ICI NEW ZEALAND LIMITED
CORPORATE RESEARCH LABORATORY
BIODETERIORATION SERVICE

REPORT NUMBER: CRL/3/88:7/7021

PRELIMINARY MICROBIOLOGICAL AND PHYSICO-CHEMICAL TESTING OF DIESEL FUEL SUBJECTED TO THE "DEBUG" FUEL FILTER

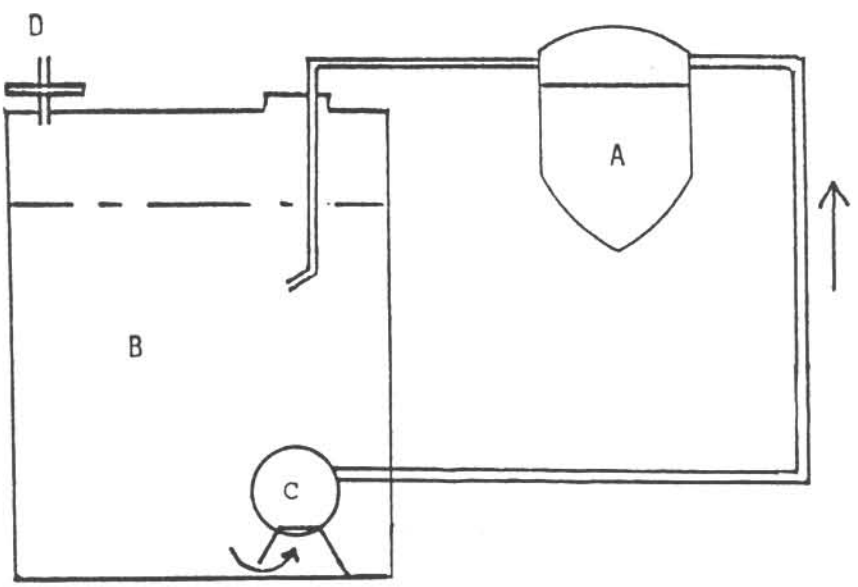
AUTHORS: G Hettige
D R Jacobson
R A Shaw

30 March 1988

ICI New Zealand Limited
CORPORATE RESEARCH LABORATORY
Seaview Road, Lower Hutt
Postal Address:
P.O. Box 30-275, Lower Hutt N.Z.
Telephone: (04) 684-324
Telex: NZ3568
Fax. (64-4) 729340
Telegrams: Impkemix Lower Hutt

FIGURE 1

DEBUG FILTER TEST RIG
SCHEMATIC DIAGRAM



A: DEBUG FILTER
B: 20L FUEL RESEVOIR
C: CIRCULATION PUMP
D: VENT FILTER

1. SUMMARY

A trial designed to test the effectiveness of the "De-Bug" Fuel Treatment Unit was undertaken by this laboratory.

Test and control fuel recirculation rigs were constructed, one with a magnetised unit, the other with an unmagnetised control. Diesel fuel with known contamination levels circulated separately through each rig. The duration of the trial was 15 days, and samples were removed from the rigs at regular intervals. These were tested for levels of fungal and bacterial contamination, turbidity, acid value and water content.

Microbiological results indicate that fuel subjected to the magnetised unit had a significantly lower fungal count than that treated in the control unit. Three different fungi were detected and measured, and all showed a rapid and dramatic decline in the magnetically-treated fuel. Fuel from the test rig remained effectively clear of fuel degrading fungi for the duration of the trial, except for two short surges of growth at 6 and 7 days. This pattern was significantly different to that of the control which had high levels of contamination for most of the trial period.

Physico-chemical testing detected no significant difference between the two units.

Further trials on fuel with very high levels of contamination are recommended so that the ability of the De-Bug to deal with such levels and its overall capacity can be determined.

2. INTRODUCTION

Various in-line filter devices based on the possible biocidal effects of magnetic fields have been developed for water treatment (e.g. "Algarid", "Hydromag"). Such devices appear to limit the growth of algae in potable water supplies, for example. There is conflicting literature evidence on the possible biocidal effects of magnetic fields on micro-organisms. However, recent work has shown that magnetic fields can affect the viability of bacteria (1).

De-Bug Filters Limited (P O Box 38100, PETONE, New Zealand) have designed and constructed a device, the "De-Bug" Fuel Treatment Unit (Patent Application #218331), based on magnetic principles, for the treatment of fuel oil, in particular, diesel.

Evidence from their own in-use trials, and from users of the De-Bug, suggests that the device can have a dramatic effect on the quality of fuel passed through it. For example, badly contaminated fuel, with much suspended insoluble material, has been shown to become clear within 48 hours of installation of the De-Bug unit on a truck fuel system. This has resulted in improved engine performance and longer conventional filter life, etc. (2)

As it has also been shown that the De-Bug does not become clogged, it is suggested that the device in some way causes the disruption of the suspended solids, in particular the fungal mycelia, to such an extent that the debris can freely pass through the normal fuel filter elements and be burned in the engine. Once the initial insoluble impurities have thus been removed, the De-Bug continues to control contamination introduced with subsequent refuelling of the vehicle, presumably for the life of the permanent magnets installed in the device (2).

Clearly, if the De-Bug device is as effective as it is claimed to be, this represents a considerable advance in fuel treatment technology, as well as providing more evidence to support theories of the effects of magnetic fields on biological systems.

However, previous efforts to support the claimed effectiveness of the De-Bug with results from properly conducted scientifically-based trials have proved inconclusive (3).

The Trial described in this report was undertaken as a confidential contract to De-Bug Filters Ltd, and was a preliminary attempt to demonstrate the efficacy of the device by running two identical test rigs simultaneously on identically contaminated fuel. One rig was "active" (magnetised), the other inactive, and the recirculated fuel was monitored for its microbiological and physico-chemical status.

This trial had a duration sufficient to demonstrate significant changes in the fuel, particularly those of a biological origin.

3. MATERIALS AND EQUIPMENT

3.1 Test Rigs

Two identical test rigs were constructed as shown in Figure 1. Rig #2 was a conventional De-Bug unit containing fully magnetised discs, while Rig #1 was identical in design but contained unmagnetised discs. This ensured that the same flow patterns and retention times occurred in both the test and control rigs.

Immersion pumps were Tecumseh "Little Giant" Model 1-7PW and flow rates around the system were adjusted to 1000ml/min (950-1050ml/min). This rate is based on typical fuel usage rates on a heavy diesel truck. The water-bath was maintained at 30°C, a typical truck fuel tank operating temperature (assuming recirculation of fuel back to the tank from the fuel injectors).

3.2 Fuel

Diesel fuel, stated to be biocide-free, was obtained from Shell New Zealand Ltd. This was dosed at the rate of about 3%v/v with a heavily contaminated fuel obtained from De-Bug Filters Ltd. The level of microbiological contamination and water content of this blend were thoroughly checked in this laboratory before use.

This blending was done in order to obtain a fuel of known levels of contamination in the "high-normal" range (see 4.1, 6.1 and Table 1).

4. EXPERIMENTAL METHODS

4.1 Fuel Preparation

A fuel bend, as described in 3.2, was held at room temperature for 7 days with occasional mixing. This ensured that the microbiological population reached equilibrium. Regular colony counts were done to check this (see 4.4).

4.2 Rig Preparation

Rigs were set up as described in 3.1 above. 10-15litres of sterile filtered diesel fuel (biocide-free) was loaded into each unit and pump-circulated with thorough mixing at 35-40°C for about 60minutes and then pumped to waste. A further 5-10 litres of the same fuel was then added to each reservoir and pumped through the De-Bug units directly to waste. This was done to ensure removal of grease and other contaminants prior to loading with test fuel of known composition.

20litres of test fuel prepared as in 4.1 was then added to each unit under reasonably aseptic conditions. The pumps were then started and the water-bath temperature raised to operating level (30°C). The temperature of the was monitored and shown to rise to the same level within 60minutes of start-up.

4.3 Rig Running and Sampling

Rigs were run continuously during the day from 0700 to 1700hours, being started and stopped with automatic timers. These timers also switched off the water bath, so that the temperature of the fuel in the reservoirs fell back from 30°C to ambient (15-20°C) overnight. This was done to simulate the overnight stop-start conditions of a truck, and allow the normal cyclic heating and cooling of the fuel and the associated condensation of water.

Flow rates were checked at least twice a day to ensure constant running conditions (flow rate set at 950-1050ml/min), and temperatures of the rigs and the water bath were also monitored.

Fuel samples were removed from both rigs to a predetermined timetable as shown in Table 1 and 2. Samples were collected aseptically from the fuel return lines in dry sterile glass bottles, 100ml being taken for microbiological testing (see 4.4) and 50ml for physico-chemical tests (see 4.5).

Further samples were taken at the end of each days running from the bottom of the De-Bug housing. This was done to check for the presence of separated water, and colony counts and water determinations were also done on this sample as described above. (These samples are referred to as "Filter Drainings" later in this report).

All residues of fuel samples were discarded after use, and not returned to the rigs. The fuel removed from the rigs as samples was not replaced, so the fuel level in the reservoirs progressively fell during the trial.

4.4 Microbiological Testing

4.4.1 Fungi and Yeasts

Duplicate volumes of fuel (50ml) were vacuum filtered aseptically on sterile 0.45micron membrane filters (Millipore type HAWG, 47mm). The filters were then placed in sterile plastic Petrie dishes and sterile molten malt-extract agar poured over them. The agar was allowed to set and the dishes incubated at 25°C for at least 5 days. The colonies were then counted and the results recorded as colony-forming units (cfu) per litre of fuel filtered. With the method used for this trial, the maximum countable level for fungi was 1000cfu/litre.

Types of fungi and yeast were determined microscopically.

4.4.2 Bacteria

Essentially the same method was used as in 4.4.1 except that a sterile 0.22micron filter was used and medium was nutrient agar. Incubation was at 25°C for 2 days.

4.5 Physico-chemical Testing

4.5.1 Turbidity

Fuel turbidity was determined on the freshly-taken samples by direct measurement in a Hach Turbidimeter (Model Ratio/XR). Results were recorded as NTU (nephelometric turbidity units).

4.5.2 Optical Density

Visible range optical absorbance (density) of the fuel samples was measured in a Varian Spectrophotometer (Model DMS 100) at a range of wavelengths (450, 500, 550nm) in 1cm cells against air reference. This was done as a further means of detecting changes in fuel turbidity, and was recorded at Optical Absorbance units as specified wavelengths.

Both Turbidity and Absorbance were measured as means of detecting changes in suspended solids levels in the fuel, especially that associated with microbiological growth. Neither method is capable of detecting very low levels of growth, nor discriminating between microbial growth and "cloudiness" or fuel haze caused by other means, e.g. water droplets suspended in the fuel, but served as a rapid non-destructive backup to the microbiological testing.

4.5.3 Acid value

Fuel samples were titrated with 0.02M.KOH (in iso-propyl alcohol) against phenolphthalein indicator to determine the acid value of the fuel. This was recorded as mg KOH/g fuel. Increases in fuel acidity, and hence acid value, often result from microbiological activity in fuels.

4.5.5 Water content

Fuel water content was measured by a standard Karl-Fischer method, and reported as ppm water in the fuel (ug water/g fuel). Filter draining samples were checked both by Karl-Fischer and by direct observation for separated water.

5. RESULTS

5.1 Microbiological

All Microbiolgical counts are listed in Table 1. It should be remembered that the maximum countable level is 1000cfu/litre. Thus, "1000cfu" could in fact be significantly higher, e.g. 5000cfu.

5.1.1 Fungi and yeast

Results from Table 1 are summarised in Graphs 1a (line graph) and 1b (bar graph). (No yeasts were detected.).

5.1.2 Bacteria

No attempt has been made to plot the results graphically as the numbers of bacteria fell away very rapidly in both test and control rigs.

5.2 Physico-Chemical

All relevant results are listed in Table 2.

5.2.1 Turbidity

Turbidity results are summarised in Graph 2.

5.2.2 Optical density

Optical density results (500nm only) are summarised in Graph 3.

5.2.3 Acid value

See Graph 5.

5.2.4 Water content

These results are depicted in Graphs 4a (normal samples) and 4b (filter drainings).

DISCUSSION AND CONCLUSIONS

6.1 Test fuel and its Microflora

The fuel used in this trial was prepared by taking normal, biocide-free diesel and dosing this with a badly contaminated fuel (supplied by De-Bug Filters). The resultant blend had a fungal count of about 1000cfu/litre and a similar count for bacteria.

The relative levels of the three main fuel-contaminating fungi, Hormoconis resinae, Paecilomyces variotii and Penicillium spp., are shown in Table 1 and are typical of a highly contaminated diesel fuel. All of these fungi have been shown to be capable of causing major problems in diesel fuel systems (4).

Pseudomonas aeruginosa was present initially at 1000cfu/l in the fuel used for the trial. This species of bacteria, whilst being a very general environmental and fuel contaminant, has not been reported to be capable of fuel degradation. Furthermore, it is very small (about 0.3microns) as compared to fungi, and would therefore be expected to pass the normal vehicle fuel filters.

It would have been preferable to have used a "naturally" contaminated fuel in the high-normal range, but it was not possible to locate such a fuel for the purposes of this trial. Fuels tested were either grossly contaminated or relatively clean. However, every effort was made to ensure that the blend finally arrived at was representative of low quality fuels, with regard to both microbiological and free water load. Furthermore, the blend was held for some days before loading into the rigs to allow and ensure equilibration of the populations in the fuel.

The methods used in this trial for counting the organisms were developed specifically for use in fuels and have been used successfully on a number of occasions in both diesel and naval bunker fuels (4, 5).

6.2 Test Rigs and Running Conditions

The test rigs were designed to simulate the conditions on a diesel truck. Hence the reservoirs were of mild steel construction, and connections were of brass. Connecting lines were of fuel-resistant plastic. The flow rate and operating temperature were typical of a truck fuel system in use.

It was decided to run the trial in a "stop-start" fashion, once more to mimic in-use conditions. As water plays a very important part in the survival and growth of micro-organisms in fuel, the cycling of the temperatures between 30°C and ambient (about 15°C) ensured that the water followed the same patterns of availability to the organisms in this trial as it might in a real situation. (see 6.5).

The pumps used performed very well and the pumping rate was constant throughout the trial. However, the recirculation rate (about 1000ml/min) was not sufficient to prevent water separating out and collecting at the bottom of the tanks and not being actively remixed with the circulating fuel. This could explain the progressive decline in water content in both rigs (see 6.5).

6.3 Microbiolgical Test Results

As may be seen from Table 1 and Graphs 1a and 1b a marked difference developed between the fungal contamination levels in the two units. This was particularly marked during the first 80hours of the trial.

The level of fungal contamination in the fuel in the magnetised unit (#2) dropped very rapidly within the first few hours of the trial and remained below 100cfu/l (a very low level) up to 15days, with the exception of two brief "spikes" or surges of contamination evident on days 6 and 7. The fuel in the control (unmagnetised) rig (#1) remained heavily contaminated for the first 80hours of the trial, more than 1000cfu/l, but then showed a more erratic pattern of contamination with periodic growth surges. These surges were more prolonged and substantial than those seen in the fuel in the magnetised rig (see Table 1 and Graphs 1a and 1b).

Intermittent growth surges are common in fuel systems, and may be explained by the break-up of clusters of spores and the likelihood of these being collected in the fuel sample.

It may be concluded from these results that the magnetised De-Bug device not only caused a dramatic reduction in the numbers of viable organisms in the fuel, but also controlled the sporadic re-occurrence of such contamination.

The fact that the contamination level in the fuel in the unmagnetised rig fell dramatically after about 80hours may be explained by the relatively poor agitation of the fuel reservoir and the progressive decline in the available water in the fuel (see 6.5).

The water content of the fuel may have fallen to below the level necessary to sustain such a high number of fungi. This could also explain the short duration of the growth surges: nonetheless, these were still less significant for the magnetised unit.

H.resinae was absent from the magnetised rig whereas it occurred infrequently in the control fuel system. P.variotii showed the highest count during the first 96hours, followed by Penicillium spp. which remained relatively high in subsequent samples from the unmagnetised rig up to Day 15.

As the fuel system contained three different fungi (all known common fuel-degrading organisms), interactions are inevitable, and cyclic growth patterns and surges can readily result.

Bacterial counts dropped rapidly in both rigs in the early part of the trial. This is characteristic of fuel bacteria (6), and may also be related in part to the water level in the system. There is therefore no evidence from these results to indicate that the De-Bug is capable of removing bacterial contamination from diesel fuel.

6.4 Turbidity and Optical Density

There was no observable difference between the turbidity or OD results from either rig, and no general trends were apparent (see Table 2 and Graphs 2 and 3).

Any changes in the fungal population must have therefore been insufficient to be detected by either of these techniques. NTU and OD measurements are normally used on microbial populations far higher that those counted in this trial (e.g. 1×10^6/ml) or for visibly cloudy solutions or suspensions. As the counts here were low by these standards and the fuel was never visibly cloudy, it is not surprising that these methods did not detect dramatic changes.

However, these methods have the advantage of speed and simplicity and are non-destructive and hence should still be considered for further trials where higher levels of suspended solids and /or microbial contamination are anticipated.

6.5 Water Content

The water level established initially in the bulk fuel sample was about 500ppm. This is regarded as a high level, and quite adequate to support fungal growth. However, as may be seen from Table 2 and Graph 4, the water content in both rigs fell to about half the initial level within the first 2 days, and then settled to about 200ppm.

As discussed in 6.2, the "stop-start" running of the rig probably resulted in water separating out from the fuel overnight as the temperature fell. Furthermore, the relatively inefficient agitation of the fuel in the tanks caused the water to remain as a separate phase after its initial separation. Alternatively, the water could have ben immobilised by reaction with the fuel container to form rust.

The fuel removed as filter drainings contained no visible free water, and the water content of these samples and their other characteristics (turbidity, fungal count, etc) confirmed it to be identical with the rest of the fuel in the system.

This suggests that the De-Bug unit was not removing water from the fuel, and therefore is not affecting the microflora by altering the balance between dissolved and micro-dispersed water in the system.

However, it is difficult to be categorical in this area without further trials with higher water levels and/or better agitation.

6.6 Acid Values

Table 2 and Graph 5 show very slight difference between the AV of the two rigs: the acid value for the magnetised rig was generally about 10% lower than the unmagnetised unit.

Both rigs gave very low acid values, and whether the small differences detected between the two is significant remains a matter of conjecture. A higher initial fuel water content or a longer trial duration may produce a more significant result.

7. GENERAL

Examination of the interiors of both units at the end of the trial showed no evidence of accumulated debris on the magnets or within the filter bodies. However, with the levels of fungi used in this trial it is unlikely that any residue would have been visible.

It has been shown that low intensity magnetic fields can affect biological systems, particularly when such fields are oscillating at relatively low frequencies (1).

The effects can be either positive or negative, depending on the intensity of the applied field, and may affect the organisms at the moment of cellular division.

The De-Bug unit does not have an oscillating field in the strict sense of the word - fixed multipole permanent magnets are used. However, the organisms may in effect be subjected to such a field by being slowly pumped over a number of magnets in series.

No attempt was made in this trial to measure the field strength or the likely frequency of "oscillation" to which the organisms are subjected. Such a study could result in design simplification or an improvement in performance.

This trial should be regarded as a preliminary technical confirmation of the effectiveness of the De-Bug Fuel Treatment Unit.

Further testing should be carried out in order to validate the effectiveness of the De-Bug against very high levels of contamination.

8. RECOMMENDATIONS

It is strongly recommended that further trials be undertaken using fuel with very much higher levels of contamination, and with a longer time-base. Positive results from such trials would greatly enhance the preliminary findings in this report, particularly with regard to the capacity of the De-Bug unit.

REFERENCES

1. Aarholt, E., Flinn, E.A.; Smith, C.W.; Phys. Med. Biol. 26, 613-621 (1981): Effects of low frequency magnetic fields on bacterial growth rates.
2. De-Bug Filter Ltd; personal communication.
3. Grayson Laboratories; personal communication to De-Bug Filters Ltd.
4. Hazzard, G.F. 1963: Fungal growths in aviation fuel system Part 4. Fungi in aviation fuel systems in Australia and the far East. Defence Standard Laboratory (Australia) Report 252, 52pp.
5. Hettige, G. and Sheridan, J.E. 1984: Mycoflora of stored diesel fuel in New Zealand. International Biodeterioration Bulletin 20 (4), 225-228.
6. Fass, R. and Miller, G. (1980): Microbiological growth in storage of jet fuel and diesel fuel. Summary of report for May 1979-April 1986. Israel Institute for Biological Research, October.

TABLE 1

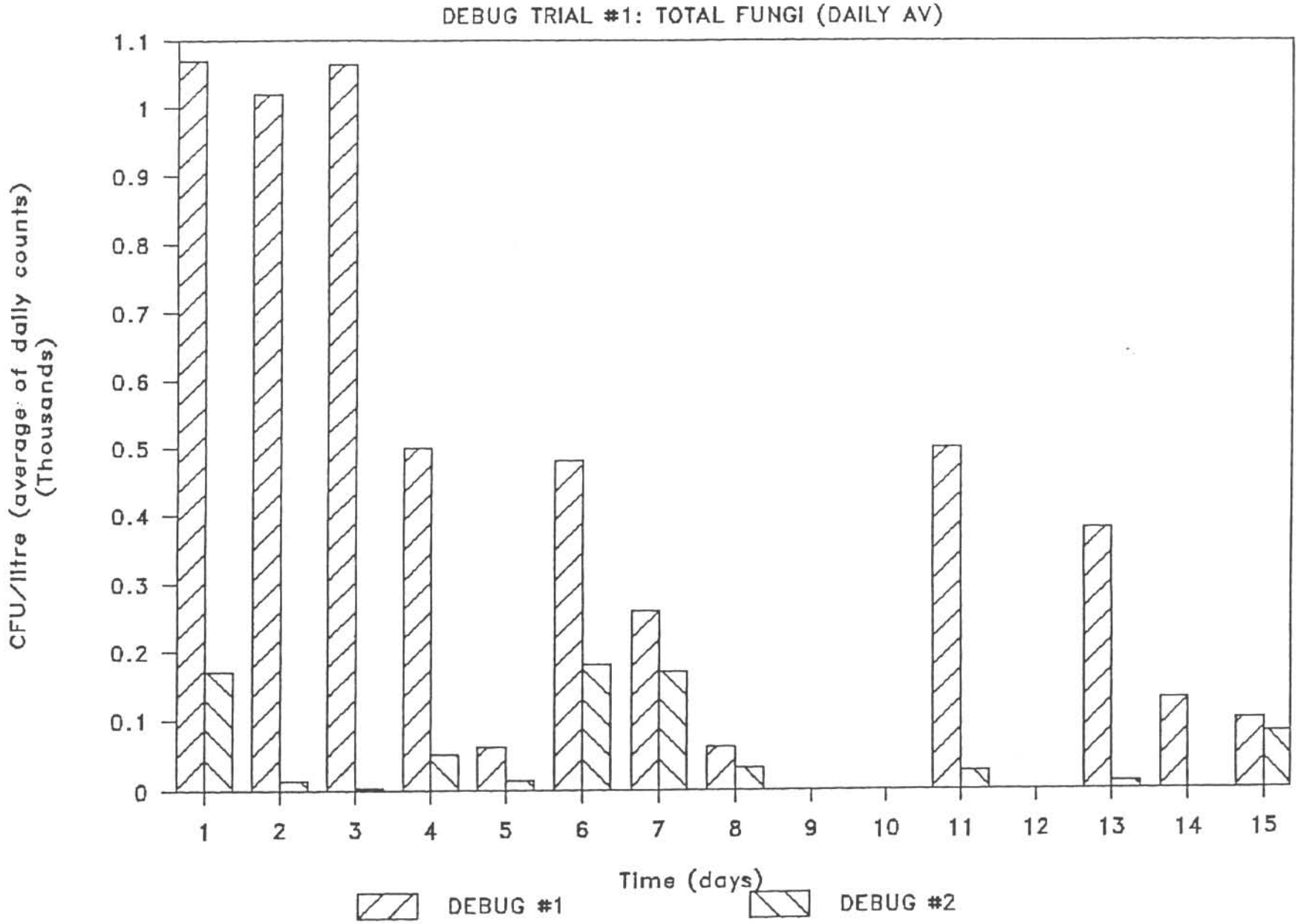
DEBUG TRIAL #1: MICROBIOLOGICAL RESULTS.

SAMPLE			UNIT #1: UNMAGNETISED					UNIT #2: MAGNETISED				
			FUNGI (COLONY FORMING UNITS PER LITRE)				BACTERIA CFU/1	FUNGI (COLONY FORMING UNITS PER LITRE)				BACTERIA CFU/1
#	DAY	TIME (HRS)	HORMOCORNIS RESINAE	PAECILOMYCES VARIOTII	PENICILLIUM spp	TOTAL FUNGI	PSEUDOMONAS AERUGINOSA	HORMOCORNIS RESINAE	PAECILOMYCES VARIOTII	PENICILLIUM spp	TOTAL FUNGI	PSEUDOMONAS AERUGINOSA
	0		430	930	550	1910	1000					
1	1	1.0	20	1000	105	1125	0	0	100	140	240	0
2	1	3.0	0	1000	0	1000	0	0	150	0	150	26
3	1	5.0	0	1000	95	1095	100	0	100	2	102	0
4	1	7.0	38	1000	0	1038	0	0	0	160	160	0
5	1	9.0	0	1000	78	1078	50	0	90	100	190	70
7	2	26.5	0	1000	30	1030	0	0	0	10	10	0
8	2	29.5	25	1000	0	1025	0	0	0	20	20	0
9	2	32.5	0	1000	15	1015	0	0	0	10	10	0
11	3	50.5	100	1000	0	1100	0	0	0	0	0	0
12	3	53.5	0	1000	0	1000	0	0	0	0	0	0
13	3	56.5	58	1000	35	1093	0	0	0	10	10	0
15	4	74.5	0	1000	0	1000	3	0	0	60	60	0
16	4	77.5	0	400	5	405	1	0	0	80	80	0
17	4	80.5	0	0	80	80	0	0	0	0	0	0
19	5	98.5	0	0	84	84	0	0	0	0	0	20
20	5	101.5	0	0	29	29	1	0	0	20	20	0
21	5	104.5	0	0	55	55	0	0	0	20	20	0
23	6	122.5	0	0	781	781	1	0	0	0	0	0
24	6	125.5	0	0	657	657	0	0	0	540	540	0
25	6	128.5	0	0	0	0	0	0	0	0	0	0
27	7	146.5	0	0	568	568	0	0	0	0	0	0
28	7	149.5	0	0	100	100	0	0	0	500	500	0
29	7	152.5	0	100	0	100	0	0	0	0	0	0
31	8	172.0	0	0	60	60	0	0	0	30	30	0
33	9	196.0	0	0	0	0	0	0	0	0	0	0
35	10	220.0	0	0	0	0	0	0	0	0	0	0
37	11	244.0	0	0	500	500	0	0	0	25	25	0
39	12	268.0	0	0	0	0	60	0	0	0	0	0
41	13	292.0	0	0	380	380	0	0	0	10	10	0
43	14	316.0	0	0	130	130	0	0	0	0	0	0
45	15	340.0	0	0	100	100	0	0	0	80	80	0

TABLE 2.
DEBUG TRIAL #1: PHYSICO-CHEMICAL RESULTS.

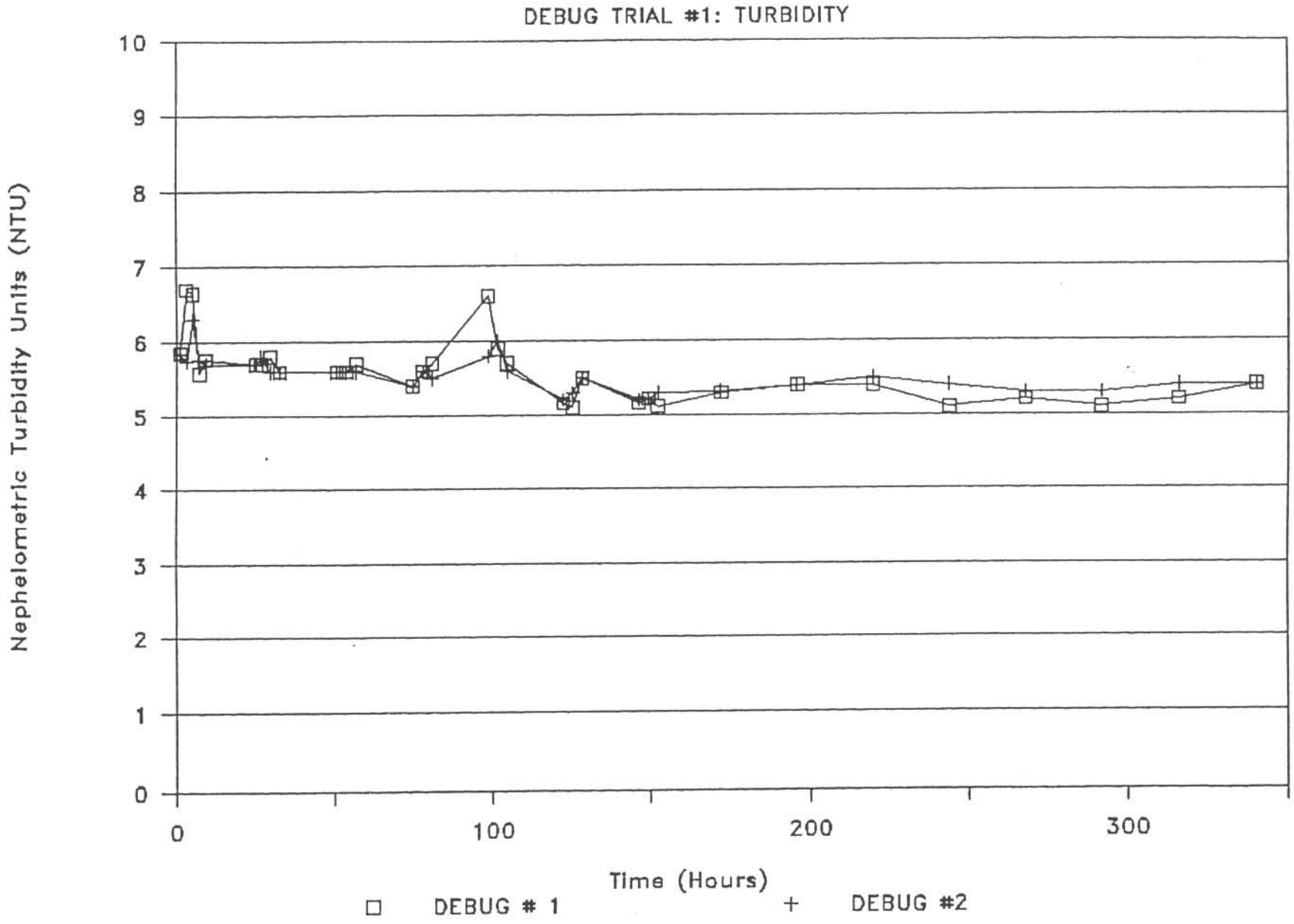
SAMPLE				UNIT #1: UNMAGNETISED						UNIT #2: MAGNETISED					
#	TIME	DAY	HOURS	ACID No	NTU	E450	E500	E550	WATERppm	ACID No	NTU	E450	E500	E550	WATERppm
			0.0	0.054	6.00	0.311	0.172	0.108	413	0.054	6.00	0.311	0.172	0.108	413
1	0900	1	1.0	0.052	5.86	0.310	0.172	0.105	356	0.054	5.85	0.312	0.174	0.109	257
2	1100		3.0	0.055	6.70	0.315	0.177	0.110	480	0.050	5.75	0.316	0.176	0.110	380
3	1300		5.0	0.055	6.65	0.320	0.184	0.120	442	0.049	6.30	0.314	0.178	0.113	433
4	1500		7.0	0.063	5.58	0.318	0.182	0.118	185	0.058	5.76	0.310	0.176	0.112	258
5	1700		9.0	0.059	5.76	0.313	0.174	0.110	303	0.052	5.70	0.311	0.177	0.113	303
6	0900	2	25.0	0.060	5.70	0.311	0.175	0.112	261	0.062	5.70	0.312	0.176	0.112	297
7	1030		26.5	0.057	5.70	0.309	0.175	0.112	282	0.059	5.80	0.309	0.174	0.111	198
8	1330		29.5	0.063	5.80	0.313	0.176	0.110	311	0.059	5.60	0.312	0.178	0.116	273
9	1630		32.5	0.062	5.60	0.317	0.183	0.120	227	0.064	5.60	0.313	0.180	0.118	236
11	1030	3	50.5	0.062	5.60	0.317	0.180	0.118	185	0.063	5.60	0.313	0.181	0.119	215
12	1330		53.5	0.059	5.60	0.315	0.182	0.120	135	0.062	5.60	0.315	0.182	0.120	126
13	1630		56.5	0.070	5.70	0.316	0.182	0.120	130	0.060	5.60	0.314	0.181	0.121	135
15	1030	4	74.5	0.058	5.40	0.321	0.184	0.119	135	0.062	5.40	0.315	0.179	0.116	126
16	1330		77.5	0.057	5.60	0.316	0.183	0.120	160	0.059	5.60	0.314	0.180	0.118	181
17	1630		80.5	0.057	5.70	0.317	0.182	0.120	135	0.059	5.50	0.315	0.182	0.121	198
19	1030	5	98.5	0.062	6.60	0.315	0.183	0.118	177	0.059	5.80	0.318	0.187	0.123	231
20	1330		101.5	0.060	5.90	0.317	0.183	0.119	194	0.062	6.00	0.319	0.183	0.122	206
21	1630		104.5	0.062	5.70	0.318	0.182	0.121	173	0.064	5.60	0.316	0.180	0.121	206
23	1030	6	122.5	0.066	5.15	0.369	0.206	0.132	202	0.064	5.20	0.362	0.204	0.132	206
24	1330		125.5	0.070	5.10	0.343	0.194	0.122	202	0.062	5.30	0.353	0.203	0.133	206
25	1630		128.5	0.072	5.50	0.335	0.195	0.126	233	0.062	5.50	0.343	0.201	0.133	231
27	1030	7	146.5	0.070	5.16	0.336	0.194	0.124	261	0.065	5.20	0.335	0.195	0.126	261
28	1330		149.5	0.065	5.22	0.336	0.193	0.125	227	0.062	5.21	0.336	0.194	0.124	240
29	1630		152.5	0.068	5.11	0.337	0.195	0.127	227	0.063	5.30	0.336	0.196	0.130	240
31	1200	8	172.0	0.070	5.30	0.325	0.190	0.125	244	0.064	5.32	0.320	0.190	0.123	168
33	1200	9	196.0	0.072	5.40	0.312	0.180	0.119	306	0.064	5.40	0.319	0.188	0.122	168
35	1200	10	220.0	0.068	5.40	0.318	0.185	0.122	194	0.063	5.50	0.318	0.185	0.124	177
37	1200	11	244.0	0.069	5.10	0.365	0.215	0.143	177	0.068	5.40	0.385	0.222	0.151	135
39	1200	12	268.0	0.068	5.20	0.377	0.224	0.153	181	0.066	5.30	0.357	0.206	0.138	164
41	1200	13	292.0	0.071	5.10	0.339	0.195	0.128	156	0.066	5.30	0.337	0.195	0.125	198
43	1200	14	316.0	0.070	5.20	0.342	0.199	0.131	248	0.064	5.40	0.365	0.212	0.145	139
45	1200	15	340.0	0.072	5.40	0.336	0.193	0.124	202	0.064	5.40	0.334	0.194	0.124	156

GRAPH 1b

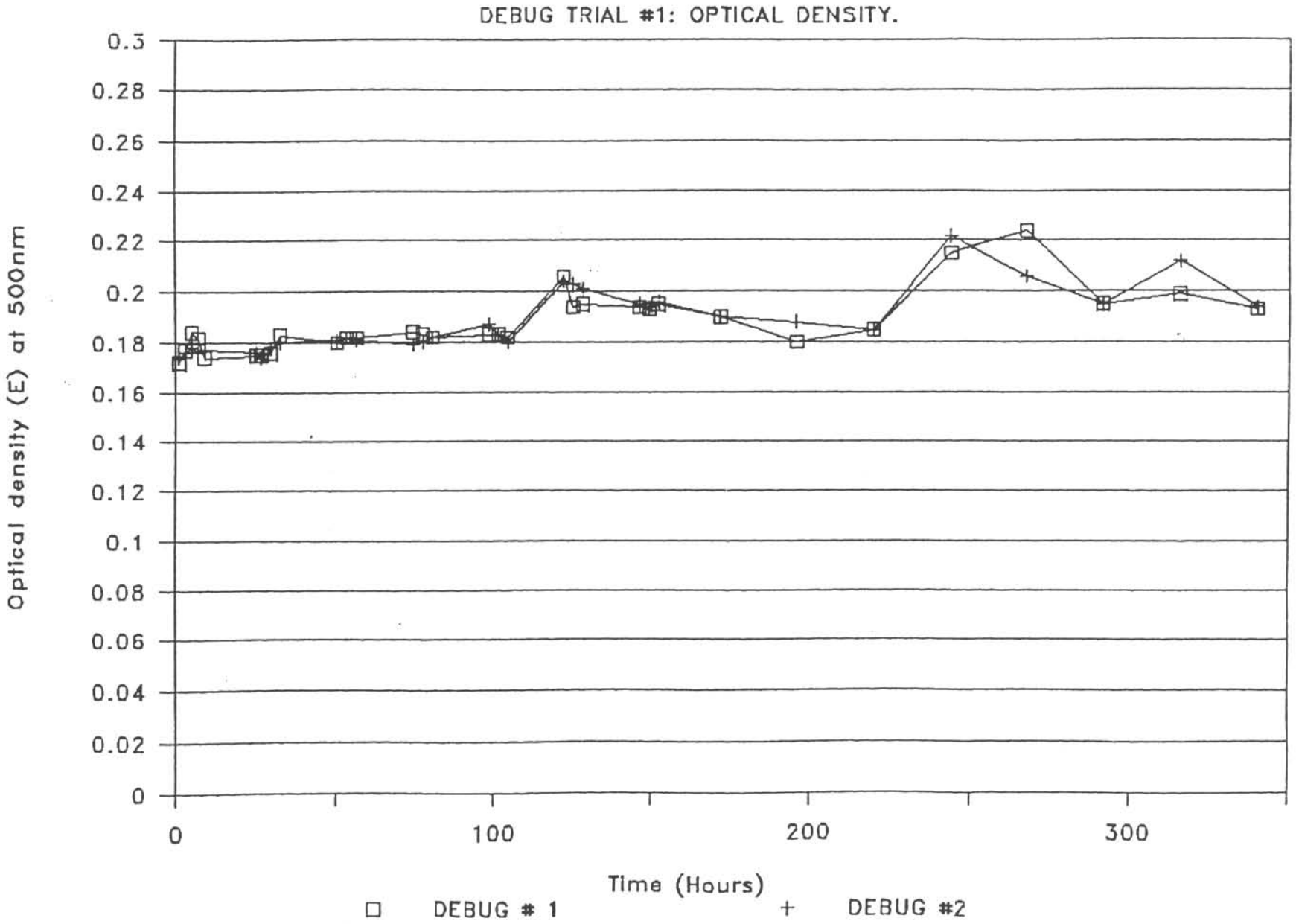


NOTE: Total fungal counts, from Table 1, are displayed here as the average of the counts for a given day.

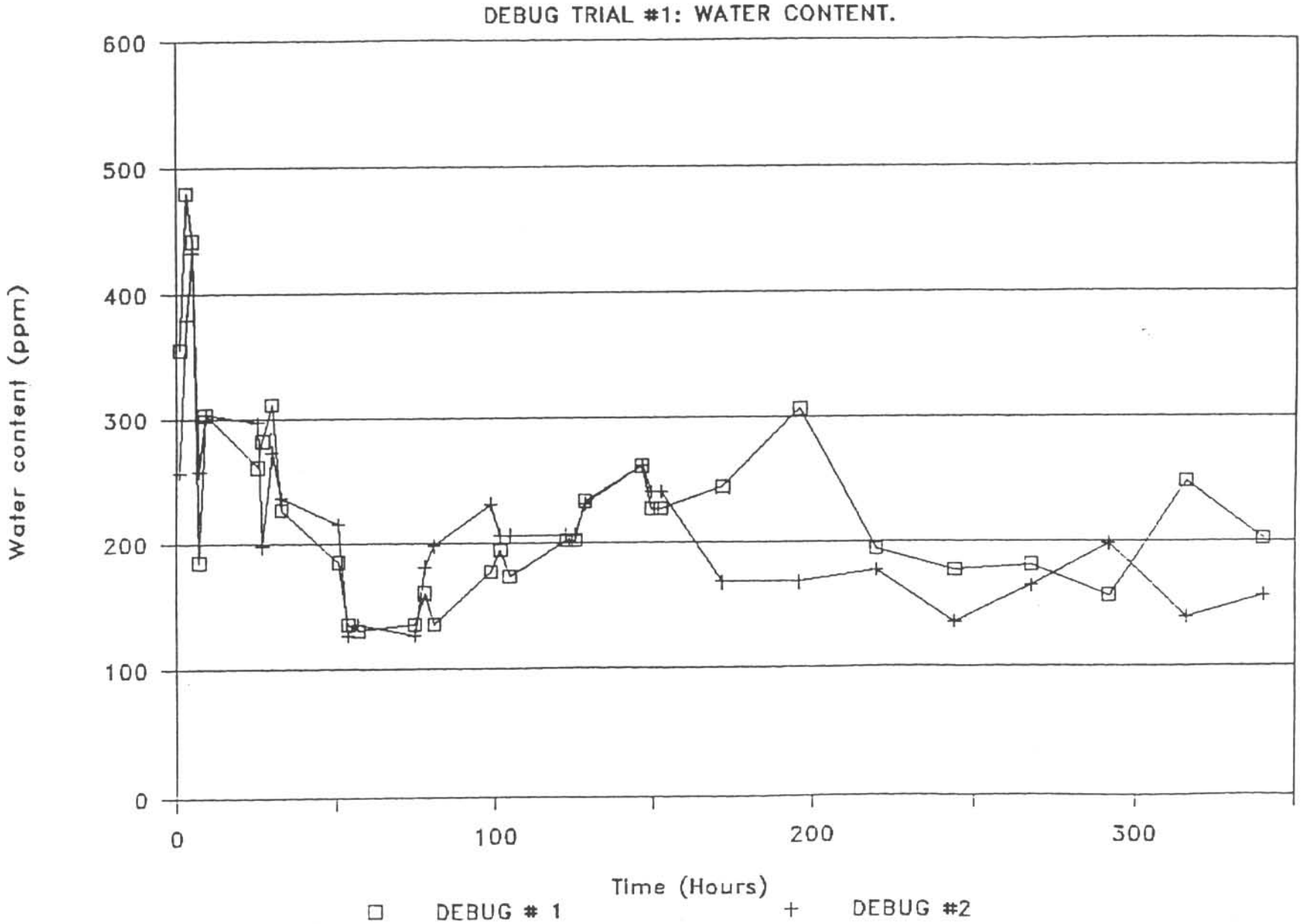
GRAPH 2.


DEBUG TRIAL #1: TURBIDITY
Nephelometric Turbidity Units (NTU)
10
9
8
7
6
5
4
3
2
1
0
0
100
200
300
Time (Hours)
□ DEBUG # 1
+ DEBUG #2

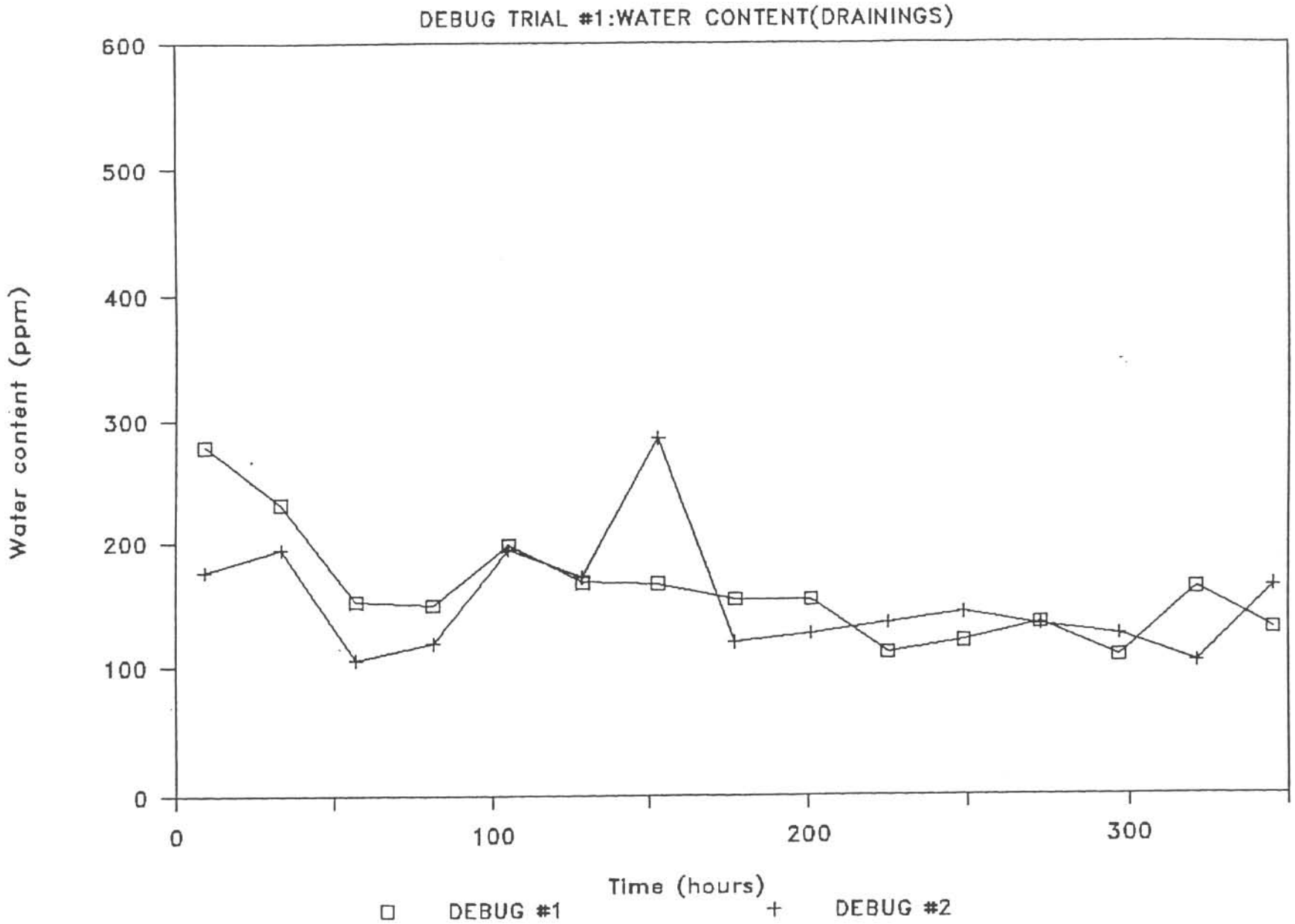
GRAPH 3.


DEBUG TRIAL #1: OPTICAL DENSITY.
Optical density (E) at 500nm
0.3
0.28
0.26
0.24
0.22
0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
0
100
200
300
Time (Hours)
□ DEBUG # 1
+ DEBUG #2

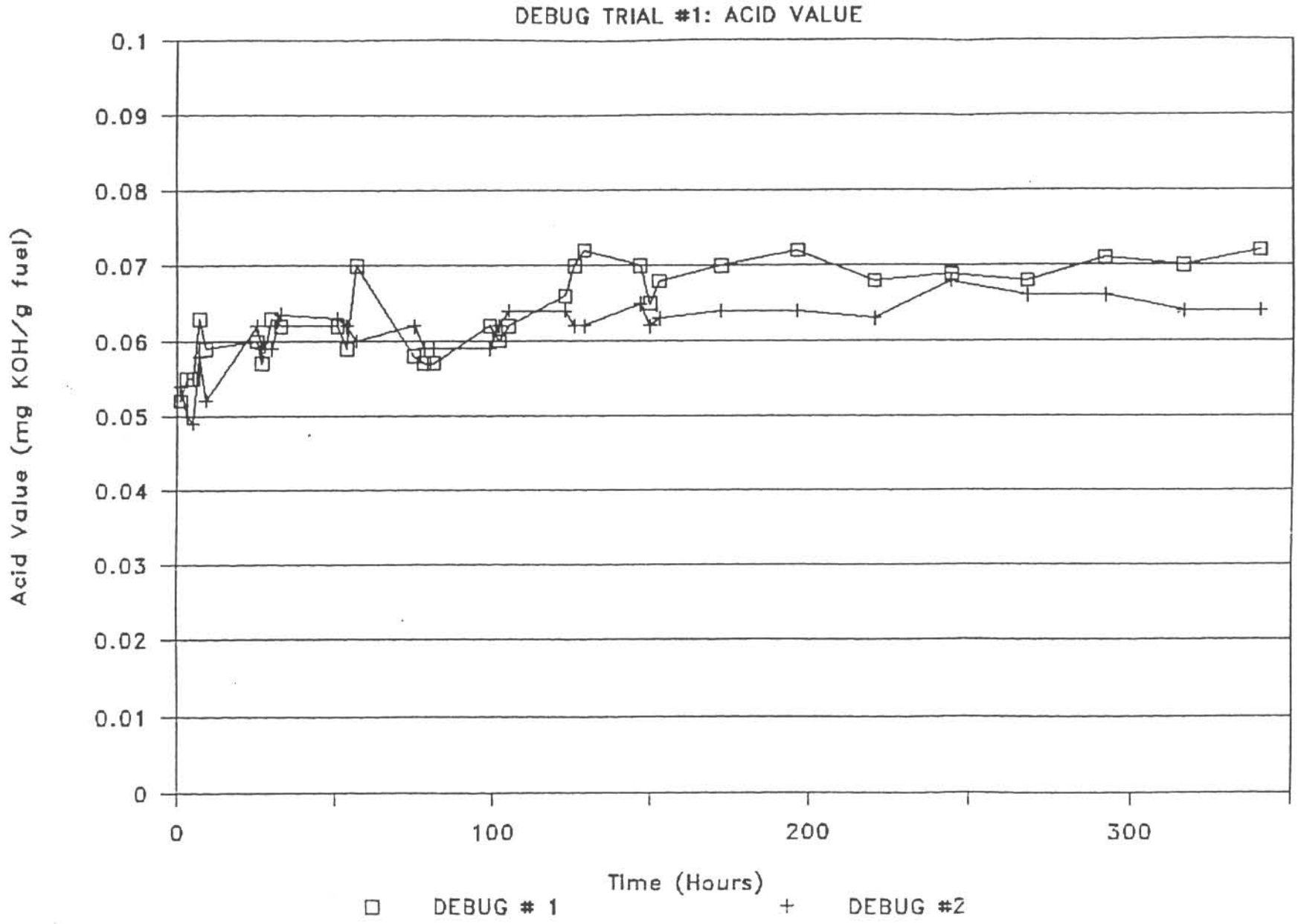
GRAPH 4a.


DEBUG TRIAL #1: WATER CONTENT.
600
500
400
300
200
100
0
Water content (ppm)
0
100
200
300
Time (Hours)
□ DEBUG # 1
+ DEBUG #2

GRAPH 4b.


DEBUG TRIAL #1:WATER CONTENT(DRAININGS)
600
500
400
300
200
100
0
Water content (ppm)
0
100
200
300
Time (hours)
DEBUG #1
DEBUG #2

GRAPH 5.


DEBUG TRIAL #1: ACID VALUE
Acid Value (mg KOH/g fuel)
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0
100
200
300
Time (Hours)
DEBUG # 1
DEBUG #2