Microbial Recovery of Metals from Spent Catalysts

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Abstract/Summary

The second quarter of 1990 was one of peripheral progress on the project of reclaiming molybdenum and nickel from spent coal liquefaction catalysts. We defined some important parameters for future research and we were able to clear up ambiguities in some of the past approaches and the problems uniquely associated with the ability of *T. ferrooxidans* to leach both Ni^{++} and molybdate from spent, alumina supported catalyst from the Wilsonville pilot project. We were also able to show that *T. ferrooxidans* was very sensitive to molybdate and extremely sensitive to tungstate, but showed relatively little sensitivity for the related elements chromate, vanadate and for the catalyst associated metal, Ni^{++} . There appears to be no negative synergistic effects between Ni^{++} and molybdate for growth, which bodes well for processes to reclaim both these metals from spent coal liquefaction catalysts. We have shown that *T. ferrooxidans* is indeed capable of leaching molybdate and Ni^{++} from spent catalysts if the catalyst is washed extensively with both an organic solvent such as tetrahydrofuran to remove the oily contaminants and an aqueous acidic medium to remove readily solubilized Ni^{++} and molybdate. It is possible to extract into an acidic medium enough molybdate from THF washed spent catalyst within 24 hr to completely inhibit the growth of all tested *T. ferrooxidans* strains. The stage is now set for the development of a molybdate tolerant strain to be used for actual leaching of the spent catalyst. We are currently seeking simpler ways of pretreating the raw spent catalyst in order to make it more amenable to microbial leaching and possibly produce an economic and feasible technology.

Technical Studies

Nickel Tolerance Studies.

We tested the tolerance of all strains obtained from INEL for Ni^{++} , since this metal is also released in addition to molybdate from the Wilsonville supported catalyst. These data are presented in Table 1. It is obvious from these data that Ni^{++} will present less of a problem than molybdate for the growth of *T. ferrooxidans* and eventual leaching of metals from spent catalysts and is tolerated, without adaptation of the strain, at very high Ni^{++} concentrations.

Table 1

Tolerance of *T. ferrooxidans* for Nickel

Growth

Nickel Concentrations

Strain	0 μM	10 μM	50 μM	100 μM	500 μM	1mM	5mM	10mM	20mM	50mM	100mM
DT-3	+	+	+	+	+	+	+	+	+	+	+
DT-5	+	+	+	+	+	+	+	+	+	+	+
DT-6	+	+	+	+	+	+	+	+	+	+	±
DT-7	+	+	+	+	+	+	+	+	+	+	±
D-26	+	+	+	+	+	+	+	+	+	±	-
A-4-1	+	+	+	+	+	+	+	+	+	+	-
A-4-2	+	+	+	+	+	+	+	+	+	+	±
F221	+	+	+	+	+	+	+	+	+	+	+

The same strains which tolerate higher molybdate concentrations (see below) show higher Ni^{++} tolerances. Ilona Fry of Battelle informed me that *T. ferrooxidans* does not like Cl^- , and since we used NiCl_2 as our source of Ni^{++} , the potential tolerances may be actually higher than those shown in Table 2. However, the levels tolerated by all of the strains are high enough that the potential leaching of spent Ni-containing catalysts should be unaffected by the Ni released. There is some effect on growth at 50 mM and 100 mM levels, but growth is only slightly retarded and the cell concentrations reach the same levels found in the controls with only a slight delay of 2-3 days.

Our final question before proceeding with more detailed tolerance experiments was to see if there was a synergistic effect between molybdate and nickel for the growth of *T. ferrooxidans*. To do this we set up a series of flasks with 62.5 μ M molybdate (the highest level readily tolerated by all of the strains tested) and varying levels of nickel up to 50 mM. Ni⁺⁺ has no effect on the growth of *T. ferrooxidans* in the presence of molybdate, and molybdate does not lower the tolerance of *T. ferrooxidans* for Ni.

Molybdate Tolerance.

Further experiments to increase the molybdate tolerance of *Thiobacillus ferrooxidans* were not successful in the second quarter. We have succeeded in the past in increasing the tolerance of strain DT-3 from a nominal tolerance of 75 μ M to 150 μ M molybdate in one strain, but further increases were not achieved during the second quarter. We have gone back and done a thorough study of the tolerances of all of the *T. ferrooxidans* strains which we have obtained from INEL and the data are presented below. This was necessary in order to be sure that the best strains will be selected for the mutagenic efforts to produce a molybdate tolerant strain. We have also considered taking the approach of using *Sulfolobus* and *T. ferrooxidans* strains obtained from the American Type Culture Collection as well as our isolates. In addition, the results presented at the First International Symposium on the Biological Processing of Coal by the Swedish group seemed to point to the inability of *Sulfolobus* to adequately leach minerals. These results must be kept in mind in determining the ultimate potential for the leaching of spent coal catalysts. We are still undecided as to whether organisms other than *T. ferrooxidans* might be useful leaching microorganisms. It is clear that they must be strong acid producers in order to adequately leach metals from the spent catalyst. In addition we have studied the effect of added organic material on the chemoautotrophic growth of *T. ferrooxidans* and found that although, in pure culture, organic matter inhibits the growth of *T. ferrooxidans*, in mixed acidic cultures, organic contents of up to 0.5% w/v glucose or yeast extract do not inhibit significantly the growth of *T. ferrooxidans* as judged by Fe⁺⁺ oxidation. Since we do not anticipate the use of pure cultures this is an important observation. However, the THF extracted oil from the spent Wilsonville catalyst is inhibitory to *T. ferrooxidans*. We think this is primarily due to the predominant presence of nitrogen heterocycles and aniline derivatives (such as aminonaphthalenes, quinolines and pyridines) which are quite toxic to microorganisms

and which also have good solubilities in acidic aqueous solutions. This problem can be overcome with an aqueous extraction of the raw spent catalyst which may be combined in one step with the washing of easily removed Ni^{++} and molybdate. We are currently testing this hypothesis.

Further experiments to increase the molybdate tolerance of *Thiobacillus ferrooxidans* have therefore been temporarily put on hold. Three strains of *T. ferrooxidans* were selected for their general higher tolerance of molybdate and then grown in the presence of the above related elements as well as Vanadium. In general we found that Cr is tolerated at very high levels relative to Mo. This was quite surprising since Cr is usually considered quite toxic to microorganisms, probably because of its high reactivity. Since the INEL broth at pH 2.5 was used, there certainly was some conversion of the Sodium Chromate supplied in the medium to Dichromate and indeed there was visible ferrous iron oxidation at high chromate concentrations. Nevertheless, visible and considerable growth of *T. ferrooxidans* was seen with much higher levels of Chromate than can be seen with Molybdate. Vanadate also was tolerated at very high levels and, as with Chromate, there was some chemical oxidation of the ferrous iron at high Vanadate concentrations. The addition of Sodium Tungstate to INEL broth at pH 2.5 revealed that *T. ferrooxidans* is extremely sensitive to Tungstate. Tungstate is tolerated at only 10% the levels Molybdate is tolerated (i.e. 10 μM for Tungstate vs. ~100 μM for Molybdate). These results are summarized in Tables 2,4,5 & 6 below.

Table 2

Molybdate Tolerances of *T. ferrooxidans* Strains

Growth

Molybdate Concentrations

Strain	0 μM	1 μM	2 μM	4 μM	8 μM	16 μM	32 μM	50 μM	62.5 μM	75 μM	87 μM	100 μM
DT-3	+	+	+	+	+	+	+	+	+	+	±	-
DT-5	+	+	+	+	+	+	+	+	+	+	+	-
DT-6	+	+	+	+	+	+	+	+	+	±	-	-
DT-7	+	+	+	+	+	+	+	+	+	-	-	-
D-26	+	+	+	+	+	+	+	+	+	±	-	-
A-4-1	+	+	+	+	+	+	+	+	+	+	-	-
A-4-2	+	+	+	+	+	+	+	+	+	±	-	-
F221	+	+	+	+	+	+	+	+	+	+	+	-

Other concentrations were also tested, including 125 μM , 250 μM , 500 μM , and 1 mM, but the above data set adequately defines the true tolerances of these s

trains. It should be noted that not only did strains DT-3, DT-5 and F221 tolerate higher levels of molybdate, but they also grew considerably faster under most "normal" conditions. The three additional strains which eventually showed some growth at the 75 μ M level did so only after extended incubation. We have also discovered that much better growth is achieved with all of these strains if the initial pH of the INEL medium is 2.5.

Jon Volkwein of the U.S. Bureau of Mines has given me the name of some people in Colorado who operate a molybdenum mine. As yet they have not been cooperative. We feel that if we can obtain some mine runoff from their operations we may be able to obtain a natural *T. ferrooxidans* strain which is resistant to molybdate instead of manufacturing one in the laboratory. Moreover, after the results shown above with Vanadate, Chromate and Tungstate, we should also investigate the possibility of obtaining runoff from a tungsten mine, especially since W appears to occur in mineral forms which are almost always tungstates. We were able to contact F. Stoll at Sandia about the potential of other metals contaminating the spent catalysts which she stated were usually Fe, Al and Ti. We checked, using atomic absorption, for the presence of Al, Ba and Ti in our INEL broth leached, washed spent catalyst from Wilsonville. It is impossible to check for Fe in the growth flasks since Fe is added at high concentrations as an energy source. We were unable to detect any of these elements (the detection limits are 2 ppm, 1 ppm and 5 ppm, respectively). In addition we found no increase in the iron content of the medium, but since the INEL broth contains 7500 ppm Fe, the possibility still exists that Fe is a contaminant. We will have a better handle on this when our ashing experiments are complete.

Experiments to modify *T. ferrooxidans*' natural sensitivity to molybdate will begin again with the addition of strains from the American Type Culture Collection (Numbers 14119 and 21834). Efforts to obtain acid mine drainage from a molybdenum mine have hit unfortunate snags, primarily due to the mine company's hesitance to release potentially environmentally sensitive samples. This avenue will be continued in the coming weeks, but the prospects do not appear good at this time. If an appropriate contact within the Bureau of Mines could be located, a useful sample might be obtained via that avenue. A similar sample from a tungsten mine could also be very helpful. We feel that this approach might lead to a natural molybdenum tolerant *T. ferrooxidans* strain. The alternative approach will be also pursued in parallel, which is to produce a tolerant strain through mutagenesis and selection.

Catalyst Studies.

Since high levels of molybdenum are released rapidly from washed and unwashed spent catalyst from Wilsonville, the acidic INEL medium will not support the growth of *T. ferrooxidans*, which are naturally tolerant to only about 100 μM molybdate. mM molybdate is released from THF washed catalyst within 48 hrs of suspension. This release is only approximately 10-20% of the molybdate present, however. We have washed this THF extracted material with INEL medium to ascertain if there is only a fraction of the molybdate on the catalyst which is readily solubilized by the INEL medium. As can be seen from Table 5, washing with INEL broth lowers the amount of molybdate and Ni solubilized by the medium to levels which can be tolerated by *T. ferrooxidans* strains. The implication is that an extensive acid pre-wash of the spent catalyst will not only remove much of the molybdenum and Ni from the spent catalyst, but also provide a suitable substrate for further leaching by *Thiobacillus*. In the future we will study both the time effect of the growth on INEL washed THF extracted spent catalyst and INEL washed raw spent catalyst.

Leaching Studies.

Table 3

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	70	138

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

Related Metal Tolerance Studies.

Table 4

Vanadate Tolerances of *T. ferrooxidans* Strains

Growth

Vanadate 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	+	+	+	+	+	+

Table 5

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 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 the Cr still remained in some form in the culture vessel.

Table 6

Tungstate Tolerances of *T. ferrooxidans* Strains

Growth

Tungstate 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	+	+	+	-	-	-

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

We have also tested the tolerance of these strains for Ni^{++} , since this metal is also released from the supported catalyst. It is obvious from these data that Ni^{++} will present less of a problem than molybdate for the growth and eventual leaching of metals from spent catalysts. It is also noted as a positive sign that Ni did not show any "negative" synergy with Molybdate, Chromate, Tungstate or Vanadate. These results are very important since it means that increasing the tolerance of *T. ferrooxidans* to molybdate will probably not involve increasing tolerance for Ni and will probably not involve increased Ni toxicity. Our final question before proceeding with more detailed tolerance experiments was to see if there was a synergistic effect between molybdate and nickel for the growth of *T. ferrooxidans*. To do this we set up a series of flasks with 62.5 μ M molybdate and varying levels of nickel up to 50 Mm. As mentioned above, Ni^{++} has no effect on the growth of *T. ferrooxidans* in the presence of molybdate, and molybdate does not lower the tolerance of *T. ferrooxidans* for nickel.

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