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The rate of die-off of *Escherichia coli* in seawater

by

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The fact that sewage bacteria such as *Escherichia coli* die in seawater has been known for many years and a good deal of research has been devoted to this subject. It is not proposed to review this work in detail as Moore (1954) gives a comprehensive review of the work done up to that time and includes a list of 113 references. At that time it seemdd to be established that a form of antibiotic activity was involved, which could be removed by sterilising or ageing the seawater. Sunlight appeared to accelerate the rate of die-off, and there was also evidence that *E. coli* died more rapidly in summer than in winter. Cioglia and Loddo (1962) found that *E. coli* is more resistant to seawater than are the pathogenic enteric bacteria, which supports the usefulness of the *E. coli* count as an index of the sanitary condition of seawater.

Apart from the 5 gallon containers used by Moore the sizes of containers used in this type of work have been 100 ml or 250 ml flasks. Although it is usually accepted that conditions in a small container are very different from those in a large one, because of the relatively greater surface areain a small flask, the author is not aware of any work having been done on the comparative rates of *E. coli* die-off in small and large containers of seawater. The tanks installation at Conway offered the possibility of doing experiments on a large scale using several thousand litres of water at a time, and this paper describes initial experiments done to study further the nature of the die-off of *E. coli* in fairly large bodies of seawater and incidentally to examine the effect of the size of the container used. The tank used in this work was "G" tank, which has a capacity of about 12,500 litres. It is approximately 3.7 m square by 0.92 m deep. It is built of concrete and as it has been in use with seawater for many years, the possibility of any bactericidal substance leaching out of the concrete is remote. As the work progressed it became apparent that the size of this tank was very suitable.

We are fortunate in having a septic tank on the site which is connected only to lavatories and not to laboratories, and which therefore yields pure sewage uncontaminated by chemicals or detergents. Normally 18 litres of sewage, filtered through filter pulp, when added to 12,500 litres of seawater gave counts of E. coli of about 50 to 150 per ml. This was a very convenient and fairly constant source of bacteria. The use of filtered sewage avoided the complication of continuous release of bacteria from disintegration of large particles, and also reduced problems of sedimentation. These were further reduced by circulating the water in the tank continuously. The pump used for this had an output of about 3,000 litres per hour, which would circulate the whole tank in about 4 hours.

This paper is mainly concerned with experiments done in May, June and July 1963, although some exploratory experiments had been done during the previous winter. In the course of all these experiments samples of the water were taken at either hourly or 2-hourly intervals and examined immediately. The method of examination was that outlined in Reynolds and Wood (1956). All samples were taken in duplicate and the counts are expressed as numbers per 20 ml. The results have been plotted on logarithmic graph paper, and for the convenience of having a common starting point have been expressed as the percentage of the original number surviving after a given period. The original number was usually in the region 1000-3000.

The preliminary experiments had indicated that the rate of die-off was greater in daylight than it was in the dark. The first experiment of the present series was designed to check this point. The tank was filled with seawater and 18 litres of sewage added during the filling. The tank took only about five minutes to fill and the great turbulence involved ensured a thorough mixing of the contents. The circulating pump was switched on and a pair of water samples was taken every two hours up to 2000, and then every hour over a total period of 48 hours. The experiment was started at 1500 B.S.T. on May 13 and there was sunshine until 1530. There was no further sunshine that day. The results are shown in Fig. 1. It will be seen that the E. coli count fell to less than 30% of the initial value by sunset of May 13, varied between 22% and 30% through the hours of darkness until shortly before sunrise on May 14, and then started to fall rapidly. By 1000 E. coli could no longer be detected and all counts from then onwards were nil. This experiment confirmed the impression, gained from the preliminary experiments, that the rate of die-off was less in the dark than in the light, even without sunshine. The second experiment was similar to the first except that the sun shone continuously until 2000. The results, shown in Fig. 2, show a very much greater death rate, with a drop to below 1% of the original count in seven hours. Again there was a flattening out of the curve about sunset but the death rate increased again soon after midnight and practically the whole population had gone by dawn.

One of the difficulties of doing this work in ths summer is that the night is short and the transition from daylight to darkness is a very gradual one. In the next experiment (Fig. 3) darkness was produced artificially at 1400 by covering the tank with a double layer of heavy black polythene. This was done immediately after the 1400 sample had been taken. A very clear-cut change in death rate was achieved within an hour, and the E. coli count remained practically constant until the experiment terminated at 2000.

Since light is such an important factor it seemed worth while to try using smaller containers in which it would be possible to control conditions more precisely. It was thought that very small containers such as glass bottles might give anomalous results due to the large area of solid surface in contact with the water, but that fairly big containers (10 or 100 litres) might give results similar to those from the main tank. In the next experiment, after the tank had been filled a 10 litre polythene bucket and a 100 litre white fibreglass bath were filled from it. The experiment was started at 1100 and the whole set-up was blacked out at 1415. The counts (Fig. 4) from "G" tank were in line with previous experiments. Those from the 100 litre fibreglass bath followed the same pattern, although the death rate was much greater. The 10 litre bucket also showed an accelerated death rate which continued practically throughout the experiment. Since light is evidently important, it is possible that the accelerated death rate in the fibreglass bath is associated with the opaque white inner surface which reflects a high proportion of radiation incident on it. The water temperature in "G" tank was about 16°C, that in the bath 17°C, and that in the bucket 18-20°C. This higher temperature may account for the anomalous results obtained in the bucket, but the evidence is not conclusive.

So far light seemed to be important, there was no clear evidence as to the effect of size of containers, and there was a suggestion that temperature might have some effect. The next experiment was planned to give more information about the effect of size of containers, and to eliminate variation of temperature between containers. "G" tank was used as the control as usual. Pint milk bottles were used as small containers. These bottles are made of clear colourless glass, are about 21 cm high and have a 3 cm diameter opening in the top. Twenty-four of these were filled from "G" tank and were suspended in it, twelve with the rim just above the general water level and twelve just submerged. The tops of all the bottles were open. Duplicate samples were taken from "G" tank, and two bottles of each type were examined at two-hourly intervals over eight hours. The results, plotted in Fig. 5, showed no difference between the death rate in "G" tank and that in the bottles whether they were submerged or not.

Since the same result could be obtained in a one pint bottle as in a 12,500 litre tank the way became open to explore the effect of light under rather more controlled conditions. In the earlier stages there had been a tacit assumption that the effective part of the light was probably ultra-violet radiation. The fact that the same death rate was obtained in a glass milk bottle as in the open tank threw some doubt on this, as one would expect very little u/v light to penetrate the glass. It was possible that sufficient came through the open top but this seemed unlikely. In the two final experiments of the present series this was explored further by using bottles some of which were clear and some of which were painted black outside. Also half of each type were closed with aluminium foil caps. The four types, clear open, clear capped, black open, black capped, were filled from "G" tank and then suspended in it to keep the temperature constant. The results of the two experiments are shown in Fig. 6 a and b. It is quite clear that the presence of an aluminium cap on the bottle makes no difference; therefore the light that is important can penetrate the glass of a milk bottle. It would also appear that the amount of light that comes in through the open top of a blacked-out bottle is insufficient to be effective. It is interesting that in both of these experiments the death rate in the clear bottles was higher than in the tank. It is thought that this may be due to the light intensity being greater in the upper 9" of the tank than in the depths. Both of these experiments were done on dull cloudy days. The previous experiment (Fig. 5) was done on a day with continuous sunshine from about 1300 onwards.

All the experiments so far have been done with seawater of specific gravity of about 1.025, and in the series here discussed the temperatures were usually in the range 12-16°C. It is clear that light plays a very important part, either directly or indirectly, in the die-off of E. coli in seawater. The effective part of the light spectrum appears to be able to pass through glass with only partially diminished intensity. No information is available yet as to what part of the spectrum is responsible for the death of E. coli. One would expect it to be some portion of the u/v wave band, perhaps towards the longer end since the radiation concerned seems to have greater powers of penetration than are usually associated with u/v light. Further experiments will be directed towards this point.

Finally it appears that experiments done in one pint (0.553 litre) bottles give the same result as those done in 12,500 litre tanks at temperatures of 10-15°C.

SUMMARY

Experiments on the rate of die-off of *E. coli* in seawater were done in a 12,500 litre tank at Conway. Filtered sewage from a septic tank was used as a source of bacteria. An inoculum of 18 litres was sufficient to give an initial count of between 50 and 150 *E. coli* per ml of seawater. Samples were taken in duplicate and examined immediately by the roll tube method. They were usually taken at hourly intervals.

It was found that numbers of *E. coli* decreased approximately logarithmically with time during daylight, but remained more or less constant during the night. The same effect could be produced by blacking out the tank. Although sunlight increased the rate of die-off, even in very dull weather a 50% reduction in numbers could occur in 4 hours, at a temperature of 13°C.

Comparisons of the rate of die-off in the 12,500 litre tank with that in one pint milk bottles at the same temperature showed that the two rates were the same over the period of the experiment. Using clear bottles and blacked-out bottles it was possible to reproduce the light and dark rates of die-off. It was noticeable that the full daylight rate was obtained in clear bottles closed with foil caps. This suggests that the part of the spectrum responsible for the high "light" rate of die-off is not the short u/v waves which are usually associated with sterilising, as they are largely stopped by glass, but longer waves.

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Fig I

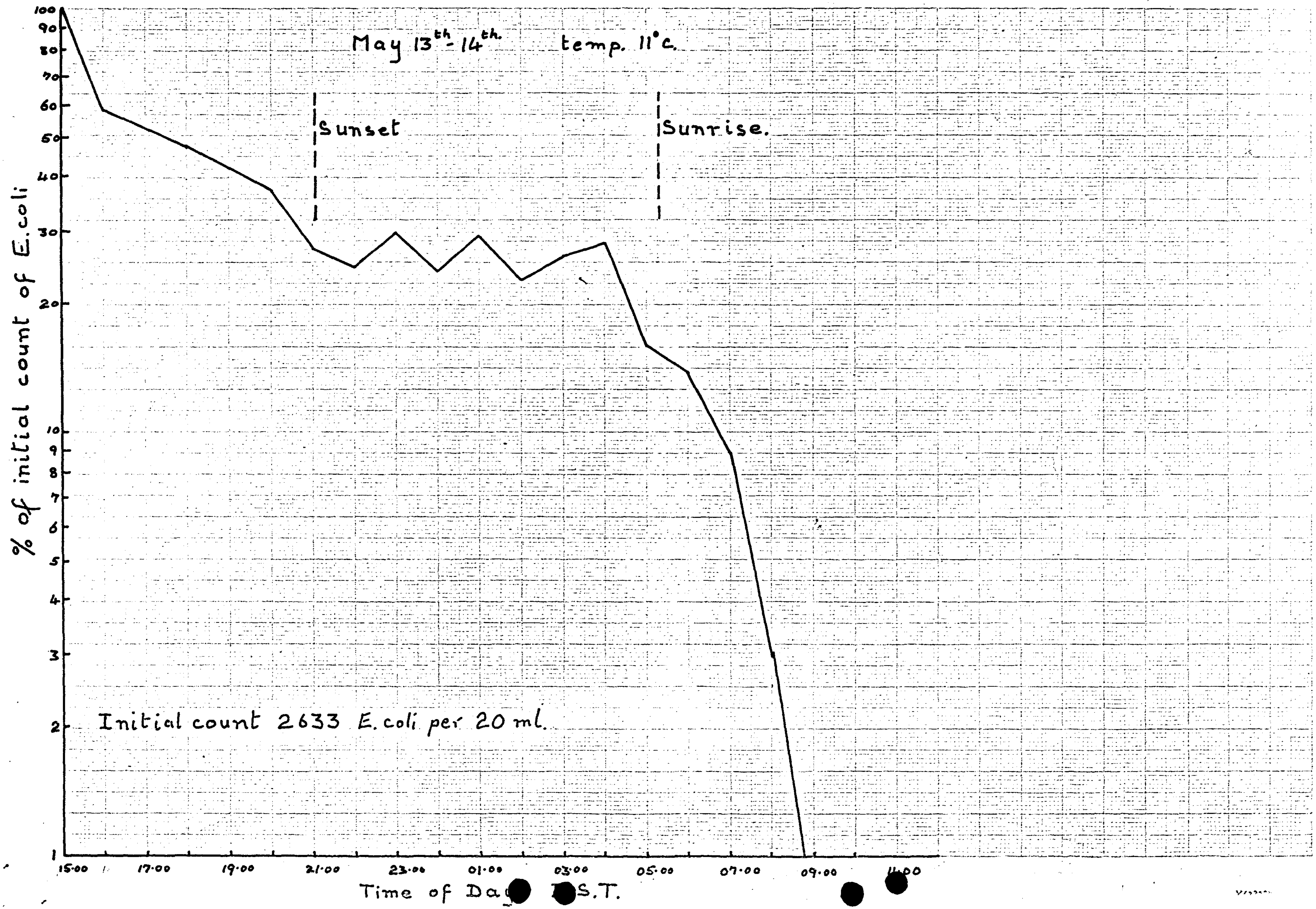

May 13th-14th temp. 11°c.
Sunset
Sunrise.
Initial count 2633 E. coli per 20 ml.
% of initial count of E. coli
100
90
80
70
60
50
40
30
20
10
9
8
7
6
5
4
3
2
1
15·00
17·00
19·00
21·00
23·00
01·00
03·00
05·00
07·00
09·00

Fig 2.

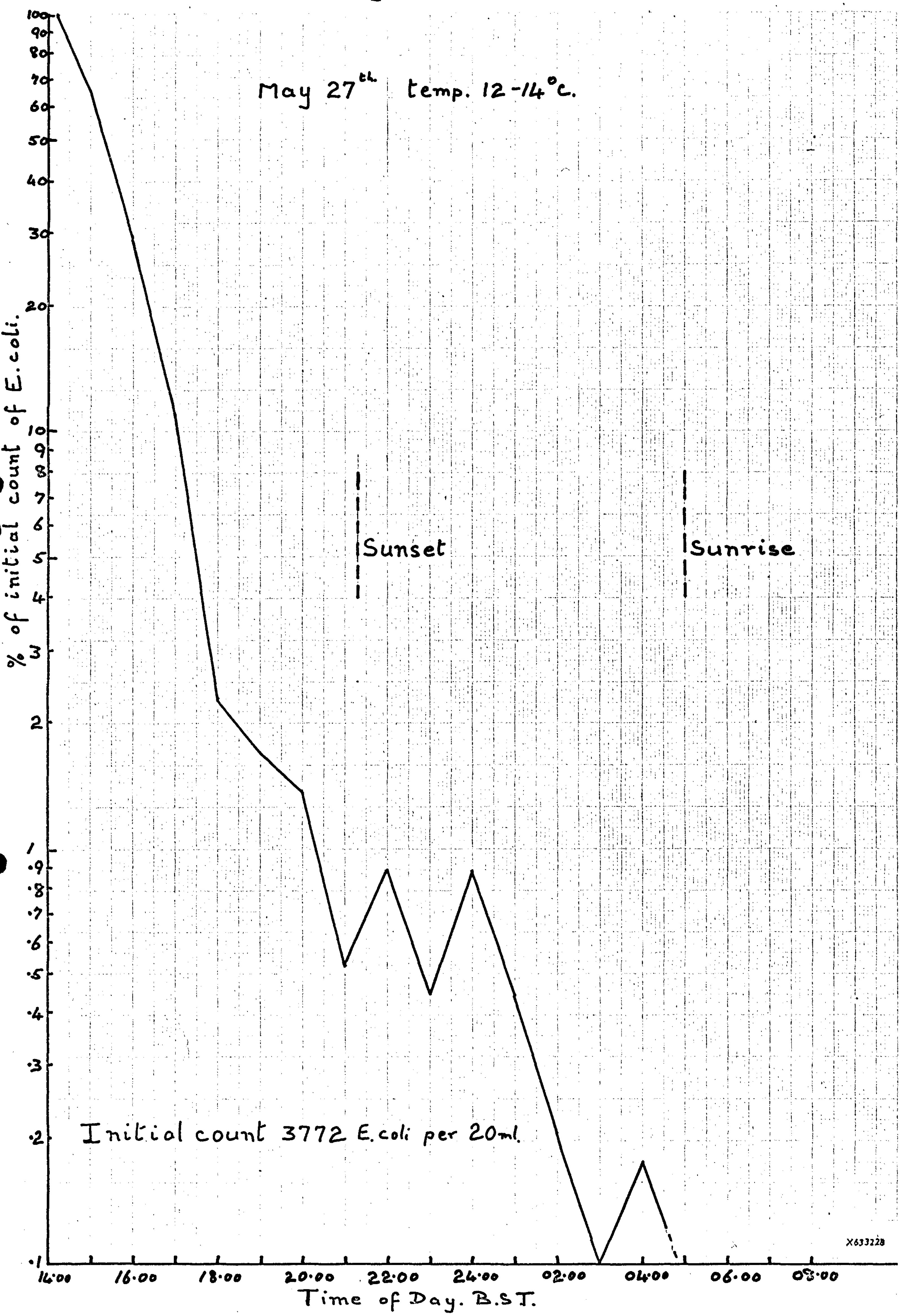
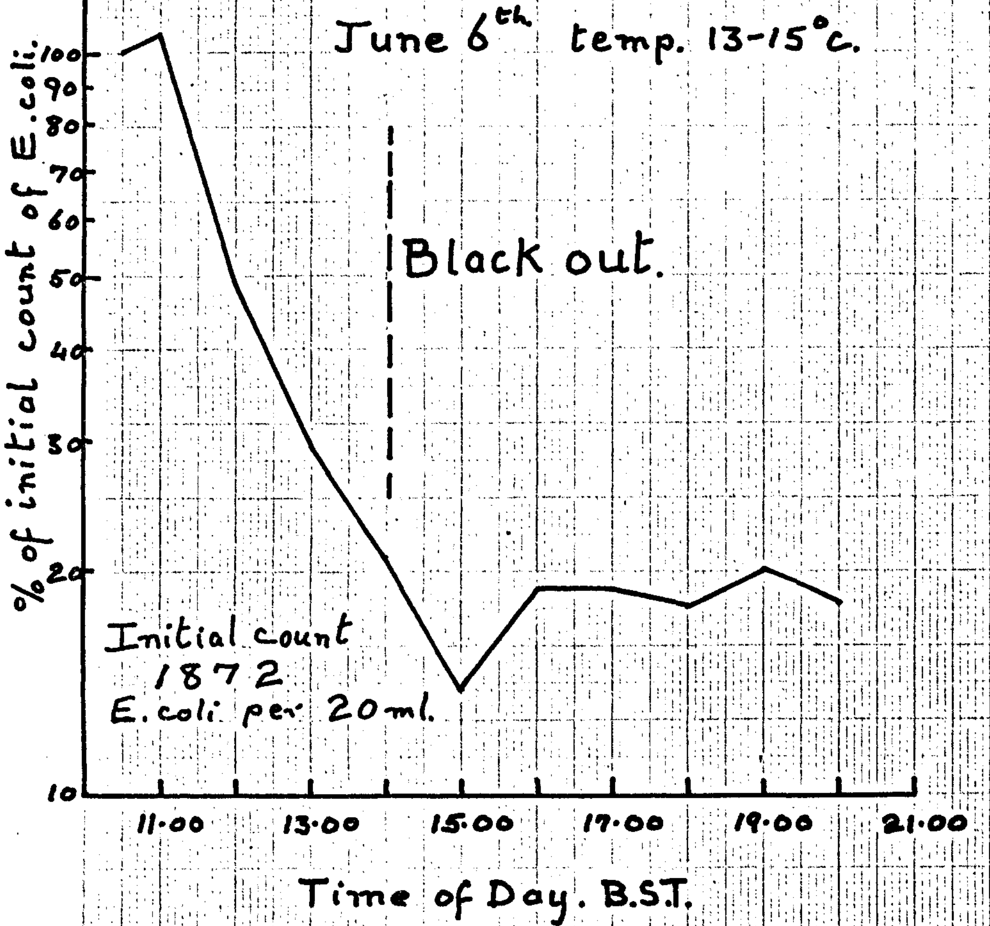
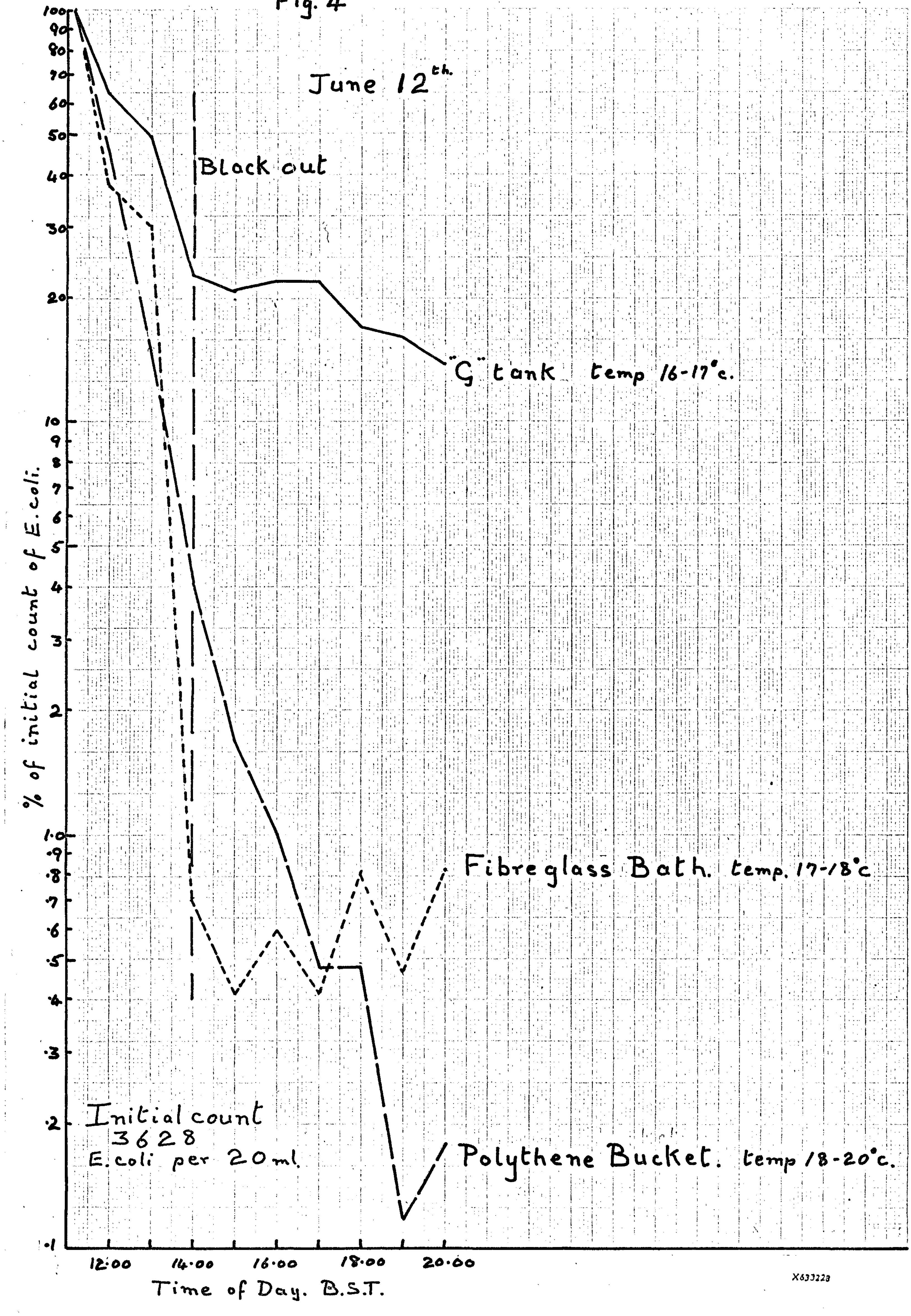

May 27th temp. 12-14°c.
% of initial count of E. coli.
100
90
80
70
60
50
40
30
20
10
9
8
7
6
5
4
3
2
1
·9
·8
·7
·6
·5
·4
·3
·2
·1
Sunset
Sunrise
Initial count 3772 E. coli per 20ml.
14·00
16·00
18·00
20·00
22·00
24·00
02·00
04·00
06·00
08·00
Time of Day. B.S.T.

Fig. 3



X833228

Fig. 4



X633228

Fig. 5.

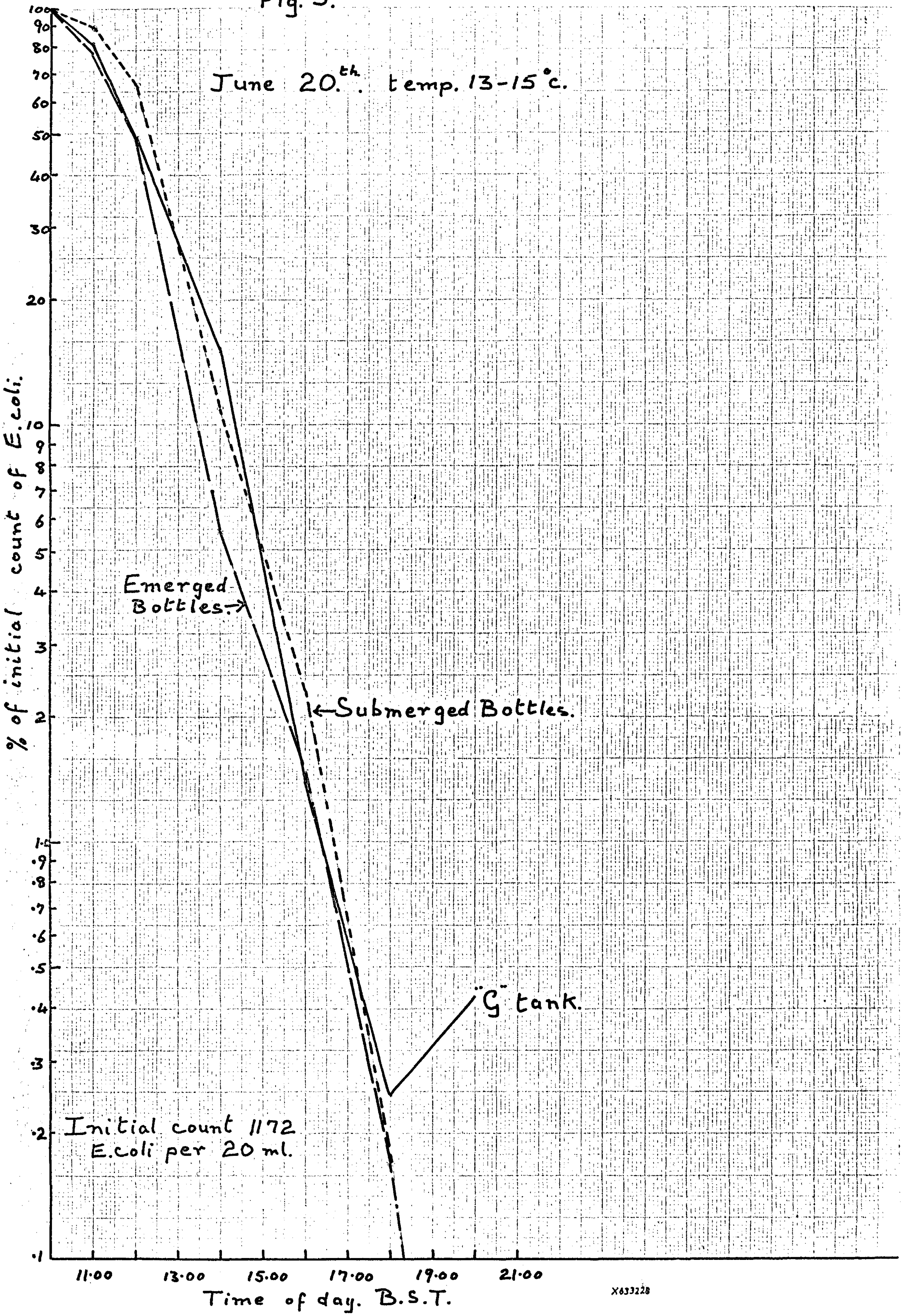

June 20th. temp. 13-15°c.
100
90
80
70
60
50
40
30
20
10
9
8
7
6
5
4
3
2
1.0
·9
·8
·7
·6
·5
·4
·3
·2
·1
% of initial count of E. coli.
Emerged Bottles.
Submerged Bottles.
"G" tank.
Initial count 1172
E.coli per 20 ml.
11·00
13·00
15·00
17·00
19·00
21·00
Time of day. B.S.T.
X633228

Fig. 6a

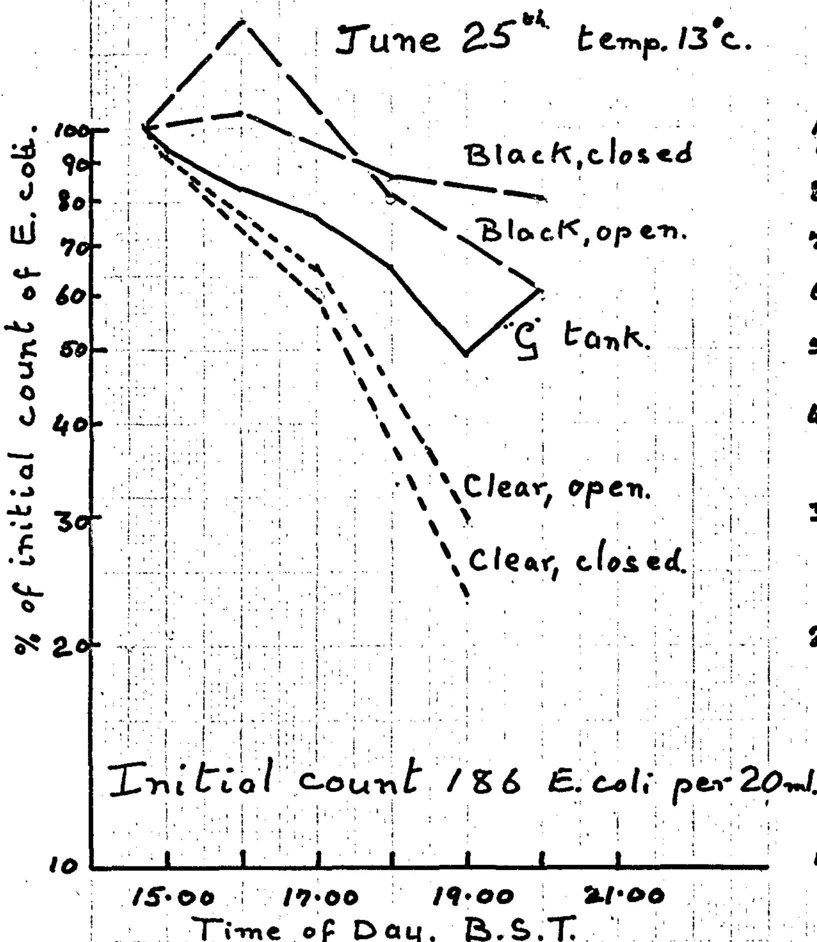
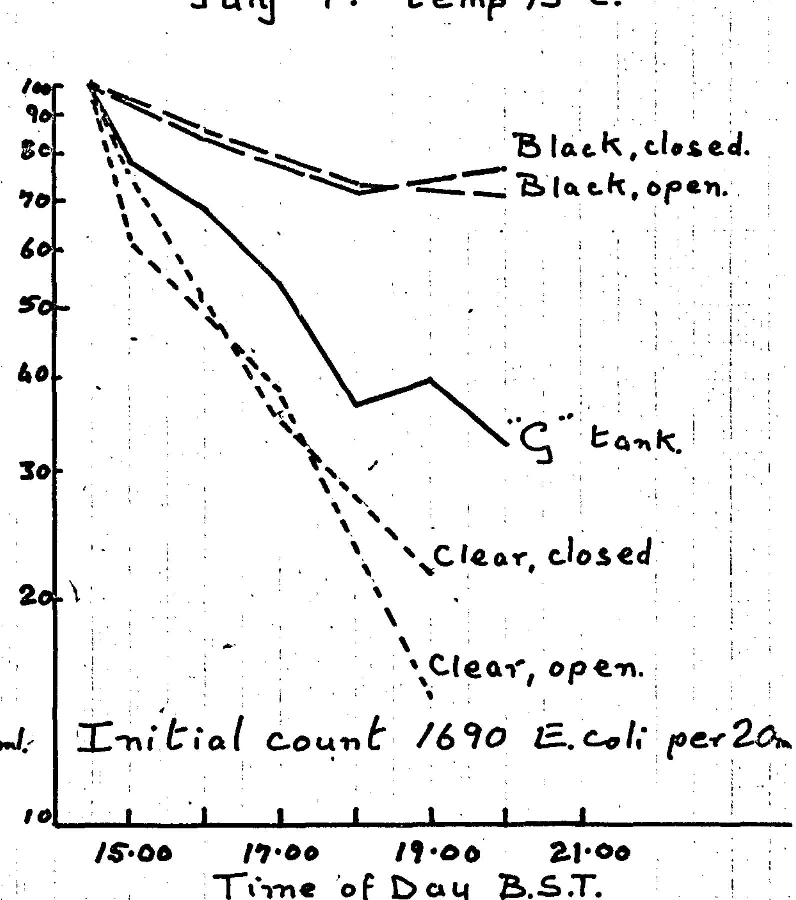

June 25th temp. 13°c.
Black, closed
Black, open.
"G" tank.
Clear, open.
Clear, closed.
Initial count 186 E. coli per 20 ml.
% of initial count of E. coli.
100
90
80
70
60
50
40
30
20
10
15·00
17·00
19·00
21·00
Time of Day. B.S.T.

Fig. 6b


July 9th temp 15°c.
Black, closed.
Black, open.
"G" tank.
Clear, closed
Clear, open.
Initial count 1690 E. coli per 20m
100
90
80
70
60
50
40
30
20
10
15·00
17·00
19·00
21·00
Time of Day B.S.T.