrate 1.5% NO ₃ No Inoculum	14.3	36.2
5	Like Condition 3, but 0.1% EDTA	31.6	35.6
6	Like Condition 4, but 0.1% EDTA	13.2	33.0
7	Citrate B, but 0.25% Citrate	27.5	14.0
8	Like Condition 7, but No Inoculum	26.7	13.9
9	Citrate B, but 0.75% Citrate	35.8	13.2
10	Like Condition 9, but No Inoculum	17.9	35.4
11	Citrate B, but 1.0% Citrate	32.0	31.1
12	Like Condition 11, but No Inoculum	13.4	35.4

° All bottles capped (facultatively anaerobic).

⁺ See Table 1.

* Previous denitrifiers combined with enrichment cultures.

Test 8

Experiments were conducted in test 8 to determine the abiotic release of Mo and Ni under conditions of no microbial growth, but carried out in Citrate Bm medium testing pH regimes from pH 5 to 9 (Table 8). This test demonstrated greater levels of Mo and Ni release correlated with more acidic conditions or lower pH. However, this pH test is currently being repeated in water only because preliminary findings from a currently run nitric acid leach indicate that the citrate present in the citrate Bm medium used in this test probably contributed to metals release.

Test 9

Test 9 was conducted to determine if varying levels of citrate at constant pH contributed to abiotic metals release. Generally, it appeared that higher levels of citrate led to greater Ni release, but led to a much greater decrease in Mo release. This latter result, the decrease in Mo release with increasing citrate levels, can not presently be adequately explained.

TABLE 8
Effect of pH on Abiotic^a Metals Release
% Mo and Ni Released After 21 Days

		Metal Release	
Sample	pH	% Mo	% Ni
1	5	51.36	72.12
2	6	45.9	72.61
3	7	39.4	59.3
4	8	42.2	57.55
5	9	40.0	41.3

- a Test was conducted in standard Citrate Bm Medium with azide added to prevent growth

TABLE 9
Effect of Varying Citrate Levels on Abiotic^a Metal Release
% Mo and Ni Released After 21 Days

		Metal Release	
Sample	% Citrate	% Mo	% Ni
1	0.5	40.0	63.0
2	1.0	30.0	67.0
3	2.5	10.0	68.0
4	5.0	10.0	68.5
5	10.0	15.0	69.0

- a Test was conducted in standard Citrate Bm with varying levels of citrate and azide added to prevent growth

Test 10

Test 10 was conducted to determine if sulfate (as a sulfur source in the medium), and three days pregrowth had an effect on metals release from spent, THF washed Shell 324 catalyst. A very interesting result (which had been noted

previously) was found for Ni release in conditions of microbial growth versus conditions of no microbial growth due to the inhibitory effects of azide. In looking at the data (see Table 10), it would appear that either azide promotes Ni release or that microbial growth inhibits Ni release as measured on day 21. However, earlier measurements (data not shown) at day 2, 5, 7 etc. indicated a rapid Ni release followed by a gradual decline in measured levels of Ni to the levels seen at day 21 shown in Table 10. This same phenomenon had been seen in prior tests also. Mass balance studies to determine the fate of the released Ni in systems where microbial growth occurs were run (see below). One possible explanation of the Ni disappearance involves Ni uptake by the denitrifiers or cell wall sorption such that upon sample sub-micron filtration prior to sample analysis, the Ni is retained by the filter. Again it can be seen that greater Mo release occurs in cases of microbial growth, and, in this experiment, higher levels of citrate led to greater levels of Mo release in both growth and non growth conditions. No conclusions about pre-growth or the effect of sulfate on metals release can be made at this time.

Test 11

Since it is now known that denitrification can occur in the presence of oxygen and that denitrification can occur at the same time as aerobic respiration, an experiment was set up wherein air was not actively excluded (i.e. uncapped) from the culture vessel. This test was conducted to determine if oxygen had an effect on metals release. The data show (see Table 11) that after 21 days 92.6 % of the Mo originally on the spent, THF washed Shell 324 was released under conditions where air was not excluded from the culture vessel. This was the highest level of Mo release noted to date for uncrushed catalyst. All cultures in this test were inoculated with our combined, mixed heterotrophic denitrifiers. Again, as noted earlier above, the percentage Ni released shown at day 21 is not a true representation of the Ni removed from the catalyst. As seen repeatedly in past experiments, and also seen in this test, microbial growth promoted greater Mo release than non-growth controls containing azide. Catalyst from sample #1 from this test as well as catalyst from some of the previous tests reported above was saved for media rejuvenation experiments reported below.

TABLE 10
Effect of Sulfate and 3 Days Pregrowth^a on Metal Release
% Mo and Ni Released After 21 Days

Sample	Condition	Metal Release	
		% Mo	% Ni
1	Citrate A ^b	51.67	14.03
2	Citrate A & Azide	41.0	43.0
3	Double Citrate A ^c	62.87	38.06
4	Double Citrate A & Azide	59.0	63.0

- a Samples 1 and 3 were grown for 3 days prior to 1.5g of catalyst being introduced. Samples 2 and 4 were also started at that time.
- b Citrate A is the same medium as Citrate Bm but supplemented with 0.5% Na₂SO₄.
- c Double Citrate A contained twice the level of all media components as Citrate A.

TABLE 11
Effect of Aerobic Versus Facultatively Grown Cultures on Metal Release
% Mo and Ni Released After 21 Days

Sample	Condition	Metal Release	
		% Mo	% Ni
1	Aerobic ^a	92.6	15.42
2	Aerobic & Azide	36.0	60.8
3	Facultative ^b	68.3	66.3
4	Facultative & Azide	52.0	65.2

- a 150 ml of Citrate Bm in 1 liter Erlenmeyer flasks, no closure, no agitation.
- b 150 ml of Citrate Bm in 200 ml bottles, loosely capped.

Test 12

Test 12 made use of catalyst previously subjected to experiments to test metals release and these catalyst samples were then tested for additional metals release after old media had been decanted and fresh media added. Table 12 identifies the samples from previous tests that were used by test number (in parentheses) followed by specific sample number. All samples received new Citrate Bm medium and were grown facultatively. Column 2 (First) represents metals release after the first medium rejuvenation measured after 3 days and column 3 (Second) represents metals release measured 13 days after a second round of medium rejuvenation.

Some very interesting and notable findings resulted from this test. Most importantly, it can be seen (Table 12) that 100% of the Mo was released from the spent catalyst for sample number 6. This is the first time we have released all of the Mo from whole uncrushed spent catalyst. It is also notable that decreasing levels of Mo were released after each rejuvenation and poor growth occurred during the final rejuvenation suggesting a limiting growth factor. After the second rejuvenation and after final measurements of metals release were made, these cultures were used to inoculate Citrate Bm subcultures that contained thiosulfate whereupon good growth occurred suggesting that during the second rejuvenation the cultures had become starved for a source of nutritional sulfur.

Test 13

It had been hypothesized that better Mo release might occur if the THF washed catalyst could be pretreated with a good chelating agent to remove Ni, a metal which is known to be quite toxic to some microorganisms. In order to test this idea samples of spent THF washed catalyst were pretreated with either nitrilotriacetic acid (NTA) or Diethylenetriaminepentaacetic acid, two good chelating agents. While the chelating agents appeared to promote Ni release (See Table 13), a generally poor release of Mo occurred, and this was attributed to the poor growth that occurred in this test which might have been caused by the toxicity of any remaining chelating agent. It is notable that sample number 1, which served as a control and had received no pretreatment with chelating agents, exhibited the best Mo release in this test.

TABLE 12
Effect of Selective Media Rejuvenations on Metal Release
% Mo and Ni Release

		Metal Release					
		Initial ^b		First ^c		Second ^d	
Sample	Condition ^a	% Mo	% Ni	% Mo	% Ni	% Mo	% Ni
1	(8) 1	51.36	72.12	66.43	81.89	73.8	85.53
2	(8) 2	45.9	72.61	63.71	83.25	68.77	86.1
3	(8) 4	42.2	57.55	58.53	71.28	61.62	73.85
4	(10) 1	51.67	14.03	64.84	45.33	69.25	46.75
5	(10) 3	62.87	38.06	73.72	56.62	77.55	64.28
6	(11) 1	92.64	15.42	100.77	28.65	N ^{Te}	NT
7	Control ^f	---	---	41.47	51.66	50.86	61.67

- a Supernatant from previous tests (8,10,11) poured off and 150 ml new citrate B (only) added. Condition = test number followed by that specific test sample number.
- b % Metal Release after 21 days in tests 8, 10, 11.
- c % Total Metal Release after 1st media rejuvenation after 3 days.
- d % Total Metal Release after 2nd media rejuvenation 13 days after 2nd rejuvenation.
- e NT reading not taken as 100% Mo release had already occurred.
- f Control started at time zero start of test 12.

TABLE 13
Effect of Chelators on Metal Release
% Mo and Ni Released After 8 and 16 Days

Sample	Condition a	Condition b	<u>8^a Day Release</u>		<u>16^b Day Release</u>	
			% Mo	% Ni	% Mo	% Ni
1	H ₂ O Only	Citrate Bm	17.0	21.0	34.0	41.0
2	H ₂ O & DTPA	Citrate Bm	11.0	41.2	21.0	49.0
3	H ₂ O & NTA	Citrate Bm	9.0	49.0	20.0	54.0
4	Citrate Bm Only	Citrate Bm	21.0	44.0	30.0	53.0
5	Citrate Bm & DTPA	Citrate Bm	14.0	50.0	20.0	54.0
6	Citrate Bm NTA	Citrate Bm	16.0	53.0	21.0	57.0

- a 1st 8 days no inoculum, DTPA and NTA at 1.0% concentration. Only Sample 4 received azide.
- b At day 8, supernatants were decanted and all samples rejuvenated with 150ml fresh, sterile Citrate Bm and inoculated with mixed denitrifiers. Metal release read after 8 more days.

Test 14

The high levels of Mo release seen in one condition in test 11 needed to be reconfirmed so test 14 was conducted to determine if we could duplicate those results. Again, it can be seen (Table 14) that the greatest Mo release occurred in the culture condition where air was not actively excluded. Interestingly, more Mo release occurred in the aerobic azide control (no growth) than in sample 3 which was grown facultatively suggesting that not only does microbial growth contribute to Mo release, but also that air somehow contributes to Mo release. As noted above previously, the low levels of released Ni measured at day 7 probably do not reflect the true amount of Ni released from the spent catalyst.

TABLE 14
Effect of Aerobic Versus Facultatively Conditions on Metal Release
% Mo and Ni Released After 7 Days

Sample	Condition	Metal Release	
		% Mo	% Ni
1	Aerobic ^a	71.0	6.81
2	Aerobic & Azide	57.55	64.63
3	Facultative ^b	44.34	46.60
4	Facultative & Azide	22.56	56.22

- a 150 ml of Citrate Bm in 0.5L Erlenmeyer flasks, no closure, no agitation.
- b 150 ml of Citrate Bm in 200 ml bottles, loosely capped.

Test 15

Test 15 was conducted to try and relate growth conditions, pH, turbidity and different sets of inocula to metals release. Table 15 gives tabular results for Mo release at day 7 and day 14 and tabular results for Ni release at day 7 only. Figures 1 through 9 give a more detailed graphic view showing measurements made for Mo release, pH and turbidity for each sample condition. Interestingly, there was not a great deal of difference in the measured levels of Mo release between the different sample conditions except for the no growth azide controls. Generally, Mo release lagged behind growth (turbidity) and the pH increased over time except for the "N" series. It is not known why the absence of active air exclusion (aerobic series) did not lead to greater Mo release in this experiment.

TABLE 15
Effect of Different Inocula and Culture Conditions on Metal Release
% Mo and Ni Release

Sample ^a	Condition	Day 7		Day 14	
		% Mo	% Ni	% Mo	% Ni
1A	Aerobic ^b	54.22	24.69	54.67	ND*
2A	Aerobic ^b	54.47	24.49	54.29	ND
3A	Aerobic ^b	56.05	39.59	58.83	ND
4A	Aerobic ^b	57.67	30.52	56.53	ND
5A	Aerobic ^b	56.35	27.92	56.50	ND
6A	Aerobic ^b	28.73	43.56	29.95	ND
1F	Facultative ^c	49.52	29.04	55.77	ND
2F	Facultative ^c	50.31	30.33	59.86	ND
3F	Facultative ^c	43.97	35.51	54.63	ND
4F	Facultative ^c	52.13	30.30	56.08	ND
5F	Facultative ^c	48.65	32.74	49.91	ND
6F	Facultative ^c	24.49	38.09	24.95	ND
1N	Anaerobic ^d	43.80	42.08	52.20	ND
2N	Anaerobic ^d	38.60	39.92	47.28	ND
3N	Anaerobic ^d	40.15	39.75	51.83	ND
4N	Anaerobic ^d	42.47	39.89	50.29	ND
5N	Anaerobic ^d	39.94	39.61	49.43	ND
6N	Anaerobic ^d	25.98	37.92	35.32	ND

- a All sample "1" inoculated newly enriched mixed denitrifiers.
Sample "2" inoculated with a mix of all heterotrophic denitrifiers.
Sample "3" inoculated with old, stored heterotrophic denitrifiers.
Sample "4" as Sample "1", but pregrown on a spent catalyst other than shell 324.
Sample "5" Inoculum was equal mix of all of above.
Sample "6" No inoculum, azide control.
- b 150 ml of Citrate Bm in 250 ml Erlenmeyer flasks, no closure, no agitation.
- c 150 ml of Citrate Bm in 200 ml bottles, loosely capped.
- d 150 ml of Citrate Bm in 200 ml bottles, prepared in an anaerobic chamber and tightly sealed.
- * Not Determined

Test 16

This test was conducted to determine if the mixed heterotrophic denitrifiers could grow in the presence of unwashed, spent Shell 324 and promote metals release. Interesting, all the cultures grew well, but Mo and Ni release was very poor (data not shown). A possible explanation for this is provided below.

Test 17

Test 17 was designed to help define the inhibitory effects of coal liquids on Mo release as test 16 had shown very poor metals release from unwashed catalyst. Sample conditions compared a control (Sample 1-Table16) which was THF washed catalyst inoculated with mixed, heterotrophic denitrifiers incubated facultatively to various other conditions. As has been noted many times previously, microbial growth (Sample 1) showed much greater Mo release than the no growth azide control (Sample 2). Interestingly, a culture vessel which had added coal liquids (Sample 3) showed relatively good Mo release even though the amount of coal liquids added was more than would contained in a culture vessel having unwashed catalyst which was done in parallel sample number 6. Therefore, while it appears that coal liquids added to THF washed catalyst slightly inhibited Mo release, coal liquids already on or within spent catalyst greatly inhibited Mo release.

Test 18

Test 18 was designed to determine if delayed inoculation along with media rejuvenation affected Mo release. This test again showed that microbial growth promoted Mo release over no growth controls and suggested that delayed inoculation led to less Mo release (Table 17).

Table 16
Effect of Added Coal Liquids on Metal Release
% Mo Released After 14 Days

Sample	Condition ^a	% Mo Release
1	Control (with denitrifier growth)	49.55
2	Azide	25.62
3	0.1 ml Coal Liquids	40.56
4	0.1 ml Coal Liquids-No Added Catalyst	0.81
5	No Citrate	6.73
6	Non THF Washed Catalyst	6.78
7	Non THF Washed Catalyst & Azide	7.05

- ^a All bottles contained 150ml Citrate Bm with 5 ml inoculum of combined mixed heterotrophic denitrifiers, and 1.5g THF washed catalyst (except for sample 6 & 7 which had ion washed catalyst). Sample 4 contained no catalyst.

Table 17
Effect of Media Rejuvenation and Delayed Inoculation on Metal Release
% Mo Released After 14 Days

Sample	Condition ^a	Metal Release % Mo
1	Control (with denitrifier growth)	52.49
2	Reinoculated & New Media After 2 Days	43.17
3	Not Inoculated & Azide	24.97
4	Inoculated After 2 Days & New Media After 2 Days	40.09
5	Never Inoculated & New Media After 2 Days	6.73

- ^a All bottles contained 150ml Citrate Bm and 1.5g THF Washed Catalyst.

Test 19

It had been suggested the no growth conditions achieved by adding azide might lead to erroneous levels of Mo released by some unknown chemical interaction of the catalyst and the azide. Therefore, test 19 was performed to determine if another means of achieving sterility, autoclaving, could be used for no growth controls. It can be seen (Table 18) that unautoclaved catalyst released more Mo than catalyst which had been previously autoclaved and slightly less Mo was released in the autoclaved azide control compared to the unautoclaved azide control.

Table 18
Effect of Autoclaving Catalyst on Metal Release
% Mo Released After 14 Days

Sample	Condition ^a	Metal Release % Mo
1	Autoclaved, Cooled, Inoculated	43.05
2	Autoclaved, Cooled, Inoculated & Azide	25.37
3	Unautoclaved, Inoculated	51.83
4	Unautoclaved, Inoculated & Azide	26.36

^a All bottles contained 150ml Citrate Bm, 1.5g THF Washed Catalyst. All were inoculated with 5 ml of combined, mixed, heterotrophic denitrifiers.

Test 20

Test 20 was designed to determine if microbial growth per se is a requisite condition for solubilization of Mo and Ni from spent Shell 324 catalyst. Controls were compared to conditions where cells had been pregrown in the presence of catalyst or without catalyst plus thiosulfate and the resulting turbid supernatant was ultra filtered to render it cell free and challenged with fresh catalyst. Table 19 (below) shows the results of this experiment. As has been noted numerous times in past work Mo release is promoted by microbial growth and cell-free supernatant has the ability to release metals. However, while pregrowth on thiosulfate had little effect on nickel release, it appeared that pregrowth on catalyst resulted in a cell-free supernatant that released Mo better than the corresponding thiosulfate pregrowth supernatant. It again appeared that microbial growth (sample 4) retarded Ni release as noted in past work, but this was later defined in mass balance experiments as a redeposition of solubilized Ni back onto the catalyst matrix.

Table 19
Activity of Cell-Free Supernatant for Metal Release
% Mo and Ni Released After 13 Days

Sample	Condition	% Mo Release	% Ni release
1	pregrown thiosulfate	37.23	32.88
2	pregrown catalyst	53.64	36.82
3	control azide	22.30	45.11
4	control with growth	45.82	11.35

Test 21

Test 21 further investigated the inhibitory effects of thiosulfate on metals release during microbial growth in the presence of catalyst as well as an additional test of metals release on crushed versus whole catalyst. After 8 days of growth, crushed and whole catalyst yielded 70% and 38% Mo release respectively. In the presence of thiosulfate, Mo release was retarded by about 10%. Interestingly, thiosulfate appeared to have little effect on Ni release and Ni redeposition on the catalyst matrix (to be discussed later) did not occur using crushed catalyst (see Table 20 below).

Table 20
Effect of Thiosulfate on Metals Release
% Metals Release After 8 Days of Growth

Sample	Condition	% Mo Release	% Ni release
1	Control	44.63	9.44
2	Azide Control	19.90	40.10
3	0.5% Thiosulfate(TS)	33.09	28.50
4	0.5% TS + Azide	23.74	37.24
5	1.5% TS	32.89	24.19
6	1.5% TS +Azide	26.83	33.63
7	Crushed	70.27	55.54
8	Crushed + Azide	37.87	57.75

Test 22

It was reasoned that better biosolubilization of metals by microbial growth might occur if the THF Shell 324 catalyst was pretreated by nitric acid because the acid per se might leach some metals as well as alter the oxidation state of the metals in the catalyst matrix leading to better metals release during subsequent microbial growth. In this initial test, better leaching appeared to occur (data not shown), but the data was hard to interpret as the nitric acid solubilized a great deal of the aluminum support matrix which led to interference in Mo release measurements. A method for accurately measuring Mo release despite the presence of aluminum was developed and this experiment was later repeated (see below).

Test 23

Test 23 was a mass balance experiment that measured levels of Mo and Ni released after 7 days of microbial growth, either aerobically or facultatively, and also measured metals retained on the catalyst in an effort to account for all the metals. No metals were detected that might have been retained with cell biomass after micro filtration of sample aliquots prior to AA analysis. An Inorganic Ventures procedure was used to analyze the filters to prepare samples of culture supernatants for testing and was also used to analyze total metals content of the catalyst after the 7 days of microbial growth. It can be seen (Table 21) that in all cases close to 100% of the metals could be accounted for. It had been noted in the past that during the course of an experiment, solubilized levels of Ni in the supernatant rose then declined during the period of microbial growth. From the data gathered in this experiment, it now appears that solubilized Ni is redeposited upon the catalyst matrix during the course of microbial growth.

Table 21
% Metals Solubilized or Retained
in Catalyst After 7 Days of growth

Sample	Condition	% Mo		% Ni	
		Released	On Catalyst	Released	On Catalyst
1	Aerobic	27.77	71.72	16.27	80.40
2	Aerobic + Azide	14.76	79.15	61.73	45.64
3	Facultative	27.64	71.62	25.76	68.82
4	Facultative + Azide	12.82	78.75	48.16	52.83

Test 24

Since it had been previously noted in certain experiments that more Mo was solubilized in growing cultures where air was not actively excluded (aerobic conditions), an experiment was performed to compare Mo and Ni release using cultures that were grown facultatively, aerobically and aerobically with active aeration. It can be seen (Table 22) that active aeration seemed to retard Mo release and much lower levels of solubilized Ni were present in the supernatant of the actively aerated culture analyzed at day 8. pH data is also included for this experiment (Table 23) and to illustrate the Ni redeposition phenomenon (discussed above) Ni analyses in ppm are provided for days 3, 6, and 8 of microbial growth (Table 24).

Table 22
Effect of Active Aeration on Metals Released
% Metals Released on Day 8

Sample	Condition	% Mo Released	% Ni Released
1	Facultative	50.41	36.15
2	Facultative + Azide	24.07	34.18
3	Aerobic	52.25	16.11
4	Aerobic + Azide	23.11	38.56
5	Aerated	41.02	4.40
6	Aerated + Azide	29.61	51.99

Table 23
pH Data for Test 24

Sample	Day 0	Day 3	Day 6	Day 8
1	6.89	6.95	7.04	7.28
2	6.89	6.99	7.08	7.16
3	6.89	7.20	7.55	7.12
4	6.89	6.97	7.05	7.10
5	6.89	8.65	7.54	7.12
6	6.89	6.95	7.05	7.00

Table 24
Solubilized Ni in ppm

Sample	Day 3	Day 6	Day 8
1	59	80	88
2	52	57	84
3	68	78	39
4	64	87	105
5	58	19	16
6	90	133	176

Test 25

Test 25 was essentially a rejuvenation experiment wherein catalyst was subjected to two rounds of microbial growth; however this test employed unwashed Shell 324 catalyst. Unwashed catalyst was used because it was thought that microbial action in the first round of growth might remove enough of the coal liquids to allow biosolubilization of the Mo and Ni during the subsequent round of growth where the spent medium was decanted and fresh media added. Both citrate Bm medium and a citrate Bm medium with low citrate levels (0.25%) were employed, the idea being that limited carbon levels would increase the pressure on the microbial consortium to utilize the coal liquids as a carbon source. There were no marked differences in metals release between these two incubation conditions (data not shown) and metals release was generally less than 10% total after both rounds of growth. It appears that coal liquids removal is essential for subsequent microbial biosolubilization.

Test 26

Test 26 was set up to study the effect of pH alone in sterile citrate Bm media as it had been noted (see Test 24 above) that the pH of the cultures varies with time. A variety pH regimes were tested and pH was found to affect Ni release, but different pH regimes had little if no affect upon Mo release (see Table 25 below).

Table 25
Effect of pH on % Metals Release

pH	% Mo Release	% Ni Release
4	22.60	16.79
5	22.86	14.64
6	23.90	13.35
7	26.87	7.75
8	24.93	1.25

Test 27

Test 27 was a quite an extensive test designed to determine if a Ni or Mo releasing "factor" is produced during microbial growth, and, if so, would it function as a metal releasing agent in the absence of cells. This test also investigated if the putative metal releasing factor (MRF) is induced and produced in the culture to a greater extent if cells are grown in the presence of catalyst without an added sulfur source versus cells grown without catalyst with thiosulfate as a sulfur source. Except for the one control (sample A), all the metals release in this experiment was mediated by cell-free supernatants and secondary growth was precluded in the already grown cultures by centrifugation to obtain the cell-free supernatant, ultra microfiltration of the supernatant, and finally the addition of sodium azide. All experiments utilized citrate Bm medium. The following key provides the conditions of the 11 samples listed in Table 26 (see below). Metals release was followed daily for 3 days after challenging supernatant with catalyst.

Key

Sample Pre-Test Growth Conditions

- 1, 2, 3 Grown for 2, 3, and 4 days respectively with thiosulfate, no catalyst present^a
- 4, 5, 6 Grown for 2, 3, and 4 days respectively with catalyst^b
- 7, 8, 9 Grown for 2, 3, and 4 days respectively with catalyst^c
- A Positive control, i.e. regular microbial growth
- B Negative control, i.e. inoculated but no growth due to azide addition
- a Supernatant challenged with fresh THF washed catalyst
- b Supernatant challenged with catalyst with which the cells had been grown
- c Supernatant challenged with fresh THF washed catalyst

Table 26
Test for Metals Releasing Factor

Sample	Mo ppm				Ni ppm			
	Day 1	Day 2	Day 3	% Total	Day 1	Day 2	Day 3	% Total
1	192	209	198	24.77	35	45	54	22.11
2	209	225	235	29.23	33	41	48	19.69
3	79	119	225	27.08	17	21	40	16.08
4	127	142	181	22.24	49	51	57	23.56
5	186	235	243	27.12	60	68	72	29.33
6	248	257	260	32.44	63	68	71	29.43
7	239	247	352	29.14	82	91	98	18.56
8	260	436	443	36.24	90	95	102	15.11
9	302	359	381	25.89	74	84	87	8.04
A	63	95	186	22.36	39	48	53	21.82
B	61	74	99	12.11	44	60	66	27.11

It is important to note that both Mo and Ni contained in the cell-free supernatants that were released by the pre-growth prior to challenging the supernatant with catalyst have been accounted for (subtracted) so that the values shown in the columns in Table 26 represent additional metals release. The results suggest that the cell-free supernatant contains a factor, (a metal releasing factor) MRF, that biosolubilizes Ni and especially Mo within a very short time frame (24 hours) as evidenced by comparing samples 1-9 to control samples. Several attempts were made to characterize/identify this factor.

Test 28

Test 28 was a simple straightforward test designed to determine if the mixed heterotrophic denitrifying cultures we were using routinely relied on the reduced sulfur present in the catalyst matrix as a nutritional sulfur source. Good growth occurred in citrate Bm medium if the catalyst was present whereas very poor growth occurred in citrate Bm medium in the absence of catalyst; however good growth could later be induced in this later condition by the addition of thiosulfate alone suggesting that the cultures were relying upon the catalyst as a source of nutritional sulfur.

Test 29

Test 29 was a repeat of test 27 using crushed Shell 324 catalyst and again cell-free supernatant released all of the biosolubilized metals within 24 hours. Sample 1 was crushed catalyst and sample 2 employed whole catalyst (see Table 27).

Table 27
Effect of Supernatant on Crushed Versus Whole Catalyst

Sample	ppm Mo			ppm Ni		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
1	738	589	589	194	192	200
2	561	430	453	143	142	142

Test 30

Test 30 tested the idea of pretreating the catalyst for 3 days with 1 molar nitric acid prior to biosolubilization by microbial growth. It was expected that the acid treatment would chemically leach a considerable amount of Ni and might alter the Mo oxidation state of the Mo remaining in the catalyst matrix leading to better biosolubilization. After three days of nitric acid treatment about 75% of the Ni and 25% of the Mo was removed from the catalyst matrix whereas much lower levels were removed in water controls (data not shown). However, after pre-treatment and 8 days of microbial growth up to 90% of the Ni and 100% of the Mo had been solubilized (see Table 28 below).

Table 28
Effect of Nitric Acid Pre-Treatment

Sample	Condition ^a	% Release Mo	% Release Ni
1	H ₂ O	47.78	34.46
2	H ₂ O + Azide	25.21	37.85
3	Acid	98.84	90.51
4	Acid + Thiosulfate	118.16	83.91

^a Catalyst was either soaked in water or 1 M nitric acid for three days and the acid or water poured off and replaced with citrate Bm medium and inoculated with mixed denitrifiers and incubated for 8 days.

Test 31

Since nitrite is transiently produced by denitrifying bacteria respiring with nitrate, it was thought that nitrite could be the metals releasing factor (MRF) discussed above. A straight chemical test comparing nitrate to nitrite showed that nitrite is not the MRF discussed above (data not shown).

Test 32

Test 32 was a straight chemical test designed to determine if various levels of the sodium ion had different effects upon Mo and Ni release. High levels of the sodium ion appear to retard Ni release, but had little effect upon Mo release (data not shown)

Test 33

Test 33 was a repeat of Test 27, the cell-free supernatant experiment, except it involved only two conditions. One condition tested supernatant along with added nitrite (1%), while the second condition tested supernatant alone (sample 2). Some differences can be noted in this experiment. See Table 29 below for results.

Table 29
Effect of Supernatant Plus Added Nitrite

Sample	ppm Mo			ppm Ni			% Mo Release	% Ni Release
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 3	Day 3
1	517	742	784	125	156	167	50.28	28.08
2	517	698	687	66	83	151	39.4	19.08

Test 34

This test was performed to determine if an alternative solvent besides THF could be used to remove coal liquids and not interfere with microbial bio-leaching of the Mo and Ni from spent Shell 324 coal liquefaction catalyst. The alternative solvent used was unleaded gasoline. Even though the gasoline removed coal liquids well, and the resulting catalyst was allowed to air dry, enough residual gasoline remained to inhibit subsequent microbial bioleaching (see Table 30).

Table 30
Effect of gasoline pretreatment

Sample	Condition	% Release Mo	% Release Ni
1	THF Washed	43.70	40.04
2	gasoline washed	23.08	11.71

It is not known why metals release from gasoline washed catalyst was poorer than THF washed catalyst, but an explanation could be that the culture used had acclimated to residual THF through routine subculturing. In this regard, cultures will now also be routinely maintained on gasoline washed catalyst in order to obtain strain tolerance toward gasoline washed Shell 324.

Test 35

Test 35 was a repeat of test 30 wherein catalyst was further pretreated with 1 M nitric acid and results similar to those shown in table 28 were obtained.

Test 36

Test 36 was essentially a repeat of the cell-free supernatant experiments done earlier (see above) except that in this experiment the supernatant had been concentrated through freeze-drying or lyophilization. The lyophilization had not been taken to completion though, so the supernatant employed in this experiment was about one half the original starting volume; therefore it was about 2X concentrated. Interestingly, (see below Table 31), the concentrated, cell-free supernatant released the majority of the Mo in seven days and about twice as much Mo was released as had been seen in previous experiments (see Table 19, above). The concentrated cell-free supernatant clearly released more Mo than the positive growth control.

Table 31
Release of Metals by Concentrated Supernatant

Sample	Condition	% Mo Release	% Ni Release
1	growth	21.66	34.21
2	supernatant	70.76	37.02

Test 37

Test 37 was performed to determine if culture inoculum age affects metals release and the test also compared metals release among 5 different enrichment cultures we routinely carry. The test involved ten bottles, 5 of which were inoculated from 5 day old cultures versus 5 bottles which were inoculated from viable one month old cultures. The results (see Table 32 below) show not only that age of inoculum is an important factor in metals release, but also that there exists a quite large variation in the amount of metals released by the different enrichment cultures.

Table 32

Sample	Condition ^a	% Mo Release	% Ni Release
1	5 day	27.59	29.81
2	5 day	32.43	34.96
3	5day	49.97	45.79
4	5 day	37.19	38.58
5	5 day	39.46	45.21
6	month	50.76	45.88
7 ^b	month	60.08	45.54
8 ^b	month	53.09	49.69
9	month	45.21	45.33
10 ^b	month	58.54	52.04

a Samples 1-5 refer to enrichment cultures maintained and named 1-5, samples 6-10 refer to these same enrichment cultures respectively but had been inoculated three weeks earlier. Metals release after 6 days growth for all samples.

b These samples showed 100% Mo release by day 12

Test 38

Test 38 was a repeat of experiments conducted in the past and it reconfirmed the findings that cell-free supernatant releases metals quickly and more efficiently than culture transfer and or fresh medium rejuvenation (data not shown).

Test 39

Test 39 was repeat experiment of earlier findings that crushed catalyst released metals faster than whole catalyst and that crushed catalyst generally released a greater quantity of metals. Earlier findings were reconfirmed (data not shown).

Test 40

Test 40 tested for metals release and compared culture turbidity (growth) so that growth, metals release, and release from different media/catalyst ratios could be compared and contrasted. All samples contained 100 ml of medium, but had different levels of catalyst. See Table 33 below for results.

Table 33
Effect of Medium/Catalyst Ratios

Sample	Condition ^a	% Mo Release	Turbidity
1	10.00	34.77	0.112
2	5.00	38.79	0.336
3	2.50	38.24	0.284
4	1.00	57.83	0.419
5	0.10	78.44	0.412
6	0.01	76.00	0.568
7	0.001	40.00	0.362
8	0.00	0.00	0.536

a Refers to grams of catalyst contained within the sample bottle, all bottles contained 100 ml Citrate Bm medium

As can be seen from the data in Table 33, there appears to be an optimum medium/catalyst ratio wherein the greatest percentage Mo release occurs. This ratio is bracketed by catalyst to medium ratios of 1:100 to 1;10,000. It can also be seen that density of growth (turbidity) is also affected by the level of catalyst present. The levels of Ni release did not correlate with any pattern (data not shown).

Test 41

Test 41 involved setting up sand pack columns interspersed with Shell 324 and running Citrate Bm medium through the columns on a continuous basis. An attempt was made to optimize this continuous bio-leaching system, but it appeared that about 50% of the Mo bio-solubilized within the first 24 hours of inoculation and dropped rapidly after that.

Test 42

Test 42 was a quick test to determine if a newly obtained thermophilic denitrifier (pure culture) could bio-solubilize Mo and Ni from spent catalyst. /The pure culture was Thermothrix thioparus. It was unable to bio-leach any metals.

Test 43

Test 43 was a retest of the idea that non-pretreated (unwashed with THF) might be used and successfully bio-leached. It was reconfirmed that not using the pretreatment greatly hinders metals release. See Table 34 below.

Table 34

Sample	Condition	% Mo Release	% Ni Release
1	washed	39.90	62.81
2	" + Azide	66.04	34.46
3	unwashed	12.25	13.71
4	" + AZIDE	11.79	12.71

It is now presumed from experiments performed that the poor metals release from unwashed catalyst is probably not due to any toxic factor in the unwashed catalyst that led to poor growth and consequently low levels of metals release. This assumption was made because good growth can be achieved in the presence of unwashed catalyst as long as some washed catalyst is also included in the growth medium.

Test 44

Test 44 was a repeat experiment to again show the difference in Mo release from spent coal liquefaction catalyst using cultures of heterotrophic denitrifiers grown under microaerophilic versus strictly anaerobic conditions, and using washed versus unwashed catalyst. Table 35 (below) shows the results of this experiment which measured Mo release after a nine day incubation period. The microaerophilic incubation released the most Mo and it was seen again that unwashed catalyst had low levels of Mo release compared to THF pre-treated catalyst incubations.

Table 35
Microaerophilic Versus Anaerobic Metals Release

Sample	Condition	% Ni Release	% Mo Release
1	MA ^a washed	36.67	52.82
2	MA unwashed	10.79	11.53
3	A ^b washed	31.44	32.54
4	A unwashed	9.10	7.34

a-microaerophilic

b-anaerobic

Test 45

Test 45 was a repeat experiment of a test done last quarter in which cell-free supernatant, which is thought to contain a metals releasing factor (MRF), is used to effect metals solubilization. However, in this particular test, THF washed catalyst

Effect of Cell-Free Supernatant Age on Metals Release TEST 45

was challenged with cell-free supernatant derived from cultures that had been grown 3, 4, 5, 6, and 7 days in the presence of catalyst. The results reported below (Table 36) reflect the average of duplicate incubations and metals release after 24 hours.

Table 36
Effect of Cell-Free Supernatant Age on Metals Release

Sample	Supernatant Age	%Ni Release	%Mo Release
1	3 days	18.33	19.56
2	4 days	22.08	28.07
3	5 days	23.13	29.88
4	6 days	31.67	17.19
5	7 days	8.75	31.38

It can be seen from the data presented in Table 36 that there is an difference in metals release depending upon the age of the cell-free supernatant used to biosolubilize the catalyst in a 24 hour period. It is not known why so little Ni was solubilized using the 7 day old supernatant, but that this could possibly arise from redeposition of Ni that was discovered in earlier research where Ni was initially solubilized by growing cultures, only to be redeposited into the catalyst matrix.

Test 46

Test 46 was an experiment designed to determine the optimum catalyst to medium ratio that would yield the greatest percentage metals release. It appears that from the data presented below (Table 37), that at around a ratio of 5 grams of catalyst to 100 ml of medium there is a drop in percentage efficiency metals removal, although greater ppm metals were solubilized in incubations that contained a greater mass of catalyst. All samples contained 100 ml of medium, but different amounts of catalyst. The results presented are values obtained after a 5 day incubation period.

Table 37
Metals Release Dependant Upon Catalyst Load

Sample	Condition	ppmNi	%Ni	ppmMo	%Mo
1	1.00g	36	14.85	223	26.91
2	2.50g	88	14.57	518	24.98
3	5.00g	141	11.60	677	16.45
4	7.50g	182	9.96	1015	16.42
5	10.00g	200	8.24	1308	15.88

It should also be noted that turbidity measurements taken on day 5 indicated a range of turbidities starting at 1.067 for the sample containing the least amount of catalyst, and the turbidity measurements declined in value (less growth) as the weight of the catalyst added was increased to a low of 0.585 for sample 5 (all data not shown).

Test 47

Test 47 determined the effect of 3 treatments upon either unwashed or THF washed catalyst samples. Samples 1-3 were THF washed catalyst, and samples 4-6 were replicates of 1-3 except unwashed catalyst was used. Sample 1 was treated with uninoculated Bm medium for 24 hours at which time a sample was taken for metals removal measurement. Then new medium was added and it was inoculated and incubated for 12 days. Sample 2 was treated similarly to sample 1 except the 24 hr treatment prior to medium addition and inoculum was 3 % hydrogen peroxide. Sample 3 was treated similarly to sample 1 except the 24 treatment prior to medium addition and inoculum was a very dilute solution of nitric acid the pH of which measured 2. Samples 4-6 were treated as samples 1, 2, and 3, but used non solvent washed catalyst. Total % metals removal, after pretreatment and a 12 day incubation period is reported below in Table 38.

Table 38
Pre-Treatment of Washed and Unwashed Catalyst

Sample	Condition	%Ni Released	%Mo Released
1	BM	56.90	66.41
2	H ₂ O ₂	82.17	121.89
3	Nitric	56.81	73.84
4	BM	9.06	14.84
5	H ₂ O ₂	28.73	45.06
6	Nitric	12.54	24.85

It is evident from the results obtained in test 47 that various pre-treatment regimens can greatly influence Ni and Mo release when the pre-treated catalyst is later bio-leached. Also, it was found that the different pre-treatment regimens release different levels of metals prior to subjecting the catalyst to bio-leaching, with 3% H₂O₂ pre-treatment leading to the greatest pre-release (data not shown).

Test 48

Test 48 was a simple repeat experiment to reconfirm that the medium, citrate Bm solubilized metals better than deionized water. Both samples were not inoculated and no growth occurred in either sample. Metals release was about twice as great in the citrate Bm medium which reconfirmed earlier findings (data not shown).

Test 49

Test 49 examined metals solubilization using different concentrations of hydrogen peroxide using THF washed, and unwashed catalyst and the effect of added iron in this system. Iron, in the form of ferrous sulfate was added to some samples as it was thought iron might generate free radicals in the presence of peroxide. However, the addition of iron had only one marked effect and this was in the case of unwashed catalyst. All sample conditions contained 1 gram of catalyst with metals release measured after 48 hours. See Table 39 below.

Key to Sample Condition for Table 39:

Sample #

1 THF washed in 100ml 3% H_2O_2

2 THF washed in 100ml 3% H_2O_2 with 0.05 g Ferrous Sulfate

3 Unwashed in 100ml 3% H_2O_2

4 Unwashed in 100ml 3% H_2O_2 with 0.05 g Ferrous Sulfate

5 THF washed in 100ml 10% H_2O_2

6 Unwashed in 100ml 10% H_2O_2

7 THF washed in 100ml dH_2O

8 Unwashed in 100ml dH_2O

Table 39
Effect of H_2O_2 and Iron On Metals Release

Sample	%Ni Release	%Mo Release
1	74.38	98.15
2	76.46	98.38
3	38.98	49.05
4	10.58	2.91
5	74.38	100.96
6	59.88	71.79
7	26.13	16.28
8	2.00	0.36

At the present time, it is unknown why the inclusion of ferrous iron along with H_2O_2 to treat unwashed catalyst so greatly inhibited metals solubilization (sample #4).

Effect of H₂O₂ and Ferrous Sulfate Extraction on Metals Release TEST 49

Test 50

Test 50 was a re-examination of gasoline an alternative to THF as a solvent for removing coal liquids prior to bio-solubilization of the catalyst metals. Regular unleaded gasoline had been tested previously, but subsequent metals bio-solubilization was very limited. This test differed in that the catalyst was challenged with a mixed, heterotrophic, denitrifying culture that had been adapted to gasoline washed catalyst (i.e. eventually the culture grew well in the presence of gasoline washed catalyst). Even though the culture grew quite well in this test, metals solubilization after 2 weeks did not exceed 10% which was similar to the prior test (data not shown).

Test 51

Test 51 was a re-examination of H_2O_2 as a metals releasing agent that was used on both THF washed and unwashed coal liquefaction catalyst. Results obtained were similar to that reported in Test 49. Virtually all the Mo and about 75% of the Ni were removed from the catalyst matrix within 24 hours, but only about 50% of the Mo and Ni were removed from the unwashed catalyst after a period of 4 days.

Test 52

Test 52 was performed using unwashed catalyst and was carried out to determine if the oxidizing nature of H_2O_2 is an important part of its metals releasing activity. Tests were performed with 3% H_2O_2 (one gram of catalyst) and either 100 ml of 3% H_2O_2 or 97 ml of 3% H_2O_2 along with 3 ml of 37% formaldehyde which can act as a reducing agent. Whereas about 50% of the Mo was released in 2 days in the test with only H_2O_2 present, only about 25% of the Mo was released in the test bottle that contained 3 ml of the 37% formaldehyde.

Test 53

Test 53 was performed using 3% H_2O_2 and varying amounts of THF washed catalyst to determine the greatest mass of catalyst that can be effectively leached in a fixed period of time. The results shown below (Table 40) suggest that metals solubilization becomes less efficient when the ratio of 5 grams catalyst to 100 ml of H_2O_2 is exceeded.

Table 40
Effect Catalyst H_2O_2 Ratio^a

Sample	Catalyst Weight	%Ni Release	%Mo Release
1	10.0	65.35	54.23

Effect of Catalyst Concentration on Metals Release TEST 53

2	5.0	62.06	98.42
3	2.5	74.14	94.50
4	1.0	79.02	106.36
5	0.5	62.67	94.40

a-metals release measured after 24 hours

Test 54

Test 54 was a re-examination of the effect of catalyst size as it pertains to metals release similar to tests conducted earlier, but, in this case, using 3% H₂O₂ instead of microbial cultures. Again it was found, as in the previous tests, that decreasing catalyst particle size greatly facilitated metals release, but that this difference was only noticeable when analysis of samples were time compressed; i.e. differences could be noted 1 hour after exposure of the various catalyst sized particles to 3% H₂O₂ treatment, but differences were much less noticeable when sample analysis was performed 8 hours after initiation of the experiment. See Table 41 below for results obtained after 1 and 8 hour treatments.

Table 41
Effect of Particle Size During H₂O₂ Treatment^a

Sample	Condition	%Ni Release	%Mo Release
1	>30 mesh	37.08	39.50
2	<30 mesh	53.75	64.00
3	<60 mesh	55.83	60.75
4	not ground	20.83	19.25
5	>30 mesh	71.90	90.18
6	<30 mesh	70.85	91.40
7	<60 mesh	69.50	89.66
8	not ground	69.52	70.55

a-first 4 samples, metals release at 1 hour; samples 5-8 same as 1-4, but release measured at 8 hours

Test 55

Test 55 was designed to test different solvents for removing coal liquids prior to subsequent H₂O₂ leaching, analogous to previous tests using bio-leaching. The solvents tested were THF, xylene, acetone, and methanol. As in previous tests, THF was found to be the superior solvent for pre-treatment prior to leaching (See Table 42 below).

Effect of Particle Size on 3% H₂O₂ and Metals Release TEST 54

**Effect of Solvent Washes on 3% H₂O₂ Metals Release
TEST 55**

Table 42
Effect of Different Solvents in H₂O₂ Leaching^a

Sample	Condition	%Ni Release	%Mo Release
1	THF	70.15	98.20
2	Xylene	32.92	38.75
3	Acetone	67.92	65.13
4	Methanol	52.50	36.60

a-metals release measured after 24 hours contact time

It is highly possible that these alternative solvents might yield results in the H₂O₂ leaching system if longer periods of pre-solvent treatment had been employed. The catalyst samples in this test were only washed for 24 hours prior to treatment and a longer washing period or catalyst washing using a soxhlet apparatus might have yielded better results.

Test 56

Similar, independent, in-house research using both 3% and 30% H₂O₂ diluted to 3% showed differences in leaching ability towards certain sulfide ores and catalysts other than Shell 324. It was thought that the use of 2 different H₂O₂ stabilizers, phosphoric acid and acetanilide might account for these differences or that, upon dilution of the 30% H₂O₂, additional stabilizer might need to be added. However, tests using either 3% H₂O₂ or 30% H₂O₂ diluted to a 3% concentration showed no differences using Shell 324 as the test catalyst (data not shown).

Test 57

Test 57 was designed to determine the number of times an aliquot of 3% H₂O₂ could be re-used to leach a new 1 gram quantity of THF washed catalyst. Obtaining this information was deemed important since the economics of the H₂O₂ leaching process would be increased if the H₂O₂ could be re-used or re-cycled several times. The data shown below indicate that H₂O₂ can be re-used several times (see Table 43). In the experimental data shown below, sample 1 represents 1 gram of THF washed Shell 324 contacted with 100 ml of 3% H₂O₂ for a 24 hour period. After the 24 hour contact period, a 5 ml aliquot was removed for analysis of the Ni and Mo leached into solution. The remaining 95 ml of this leachate was then challenged with a new 1 gram sample of THF washed catalyst and this was sample 2 and was likewise analyzed after 24 hours. This process was repeated for samples 3,4,5 and 6 except that sample 5 was analyzed after 3 days contact time and sample 6 was analyzed after 8 days contact time. It should be noted that each successive test sample had 5 ml less H₂O₂ because a 5 ml aliquot was removed at each time point before further re-use of the remaining H₂O₂ solution which contained successively higher levels of leached Ni and Mo.

Concentration of Metals Release by Reuse of 3% H2O2 TEST 57

Concentration of Metals Released by Reuse of 3% H_2O_2 Test 57

Table 43
Re-Use of H₂O₂ Leachate

Sample	Volume	PPM Ni	% Ni Rel	PPM Mo	% Mo Rel
1	100	177	72.63	718	89.75
2	95	364	70.33	1451	82.56
3	90	565	67.79	2297	86.11
4	85	736	48.79	3162	77.55
5	80	891	36.33	4649	128.94 ^a
6	75	1237	89.56	5357	37.32 ^b

a-measured after 3 days contact time

b-measured after 8 days contact time

Test 58

Test 58 was a retest of the thermophilic enrichment culture (started from inocula obtained from Yellowstone National Park) which, upon initial enrichment, had not shown significant bio-leaching ability. After initial tests showed little bio-leaching ability, the enrichment culture was routinely cultivated for several months in the presence of catalyst in an attempt to improve its tolerance of the catalyst and perhaps improve the bio-leaching capacity of the enrichment consortium. No significant improvements in bio-leaching ability were noted for this thermophilic enrichment culture.

General Discussion and Conclusions

Individual test results and their importance have been separately discussed in each section of the test program. This was done because of the many variables and conditions which have been investigated, each of which is important in effecting or retarding the release of the metals from the catalyst.

An overall examination of these findings show that the release of Mo is dependent upon many parameters, but is dominated by microbial growth in a bio-leaching system. Such microbial Mo release is a rapid process usually requiring less than 1 week for 90% of the releasable Mo to be solubilized. Efforts performed in the research presented here centered on optimizing the parameters that stimulate microbial growth and action. It is suggested that if further research is continued, it should pursue those medium components and incubation conditions which best promote the microbial release of Mo. Another avenue of research (since Ni is toxic to many microorganisms) should investigate the possibility of promoting Mo release microbially by first leaching Ni (using leaching techniques other than chemical chelation) and then subjecting the preleached catalyst to microbial Mo

release. An example of this would be the highly efficient H_2O_2 extraction technology demonstrated for this project. Also, although not demonstrated in this project, the products of microbial growth (H_2O_2 ?) rather than microbial growth per se may be important in this Mo release system. In this regard, further efforts should be made at determining the role of air (oxygen) in its suggested importance in furthering Mo release. It was found in this research that thermophily conferred no advantage in this bio-leaching technology, but further efforts should be made to study thermophiles obtained from appropriate highly selective habitats such as thermal areas of high metal sulfide content.

While the majority of the research performed for this project centered on the physio-chemical parameters that optimized microbial bio-leaching of metals from spent coal liquefaction catalyst, many other factors were investigated, and in many cases it was found that 100% of the Mo on spent catalyst could be solubilized. One of the theories generated by these investigations was the idea that not only is microbial growth important in a metals solubilization system, but also it may be that a product of microbial growth can serve as a metals releasing agent. Attempts were made to isolate or characterize a metals releasing agent or metals releasing factor (MRF) and those attempts were largely unsuccessful; however, supernatant, concentrated by lyophilization, revealed a metals releasing ability. The metals releasing factor was never characterized, but a well known by-product of microbial metabolism, H_2O_2 , was chosen as microbial metabolic by-product that merited further investigation.

Experiments testing the ability of H_2O_2 to solubilize metals were surprisingly successful (see above). Not only were 100% of the Mo and 75% of the Ni leached into solution in typical tests, but also it was found that the action of H_2O_2 in its metals leaching ability was quite rapid. 3% H_2O_2 could usually leach about 100% of the Mo and about 75% of the Ni in 24 hours, and whereas bio-solubilization with heterotrophic denitrifying also could approach this high level of solubilization, the biological process usually required a period of more than a week. Tests revealed that both systems worked best when the liquid to catalyst ratio was near 100 to 1. The price of the Citrate Bm medium (retail chemical costs) and the price of 3% H_2O_2 are about equivalent; however, since the 3% H_2O_2 system performs the necessary leaching about 10 times faster, it would be more economically feasible to recycle Mo and Ni using the 3% H_2O_2 system. This economically superior method is further favored due to the absence of labor that is involved in bio-leaching system where time and labor must be expended to prepare the appropriate medium.