AN EXPERIMENTAL STUDY OF THE EFFECTS OF SEWAGE SEEDING ON THE BIOCHEMICAL OXYGEN DEMAND OF A STERILE INDUSTRIAL WASTE.

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THESIS

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INTRODUCTION

The importance of the biochemical oxygen demand (B. O. D.) test cannot be questioned in its application to sewage treatment plant operation, water treatment plant operation, stream surveys and the treatment of industrial wastes. The test is used to determine the efficiency of a waste treatment plant or of a unit of such a plant, to determine the extent of pollution of a stream and to compare the strengths of different domestic and industrial wastes.

This thesis is chiefly concerned with the application of the test to industrial waste analyses. In the design of sewage treatment plants the engineers are often confronted with the problem of determining the population equivalent of some contributing industrial waste. This is usually done by determining the B. O. D. of the waste and calculating the population equivalent in terms of domestic sewage having the same B. O. D. requirements. Large contributions from industries have an important bearing on the capacities of sewage treatment plants and it is important that the B. O. D. determination of any industrial waste be accurate.

The biochemical oxygen demand determination is a measure of the oxygen required to oxidize the organic matter of a certain sample (1). The oxidation is brought about by bacteria in a sample which is held at some predetermined temperature for the required incubation period; frequently for five days. Eight ounce glass stoppered bottles are used for incubating the sample which is prepared by making a proper dilution of the waste being used. Several dilutions are usually required so that after the incubation period a few parts per million of oxygen will be left in the bottles. Sterile industrial wastes must be inoculated with the proper organisms. To do this, various amounts of sewage are added as seeding to the bottles and then incubated for the desired period at some constant temperature. Bottles containing the various amounts of seeding and diluting water with no waste are also incubated to eliminate the B. O. D. of the seeding material that has been added. Similar sets of incubation bottles are set up for each day that the B. O. D. is to be determined. After the incubation has proceeded the proper number of days, one set of bottles is removed from the incubator and the dissolved oxygen contents of the incubations are determined by chemical methods. The difference between the oxygen content of the diluting water and the dilution is a measure of the B. O. D. of the waste.

However, the test as it is now made, has not proven entirely satisfactory for the determination of the B. O. D. of industrial wastes. Many discrepencies are apparent due to several factors among which the most important is the seeding given to the dilution prior to incubation.

HISTORICAL

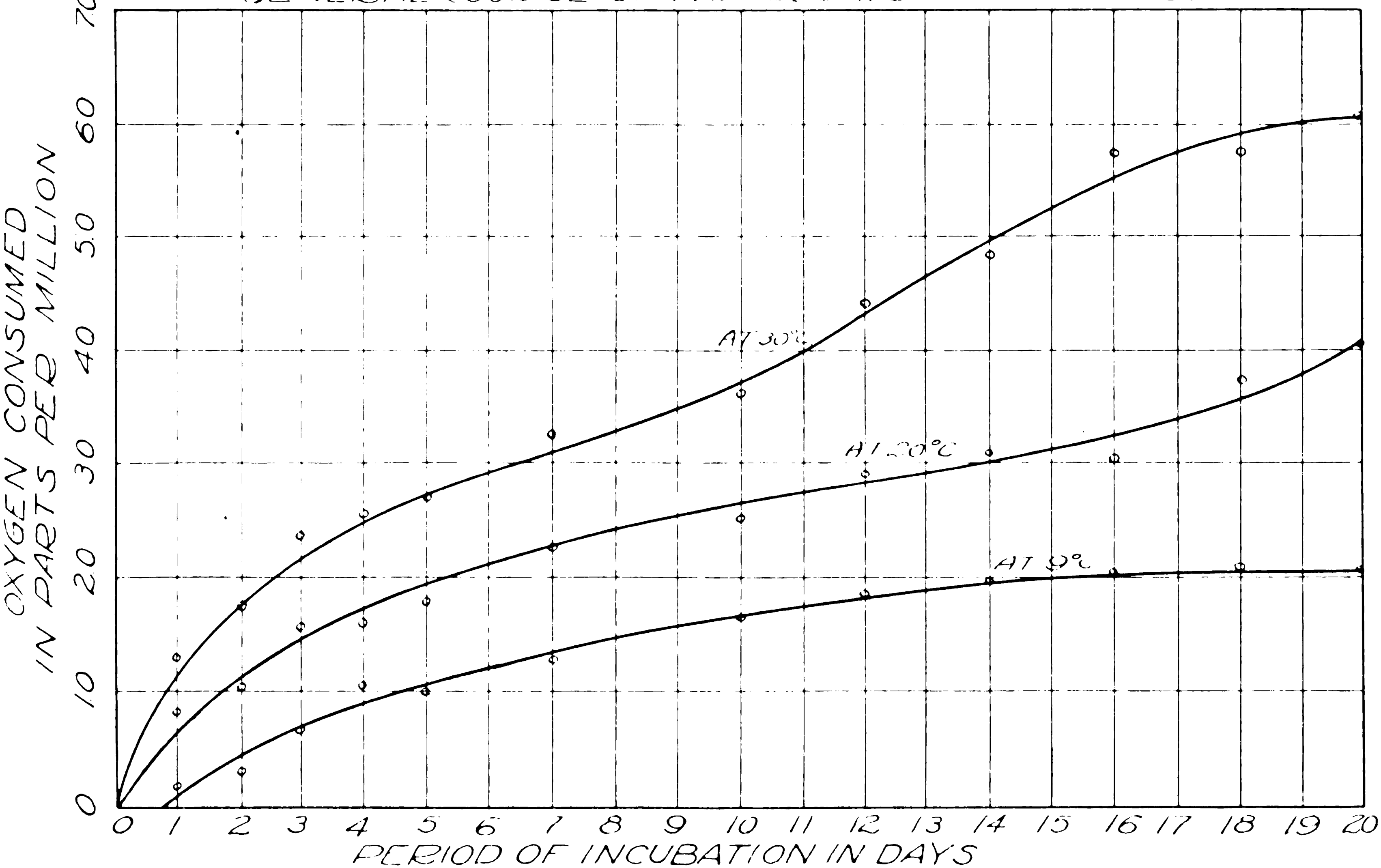
The biochemical oxygen demand determination is by no means a new test for it was used as early as 1870 by a British Rivers Pollution Commission. In Germany it was used as early as 1900 and in France as early as 1885. The test in its present modified form has been used in the United States since about 1915.(2)

From experiments conducted by Theriault and confirmed by others, it has been found that the deoxygenation of an organic waste proceeds in two distinct stages--first the carbonaceous stage where the carbonaceous matter is oxidized and then the second or nitrification stage where the nitrogenous matter is oxidized. The general course of the deoxygenation curve is shown in the accompanying graph which is taken from Theriault. The curves are plotted from data of the same sample incubated at different temperatures for varying lengths of time.

Phelps proposed a formula many years ago which fits with reasonable accuracy the experimental results of deoxygenation. The formula is based on the assumption that the rate of deoxygenation at any instant is directly proportional to the amount of organic matter present in the sample and is applicable only to the first stage of deoxygenation.

Without going into the derivation of Phelps formula which can be found elsewhere(3) it may be stated as follows:

GENERAL COURSE OF THE DEOXYGENATION CURVE



$$X_t = L - L_t = L(1 - 10^{-K_1 t})$$

Where L_t equals the B. O. D. in p.p.m. remaining after time t. Where L equals the B. O. D. in p.p.m. exerted by the organic matter during first stage.

$L - L_t = X_t$ oxygen in p.p.m. used up in t days, as determined by the B. O. D. test.

K_1 = deoxygenation constant.

The deoxygenation constant for any temperature has been found to vary with the 20° temperature constant as the following formula;

$$K_{1(T)} = K_{1(20)}\left[1.047^{(T - 20)}\right]$$

Where $K_{1(20)} = 0.100$

The temperature also affects the magnitude of the first stage B. O. D., and the first stage demand at any temperature T bears the following relation to the 20° demand:

$$L_T = L_{20}\left[1 + 0.02(T - 20)\right]$$

These formulas can be used to change any oxygen value for one temperature and a given period of incubation into terms of the oxygen value under an entirely different set of conditions. They are applicable to recently polluted samples.

There are a number of factors which affect the B. O. D. determination; the pH of the medium, the amount and kind of bacteria present and the mineral content of the sample and/or diluting water.

In general the advantage of the test over a strictly chemical test like the potassium permanganate oxygen consumed test is that it is very similar to the natural stream conditions or conditions in the diluting body of water. The disadvantages of the test are that there are often toxic substances which inhibit bacterial growth especially in some inductrial wastes, long periods of incubations at constant temperatures which require a high degree of mechanical control and rather close attention(5) and also there may be, particularly in industrial wastes, certain chemical constituents which will interfere with the dissolved oxygen determination by the Winkler method. Theriault(6)(7) and Theriault and McNamee(8) have successfully modified the Winkler method to give correct values for dissolved oxygen when interference is encountered due to iron salts, nitrites, organic matter, hypochlorites and sulphite wastes. Eldridge and Mallmann(4) have found that a synthetic diluting water with a mineral content very similar to the mineral content of river waters will give higher B. O. D. values than distilled, bicarbonate, carbonate or phosphate waters. They also found that the pH of their dilutions dropped slightly during the oxidation period in dilutions made with tap water and distilled water, but in dilutions with other waters there was practically no change. Their results show that the initial pH has some effect on the B. O. D. results, but that it is not necessarily a limiting factor.

Eldridge(9) has found that in making dilutions of sterile material and seeding with sewage that the B. O. D.

varies almost directly with the amount of seeding up to about seven days incubation.

Holderby and Lea[10] have found that the B. O. D. is substantially affected by the ratio of organic carbon to total nitrogen. With ratios of carbon to nitrogen as large as 120 to 1 the rate of oxidation is very slow, but if the ratio be reduced to 5.7 to 1 the five day oxygen demand values would be at a maximum.

Much uncertainity surrounds the test especially when seeding material must be added as pointed out by Eldridge[11] who shows that the five day B. O. D. of a paper mill white water increases in higher dilutions and increases with an increasing amount of seeding. These same results have been found to apply to a sterile milk waste as is shown in the accompanying experimental and data portions of this report.

EXPERIMENTAL

This study was made to determine, if possible, the correct amount of sewage seeding to be added to a sterile industrial waste sample to get the proper B. O. D. of the waste. The experiment was divided into three trials, each of which extended over a twenty-day incubation period using an incubation temperature of $20^{\circ} \pm 1^{\circ}$ Centigrade.

Milk waste was selected because it eliminated many factors which affect the B. O. D. determination. This waste is homogeneous, has an optimum pH value, is easily obtainable and easily made sterily. A factory waste of milk diluted with distilled water and containing 0.5 per cent whole milk was made up and autoclaved for each trial. In trial No. 1, 1.0 per cent and 0.5 per cent dilutions were made of this factory waste and in trials No. 2 and No. 3 0.75 per cent and 0.5 per cent dilutions were used.

Eight ounce glass stoppered bottles were calibrated and used for the incubation of the samples. Bottles were selected which did not vary more than five cubic centimeters from 250 cubic centimeters capacity. The samples were incubated in a water bath at 20°C. for periods of 2, 5, 10, 15 and 20 days. At the end of these periods the dissolved oxygen content was determined by the Winkler method.

The sodium bicarbonate diluting water(1) used was prepared by adding six grams of sodium bicarbonate to five gallons of distilled water and bubbling air through the contents until the dissolved oxygen content is above 7.0 p.p.m. It has been pointed out by Eldridge and Mallmann(4) that this diluting water does not give as high B. O. D. values as other waters, but it has been temporarily accepted as a standard in sewage treatment plants and gives results comparable to practice in this part of the country.

The effluent of the East Lansing Imhoff tanks was filtered and used as a seeding material.

The following dilutions and seedings were made for the 2, 5, 10, 15 and 20 day B. O. D. determinations.

TRIAL NO. 1

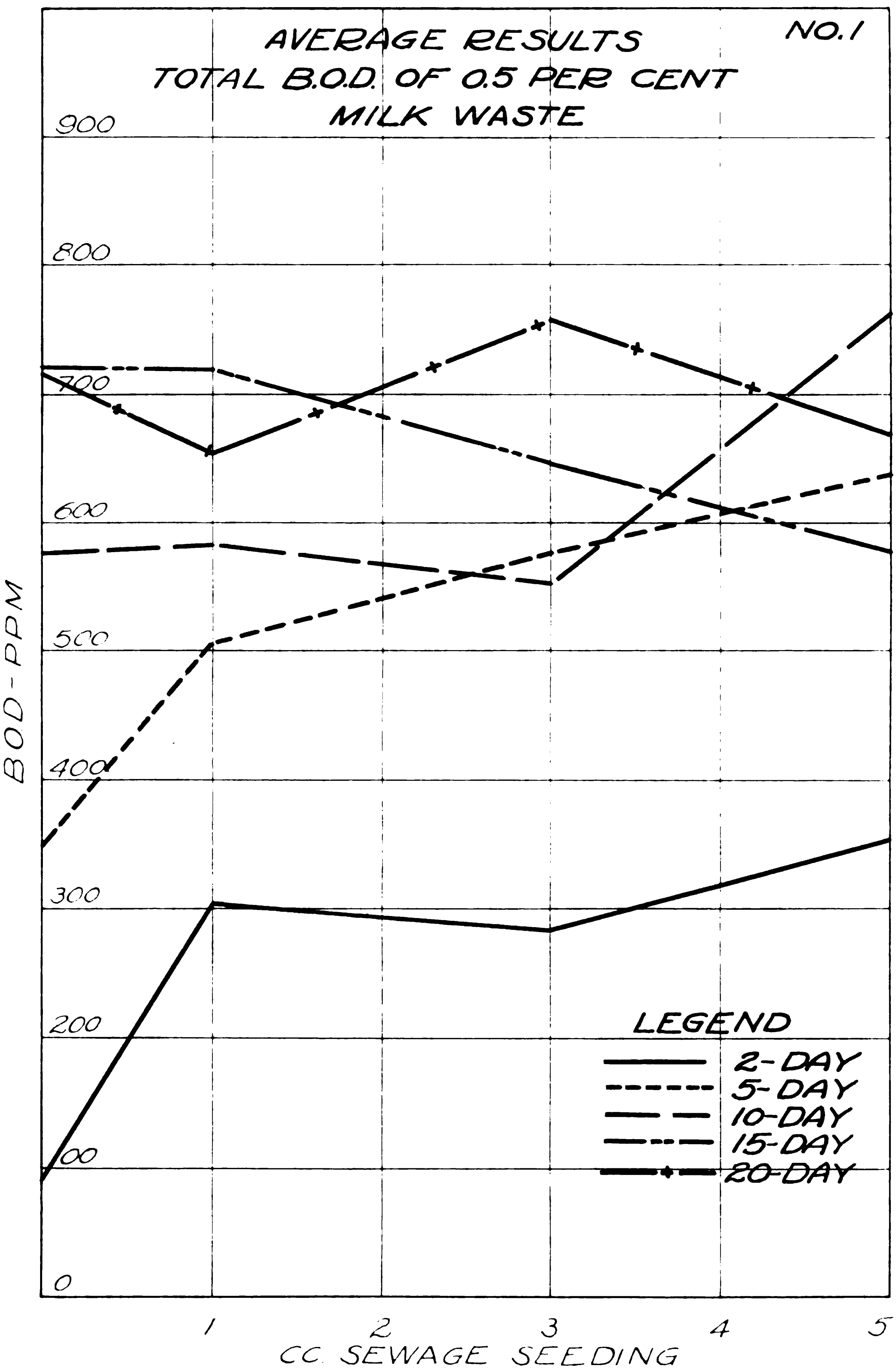
1-	Diluting Water	-	1.0 per cent waste	-	0.5 per cent waste	-	each	with	no	seed.
2-	"	-	"	-	"	-	"	"	1cc.	"
3-	"	-	"	-	"	-	"	"	3cc.	"
4-	"	-	"	-	"	-	"	"	5cc.	"

TRIALS NO. 2 AND NO. 3

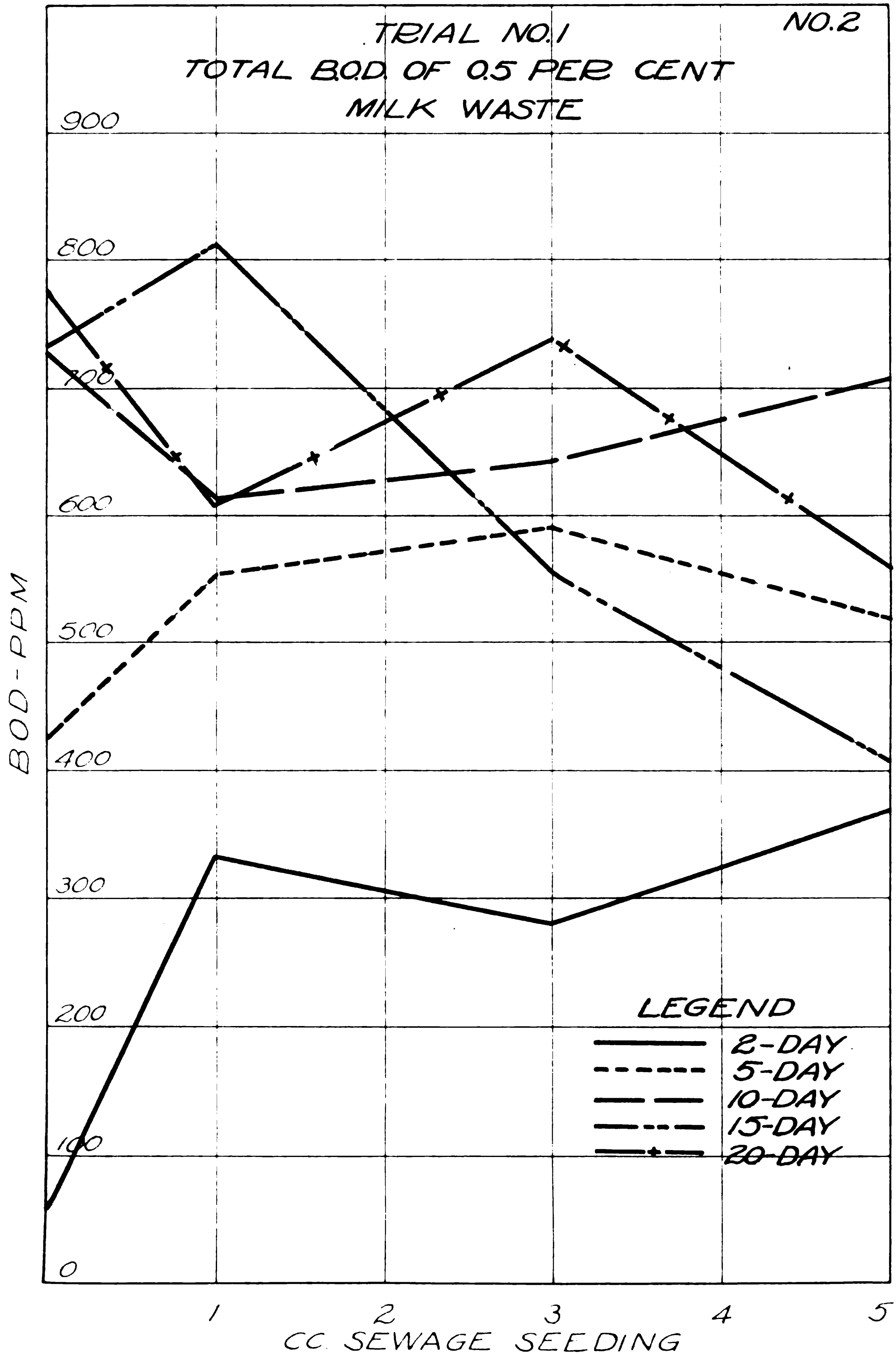
1-	Diluting Water	-	0.75 per cent waste	-	0.5 per cent waste	-	each	with	no	seed.
2-	"	-	"	-	"	-	"	"	1cc.	"
3-	"	-	"	-	"	-	"	"	3cc.	"
4-	"	-	"	-	"	-	"	"	5cc.	"

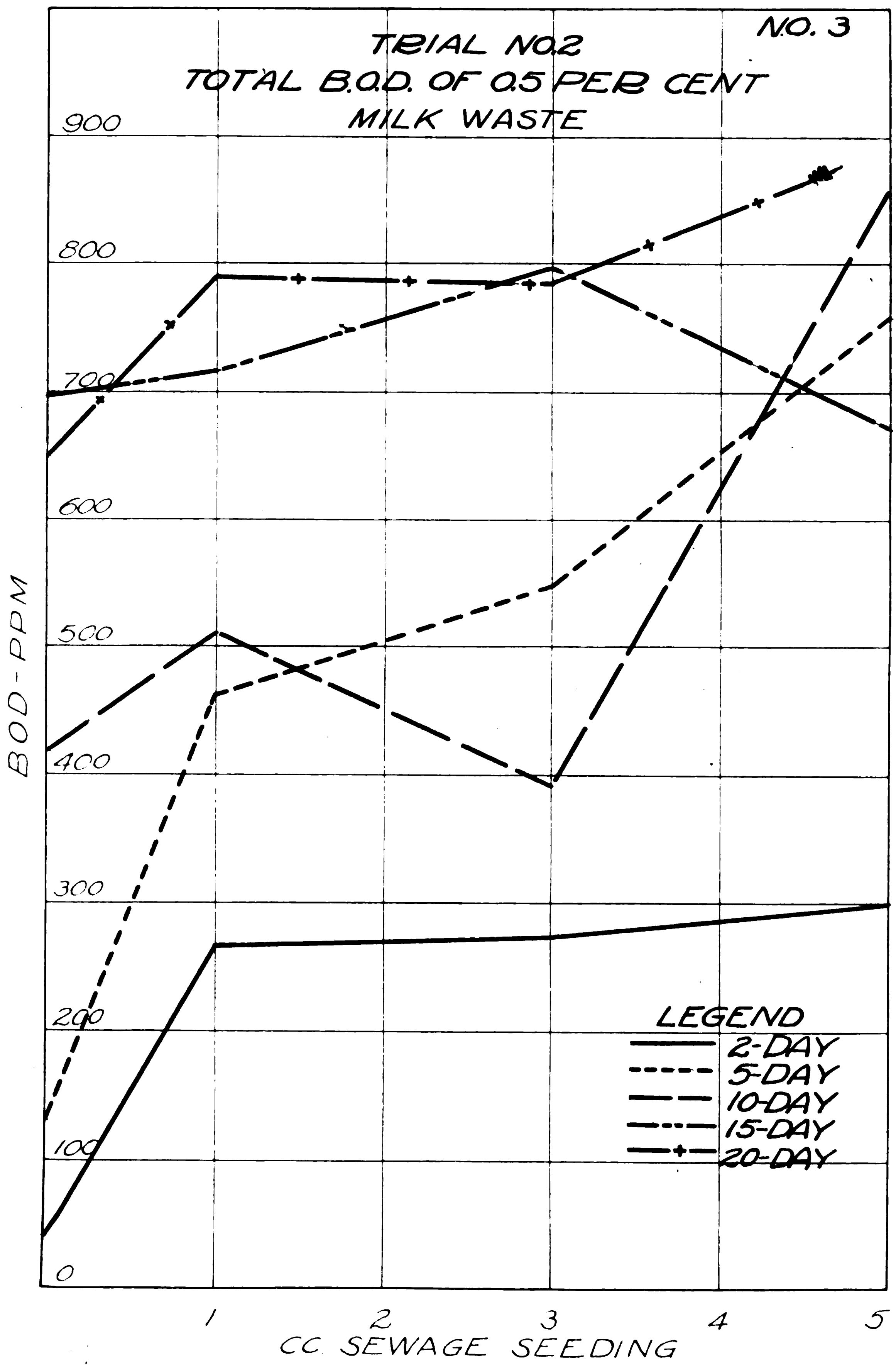
Bacterial counts were made of the sewage seeding material and of the dilutions just before the chemicals were added to make the dissolved oxygen test. These give the number of organisms added to each B. O. D. bottle and the bacterial population of the samples after the incubation periods of 2, 5, 10, 15 and 20 days. These bacterial counts are plotted against the days incubation for trial No. 2 on graphs No. 9 and No. 10. Graph No. 11 compares the bacterial count and the B. O. D. of the same sample. Graph No. 12 gives the daily B. O. D. values plotted against the days incubation.

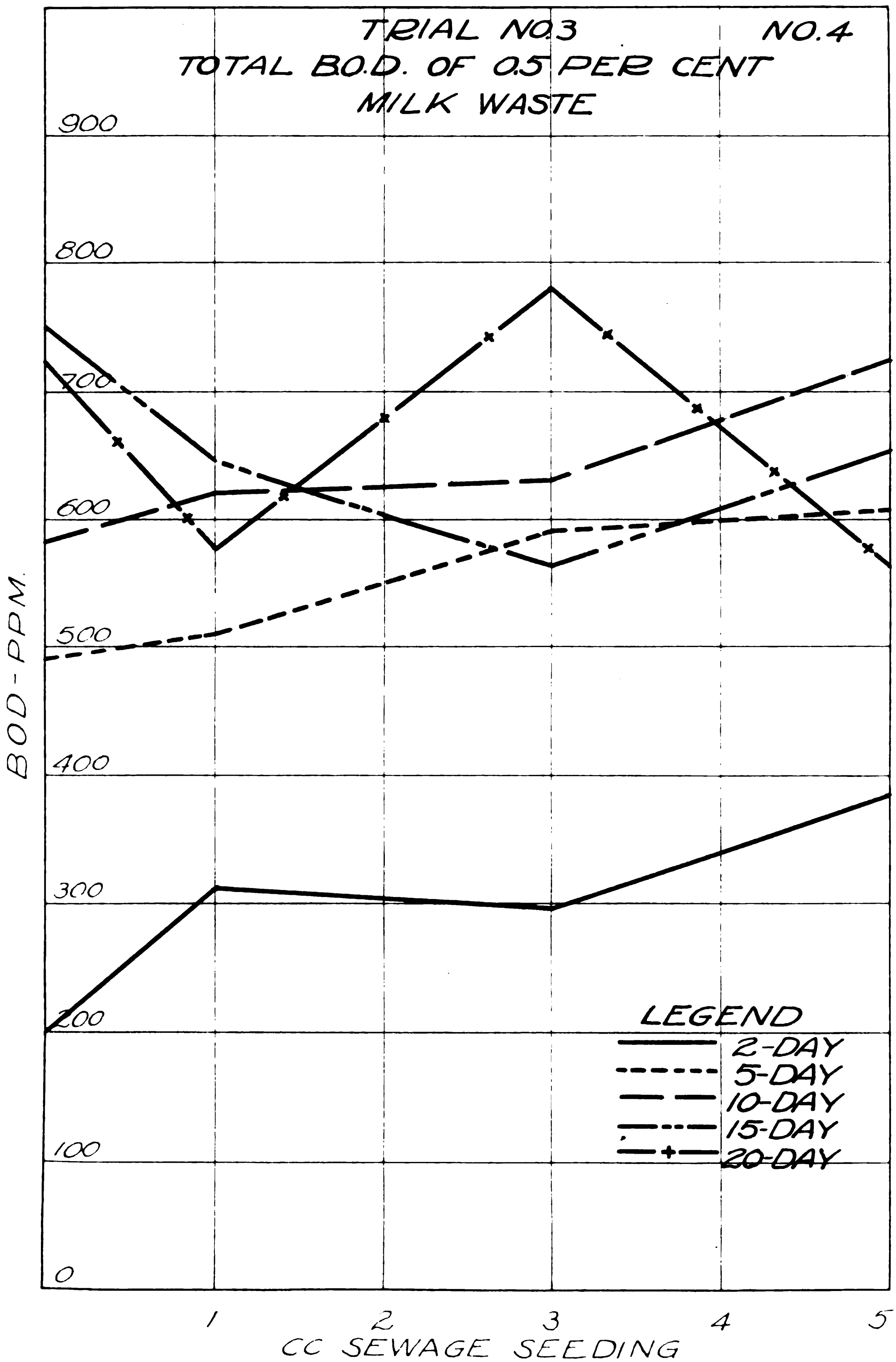
Graph No. 5 shows the average effects of sewage seeding on the B. O. D. of a 0.5 per cent milk waste using various periods of incubation and is a composite of graphs No. 6, No. 7 and No. 8 which in turn are the respective graphs of trials No. 1, No. 2 and No. 3.

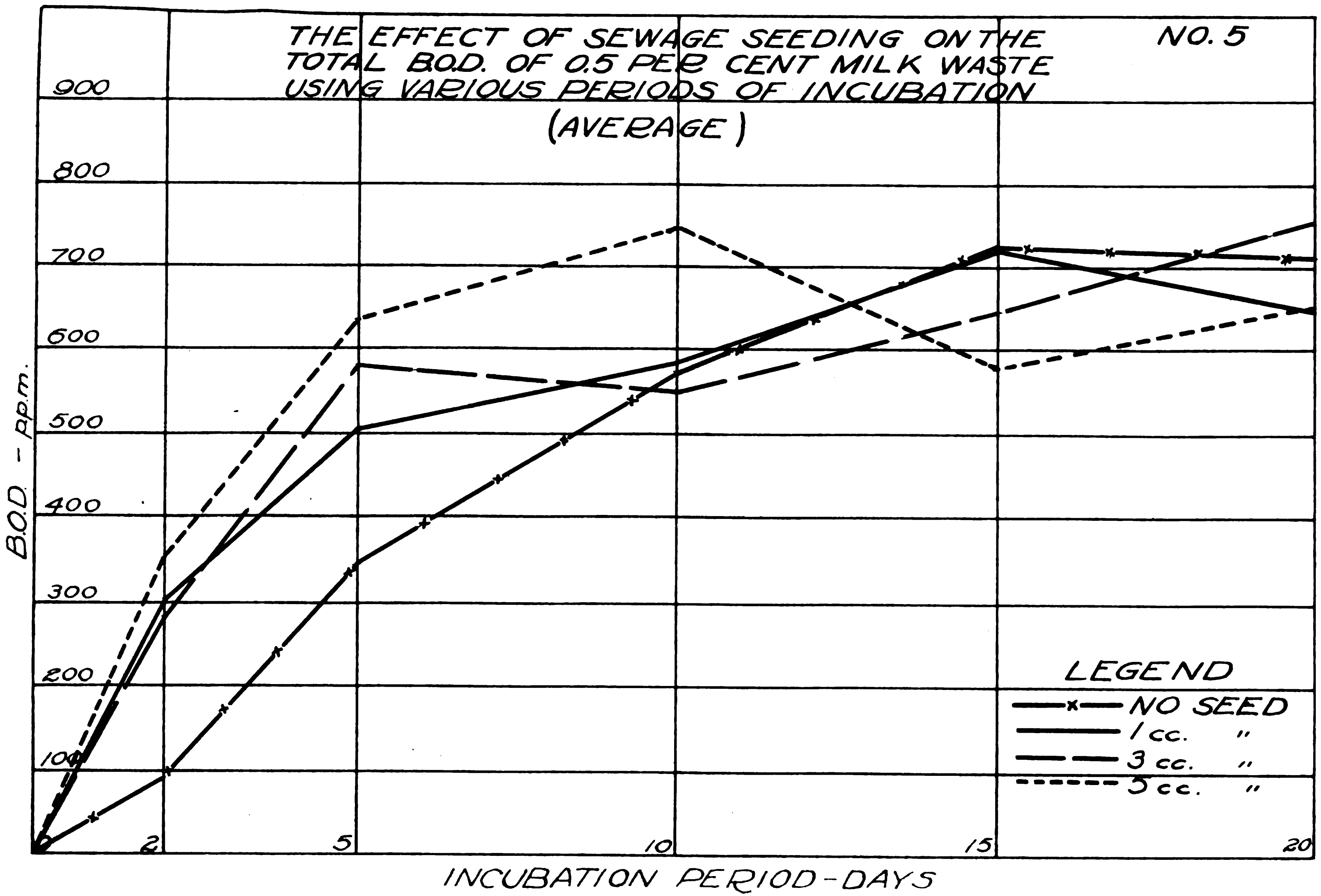


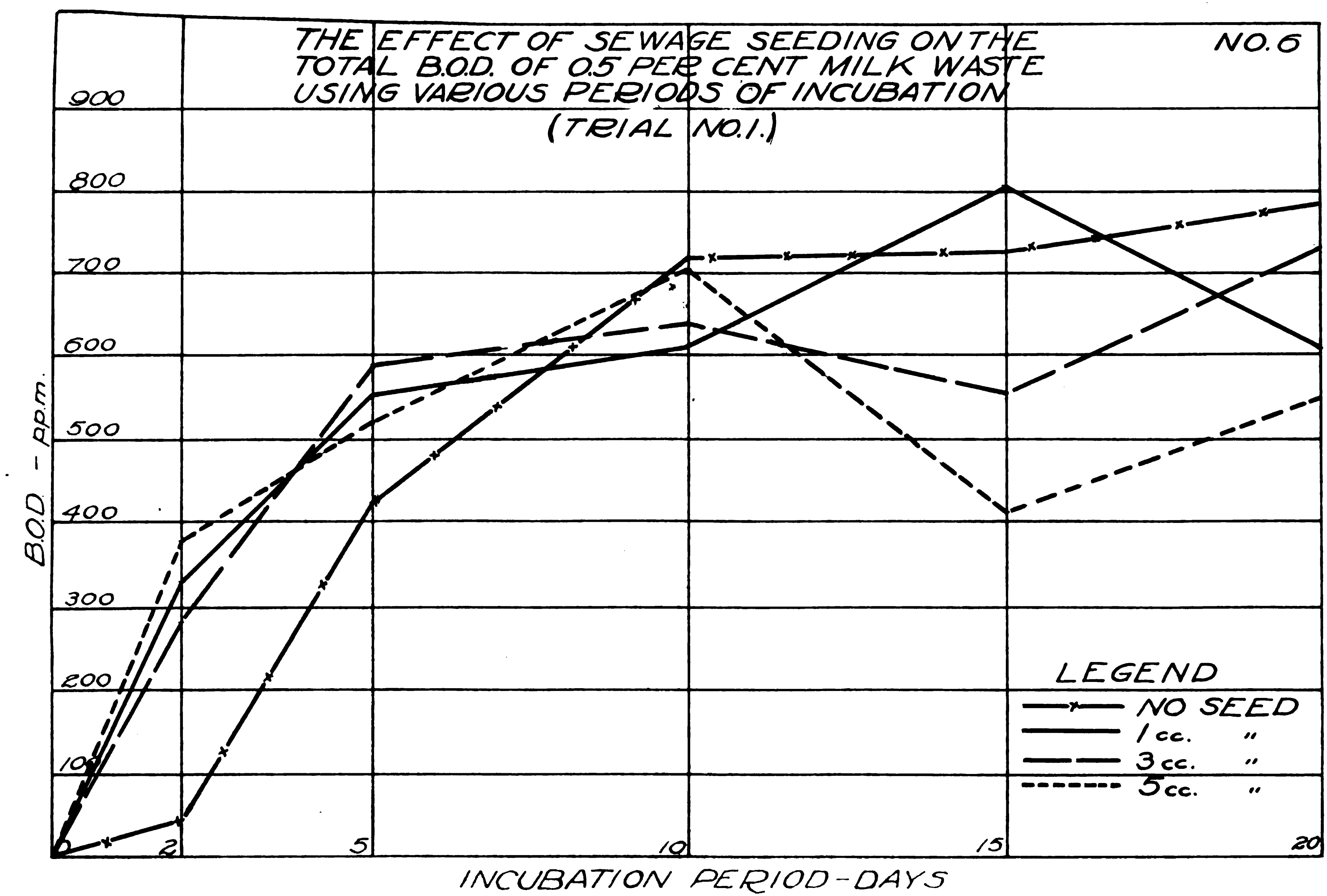
AVERAGE RESULTS
TOTAL B.O.D. OF 0.5 PER CENT
MILK WASTE
NO. 1
900
800
700
600
500
400
300
200
100
0
BOD - PPM
1
2
3
4
5
CC. SEWAGE SEEDING
LEGEND
2-DAY
5-DAY
10-DAY
15-DAY
20-DAY

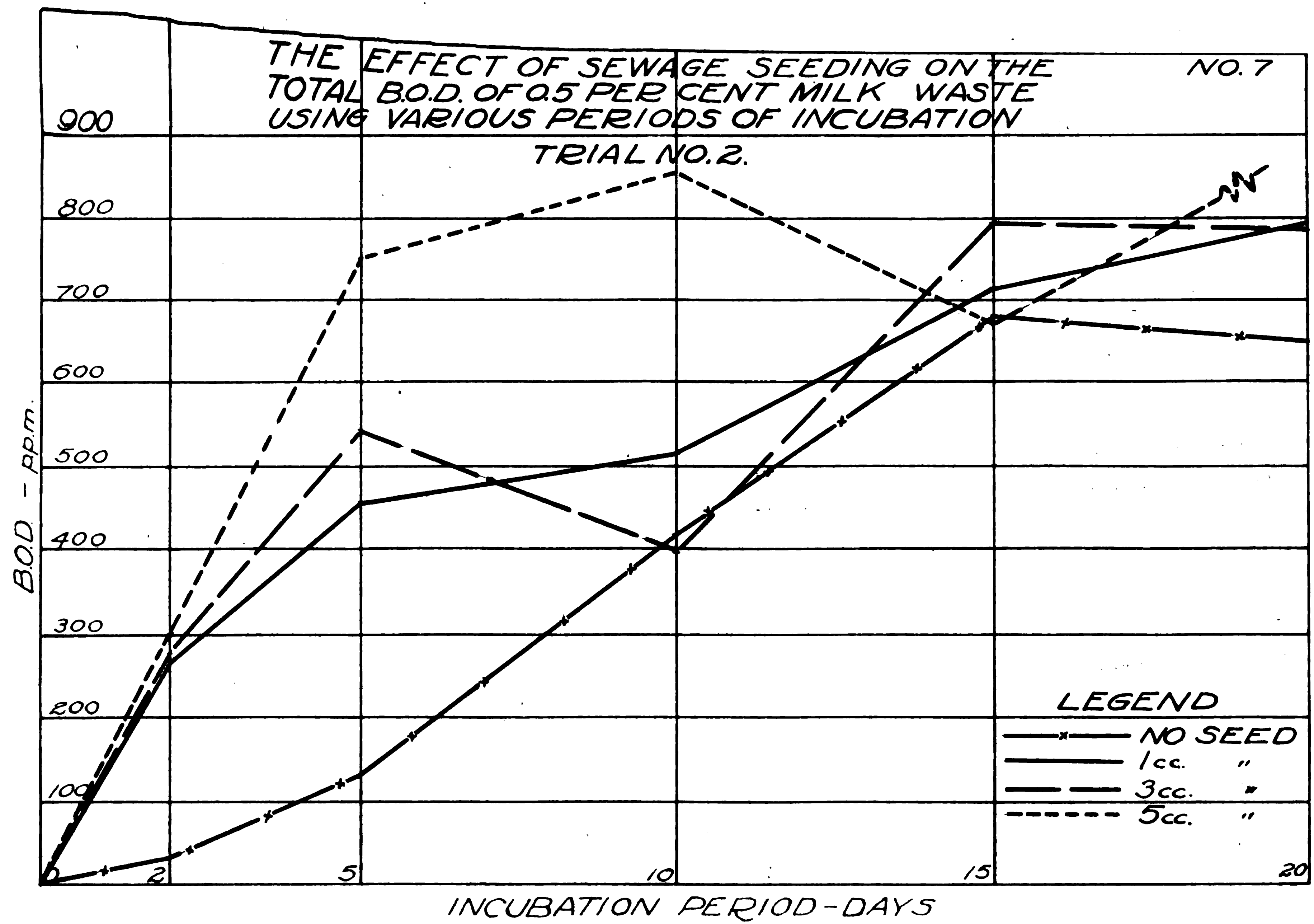

NO.2
TRIAL NO.1
TOTAL B.O.D. OF 0.5 PER CENT
MILK WASTE
900
800
700
600
500
400
300
200
100
0
BOD - PPM
1
2
3
4
5
CC. SEWAGE SEEDING
LEGEND
2-DAY
5-DAY
10-DAY
15-DAY
20-DAY

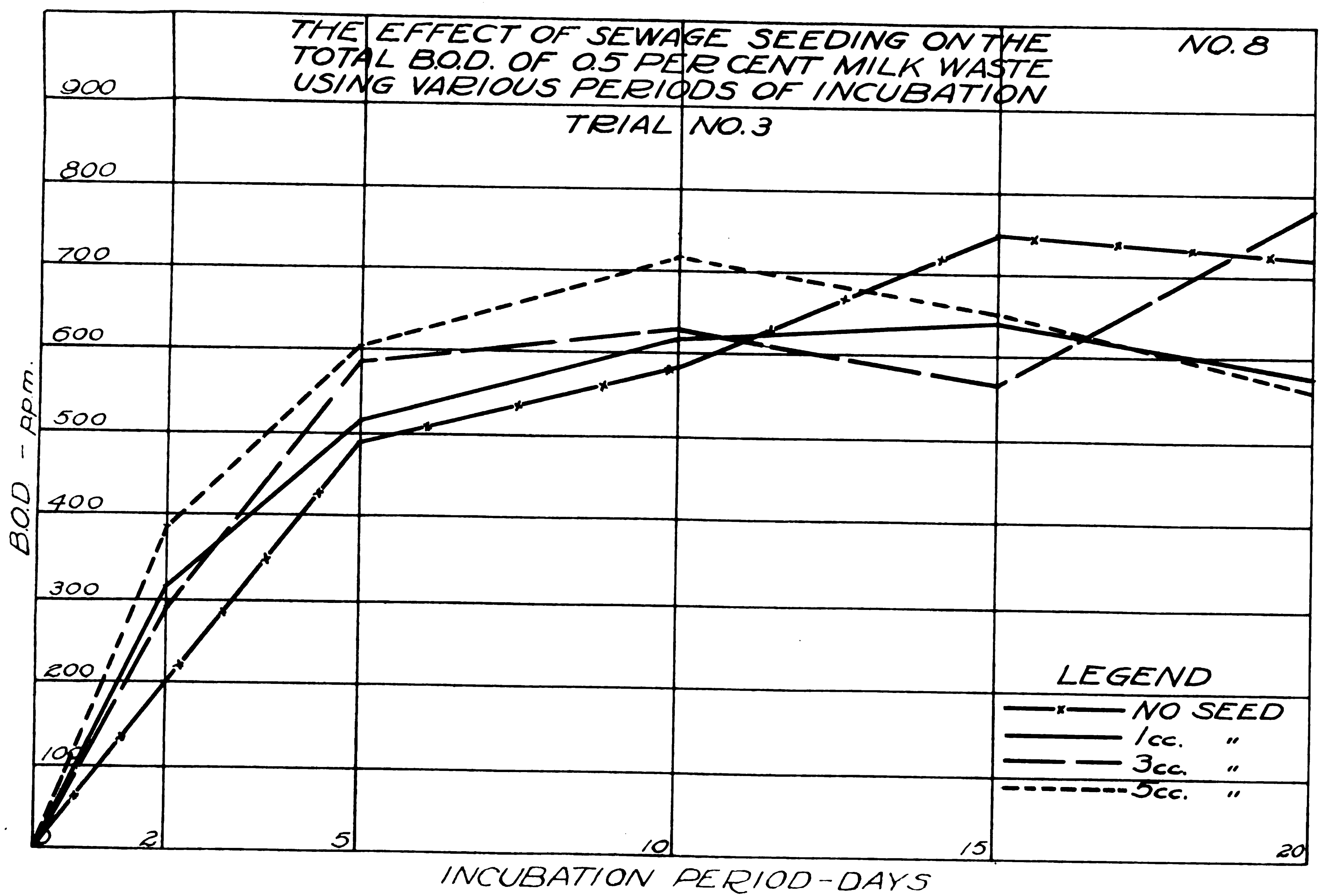

NO. 3
TRIAL NO.2
TOTAL B.O.D. OF 0.5 PER CENT
MILK WASTE
900
800
700
600
500
400
300
200
100
0
BOD - PPM
1
2
3
4
5
CC SEWAGE SEEDING
LEGEND
2-DAY
5-DAY
10-DAY
15-DAY
20-DAY

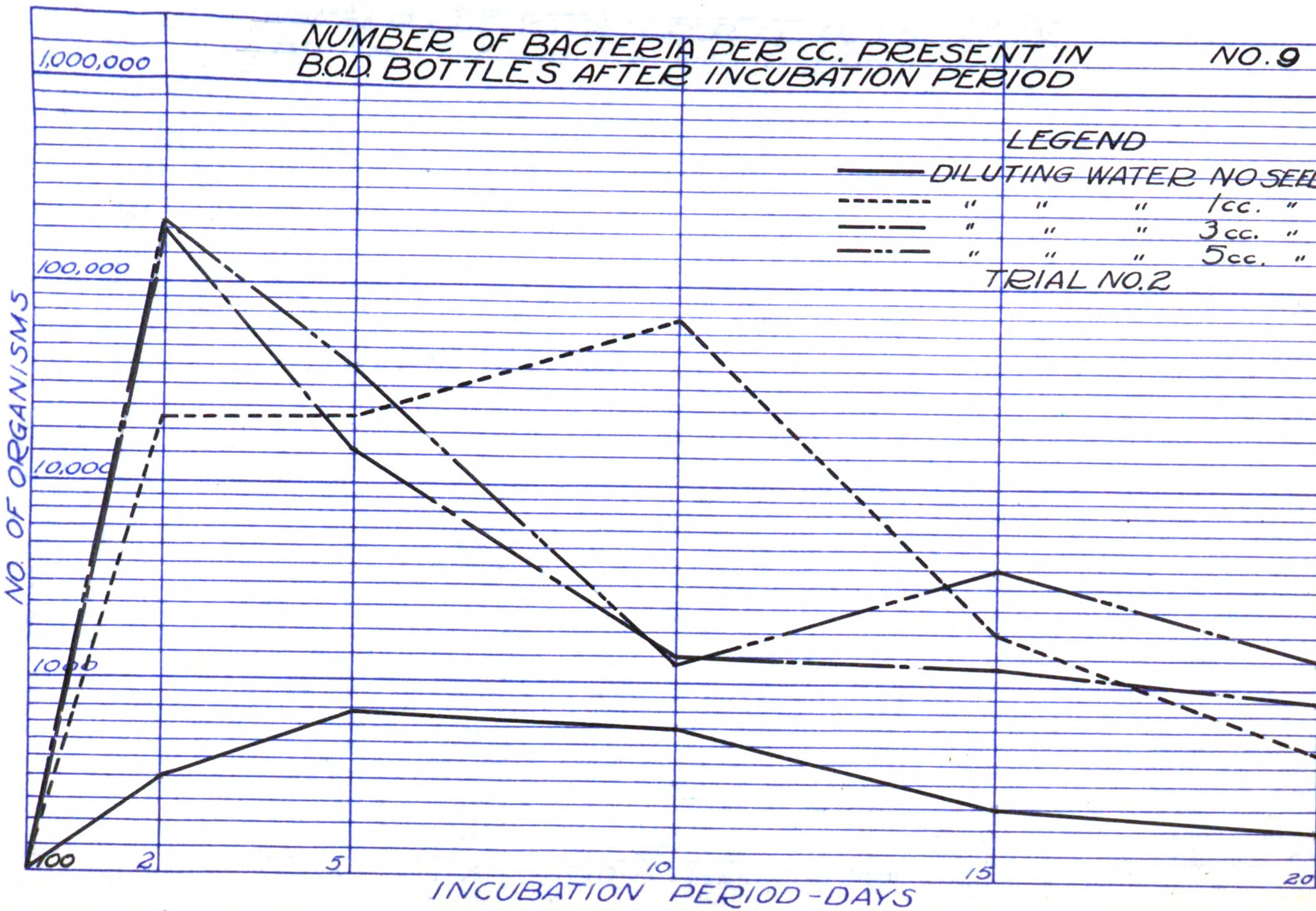

TRIAL NO.3
NO.4
TOTAL B.O.D. OF 0.5 PER CENT
MILK WASTE
900
800
700
600
500
400
300
200
100
0
BOD - PPM.
1
2
3
4
5
CC SEWAGE SEEDING
LEGEND
2-DAY
5-DAY
10-DAY
15-DAY
20-DAY

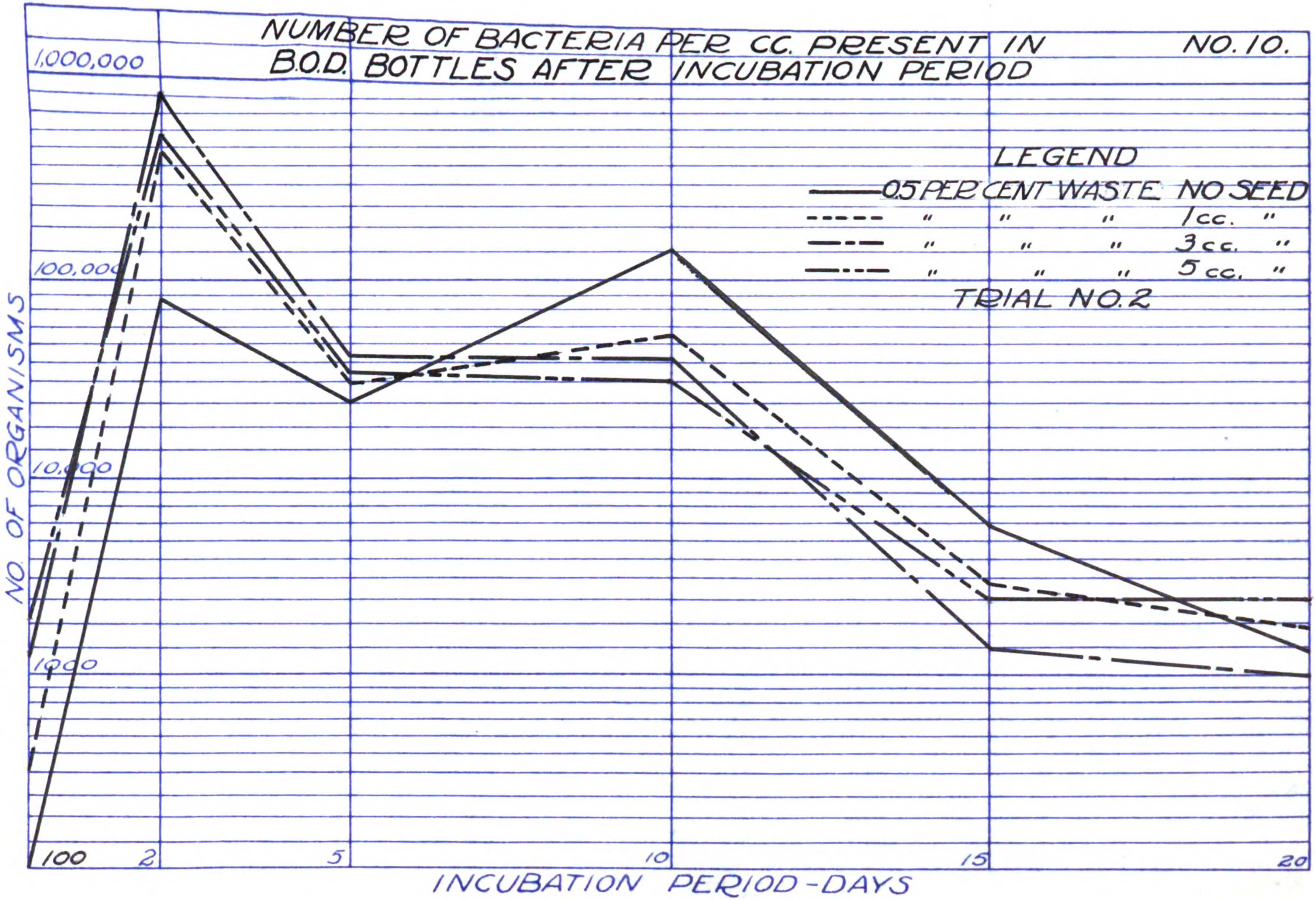

THE EFFECT OF SEWAGE SEEDING ON THE TOTAL B.O.D. OF 0.5 PER CENT MILK WASTE USING VARIOUS PERIODS OF INCUBATION
(AVERAGE)
NO. 5
B.O.D. - p.p.m.
900
800
700
600
500
400
300
200
100
2
5
10
15
20
INCUBATION PERIOD-DAYS
LEGEND
NO SEED
1 cc. "
3 cc. "
5 cc. "


THE EFFECT OF SEWAGE SEEDING ON THE
TOTAL B.O.D. OF 0.5 PER CENT MILK WASTE
USING VARIOUS PERIODS OF INCUBATION
(TRIAL NO.1.)
NO.6
900
800
700
600
500
400
300
200
100
0
2
5
10
15
20
B.O.D. - p.p.m.
INCUBATION PERIOD-DAYS
LEGEND
NO SEED
1 cc. "
3 cc. "
5 cc. "

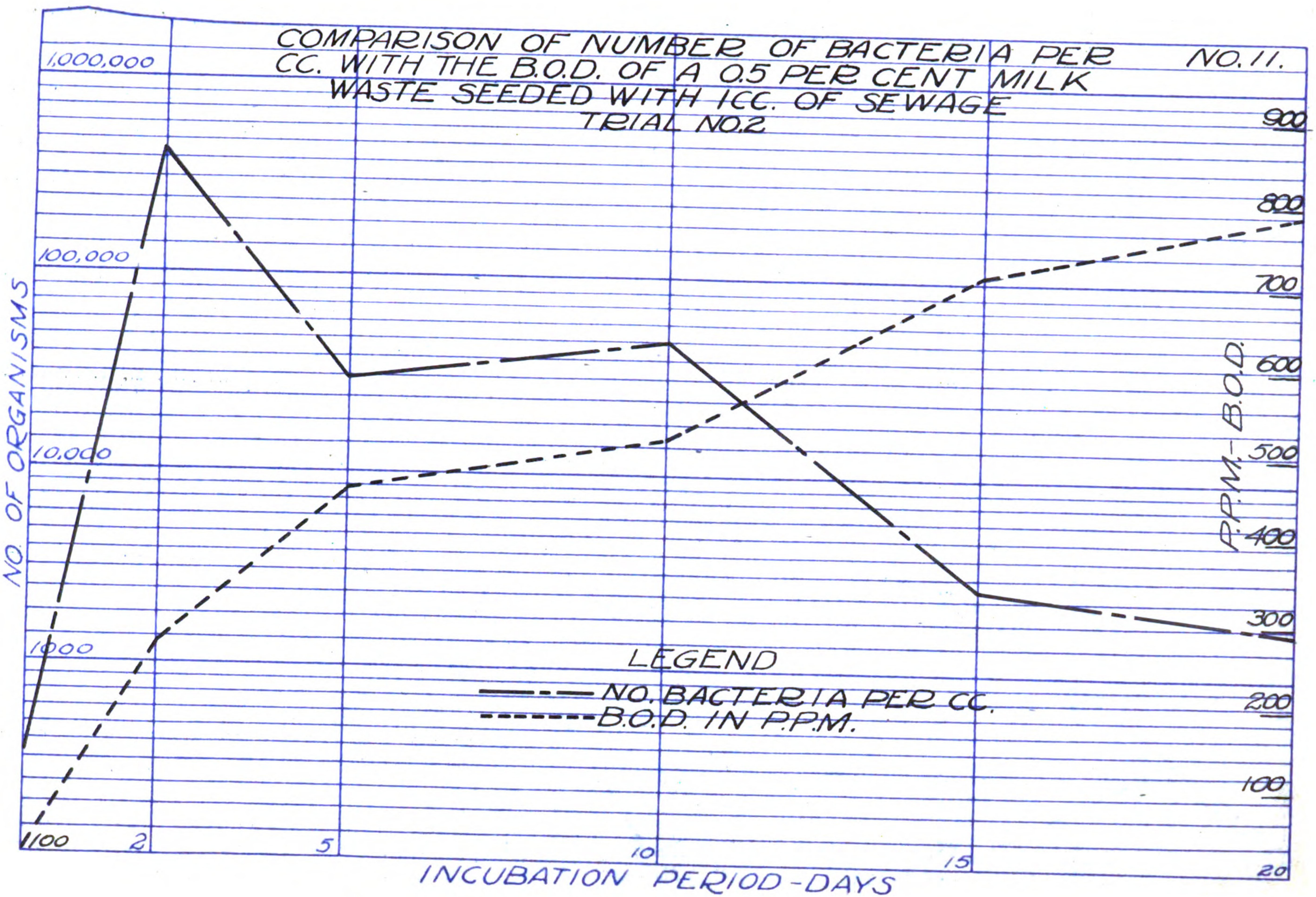

THE EFFECT OF SEWAGE SEEDING ON THE TOTAL B.O.D. OF 0.5 PER CENT MILK WASTE USING VARIOUS PERIODS OF INCUBATION
NO. 7
TRIAL NO. 2.
B.O.D. - p.p.m.
900
800
700
600
500
400
300
200
100
0
2
5
10
15
20
INCUBATION PERIOD-DAYS
LEGEND
NO SEED
1cc. "
3cc. "
5cc. "

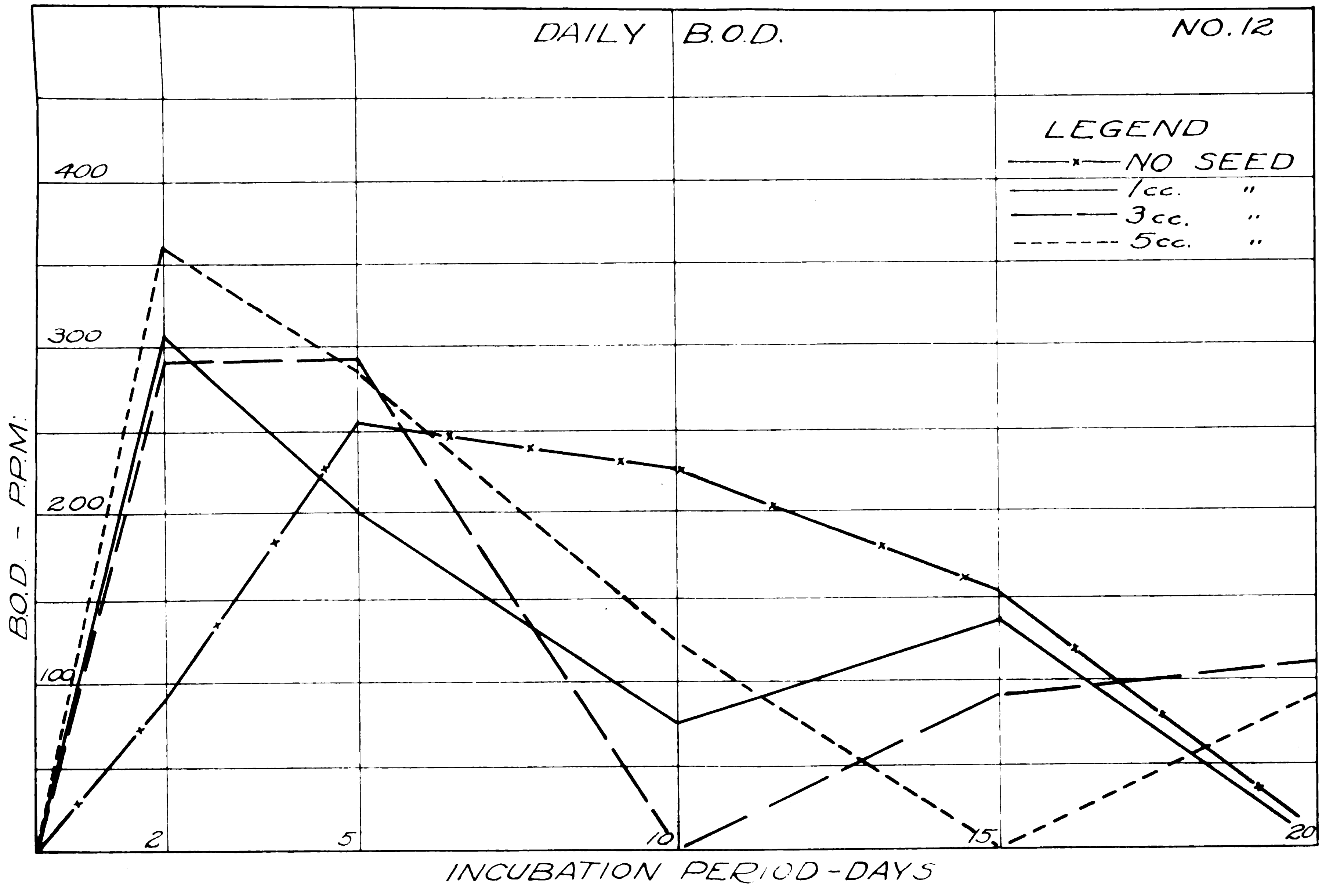

THE EFFECT OF SEWAGE SEEDING ON THE
TOTAL B.O.D. OF 0.5 PER CENT MILK WASTE
USING VARIOUS PERIODS OF INCUBATION
NO. 8
TRIAL NO. 3
B.O.D - ppm.
900
800
700
600
500
400
300
200
100
0
2
5
10
15
20
INCUBATION PERIOD-DAYS
LEGEND
NO SEED
1cc. "
3cc. "
5cc. "


NUMBER OF BACTERIA PER CC. PRESENT IN
B.O.D. BOTTLES AFTER INCUBATION PERIOD
NO. 9
LEGEND
DILUTING WATER NO SEED
" " " 1cc. "
" " " 3cc. "
" " " 5cc. "
TRIAL NO. 2
1,000,000
100,000
10,000
1000
100
2
5
10
15
20
NO. OF ORGANISMS
INCUBATION PERIOD-DAYS



NUMBER OF BACTERIA PER CC. PRESENT IN B.O.D. BOTTLES AFTER INCUBATION PERIOD
NO. 10.
LEGEND
0.5 PER CENT WASTE NO SEED
" " " 1cc. "
" " " 3cc. "
" " " 5cc. "
TRIAL NO. 2
1,000,000
100,000
10,000
1000
100
NO. OF ORGANISMS
2
5
10
15
20
INCUBATION PERIOD - DAYS


COMPARISON OF NUMBER OF BACTERIA PER
CC. WITH THE B.O.D. OF A 0.5 PER CENT MILK
WASTE SEEDED WITH 1CC. OF SEWAGE
TRIAL NO.2
NO. 11.
1,000,000
100,000
10,000
1000
NO. OF ORGANISMS
900
800
700
600
500
400
300
200
100
P.P.M.-B.O.D.
LEGEND
NO. BACTERIA PER CC.
B.O.D. IN P.P.M.
1
100
2
5
10
15
20
INCUBATION PERIOD-DAYS


DAILY B.O.D.
NO. 12
LEGEND
NO SEED
1cc. "
3cc. "
5cc. "
400
300
200
100
B.O.D. - P.P.M.
2
5
10
15
20
INCUBATION PERIOD-DAYS

DISCUSSION

It is clearly shown by graph No. 1, which is a composite of graphs No. 2, No. 3 and No. 4, that for the 2, 5 and 10 day incubation periods there is a marked increase in the B. O. D. of the waste with an increase in the amount of seeding materials. The curves for the 15 and 20 day incubations have shown a tendency to flatten out and even to decline with the amount of seeding. Such decline cannot be explained by the writer.

Graph No. 5, being a composite of graphs No. 6, No. 7 and No. 8, shows again that the B. O. D. of a 0.5 per cent milk waste varies directly as the amount of seeding nearly up to the tenth day of incubation. From this point on, the curves tend to come closer together and some even cross each other. The fact that the curve for the five cc. of seeding declines between the tenth and fifteenth days of incubation cannot be explained. It is apparent that the five day B. O. D. results are not dependable because of the wide variations in values from 345 to 635 p.p.m. for the same waste with amounts of sewage seeding varying from nothing to 5 cc.

Graph No. 9 shows the number of bacteria per cc. present in the B. O. D. bottles after the incubation period for the bottles which contained only diluting water and seeding material. The curves representing the growth in the bottles with no seed and with 1 cc. of seed show the typical lag in the growth to maximum number of organisms, while the other curves show a rapid increase in the first two days and a gradual decline over the remainder of the period. The lag in the development of growth in the samples with no seed and a small amount of seed is present but not so pronounced in the dilutions of the 0.5 per cent milk waste as shown by graph No. 10.

Graph No. 11 compares the number of bacteria with the B. O. D. of a sample containing 1 cc. of sewage seeding, over the twenty-day incubation period. It shows how the number of organisms present in a B. O. D. bottle increases rapidly to a maximum in the first one or two days, then as the relative amount of food per organism decreases the bacterial population must and does decrease steadily over the remainder of the incubation period. As the number of organisms decrease because of a decrease of food or oxidizable organic matter the daily B. O. D. values decrease. In other works the increment of increase in B. O. D. values decreases during the incubation period. This fact is clearly shown by graph No. 12 which gives the increment of increase of B. O. D. values for each day, over the value for the preceeding period of incubation.

CONCLUSIONS

1. With this waste selected it was found that the B. O. D. increased in direct proportion to the amount of sewage seeding.

2. The bacterial population of the B. O. D. samples

was found to increase rapidly in the first one or two days to a maximum then gradually drop as the incubation period proceeded.

3. From the results obtained no definite amount of seeding can be said to be the proper amount to give the exact value of the B. O. D. of this waste.

4. It is quite certain that by using the 5 day B. O. D. values we are liable to a wide variation in results as clearly shown by graph No. 5.

5. It was found that the daily B. O. D. curves follow the same general trend as do the curves for the number of organisms present in the B. O. D. bottles as shown by comparing graphs No. 10 and No 12.

TABULATED DATA

Trial No. 1.

Seeding material count - 33,000 bacteria per cc.

0.5 per cent milk waste

Days incu-bation	B. O. D. - p.p.m. No seed	1cc. seed	3cc. seed	5cc. seed
2	44	323	230	368
5	420	552	533	520
10	722	608	636	704
15	723	812	556	412
20	734	604	732	560

Trial No. 2
Seeding material count - 110,000 bacteria per cc.

0.5 per cent milk waste

Days incu- bation	B. O. D.-p.p.m. No seed	1cc. seed	3cc. seed	5cc. seed
2	36	263	272	300
5	132	456	540	752
10	416	516	392	856
15	696	716	726	653
20	643	792	734	830

Days incu- bation	No. of Bacteria per cc. No seed	1cc. seed	3cc. seed	5cc. seed
2	84,000	550,000	900,000	690,000
5	30,000	40,000	53,000	45,000
10	150,000	66,000	51,800	40,000
15	6,900	3,600	1,600	3,000
20	1,500	2,000	1,000	3,000

Trial No. 3

Seeding Material Count - 93,000 bacteria per cc.

0.5 per cent milk waste

Days incu- bation	B. O. D. - p.p.m. No seed	1cc. seed	3cc. seed	5cc. seed
2	200	312	296	334
5	433	512	533	604
10	534	616	623	720
15	743	640	564	643
20	724	576	734	560

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