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THE MICROBIOLOGY OF HEAVY WATER IN THE HIFAR REACTOR

by

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ABSTRACT

The high flux research reactor HIFAR contains ten tons of heavy water which acts as moderator and primary coolant. Over an eighteen months period regular microbiological examinations have been carried out on samples of heavy water taken from various parts of the circuit. The heavy water circuit provides an interesting opportunity for the study of microorganisms because of the high isotopic purity (greater than 99.6 per cent.), and the high chemical purity of the heavy water in the reactor. Furthermore, during its passage through the reactor core the water and suspended bacteria are subjected to intense irradiation, the neutron flux being approximately 10^{14} neutrons cm^{-2} sec^{-1}.

The presence of bacteria in the heavy water circuit has been demonstrated and experimental results and methods used are discussed. Some evidence is presented to show that the ion-exchange resin bed contributes nutrients to support bacterial growth.

CONTENTS

1. INTRODUCTION

In January 1958 after very careful cleaning and drying of the moderator circuit of the reactor HIFAR (Watson-Munro 1958), ten tons of high purity heavy water were loaded. The Research Establishment's Analytical Chemistry Section had to ensure that the purity of this heavy water was maintained. As part of this work it was decided to carry out a bacteriological examination of the heavy water over a period of some months. A preliminary examination showed that living bacteria were present in the heavy water circuit of HIFAR and a brief account was published (Davis and McPherson 1961). Only two other references to the presence of bacteria in reactor cooling circuits have appeared in the literature (Fowler et al. 1960 and Ophel 1960) and both were concerned with bacteria in light water circuits.

Although no serious problems attributable to microorganisms were envisaged it was appreciated that bacteria could possibly cause interference to the operation of the ion-exchange resin column in the reactor heavy water circuit. Such interference could occur either by microbial growth in the resin bed restricting water flow or by breaking down the resin beads and altering their action.

2. SAMPLING

It was difficult to devise a sampling method which would meet the requirements of the microbiologist, reactor engineer, and health physicist. The system finally adopted was to install a sealed Perspex box (Figure 1) surrounding the outlet point and having a three inch diameter port on the floor and one wall of the box. The sampling point chosen is on a bypass line from the weir overflow pipe and water circulated through this line returns to the overflow pipe and to the D_2O storage vessel for recirculation (Figure 2). The interior of the box is kept saturated with formalin vapour which is able to diffuse into the heavy water as far back as the valve (see Figure 1), that is, about 10 ml of heavy water will be contaminated with formalin. Heavy water samples were collected in sterile Pyrex test tubes of 50 ml capacity sealed with ground glass stoppers. Whenever heavy water was sampled, four of these tubes were filled and the bacteriological examination was carried out on the fourth.

To ensure that the sample used for bacteriological examination would be free of formalin four tubes were filled and the heavy water was examined polarographically. The first tube contained 100 : 10 p.p.m. and tubes 2, 3, and 4 less than 0.1 p.p.m. of formalin.

Ideally, the bypass line should have had heavy water circulating through it continuously but this was not possible until September 1960. In the earlier work the line was left flushing at the rate of 50-150 ml per minute until temperature rise indicated that the static water in the bypass had been flushed away.

3. EXPERIMENTAL METHODS

The microbial content of the HIFAR heavy water circuit was at first measured by the most probable number technique but later 1 ml aliquots of the heavy water were used to obtain the total viable count. Unless otherwise stated the results were obtained using Difco nutrient agar reconstituted in demineralised light water and incubated at 37 °C for forty eight hours.

Using standard techniques, attempts were made to isolate thermophilic microorganisms, anaerobes, yeasts, and fungi. Attempts were made, also, to isolate sulphate-reducing bacteria using media recommended by the Society of American Bacteriologists (1957).

Dehydrated media, reconstituted in heavy water were also used at times in attempts to isolate any mutants requiring deuterium. In these experiments we used the Lederberg replica plating technique (Lederberg and Lederberg, 1952).

4. RESULTS

In Figure 3 the number of organisms per ml is plotted against reactor power level (in megawatt days/day); exact figures are given in Table 1. The temperature of the heavy water in the reactor aluminium tank (R.A.T.) approximately 40 °C. These bacterial numbers are only to be taken as an indication of the bacterial content of the heavy water circulating through the reactor because the water samples are taken from a by-pass line which is not continuously flushed. It is interesting to note that at the time this work was carried out the tritium content of the heavy water was 0.8 c/l and the organisms encountered tolerated this level of activity well. The graph (Figure 3) indicates that a rise in bacterial numbers occurred some days after the commencement of each power run.

During the October/November 1960 power run we were able to circulate heavy water through the by-pass line continuously, thus enabling us to measure more directly the bacterial population of the water leaving the R.A.T. during a power run. Prior to the October 1960 start-up, which followed an extended shutdown of five weeks, the bacterial count was 390 organisms/ml and this figure dropped rapidly as the reactor was raised to a power level of 10 megawatts. However, organisms could still be isolated at the rate of 0-3 organisms/ml throughout the run, though it is likely that these latter isolations were of organisms which had passed through the R.A.T. receiving only minimal irradiation or, possibly, which had been held by constrictions in the weir overflow pipe-line. The former explanation is feasible because of the turbulence within the R.A.T. The dose rates encountered in the R.A.T. are given in Appendix I.

During the same power run tests were made at the inlet and outlet points to the ion-exchange bed. Water passing over the weir runs into the D_2O storage vessel (Figure 2) and from there is pumped through the ion-exchange bed back to the main circulating pumps. The sampling points, though not ideal for bacteriological purposes, do enable a comparison to be drawn as conditions in both lines and valves are identical. The results of three separate determinations showed that the water entering the ion-exchange column contained 1000 organisms/ml and the water leaving contained 10,000 organisms/ml.

Before HIFAR first reached criticality on the twenty-fifth of January, 1958 microbiological examination of the heavy water revealed the presence of numerous species of bacteria, the most prolific being Pseudomonas aeruginosa. However after criticality was reached the only organisms isolated in any number were a non pigment producing Gram-negative rod. Further investigation led us to place this organism among the Achromobacter and it possesses most of the characters of the species liquefaciens, although it grows quite well at 37 °C. Other organisms isolated include a bacillus species, a Gram-positive coccus, and a Gram-negative rod which on culture produces a pale red-brown pigment. Attempts to isolate thermophiles, anaerobes, yeasts, fungi, and sulphate reducers were not successful. Mutants requiring deuterium were not encountered.

By July 1958 the variety of organisms had decreased and since that time heavy water samples and ion-exchange resin samples taken from HIFAR have always given rise to cultures containing only two organisms: Achromobacter and bacillus, the former predominating.

5. DISCUSSION

The above results show that some bacteria can survive and reproduce in a high purity heavy water environment without prior adaptation. Further, they do this in the somewhat unfavourable environment of a nuclear reactor heavy water circuit practically free of nutrients, deficient in oxygen, and intermittently exposed to high levels of radiation. That the most important site of bacterial growth is within the mixed bed ion-exchange column is borne out by the fact that ten times as many organisms leave the bed as enter it. The mixed-bed ion exchange contains 1 cu.ft of 1:1 Zeokarb 225 and Deacidite FF (Permutit Co.) in the D^+ and OD^- forms. The resin was placed in the circuit on 13th January, 1960. It is probable that the bacteria draw their carbon, nitrogen, and trace nutrient requirements from the resin. Appreciable resin breakdown takes place when ion exchange resins are irradiated (Nater 1958, 1959) resulting in the release of traces of soluble organic compounds containing nitrogen, sulphur, and other elements, depending on the type of resin and irradiation conditions.

6. CONCLUSIONS

We feel that in spite of the sampling difficulties, some conclusions may validly be drawn from the first group of results (Figure 3). The regular cycle which occurs and the impossibility of external contamination of the sampling outlet valve lead us to suggest that there is a cyclic build-up of organisms in the normally stagnant D_2O sampling line. The increase could reasonably be explained if we postulate that the high level of radioactivity within the R.A.T. results in the formation of new compounds in solution in the D_2O, capable of serving as nutrients.

7. ACKNOWLEDGMENTS

The perspex sampling box and apparatus for the Lederberg replica plating technique were constructed by Mr. F.R.Harrison. The photographs were taken by Mr. A.A.Dickes. The polarographic analyses were carried out by Mr. T.M.Florence.

7. REFERENCES

Davis, P.S., and McPherson, G.G., (1961). – Bacteria in Reactor D_2O. Nucleonics 19 (5): 6.

Fowler, E.B., Christenson, C.W., Jumey, E.J., and Schafer, W.D., (1960). – Bacterial "infection" of the Omega West Reactor. Nucleonics 18 (4): 102–105.

Lederberg, J., and Lederberg, E.M., (1952). – Replica plating and indirect selection of bacterial mutants. J. Bact. 63: 399–406.

Nater, K.A., (1958). – Proc. Conference on Peaceful Uses of Atomic Energy, Geneva. A/CONF. 15/P/1474.

Nater, K.A., (1959). – JENER Public. 17.

Ophel, I.L., (1960). – Reactor Antibiotics, Nucleonics 18 (10): 6–7.

Society of American Bacteriologists (1957). – "Manual of Microbiological Methods". (McGraw Hill: New York).

Watson-Munro, C.N., (1958). – Divergency data for HIFAR. Atomic Energy 1 (2): 3–8.

TABLE 1

Viable Counts of Heavy Water Samples Taken from HIFAR During the Period February 2 – May 26, 1960

Date	Organisms/ml	Reactor Power Level Megawatt days/day
2.2.1960	12	Shutdown
3	15	"
4	28	"
5	36	2.2
6	–	6.7
7	–	6.4
8	8	7.2
9	52	8.5
10	188	9.4
11	188	7.2
12	60	Shutdown
13	–	–
14	–	"
15	–	"
16	–	"
17	100	"
18	–	"
19	12	"
20	–	1.5
21	–	Shutdown
22	16	"
23	–	"
24	18	"
25	–	1.8
26	20	8.0
27	–	8.0
28	–	8.0
29	16	8.0
1.3.1960	–	8.0
2	300	8.0
3	–	8.0
4	90	8.0
5	–	8.0
6	–	5.4
7	60	Shutdown
8	–	"
9	36	"
10	–	"
11	22	"
12	–	2.7
13	–	6.0
14	25	6.0
15	–	5.3
16	432	8.5
17	–	9.0
18	18	9.0
19	–	9.0
20	–	9.0
21	10	7.5
22	–	10.0
23	28	9.0
24	–	9.0
25	32	9.0
26	–	10.0
27	–	4.5
28	18	10.0
29	–	9.5
30	24	6.0
31	–	10.0

Date	Organisms/ml	Reactor Power Level Megawatt days/day
1.4.1960	–	10.0
2	–	10.0
3	–	10.0
4	–	Shutdown
5	116	"
6	12	"
7	28	10.0
8	18	10.0
9	–	10.0
10	60	10.0
11	–	10.0
12	90	10.0
13	–	10.0
14	–	10.0
15	–	10.0
16	–	10.0
17	–	10.0
18	–	10.0
19	6	10.0
20	–	10.0
21	228	10.0
22	344	10.0
23	–	10.0
24	–	10.0
25	–	10.0
26	40	10.0
27	18	Shutdown
28	–	"
29	4	1.5
30	–	10.0
1.5.1960	–	10.0
2	6	10.0
3	–	10.0
4	12	10.0
5	–	10.0
6	76	10.0
7	–	10.0
8	–	10.0
9	108	10.0
10	–	10.0
11	28	10.0
12	–	7.5
13	38	2.2
14	–	10.0
15	–	10.0
16	–	4.5
17	44	Shutdown
18	–	"
19	25	"
20	–	4.6
21	–	4.0
22	–	10.0
23	128	10.0
24	–	10.0
25	80	10.0
26	30	10.0

APPENDIX I

AVERAGE NEUTRON FLUX AND γ DOSE RATE IN THE REACTOR ALUMINIUM TANK

The following neutron fluxes and γ dose rates were calculated by Dr. J.L. Symonds, Physics Division, A.A.E.C. They represent the average of these quantities over the whole volume of heavy water in R.A.T. No figures for the β contribution to the total dose rate are included since a rough calculation indicated that this contribution is small.

It is felt that the quoted average γ dose rate is of reasonable accuracy (probably better than ± 30%); because of the complicated flux patterns in the reactor the neutron flux figures given are probably, at best, reasonable estimates. The power level chosen was 1 megawatt; to obtain dose levels at other powers the dose may be assumed to be directly proportional to power.

Average γ –dose rate	=	9.4×10^6 r/hr
Average thermal neutron flux	=	4×10^{12} n cm^{-2} sec^{-1}
Average fast neutron fluxes:		
E < 0.5 MeV	=	2.3×10^{10} n cm^{-2} sec^{-1}
E 0.5–1 MeV	=	3×10^{10} "
E 1 – 2 MeV	=	5×10^{10} "
E 2 – 3 MeV	=	3×10^{10} "
E >3 MeV	=	3.5×10^{10} "

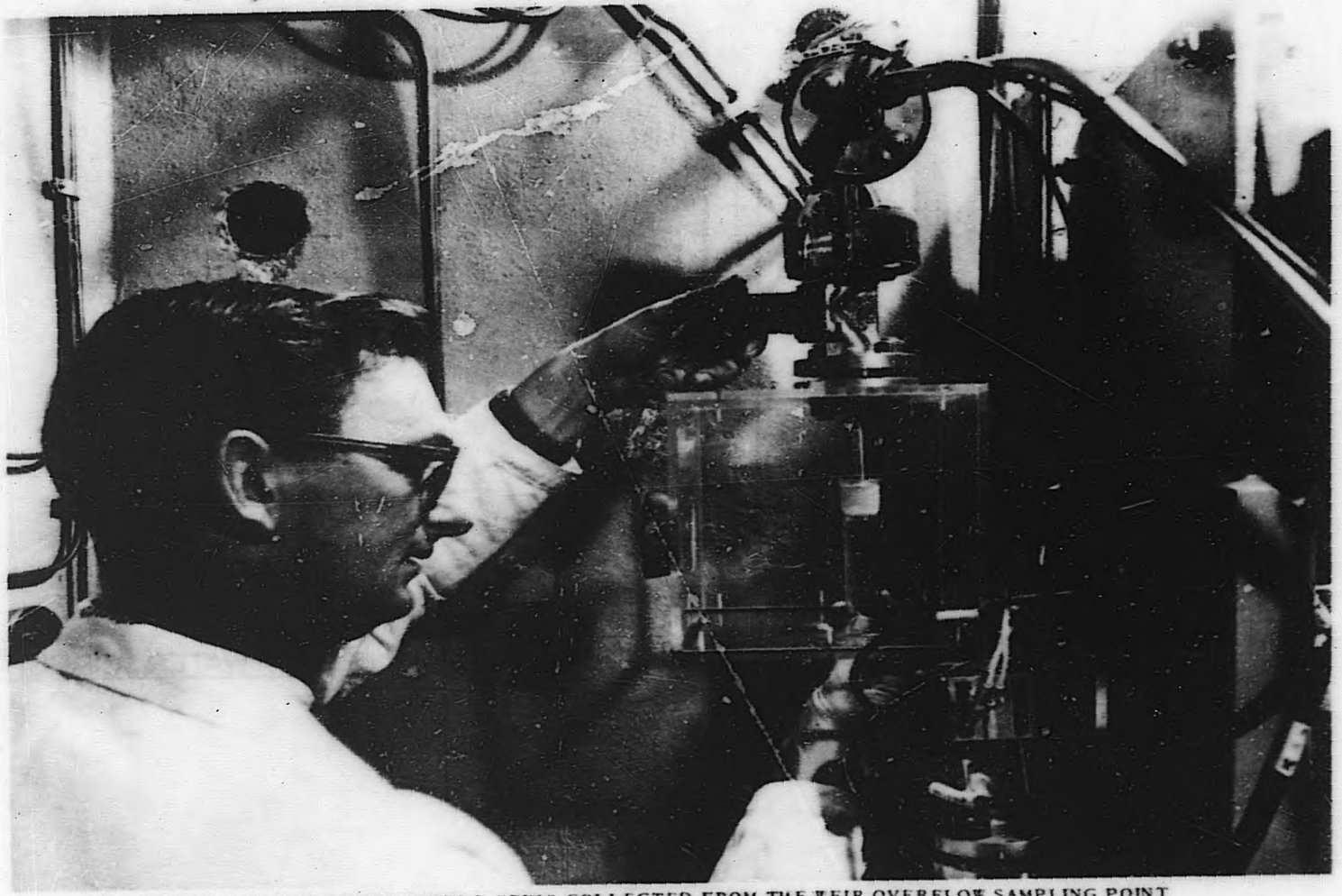
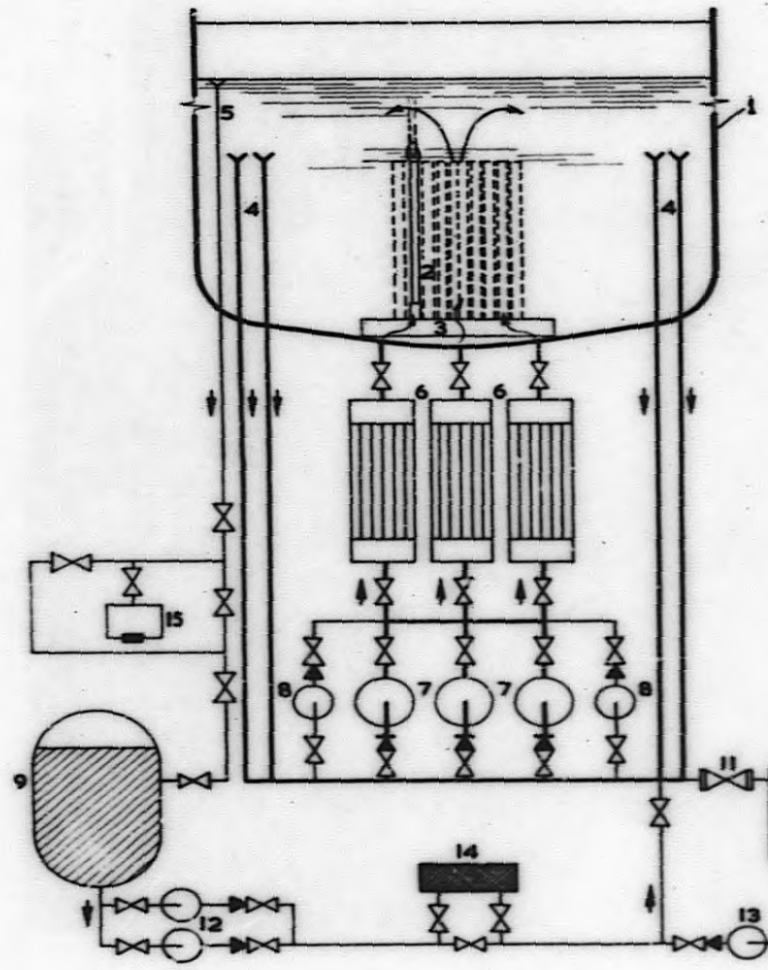


FIGURE 1 HEAVY WATER SAMPLE BEING COLLECTED FROM THE WEIR OVERFLOW SAMPLING POINT



1..... ALUMINIUM TANK
2..... FUEL ELEMENTS
3..... HEADER PLATE
4..... D_2O OVERFLOW PIPES
5..... D_2O LEVEL PIPE
6..... HEAT EXCHANGERS
7..... MAIN D_2O PUMPS
8..... EMERGENCY D_2O PUMPS
9..... D_2O STORAGE VESSEL
10..... PARTIAL DUMP TANK
11..... PARTIAL DUMP VALVE
12..... D_2O LEVEL PUMPS
13..... TRANSFER PUMPS
14..... ION EXCHANGER
15..... D_2O SAMPLING BOX

FIG. 2 HIFAR HEAVY WATER CIRCUIT

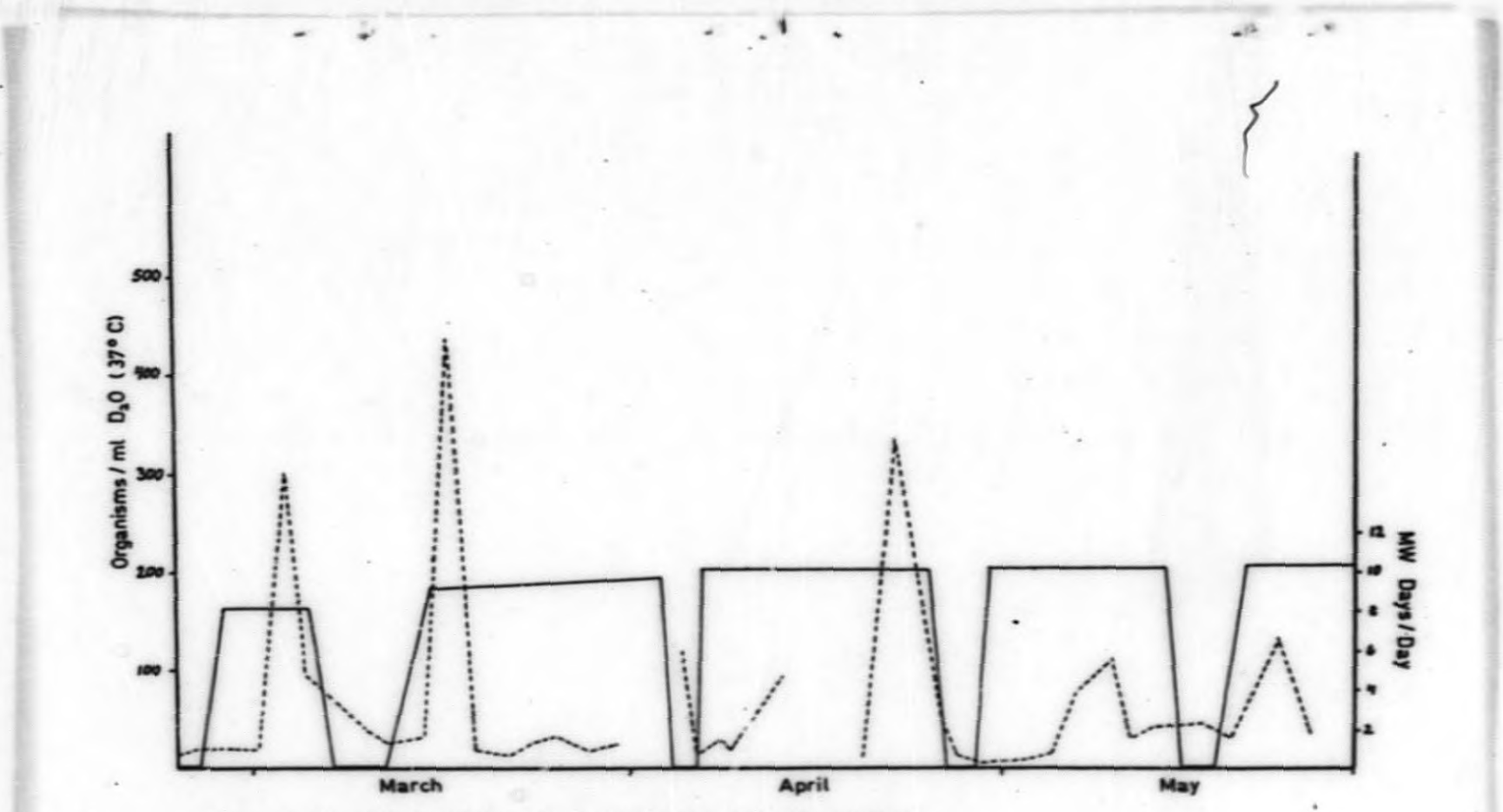


Figure : Microbial Variation in Heavy Water Over Five Power Runs.
Organisms/ml (-----); Power Level (———).

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