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Quarterly Report

Microbial Recovery of Metals from Spent Coal
Liquefaction Catalysts

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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. The continuity of the research program was maintained during the period of principal investigator replacement. This transition period allowed the project data to be reviewed and research plans were formulated to gain new insights into the mechanisms involved in metals release.

Introduction

The work initiated in the first quarter of 1993 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. To reduce the number of solvent systems the data were examined and two solvents selected for continued testing. The two solvent systems which were chosen and have been employed are the isopropanol and a tetrahydrofuran (THF) extractive solvents. These solvents were selected as representative systems following consideration of their comparative attributes including cost, ease of handling, and their 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 will be 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 has concentrated on using THF as the solvent system allowing work to proceed rapidly. However, additional work will still be directed toward defining any solvent system superior to THF in terms of aiding microbiological release of metals and economic feasibility. These studies include measurements of metal release at various catalyst-media ratios for both the tetrahydrofuran and isopropanol washed catalysts. The importance of the catalyst-media ratios is assuming a greater importance since it is desired to reduce the media volume to the lowest level possible while still retaining good metals release.

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 is being more fully investigated.

Multiple microbial metal releasing experiments with a series of comparative growth systems have been 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). The growth studies were conducted with both isopropanol and THF solvent extracted catalysts and with various concentrations of media additions and include various ratios of catalyst-media ratios. Later tests investigated only THF washed catalyst. Metals released at shorter time periods (every two days) were determined and comparative responses related to microbial growth; e.g. (turbidity), pH changes, and gas production.

TABLE 1
MEDIUM B

1.0g KH_2PO_4
1.8g Na_2HPO_4
10.0g NH_4NO_3
5.0g Na_3 Citrate
1.0ml SL-4 trace metals (See Table 2)
1L dH_2O

Use 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.

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 have been obtained from the American Type Culture Collection (*Bacillus stearothermophilus* ATCC 12016) and the cultures are being grown at 60°C for initial 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 is being undertaken. In addition, 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 (ongoing), and we hope 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.

All current work will center 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 is being 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 150 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.

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 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 are in progress. In addition, the effects of non chelating carbon sources such as lactate to enhance microbial growth, but which have no chelating power, are being 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 current 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 2 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 are now in progress to investigate this possibility and preliminary results indicate that the Ni is associated with the cell biomass.

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 + Crusted 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> ^o	<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

^o All bottles capped (facultatively anaerobic).

⁺ See Table 1.

* Previous denitrifiers combined with enrichment cultures.

Future Research

The release of Mo is dependent upon many parameters, but is dominated by microbial growth. Such microbial Mo release is a rapid process usually requiring less than 1 week for 90% of the releasable Mo to be solubilized. Efforts will center on optimizing the parameters that stimulate microbial growth and action. We will continue to pursue those medium components and incubation conditions which best promote the microbial release of Mo. Also, since Ni is toxic to many microorganisms, we will investigate the possibility of promoting Mo release microbially by first leaching Ni (an example being chemical chelation) and then subjecting the preleached catalyst to microbial Mo release.

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