Title: Anaerobic Digestion Toxicity
1) Hydrogen and carbon monoxide monitoring (Process Control)
2) Microtox (Screening)

PI: M. Switzenbaum

Objectives: One of the problems in any biological wastewater treatment system is process reliability for the treatment of wastewaters containing toxic materials. This is especically true for anaerobic systems. The study proposed here addresses toxicity of anaerobic systems for both process control, and screening of influents.

1) Hydrogen,CO Monitoring (Process Control)
At the present time in our laboratory, an investigation is being conducted which has the purpose of developing an early warning system for anaerobic digestor upsets. The key to this system is hydrogen measurement since hydrogen is a key intermediate in anaerobic methane fermentation. In a well operating digester, hydrogen should never accumulate. By monitoring hydrogen it may be possible to prevent major upsets from occuring. The present study uses wastewater sludge, which is a poorly defined substrate. The study proposed here compliments the ongoing study by using enrichment cultures with well defined substrates. Also in our present work, we find CO accumulaton in certain upset conditions. Therefore we propose to monitor both CO and hydrogen in enrichment cultures with a number of known anaerobic methane fermentation toxicants.

2) Microtox (Screening)
The Microtox bioluminescence toxicity analyzer is a fast, reliable and reproducible method. Correlations between Microtox, and other bioassay tests have been established. At the present time in our laboratory, Microtox is being compared with the Anaerobic Toxicity Assay (ATA) which is a well known screening test for industrial wastes. The second part of this study will use the same enrichment cultures and compare Microtox with hydrogen and CO accumulation.

Procedure: The protocol for hydrogen and CO monitoring has been developed, as well as the Microtox procedures. For this project three fill and draw reactors will be used with three different substrates-acetate, formate, and lactose. This will result in three different populations. The acetate reactor will select for methanogens which use acetate, while the formate reactor will select for hydrogen using methanogens. The lactose reactor will result in a mixed population of fermentative, acetogenic, and methanogenic bacteria.

Alliquots will be taken from each of the three reactors and put into serum bottles, and then be spiked with known toxic agents such as heavy metals and toxic organics. CO and hydrogen will be monitored over time along with methane production. These results will be correlated to parallel Microtox testing.

Expected Results: A technical report will be prepared from the results of this study. In addition, papers will be published. This work provides a means of continuing work previosly funded by MDWPC, and supplements an ongoing effort of research sponsored by the New York State Energy Research and Development Authority.

Cost: $46,000

Name of Project: Anaerobic Digestion Toxicity: Process Control and Toxicity Screening

Faculty Advisor: M. S. Switzenbaum

Students: Kajsa Norgren (M.S. Student)
Eugenio Giraldo (M.S. Student)
Doris Atkinson (M.S. Student)

Progress Report: May, 1987

1. Microtox Study. Report is in preparation and will be done June 1.

2. H_2/CO Monitoring. Both the acetate and sucrose enrichment cultures have been started. Both students are learning the analytical techniques. A copy of the serum bottle protocol is enclosed.

Research Timetable: (3 year study)

On schedule (see January 1987 progress report)

Project: Anaerobic Digestion Toxicity

Faculty Advisor: Dr. Michael S. Switzenbaum

Students: Eugenio Giraldo and Kajsa Norgren

Progress Report: April 1988

This report summarizes progress over the past three months on the anaerobic digestion toxicity study. During this time, experimental data has been collected and processed.

Acetate Cultures.

The sucrose culture used for the preliminary testing had to be abandoned, since problems with excessive wall growth occurred again. A new reactor was set up, and this new sucrose enrichment culture was used as inoculum for the serum bottle studies for acetoclastic methanogens. Acetate was used as feed, along with the necessary nutrients. In this way the methane produced is attributable just to acetoclastic methanogens. Toxicity of Cu, Ni, and Cd has been tested so far. At various time intervals, the following were measured: hydrogen, carbon monoxide, methane, carbon dioxide and total gas production. pH was recorded at the end of each experiment.

We are now testing the three remaining toxicants (Zn, BES and formaldehyde), and processing the data collected so far.

Sucrose Cultures.

The serum bottle test for formaldehyde was performed. With this toxicant, the series of toxicants to be tested has been completed. Additional runs may be necessary for some of the previously tested toxicants in order to get a more detailed observation of the whole range of effects generated. The plotted data of the variables Methane production, Carbon monoxide concentration, and Hydrogen concentration are shown for the different toxicants tested (Cu, Ni, Zn, Cd, Bromoethanesulfonic acid(BES)) in the attached figures.

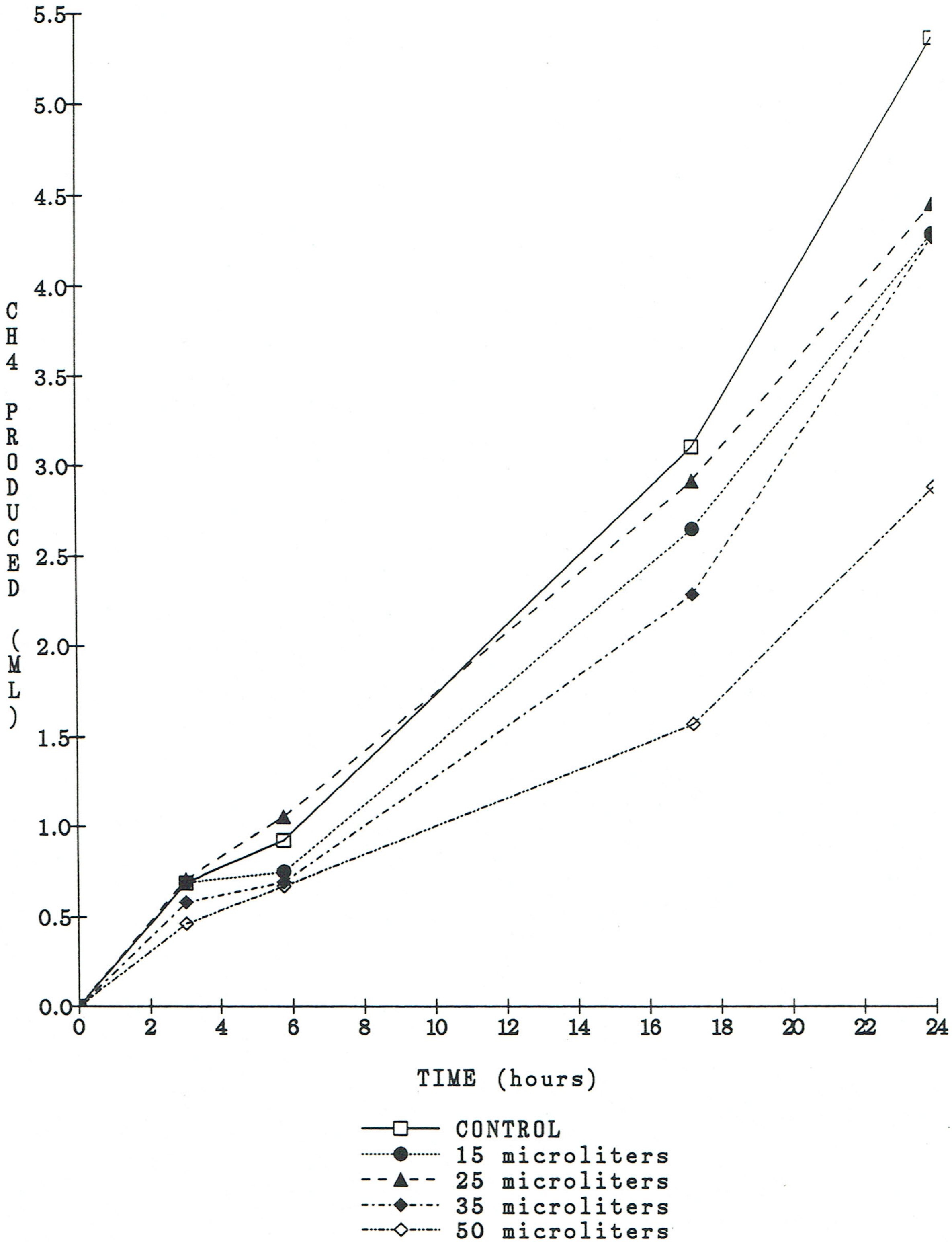
A 1.5 liter semi-continuous reactor has been built for the on-line study. It is currently being tested and calibrated .

Future Plans.

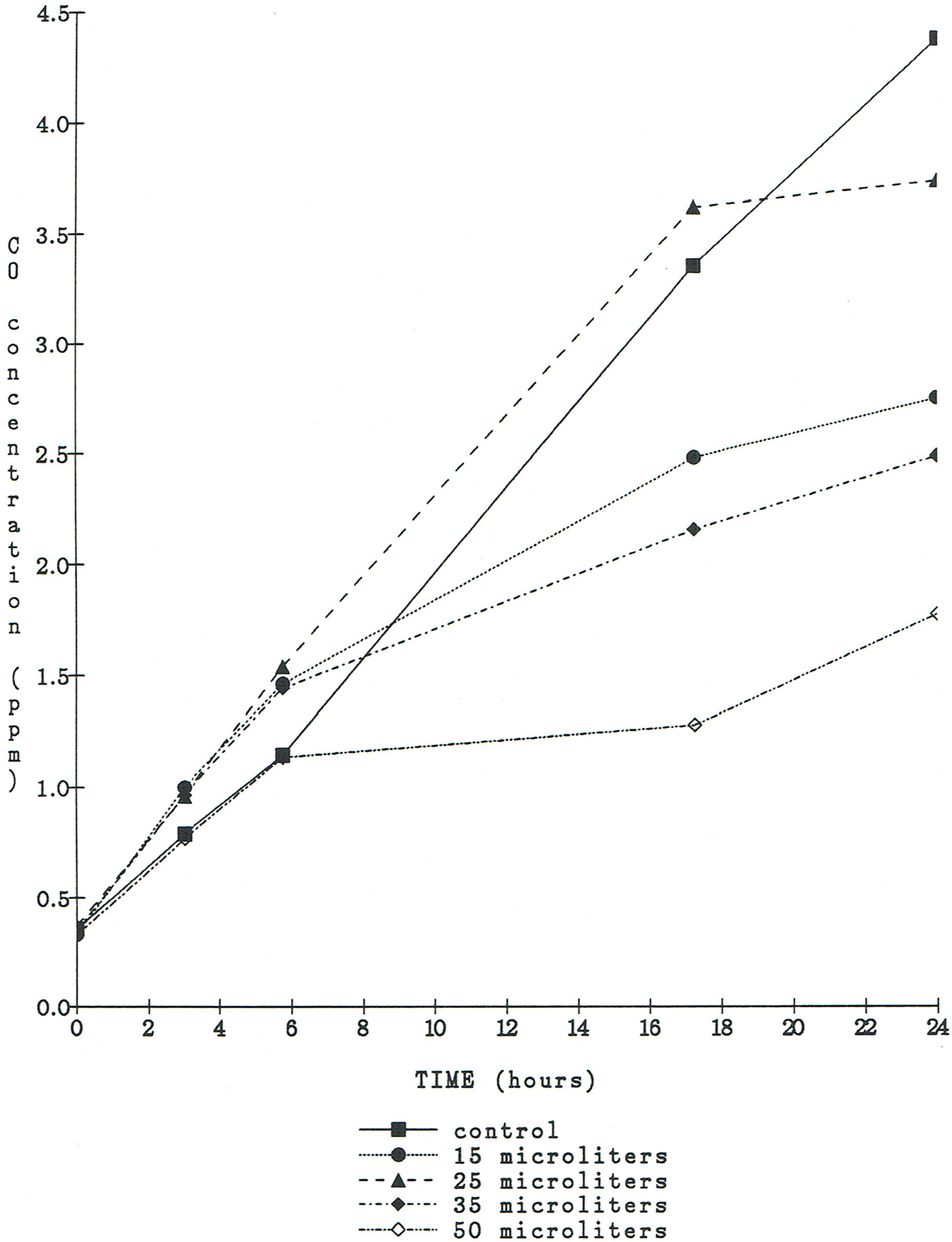
The following will be accomplished:

1. The toxicants Zn, BES, and formaldehyde will be tested using the acetate cultures.
2. The necessary extra serum bottle runs for the sucrose cultures will be done.
3. We shall do several on-line runs with the sucrose cultures.

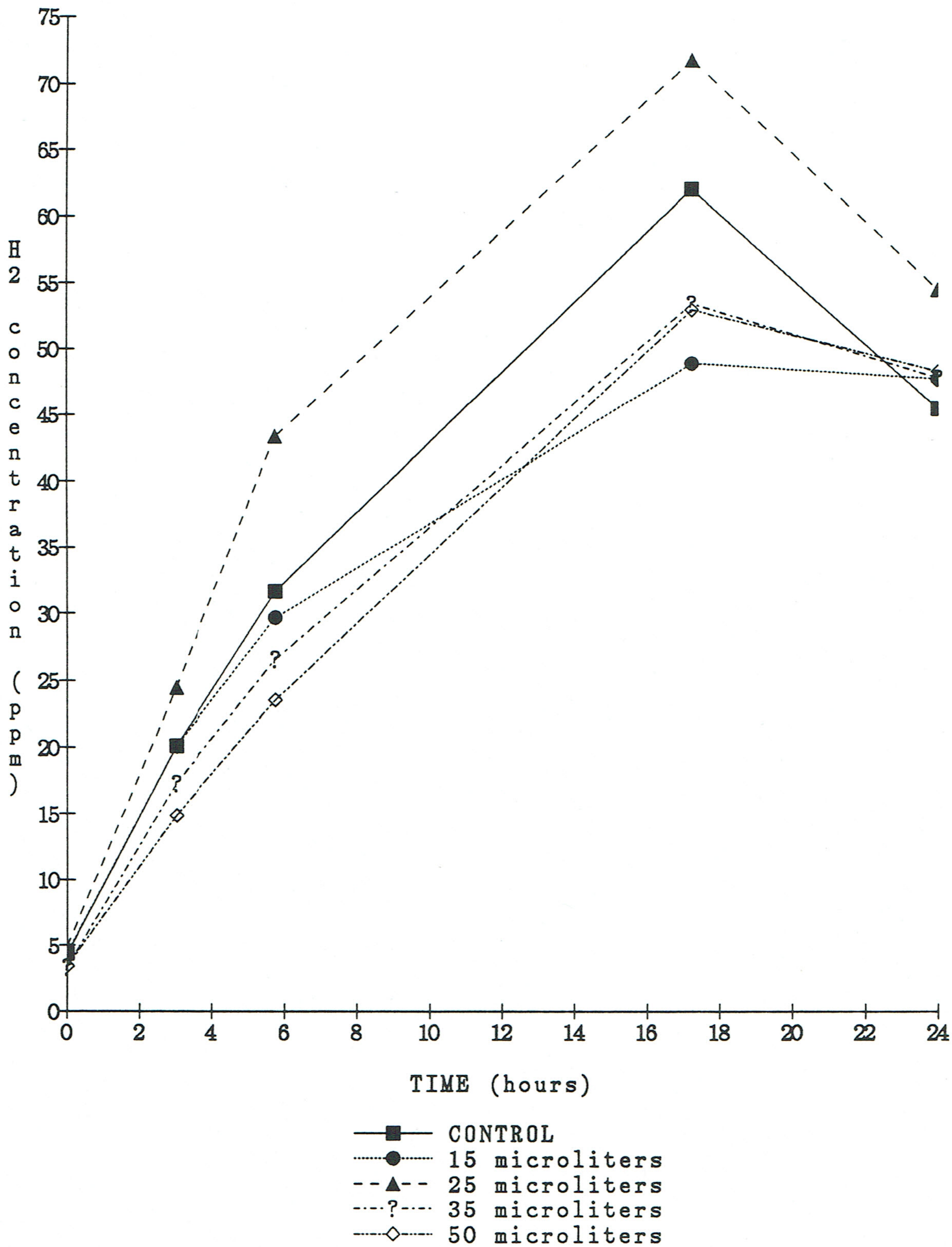
ZINC INHIBITION OF A SUCROSE ENRICHMENT CULTURE



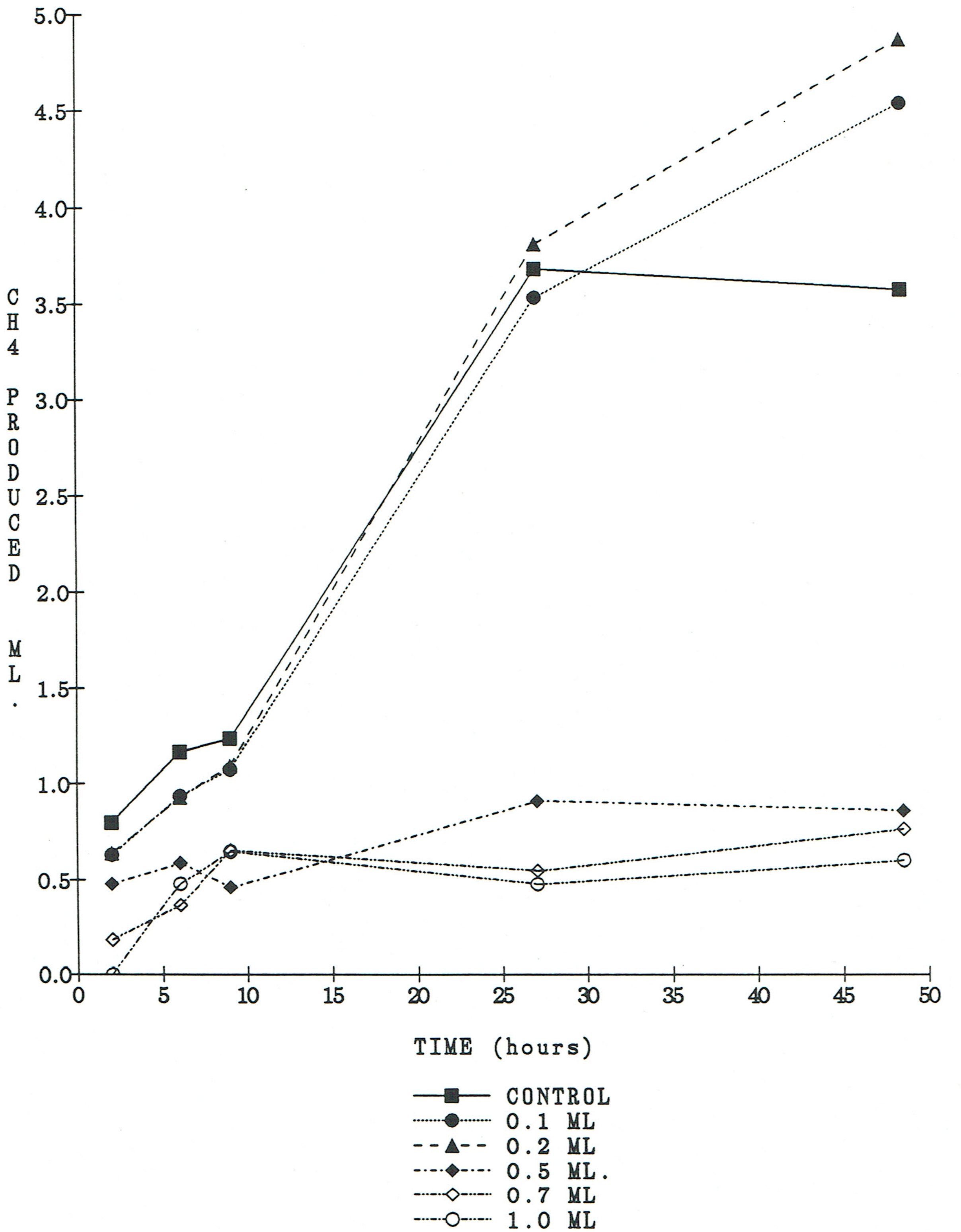
RESPONSE OF CO TO INCREASED LEVELS OF ZINC.



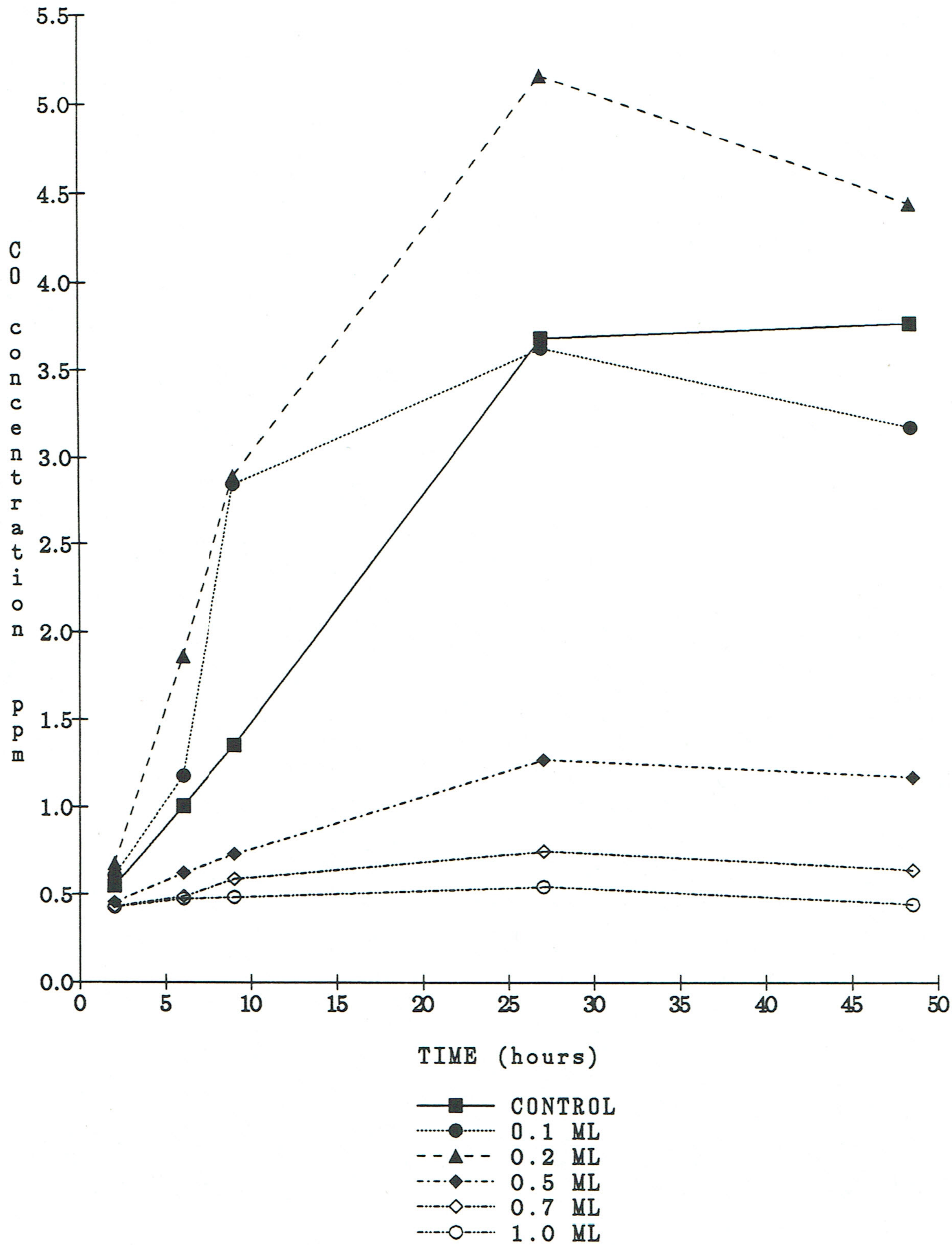
H2 RESPONSE TO INCREASED ZINC CONCENTRATION



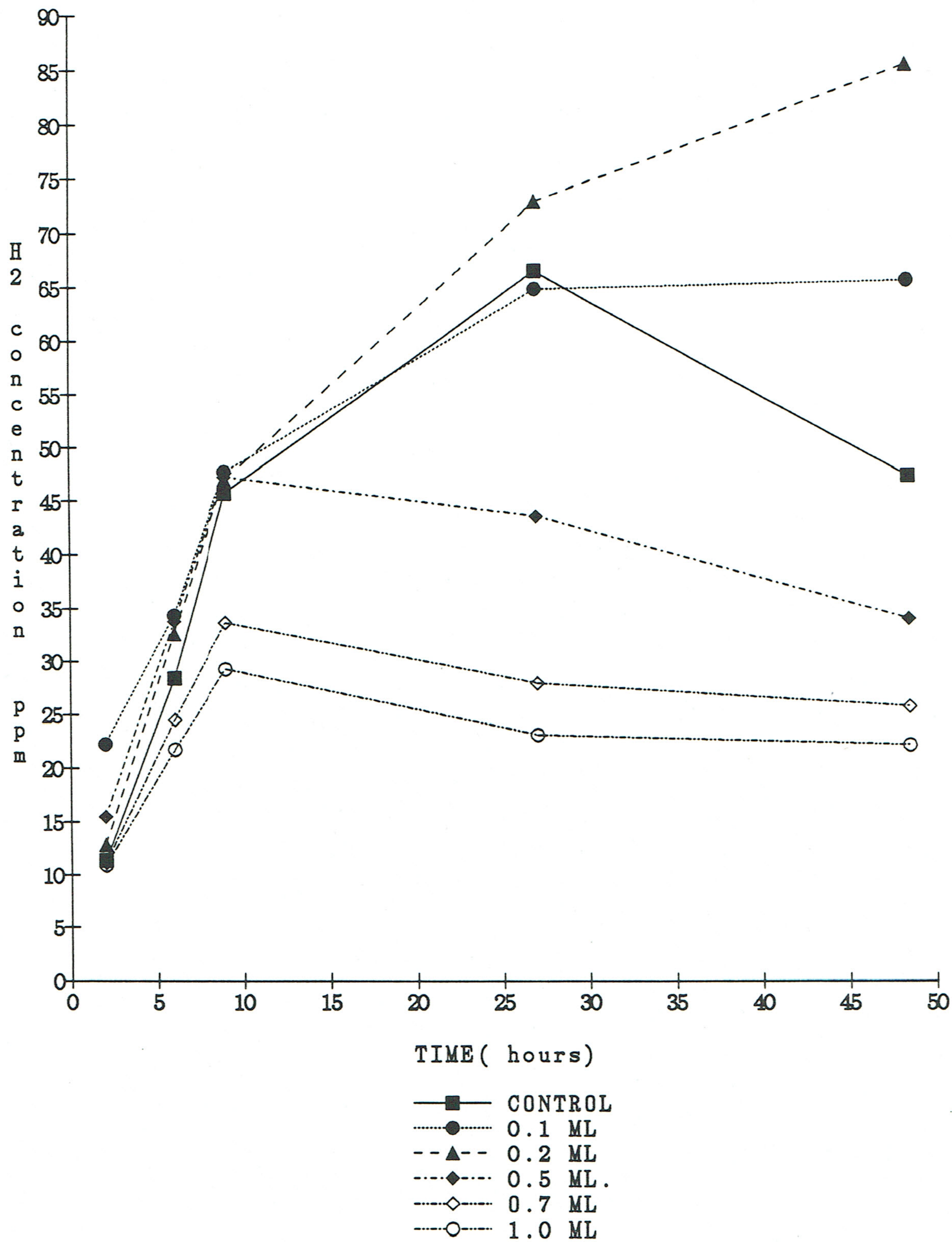
NICKEL INHIBITION OF AN ANAEROBIC SUCROSE ENRICHMENT CULTURE



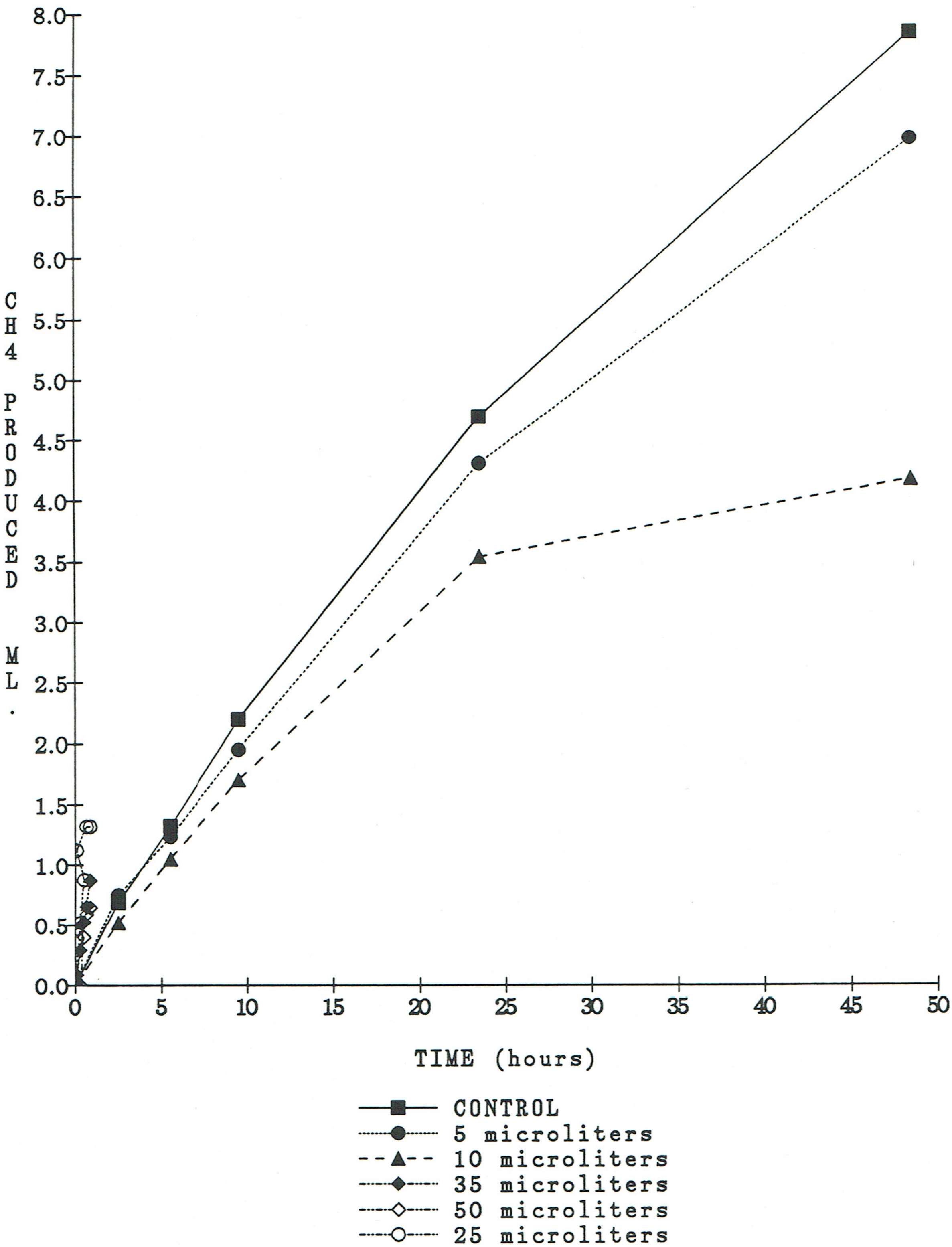
RESPONSE OF CO TO INCREASED CONCENTRATIONS OF NICKEL.



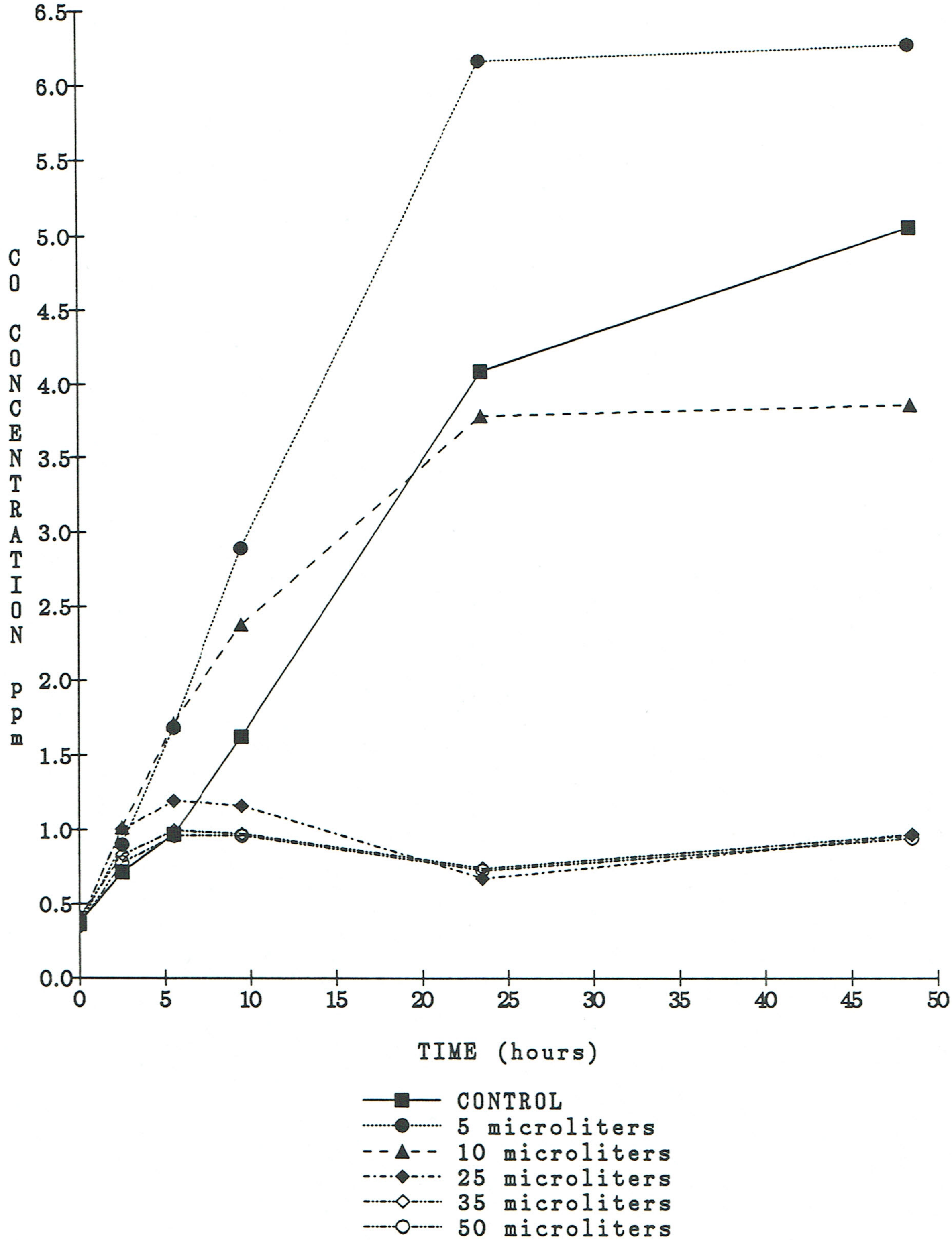
RESPONSE OF H2 TO INCREASED CONCENTRATIONS OF NICKEL.



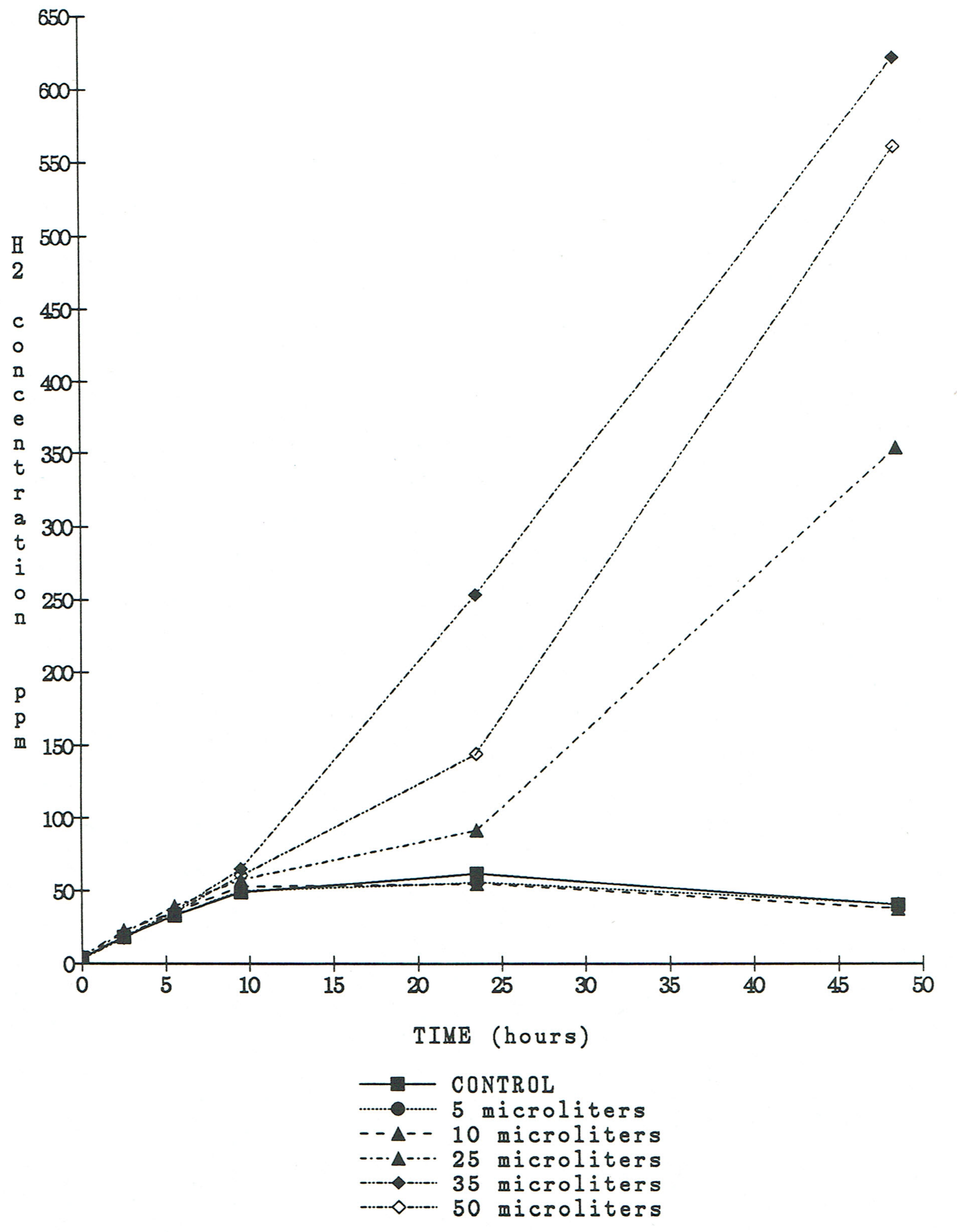
CADMIUM INHIBITION OF AN ANAEROBIC SUCROSE ENRICHMENT CULTURE



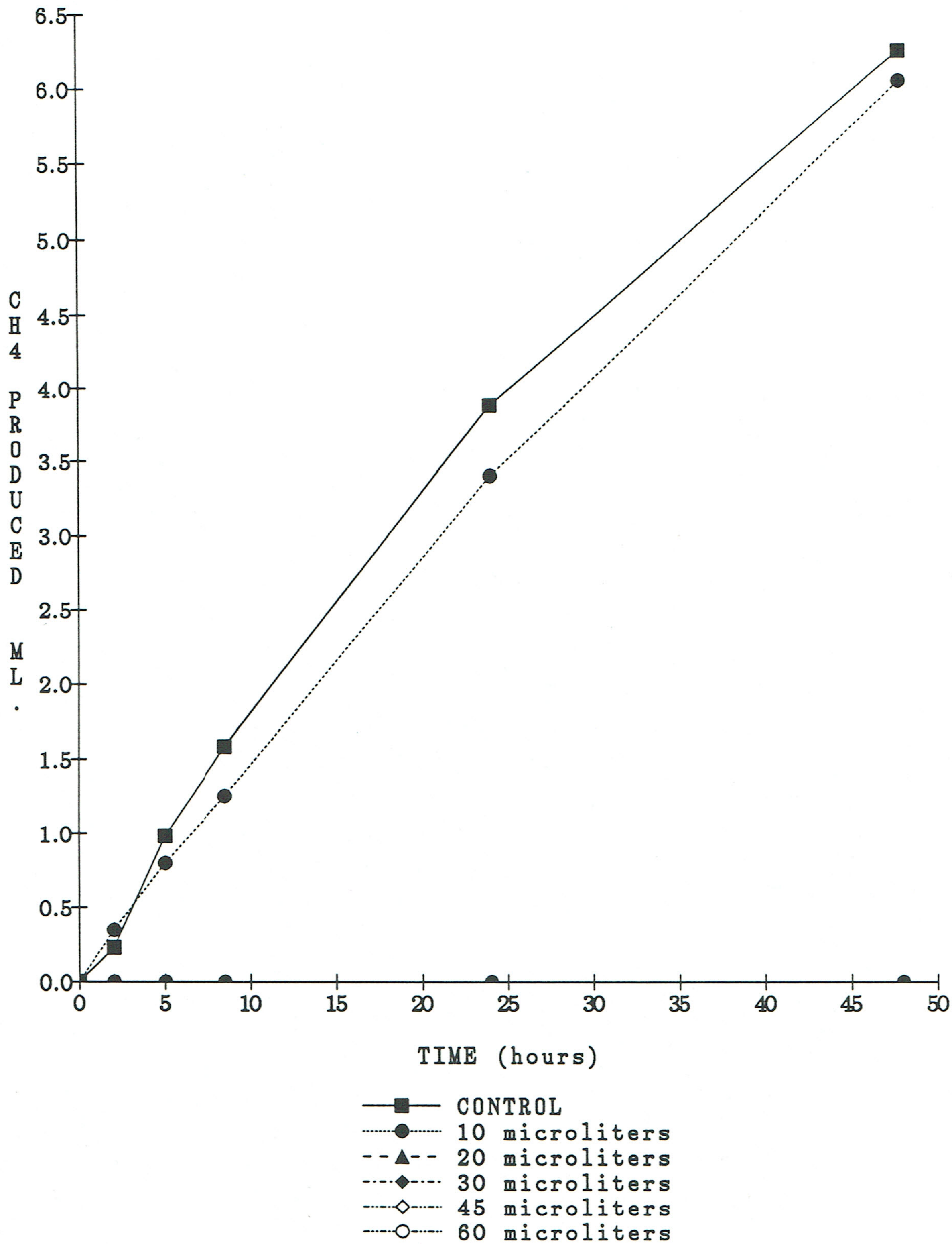
RESPONSE OF CO TO INCREASED CONCENTRATIONS OF CADMIUM.



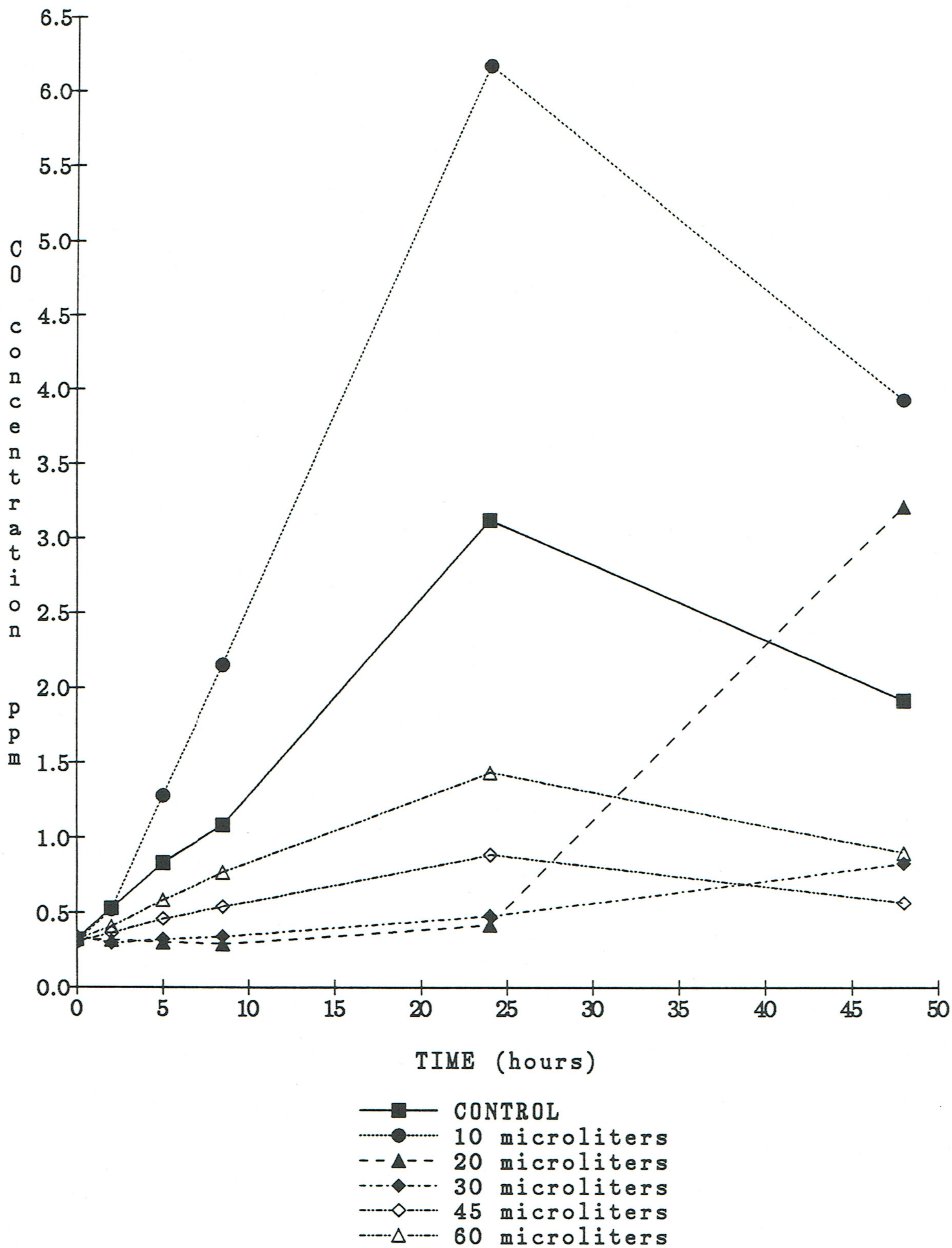
RESPONSE OF H2 TO INCREASED CONCENTRATIONS OF CADMIUM.



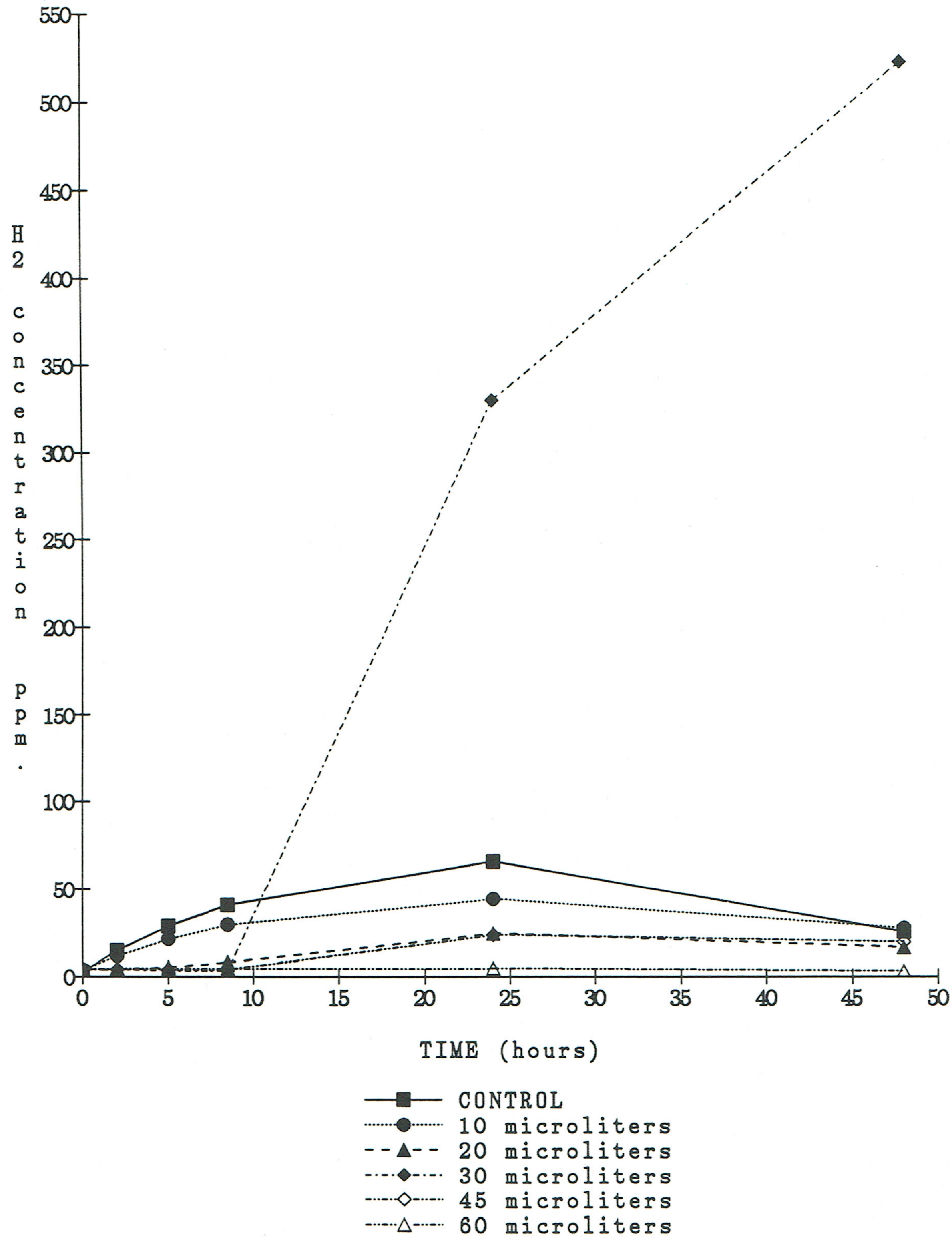
COPPER INHIBITION OF AN ANAEROBIC SUCROSE ENRICHMENT CULTURE



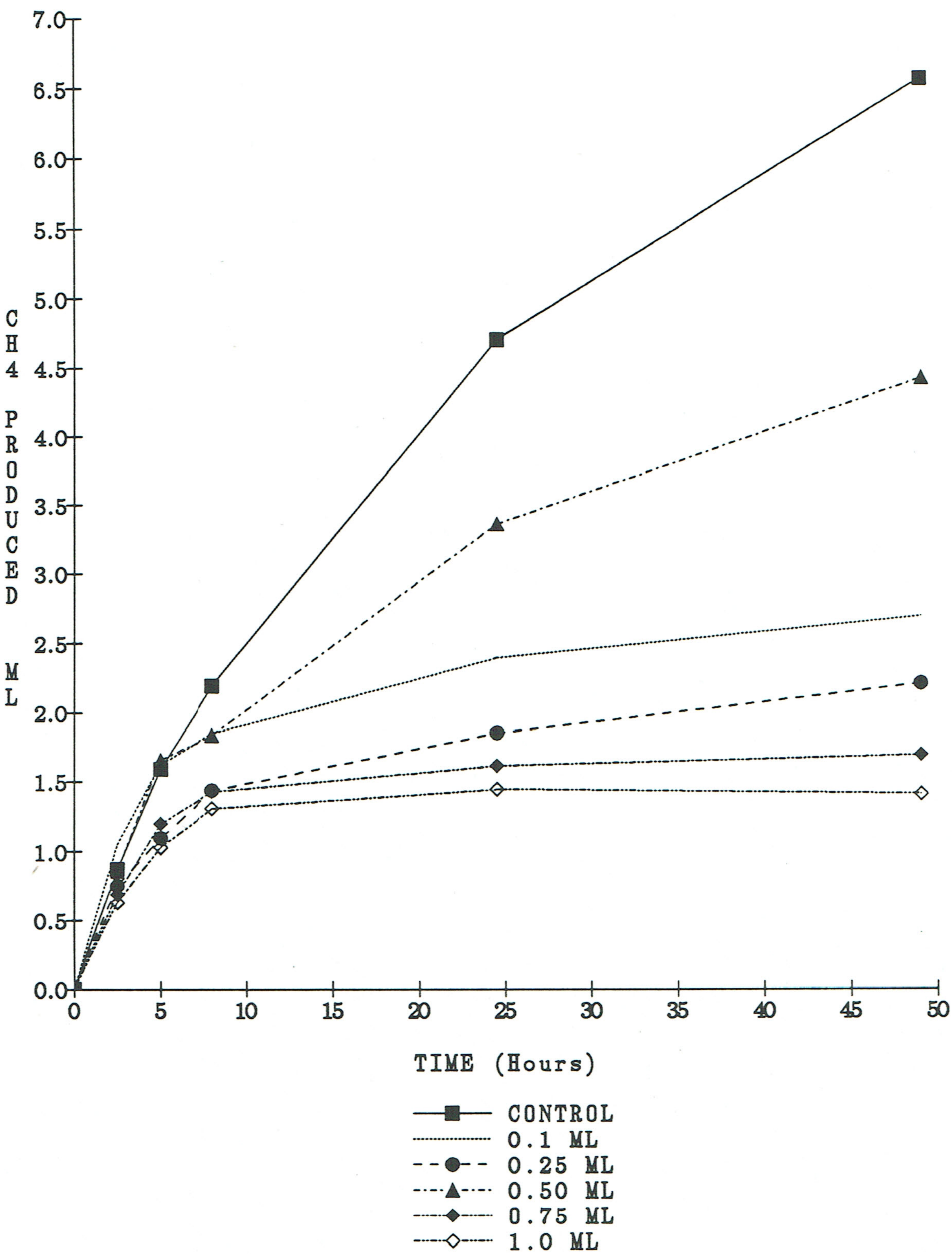
RESPONSE OF CO TO INCREASED CONCENTRATIONS OF COPPER



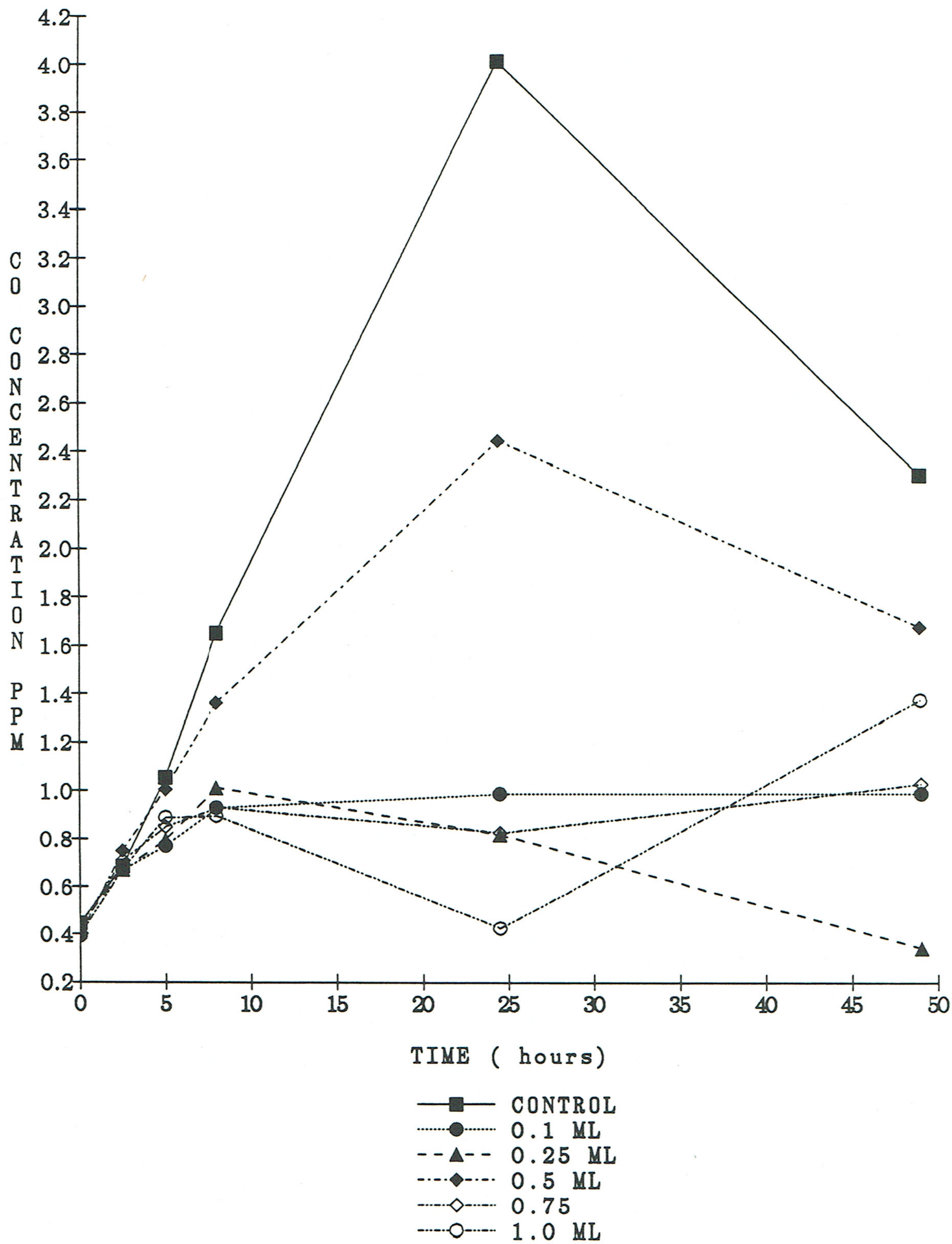
RESPONSE OF H2 TO INCREASED LEVELS OF COPPER.



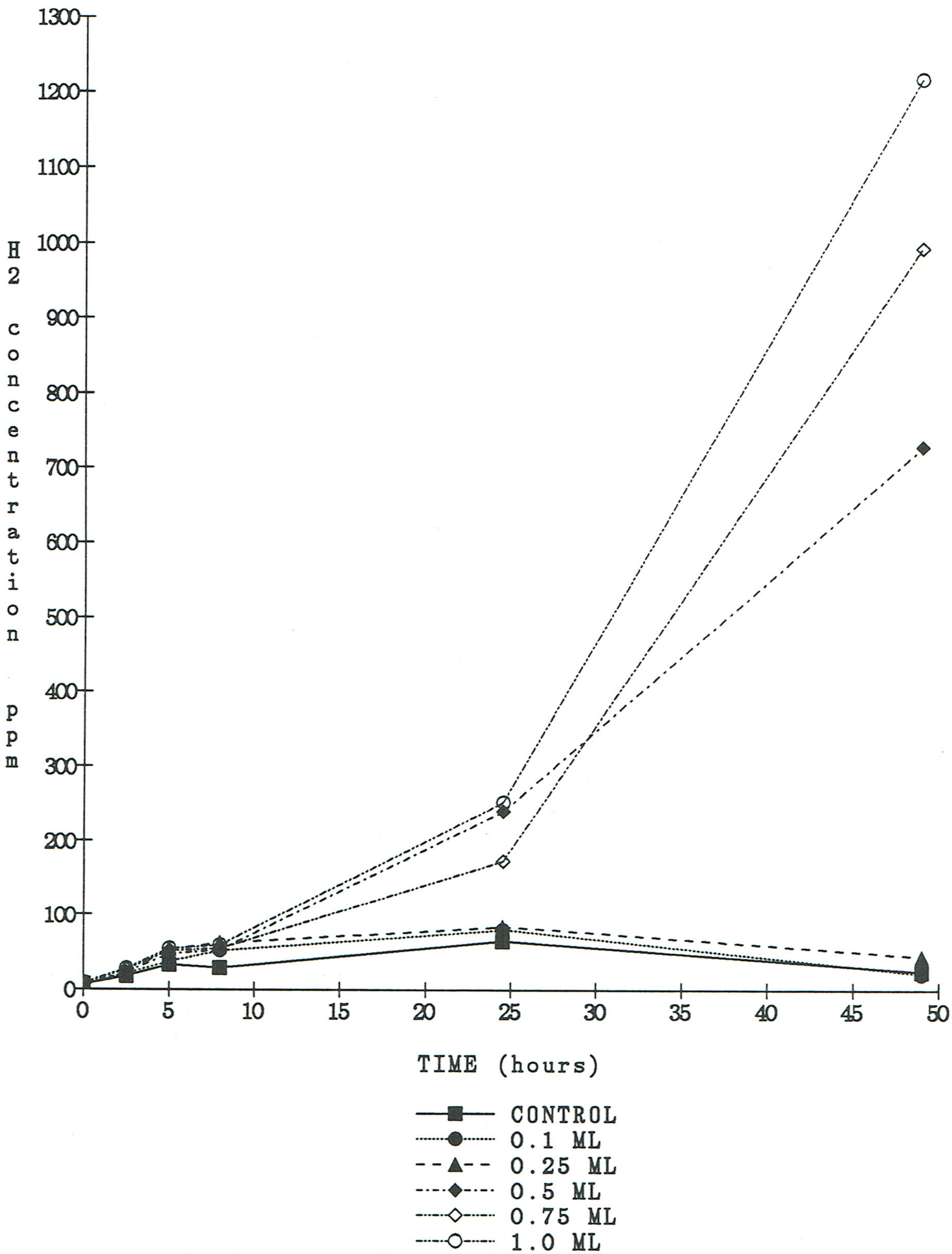
BES INHIBITION OF AN ANAEROBIC SUCROSE ENRICHMENT CULTURE.



RESPONSE OF CO TO INCREASED CONCENTRATIONS OF BES



RESPONSE OF H2 TO INCREASED CONCENTRATIONS OF BES



Name of Project: Variation of Heavy Metals Concentrations in Municipal Wastewater Treatment Plant Sludges
Faculty Advisors: M. S. Switzenbaum, J. K. Edzwald, and W. R. Knocke
Student: George Bacon (M.S. Student)
Research Engineer: Dan Wagner
Progress Report: April, 1988

Work on this project has progressed in four areas: revision and publication of the sludge-metals literature review, final development of sample preparation methods, completion of a statistically based sampling plan, and collection and analysis of sludge and compost.

George Bacon incorporated MDWPC comments into the final revisions of a literature review titled "Variation in Heavy Metals Concentrations in Municipal Wastewater Treatment Plant Sludges". The report was published and advanced copies sent to the Division. The balance of the Division copies are being held at UMass for pickup.

Preparation of a homogeneous sludge sample requires a lengthy seven step procedure (Table 1). Sieving finished compost and mixed compost separates the sludge material from wood chips and fragments. Grinding produces a fine homogeneous powder which can be readily weighed. We have reduced preparation time by several improvements in the grinding and filtering steps. However, each sample still takes about one hour to prepare and analyze.

The sludge metals project has two primary aims: 1) to determine whether heavy metals are concentrated or diluted by the composting process, and 2) to check the validity of compliance monitoring sampling methods for measuring metal concentrations. We have developed and implemented a statistically based sampling regime to accomplish these aims (Table 2). Samples of parent sludge being loaded into a designated compost bin are collected over a 3 to 4 day period. After a ca. 30 day incubation, finished compost-wood chip mixture samples are taken as the bin is unloaded. Every other bin (3 to 4 per month) are designated as study bins. George Heisler, Chief Operator of the Hoosac Water Quality District Wastewater Treatment Facility, and his staff make all the parent sludge and finished compost mixture collections.

Two other types of compost, cured compost and stockpiled mixture are collected every other week for comparison to parent sludge and finished compost mixture from the designated study bins (Table 2). Finished compost is stockpiled for curing for up to 6 months before distribution. Metal measurements are made on a composite sample from this pile for compliance monitoring. Compost mixture is stockpiled during the winter months, gradually sieved during the spring, and added to the curing finished compost pile. Thus, comparison of designated lots of compost produced by the plant over a known time period can be easily compared to compost ordinarily collected for compliance monitoring of sludge heavy metals. Mike Sutherland from the UMass Statistical Consulting Center has reviewed the sampling plan and has agreed it is robust enough for good statistical testing of the validity of compliance monitoring samples.

Preliminary analysis of sludge and compost samples shows considerable variation in sludge metals concentrations (Tables 3, 4, and 5). Variation in the parent sludge placed in a study bin over three days was greater than the variation of compost from the same bin (Table 3, Bin A). Variation in metals concentrations, especially Cd, over the two months of sampling was also evident (Table 4). For Bin A there was no consistent pattern of dilution or concentration for the six metals tested. Composite samples taken on from the cured compost pile were fairly similar (Table 5). Concentrations of Cd, Cr, Pb, and Zn were much higher in cured compost than in parent sludge generated at the plant on the same dates (compare Table 4 and 5).

Intensive sampling as shown in Table 1 will continue through June 88 with monthly sampling through August 89 (see Research Timetable).

Research Timetable:

March 88-May 88	-parent sludge and finished compost sampling and analysis (3 to 4 study bins/month)
June 88	-sampling and analysis and finished compost from study bins started in May
July 88	-data reduction and analysis
August 88	-final report preparation
June 88-August 89	-percent sludge and finished compost sampling and analysis (one study bin/month)

Table 1. Sample preparation summary for sludge, finished compost and compost mixture. Note: the sieve step is only required for finished compost and compost mixture.

DRY:	-dry sample at 103 °C for 24 hours
SIEVE:	-add twelve porcelain balls to sieve and shake for 10 min -collect fraction that passes a 2 mm screen
GRIND:	-grind compost to a fine powder with mortor and pestle -clean motor and pestle with a stiff brush between samples
REDRY:	-redry sample at 103 °C for 2 hours
WEIGH:	-weigh out 0.5 g samples into digestion tubes
DIGEST:	-add about 3 ml water and 6 ml conc. HNO_3 to tube -heat and reflux for 15 min -cool and bring to 50 ml mark with deionized water
FILTER:	-filter samples directly into clean polyethylene storage bottles with the four position Buchner funnel manifold -filter two samples through each Whatman GFC pad with between sample rinsing with 10% HNO_3
MEASURE:	-measure metals concentrations by atomic absorption against 10% HNO_3 mixed metal standards

Table 2. Sampling regime for sludge and compost at the Hoosac Water Quality District Wastewater Treatment Facility.

SAMPLE TYPE	SAMPLING REGIME	SAMPLE SIZE (ml)
Parent Sludge*	-four samples taken per day for the 3 to 4 days during sample bin filling, one replicate per sample	200
Finished Compost** Mixture	-four samples taken at halfway point and four at end point of study bin unloading, one replicate per sample	450
Stock Piled+ Mixture	-three replicate samples drawn from a 10 gal composite	450
Cured Compost+	-three composite samples	450

*three or four bins per month are designated for sampling

**bins are unloaded about 30 days after start up

+samples taken every two weeks

Table 3

DATA SUMMARY

BIN	DATE COLLECTED	TYPE	SAMPLE #	DIGEST #	DILUT. #	METALS CONCENTRATIONS (mg/kg dry wt.) Cd	Cr	Cu	Ni	Pb	Zn
A	01/21	S	638	138	794	42	75	425	167	59	276
A	01/21	S	639	454	768	40	73	404	159	56	286
A	01/21	S	640	413	746	41	74	415	148	67	258
A	01/21	S	635	114	785	36	69	404	155	50	236
A	01/21	S	636	427	757	42	77	428	161	61	272
A	01/21	S	637	414	765	42	76	419	165	67	267
A	01/21	S	632	63	780	40	75	404	159	56	262
A	01/21	S	633	470	725	41	74	415	157	57	261
A	01/21	S	634	445	766	40	74	392	149	57	260
A	01/22	S	665	97	739	42	96	484	167	67	273
A	01/22	S	664	411	746	41	98	492	166	68	269
A	01/22	S	663	459	790	41	98	465	164	52	255
A	01/22	S	662	112	772	40	96	469	162	59	269
A	01/25	S	661	448	741	45	98	467	250	57	268
A	01/25	S	660	435	725	46	92	481	254	62	262
A	01/25	S	659	419	726	46	93	462	269	55	254
A	01/25	S	658	417	755	48	92	498	255	58	267
A	03/01	C	897	100	732	47	94	453	162	66	287
A	03/01	C	898	83	758	49	98	473	171	68	298
A	03/01	C	899	102	740	46	91	413	156	71	284
A	03/01	C	900	67	765	53	107	444	180	86	336
A	03/01	C	893	149	774	43	90	434	168	72	312
A	03/01	C	894	444	744	47	93	440	166	67	283
A	03/01	C	895	454	759	48	94	437	161	74	297
A	03/01	C	896	412	760	45	98	439	165	78	307

BIN	DATE COLLECTED	TYPE	SAMPLE #	DIGEST #	DILUT. #	METALS CONCENTRATIONS (mg/kg dry wt.) Cd	Cr	Cu	Ni	Pb	Zn
B	01/27	S	654	432	778	45	92	461	235	48	261
B	01/27	S	655	92	767	47	88	488	239	57	255
B	01/27	S	656	423	733	47	90	483	242	59	265
B	01/27	S	657	118	777	47	85	498	246	58	268
B	01/28	S	653	453	756	44	87	474	228	57	273
B	01/28	S	652	446	793	43	97	484	222	60	256
B	01/28	S	651	119	730	45	92	499	233	54	274
B	01/29	S	650	473	748	42	95	477	213	71	317
B	01/29	S	649	436	731	42	92	465	208	53	261
B	01/29	S	648	458	751	41	90	478	212	50	255
B	02/01	S	645	447	766	40	84	499	246	71	301
B	02/01	S	644	455	783	40	89	503	249	68	274
B	02/01	S	647	466	781	39	91	493	249	88	275
B	02/01	S	646	89	785	41	105	533	266	70	292

Table 3. cont.

BIN	DATE COLLECTED	TYPE	SAMPLE #	DIGEST #	DILUT. #	Cd	Cr	Cu	Ni	Pb	Zn
						METALS CONCENTRATIONS (mg/kg dry wt.)					
C	02/09	S	681	408	757	31	79	472	218	53	246
C	02/09	S	641	120	789	33	84	479	217	62	602
C	02/09	S	642	422	749	32	81	501	215	63	289
C	02/09	S	643	131	786	32	73	514	223	55	266
C	02/10	S	677	464	738	33	74	587	230	61	301
C	02/10	S	680	456	788	33	68	540	208	59	264
C	02/10	S	679	93	727	33	74	538	214	68	264
C	02/10	S	678	154	747	33	69	558	214	59	269
C	02/11	S	676	114	750	33	71	533	214	56	264
C	02/11	S	675	406	764	34	73	546	208	61	268
C	02/11	S	674	440	743	32	78	523	211	57	258
C	02/11	S	673	65	763	34	69	557	214	58	284

BIN	DATE COLLECTED	TYPE	SAMPLE #	DIGEST #	DILUT. #	Cd	Cr	Cu	Ni	Pb	Zn
						METALS CONCENTRATIONS (mg/kg dry wt.)					
D	02/19	S	672	404	773	27	74	534	203	69	274
D	02/19	S	671	461	780	28	76	546	215	61	285
D	02/19	S	670	150	776	27	77	535	212	62	268
D	02/22	S	669	401	784	27	70	530	275	64	282
D	02/23	S	1001	409	768	25	69	518	263	64	270
D	02/23	S	1002	115	737	24	75	518	258	69	261
D	02/23	S	1003	151	794	24	73	530	268	69	273
D	02/23	S	1004	451	771	28	75	519	261	67	268
D	02/24	S	1005	443	762	27	72	528	260	62	268
D	02/24	S	1006	472	736	29	78	541	262	66	276
D	02/24	S	1007	63	787	28	74	553	253	67	272
D	02/24	S	1008	424	796	29	76	538	254	63	281

Table 4.

BIN SUMMARY

BIN	SAMPLE TYPE	METALS CONCENTRATIONS (mg/kg dry wt.) Cd	Cr	Cu	Ni	Pb	Zn
A	S	43	90	459	196	60	265
	C	47	96	442	166	73	301
B	S	43	91	488	235	62	273
	C						
C	S	33	74	529	216	59	298
	C						
D	S	27	74	533	249	65	273
	C						

Table 5.

RANDOM SAMPLES SUMMARY

SAMPLE No.	DATE COLLECTED	SAMPLE TYPE	METALS CONCENTRATIONS (mg/kg dry wt.) Cd	Cr	Cu	Ni	Pb	Zn
374	01/21	CP	83	123	427	191	93	360
707	02/10	CP	95	165	508	196	98	371
280	03/01	CP	92	149	505	198	101	361
706	03/01	CP	102	152	540	207	109	375