THERMAL PROCESSING

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Abstract

The effect of temperature on the thermal destruction rate of P.A.3679 in cured ham medium was measured.

Ham containing 2.8% salt, 200 ppm sodium nitrite and 0.5% sodium tripolyphosphate was inoculated with P.A.3679, stuffed into tubes, sealed and heated in steam at 100-121.1°C for various time periods. The number of surviving spores was determined using the most probable number technique.

D values were determined at 5 temperatures for a number of spore crops. Logarithms of these D values were plotted against temperature to obtain Z values of 8.6-10.5°C.

Omission of salt, nitrite, phosphate or all three from the media did not appreciably effect the D value at 115.2°C.

Ham at various levels of salt, sodium nitrite and pH were inoculated at a level of 10⁵ spores P.A.3679/gm and heated at 115.2°C for 30 minutes. These were examined periodically for outgrowth and the time at which this occurred was recorded. The percentage of the tubes which did not exhibit visible signs of outgrowth after 150 days was plotted against pH with salt at 2.6% and nitrite at 150 ppm; against salt with pH at 5.8 and 6.4 and nitrite at 150 ppm; against nitrite with pH at 5.8 and 6.4 and salt at 2.6%.

Outgrowth increased with pH increase, decreased with salt and nitrite increase. The effect of nitrite in the range of 0-400 ppm was similar to the effect of salt in the range of 0-4%. The effect of pH in the range of 5-7 was greater than that of either the salt or nitrite concentration.

SCHINKENHERSTELLUNG UNTER HITZE

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Zussammenfassung

Die Wirkung von Temperatur auf die Zerstoerungsgeschwindigkeit von P.A. 3679 wurde im eingepoekelten Schinken gemessen.

Schinken, der 2.8% Salz, 200 ppm Chilesalpeter, und 0.5% Tripolyphospat enthaelt, wurde mit P.A. 3679 inokuliert, in Reagenzglaeser verpackt, verschlossen und mit Dampf auf 100 - 121.1°C fuer verschiedene Zeitspannen erhitzt. Die Anzahl der uerberlebenden Keime wurde mit der "Wahrscheinlichkeitsnummer" (most probable number technique) bestimmt.

D-Werte wurden bei 5 Temperaturen fuer eine Anzahl von Keimen ermittelt. Logarithmen von D-Werten wurden gegen Temperaturen ausgewertet, um die Z-Werte von 8.6 - 10.5°C zu erhalten.

Der Entzug von Salz, Nitrit, Phosphat (einzeln oder gemeinsam) vom Schinken hatte keine besondere Wirkung auf die D-Werte bei 115.2°C.

Schinken mit verschiedenem Gehalt an Salz, Nitrit, und verschiedenen pH-Werten wurden mit einem Gehalt von 10⁵ Keimen P.A. 3679/gm inokuliert und auf 115.2°C fuer 30 Minuten erhitzt. Diese Proben wurden regelmaessig auf Auswuchs untersucht und die Zeiten vermerkt.

Der Prozentsatz der Reagenzglaeser der keine bemerkenswerte Anzeichen von Auswuchs nach 150 Tagen zeigte, wurde folgendermassen ausgewertet:

> pH bei 2.6% Salz und 150 ppm Nitrit Salz bei 5.8 und 6.4 pH und 150 ppm Nitrit Nitrit bei 5.8 und 6.4 pH und 2.6% Salz

Der Auswuchs erhoehte sich mit hoeherem pH Wert und wurde niedringer mit hoeherem Salz- und Nitrit-Gehalt. Die Wirkung von Nitrit im Bereich von 0 - 400 ppm war der Wirkung von Salz im Bereich von 0 - 4% aehnlich. Die Wirkung von pH im Bereich von 5 - 7 war groesser als die der Salz- oder Nitrit-Konzentration.

PROCEDE THERMIQUE

On a étudié l'influence de la température sur le taux de destruction thermique des spores de l'anaérobie putréfactif 3679 inoculées dans un milieu de culture renfermant du jambon fumé.

Le jambon contenant 2.8% de sel, 200 ppm de nitrite de sodium et 0.5% de tripolyphosphate de sodium fut inoculé avec les spores, distribué dans des tubes, scellé et porté aux températures de 100°C à 121.1°C dans un autoclave pour différentes périodes de temps. Le nombre des spores viables fut determiné au moyen de la procédure du nombre le plus probable.

Les valeurs D furent déterminées à 5 températures sur un nombre d'échantillons sporaux. Le graphique des logarithmes de ces valeurs en fonction de la température a permis de déterminer des valeurs Z de 8.6°C à 10.5°C.

L'omission de sel, nitrite, phosphate ou de tous les trois ingredients du milieu de culture n'a pas changé sensiblement la valeur D à 115.2°C.

Le jambon renfermant d'autres concentrations de sel et de nitrite de sodium à différent pH fut inoculé avec les spores (10⁵/g) et chauffé à 115.2°C pour 30 minutes. La croissance des cellules a été suivie périodiquement et le début en fut noté. On a mis en graphique le pourcent des tubes ne montrant aucune croissance visible après 150 jours contre le pH avec le sel à 2.6% et le nitrite à 150 ppm, contre le sel avec le pH à 5.8 et 6.4 et le nitrite à 150 ppm; et contre le nitrite avec le pH à 5.8 et 6.4 et le sel à 2.6%.

La multiplication cellulaire s'est accentuée avec l'augmentation du pH et a diminué avec une augmentation de la concentration de sel et de nitrite. L'effet du nitrite à des concentrations de 0 à 400 ppm fut semblable à l'effet du sel dans l'écart de 0 a 4%. Un pH variant 5.0 a 7.0 eut un plus grand effet que le sel ou le nitrite.

ТЕПЛОВАЯ ОБРАБОТКА

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Резиме:

Влияние температуры на степень теплового уничтоженил вызывающего гниение Анаэроба 3679 /ВГА 3679/ в среде законсервированной ветдины.

В ветчине, содержащей 2,8% соли, 20 мг/100 г нитрит натрия и 0,5% триполифосфат натрия, произвели посев ВГА 3679. Ветчина была расфасована в герметически закупоренные тюбики и подверглась подогреванию паром при температуре 100-121,1°С в течение различных отрезков времени. Число выживших спор было определено путем применения наиболее вероятных числовых поисмов.

Величины D определнлись при пяти температурах для несмольких урожнев спор. Лэгарифмы величин D были нанессны на график в соответствии с температурой, чтобы получить величину Z в пределах 8,6-10,5°C.

Исключение соли, нитрита, фосфата или всех трех заметно не повлияло на величину при 115,2°C.

В ветчине с разным содержанием соли, нитрит натрия и рН произвели посев ВГА 3679/г на уровне 10⁵ спор/г и подогревали при температуре 115,2°С в темение 30 минут. Периодически проверялось развитие культуры и отмечалось время этого развития. Процент тюбиков, которые не показали наглядного роста поеле 150 дней, был нанесен на график в соответствии к рН при 2,6% соли и при 15 мг/100 г нитрита; в соответствии соли к рН при 5,8 и 6,4 и при 15 мг/100 г нитрита; в соответствии нитрита к рН при 5,8 и 6,4 и при 2,6% соли.

Рост увеличился с повышением рН, уменьшился с увеличением соли и нитрита. Иличние нитрита в пределах 0-40 пг/100 г было подобным влиянию соли в пределах 0-4%. Влияние рН в пределах 5-7 было большим, чем концентрация соли или нитрита.

Experimental

1. Preparation of Spore Suspension (PA3679)

A stock culture of PA3679 was obtained from the National Canners Association, Washington, D.C. The following medium was used to prepare the spore suspension.

Beef hearts were cleaned of all visible fat and ground to the consistency of hamburger. One litre of distilled water was then added per pound of heart meat and the mixture simmered for one hour. The heated broth was then pressed through cheesecloth to separate the meat particles. The broth was chilled and the solidified fat particles were removed by filtration through cheese-cloth. The pH of the broth was then adjusted to 8.5 with sodium hydroxide. To each litre of the infusion broth was added the following ingredients:

pressed beef heart particles	65.0 g
hydrogen-reduced iron powder	5.0 g
tryptone	10.0 g
Gelatine	10.0 g
Glucose	0.5 g
potassium phosphate	4.0 g
sodium citrate	3.0 g
isoelectric casein	5.0 g

The following quantities of medium were then added to 4 containers:

- (1) 100 ml
- (2) 500 ml
- (3) 1000 ml, and
- (4) 2000 ml,

and then sterilized at 121°C for 20 minutes.

A 24 hour culture of the stock spore suspension (heat shocked at 100°C for 5 minutes) was inoculated into container 1 and incubated 24 hours at 36°C.

After incubation the first container was inoculated into container No. 2. This progression of inoculations continued to container No. 4. The final culture was transferred to 24°C for incubation and stored until a large percentage of the bacterial cells had sporulated (17 days).

The culture was then filtered through cheesecloth using sterile precautions. The spores were harvested from the filtrate by centrifugation (6000 rpm for 30 minutes). The harvested spores were then washed 4 times in sterile tap water.

Counts were determined on the spore suspensions by heating at 100°C for 5 minutes and subculturing in WYNNE medium (Difco) using a 5-tube Most Probable Number (MPN) procedure.

2. Choice of Tube Size and Heating Bath

The tube size large enough to provide a 1 gm sample size but small enough to seal easily and to quickly reach the temperature of the bath was desired.

It is difficult to determine the internal temperature of a meat emulsion in a small tube due to the difficulty of centering the thermocouple.

Heat penetration measurements were therefore conducted in three larger tubes and the Fh value calculated for the smaller tubes from the formula (1).

$$Fh = \frac{0.398}{[1/a^2) + (0.427/b^2)]} k$$
 (minutes)

Where a = radius of cylinder

b = 1/2 length of cylinder

k = thermal diffusivity

Tubes with diameters of 2.43 cm, 1.95 cm and 1.62 cm were filled with ground ham meat containing 20% pickle, using a stuffing horn. A rubber stopper was then fitted with a thermocouple as shown in Diagram I. The filled tubes were heated in a water bath at 85°C, or in an oil bath at 110°C. From this data the Fh values were obtained and substituted into the formula (1) to obtain the values for k in water and in oil. These k values were then transposed back into the formula and Fh values calculated for various tube diameters.

The Fh value was plotted as a function of tube diameter in Graph 1 for both oil and water. It was estimated from this graph that a tube of 1 cm diameter has an Fh value of 0.9 min. in water. The Fh value was 1.5 min., in oil, a value too high for satisfactory control of heat input at the higher temperatures.

It was established that steam at 115.5°C gave an Fh value similar to water. Steam was therefore chosen as the heating medium.

3. Thermal Death Time Apparatus

A thermal death time apparatus was required which would be capable of rapid change in temperature and able to hold that temperature precisely when attained. A pilot plant retort was used as a steam source. A normal household pressure cooker was modified as shown in Diagram II to provide the steam chamber for heating. A 3-way valve and a spray nozzle in the lid was provided for rapid chilling.

The Fh value obtained from the curves compares very closely with that obtained when heating in a water bath. In all subsequent tests this thermal death time heating apparatus was used.

4. Ferric Citrate Addition

Ferric citrate was tested as an indicator of $\rm H_2S$ production presumably caused by outgrowth during storage of the tubes after heating. An indicator which would give a colour change was desired so that outgrowth would be evident on visual examination.

Ferric citrate (0.2 g/kg meat) was added to a cured meat suspension containing 106 PA 3679 spores/g. Some tubes of this emulsion were given a severe cook (Fo=3) while others were pasteurized (heated to 71°C) then stored at room temperature. The pasteurized tubes turned black indicating the production of H₂S, while the sterilized tubes remained clear.

In subsequent tests ferric citrate was included in the medium.

5. Thermal Death Rate Studies

A series of tests were conducted to determine the number of organisms surviving after heating for various lengths of time at 100-121°C. The following procedure was used in all cases.

In order to obtain a uniform meat (pork) source for a series of experiments the lean meat was removed from 3 hams (uncured). The muscles were cut into 1 inch cubes, mixed well, then divided into 400 g lots and frozen until required.

To 400g of thawed pork cubes was added 100 g of pickle containing:

sodium chloride - 140 g/litre sodium nitrite - 1 g/litre sodium tripolyphosphate - 25 g/litre

This was held at approximately 5°C for 3 days then ground twice through a 1/8 inch plate.

An aliquot of the spore suspension, giving a concentration of <u>ca</u> 10⁶ PÅ3679 organisms/g, was added slowly to the ham emulsion while mixing in a small Hobart mixer. Similarly, 1% ferric citrate solution was also added to give 0.2 g ferric citrate/kg emulsion.

Tubes were weighed, 1/2 filled with emulsion and reweighed. The open ends were sealed by heating quickly in an oxygen/gas flame and crimping with forceps. The tubes were then heated for various times at temperatures of 100°C, 104.3, 110, 115.5, and 121°C. They were chilled immediately after processing by a cold water spray then immersed in ice-water to prevent the premature outgrowth of spores. They were then placed in cold 75% ethyl alcohol for 10 minutes prior to sampling. Control tubes were heated at 82.2°C for 10 minutes to activate spores prior to sampling.

A 1/100 dilution of the emulsion in the tube was prepared by adding the necessary amount of sterile distilled water to a sterile Waring Blender jar. The tube containing the emulsion was then removed from the alcohol container, flamed and placed in the blender containing sterile water and blended at high speed for 2 minutes.

After blending, appropriate dilutions were made in sterile distilled water (depending on the degree of heat treatment and on the duration of treatment).

Screw cap tubes containing 20 ml of WYNNE medium were inoculated from the appropriate dilutions in the amounts of 1, 0.1 and 0.01 ml. Sterile sodium bicarbonate solution (10%) was added (0.2 ml) to each tube at the time of inoculation. Thus, there were 5 tubes each inoculated with 1 ml of the first dilution, 5 tubes each with 0.1 ml and 5 tubes with 0.01 ml.

All tubes were incubated at 36°C for 5 days at which time they were examined for turbidity, gas production and putrid odour. The number of tubes showing growth for each dilution of the sample was noted and the numbers of spores in the sample estimated from the Most Probable Number table.

The number of survivors was determined in duplicate (2 tubes) at each time/temperature condition and the data plotted graphically.

The D value was determined for each temperature.

The logarithm of the D value was then plotted against temperature to obtain the Z value.

This procedure was repeated using a number of spore suspensions. In come cases phosphate, salt or nitrite was omitted. The data obtained is given in Tables I, II, and III.

6. Outgrowth Studies

The aim of this phase of the test series was to study the effect of pH, salt, and nitrite on the probability of outgrowth of spores of PA3679 heated in ham medium.

Pork from ham legs was mixed with pickle as previously described in all cases using 100 gms pickle to 400 gms meat and 0.2 gm ferric citrate/kg emulsion. The amount of salt and nitrite in the pickle was varied over a range of 0-4% and 0-400 pm respectively.

For those experiments in which the effect of pH was being studied, adjustment was made by the addition of 8% NaOH or 14% HCl as required while mixing in the Hobart mixer. The amount of water so added was compensated for by addition of less water in the pickle.

In all cases samples were inoculated at a level of 10^5 spores/gm with spore suspension containing about 10^7 spores/gm. This emulsion was stuffed into glass tubes as previously described and heated 30 minutes at 115.5° C. For each of the experiments, about 200 tubes were used to give 50 tubes per condition all inoculated with the same spore suspension. The tubes were held at 30° C and examined visually periodically for evidence of outgrowth. A log was kept of the number of tubes which spoiled and the time at which spoilage occurred. The data is contained in Table IV. Visual evidence of spoilage was confirmed microbiologically. Samples which did not show visual evidence of spoilage after 300 days were found to be microbiologically sterile.

Discussion

Thermal Destruction

D values at 121°C varied fairly substantially from suspension to suspension. The $D_{121^{\circ}\text{C}}$ value of suspension #1 for instance was 0.9 minutes while that of suspension #8 was 5.5 minutes. The reason for this was not clear. When Z values, however, were calculated from the data (Table II), they were reasonably consistent ranging from 8.6°C to 10.5°C about an average of 9.7°C . This is near reported values of 10°C .

The D values at 115.5°C did not vary substantially with levels of salt, nitrite or phosphate. The data is contained in Table III.

Outgrowth Studies

Table IV contains the data obtained from the outgrowth studies. Observation of this data shows that outgrowth occurred according to a pattern in which there was a lag period followed by rapid outgrowth of a certain finite percentage of the tubes. After a period of time further outgrowth did not occur and a percentage of the tubes remained stable even after 150 days.

In order to display this more clearly the data has been grouped to show the outgrowth with respect to time for various levels of ultimate outgrowth. Table V shows the actual numbers of remaining tubes, and Graph II shows the percentage of remaining tubes. The curves display a typical lag period prior to an outgrowth period. There is then a flattening of the curve and after 100 days almost no further spoilage occurs. The lag period was longer for those tests in which very limited outgrowth occurred. There was also a longer period during which growth occurred. Thus for instance the curve representing 60-79% remaining stable at 50 days shows a considerable rate of spoilage up to 100 days while the 0-19% group has practically ceased growing out at 50 days.

These data can be used to calculate the probability of outgrowth using the equation

$$P_{i,j} = \frac{(N_{ij} - N_i + 1,j)}{(d_i - d_i + 1)N_{i,j}}$$

Table VI shows the definition of j, the percent of tubes remaining at 50 days.

This gives the probability of outgrowth on a perday basis, for tubes existing at the beginning of interval i, and which occurs before the end of the interval. $N_{i,j}$ and $N_{i+1,j}$ are the number of surviving tubes at the beginning of the ith interval and at the beginning of the (i+1)th interval. Simarly, d_i and d_{i+1} are the number of days from the start of the experiment to the beginning of these two intervals. These probabilities were plotted at the midpoints of the intervals because they apply during that interval. This is shown in Graph III. It is interesting to note that the probability increases from time zero to a maximum at about 12-13 days regardless of the group being considered. This probability then falls off to a near zero level at about 150 days.

Effect of pH on Outgrowth

The experiment was run in blocks of four determinations. Each determination comprised approximately 50 tubes, for a total of about 200 tubes per block. This was the maximum that could be handled at one time. When preparing to stuff the tubes, an emulsion was made up, inoculated with the spore suspension and mixed thoroughly. It was then divided into four approximately equal portions, and each portion adjusted to the desired pH level. The tubes were then stuffed and sealed as described previously and all cooked together in the pressure vessel. In this way, the only difference between the four determinations was in the pH adjustment. The emulsion, spore suspension, cooking time and temperature were the same within blocks. Therefore, the block of four determinations could be expected to be more consistent in response relative to each other.

The results have been plotted on the attached Graph IV and the four determinations forming each block have been joined by straight lines. The pattern of the response is clearly evident from this graph. Very little spoilage occurred at the low pH levels (i.e. below 5) and almost complete spoilage at higher pH (i.e. above 7). At intermediate pH levels, the spoilage varied considerably between tests. For example, at pH 6.0, spoilage varied from 0 to 96% at 150 days.

The average response was estimated in the range from 5 to 7 pH units as follows. Six levels of pH were selected including 5 and 7 and in steps of 0.4 pH units between. At each of the pH levels, a vertical line was drawn on the graph. Where the straight lines for each block of determination crossed, the spoilage was read from the graph. These values were averaged and the averages have been plotted on the graph.

Effect of Nitrite on Outgrowth

The effect of nitrite was investigated at two levels of pH, namely 5.8 and 6.3. The experimental design was very similar to the pH series. Within each block of four determinations, the only difference was in the amount of sodium nitrite added.

The attached Graph V shows the results. The averages were determined from the results at 0, 100, 200 and 400 ppm of nitrite, without the necessity of estimating from the graph.

Effect of Salt on Outgrowth

The salt effect was investigated in the same way as the nitrite effect. Despite the variability of the results, the same type of response is clearly evident (see Graph VI). The pH had the main over-riding effect and the nitrite and salt had smaller, but yet clear and indisputable subsidiary effects. The effects of the three factors appear to be additive.

Multiple Regression Analysis (pH, Salt and Nitrite)

Within practical ranges of the factors, the effects

can be considered linear. The following ranges were chosen:

Factor	Range
рН	5.5 - 6.6
Nitrite	0 - 200
Salt	1.5 - 3.0

The percentage spoiled at 150 days was selected for these calculations and all results following within the above ranges were listed. The following was the best-fitting linear equation by the method of least squares:

$$\hat{Y} = -322 + 73.9(pH) - 0.115(N) - 24.7(S)$$

where y =estimated percent outgrowth at 150 days

pH = usual definition

N = nitrite in parts per million (ppM)

S = salt in percentage.

The multiple correlation coefficient was 0.632, and each of the regression coefficients were significant.

The standard error of estimate using the equation was 31.7%, which shows the variability of the results.

Models incorporating interaction terms were fitted to the data because, from the graphs, there was an indication of synergism between nitrite and pH. Models which included this interaction, either as a multiplication between the two factors, or as a division (nitrite/pH), did not improve the fit, except to an insignificant degree. For example, the multiple correlation improved to 0.633 (from 0.632).

Similar regression analysis was applied to the spoilage at 10, 20, 50 and 100 days, and similar results were obtained. In fact, an experiment lasting 50 or 100 days would yield sufficient data to draw valid conclusions regarding spoilage.

The heavy dotted lines or Graphs IV, V and VI show the best-fitting linear regression model.

From the equation derived one might expect a similar increase in inhibitory effect from

- a) A decrease in pH of 0.1 units $(73.9 \times 0.1 = 7.4)$
- b) An increase in nitrite of 65 ppm $(0.115 \times 65 = 7.5)$
- c) An increase in salt of 0.3% (24.7 x 0.3 = 7.4).

Spore Suspension #1

121°C		115.5°C			110°c		104.4°C		100°c	
Time (min.)	Log Survivors	Time (min.)		ivors	Time (min.)	Log Survivors	Time (min.)	Log Survivors	Time (min.)	Log Survivors
0	6.4	0	6.4	5.9	0	6.4	0	6.5	. 0	5.9
0	6.4	0	6.2	5.9	0	6.4	30	4.5	0	5.8
2	4.0	2	5.5	4.9	5	6.1	60	4.2	120	5.5
. 2	4.2	2	5.5	5.1	5	6.4	90	3.7	120	5.7
3	2.9	4	4.5	4.4	10	6.0	120	2.7	240	4.8
3	2.7	4	4.5	4.2	10	6.2	150	1.3	240	5.0
4	1.3	6	3.4	3.5	15	5.5	180	1.3	480	4.3
4	1.3	6	3.5	3.5	15	5.7			480	4.3
		8	3.5	3.0	20	5.2			960	2.3
		8	2.7	3.2	20	5.0			960	2.3
		10	2.4	2.1	25	5.0			1440	1.3
		10	2.5	2.1	25	4.4			1440	1.3
D	0.9		2.5	2.7	(2.6)	12.5		33.6		297
Log D	-0.05		0.	415		1.096		1.526		2.473

 $Z = 8.6^{\circ}C$

TABLE I

	121°C	1	15.5°C		110°C	10	4.4°C		100°C
Time (min.)	Log Survivors								
0	6.4	0	6.4	0	5.9	0	6.4	0	6.7
0	5.9	0	6.0	0	5.9	0	6.4	0	6.7
2	6.2	5	5.7	15	5.9	80	6.2	150	6.4
2	5.7	5	5.5	15	5.9	80	6.2	150	6.4
6	4.5	10	5.5	30	6.0	160	5.5		
. 6	5.0	10	5.4	30	5.4	160	5.5	300	6.0
8	3.7	20	5.0	45	5.2	240	5.5	300	5.7
8	3.0	20	4.4	45	5.2	240	5.5	540	5.4
10	2.9	30	3.7	60	4.9	320	5.0	540	5.5
10	3.0	30	3.7	60	4.9	320	5.0	810	5.2
12	2.7	40	2.4	75	4.4	400	4.5	810	4.7
12	2.5	40	2.5	75	4.4	400	4.1	1290	2.7
14	2.4			100	3.4	480	3.4	1290	2.9
14	1.9			100	3.4	480	3.4	1470	1.8
								1470	1.3
D	3.0		10.9		36.4		158		285
Log D	0.477		1.037		1.561		2.200		2.455

TABLE I

	1	21°C	11	5.5°C	1	10°C	10	4.4°C	1	.00°C
	Time (min.)	Log Survivors								
	0	6.9	0	6.9	0	6.5	0	6.1	0	6.7
	0	6.9	0	6.5	0	6.5	0	6.4	0	7.4
	1.5	5.4	5	5.5	14	6.1	80	6.4	150	6.1
	1.5	5.9	5	5.4	14	5.9	.80	5.7	150	6.7
	4	4.5	10	5.1	28	5.3	170	6.2	300	6.0
	. 4	4.3	10	4.9	28	5.2	170	6.0	300	5.7
-	5	3.3	15	4.4	42	4.5	260	3.9	540	4.9
747	5 5	3.3	15	4.5	42	4.7	260	4.1	540	4.9
1	7	2.7	20	2.9	56	4.2	350	3.7	810	2.3
	7	2.9	20	2.9	56	4.0	350	3.4	810	2.3
	10	2.1	30	2.5	70	2.7	440	2.3	1290	1.6
	10	2.0	30	2.5	70	2.7	440	1.7	1290	1.6
			40	1.3	80	2.4			1500	1.3
			40	1.8	80	2.5			1500	1.3
	D	1.9		7.4		18.6		88.8		228
1	Log D	0.278		0.870		1.270		1.948		2.358

Z = 10.20C

	121°C	115	5.5°C		110°C		104.4°c	
Time (min.)	Log Survivors							
0	6.1	0	5.4	0	5.4	0	5.7	
0	6.1	0	5.7	0	5.7	0	5.5	
2	5.4	5	4.7	10	5.4	60	5.7	
2	4.9	5	4.9	10	5.9	60	5.5	
4	4.4	10	4.5	20	5.1	120	5.0	
. 4	4.4	10	4.7	20	5.1	120	5.2	
6	4.0	15	3.7	30	4.9	210	. 4.4	
6	3.2	15	3.9	30	5.1	210	4.7	
8	3.1	20	2.9	40	4.1	270	3.8	
8	3.1	20	3.3	40	4.4	270	3.4	
10	2.5	30	1.7	60	3.7	360	3.2	
10	2.5			60	4.2	360	3.0	
-12	1.3			80	3.1			
12	1.3			80	3.1			
D	2.6		8.0		28.9		124.3	
Log D	0.414		0.902		1.461		2.095	

 $z = 9.9^{\circ}C$

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- 1244 -

TABLE I

	121°C		115.5°C			110°C		104.4°C		100°c	
	Time (min.)	Log Survivors									
	0	5.7	0	6.0	0	6.2	0	6.1	0	6.4	
	0	5.4	0	6.0	0	6.2	0	6.1	0	6.7	
	2	4.7	5	6.2	10	5.5	60	6.2	120	6.1	
	2	4.5	5	6.2	10	5.7	60	6.2	120	6.7	
	4	3.7	10	5.7	20	5.4	120	5.7	240	6.2	
	4	3.7	10	6.2	20	5.4	120	5.7	240	5.7	
	6	2.7	15	4.4	40	5.0	180	5.0	600	4.5	
	6	2.5	15	4.5	40	4.7	180	4.7	600	4.4	
	8	1.7	20	3.5	50	5.1	240	3.9	1200	2.1	
	8	1.7	20	3.5	60	4.0	240	3.9	1200	1.7	
			30	2.5	60	3.9	300	3.4	1590	1.3	
			30	2.5	80	3.1	300	3.7	1590	1.3	
			40	1.7	80	3.5	360	2.7			
							360	2.7			
	D	2.1		7.2		27.5		92.1		271	
Loc	g D	0.322		0.858		1.439		1.965		2.433	

Z = 10.00C

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Spore	Susper	nsion	#6
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TABLE I

	121°C	1	15.5°C		110°C		
Time (min.)	Log Survivors	Time (min.)	Surv	-	Time (min.)	Log Survivors	
0	5.5	0	5.4	6.1	0	5.4	
0	5.5	0	5.9	5.7	0	5.9	
6	3.5	5	5.4	4.8	60	4.7	
6	3.6	5	5.7	5.2	60	4.3	
9	2.5	10	5.0	5.2	120	4.0	
. 9	2.5	10	5.3	5.1	120	2.9	
12	1.3	15	4.7	4.7	180	2.4	
		15	4.7	4.7	240	1.3	
		20	4.3	4.2	240	1.7	
		20	4.3	4.2			
		25	4.3	2.4			
		25	3.5	2.7			
D	2.9		9	.2		55.5	
Log D	0.462		0	.964		1.744	

Spore	Suspension	#7
-------	------------	----

TABLE I

121°C		13	15.5°C	110°c		
Time (min.)	Log Survivors	Time (min.)	Log Survivors	Time (min.)	Log Survivors	
0	4.4	0	4.6	0	4.7	
0	4.1	0	4.2	45	3.9	
2	4.1	5.	4.1	90	2.8	
2	3.9	5	3.3	135	1.5	
6	2.4	15	2.7			
6	2.3	25	1.9			
10	1.0	25	1.3			
10	1.0					
D	2.9		8.5		41.6	
Log D	0.462		0.93		1.62	

 $z = 9.5^{\circ}C$

Spore Suspension #8

TABLE I

121°C		21°C		115.5°C	110°c		
	me n.)	Log Survivors	Time (min.)	Log Survivors	Time (min.)	Log Survivors	
	0	5.0	0	4.9	0	5.0	
	0	5.2	0	5.2	0	4.7	
	2	4.7	5	5.2	40	4.3	
	2	4.3	5	4.7	40	4.3	
	5	4.0	10	4.3	80	4.3	
	9	3.5	10	4.3	80	4.2	
	9	3.5	20	3.9	120	3.7	
			20	4.2	120	3.5	
			30	2.7	160	2.1	
			30	3.1			
	D	5.5		12.9		59.7	
Log	D	0.74		1.11		1.776	

 $z = 10.5^{\circ}C$

TABLE II (Summary of Table I)

THERMAL DESTRUCTION AT VARIOUS TEMPERATURES

Spore Crop	1	2	3	4	5	6	7	8
D Values					,			
121.10	.9	3.0	1.9	2.6	2.1	2.9	2.9	5.5
115.50	2.6	10.9	7.4	8.0	7.2	9.2	8.5	12.9
1100	12.5	36.4	18.6	28.9	27.5	55.5	41.6	59.7
103.30	33.6	158	88.8	124.3	92.1			
100°	297	285	228		271			
Z Values	(86)	(10.3)	(10.2)	(9.9)	(10)	(8.6)	(9.5)	(10.5)

TABLE III

EFFECT OF NITRITE, SALT, PHOSPHATE ON
D VALUE AT 115.50C

No Ni		, No	Salt	No Pho	sphate	Water	THE RESERVE THE PERSON NAMED IN COLUMN	All P	resent
Log N	Time (min)	Log N	Time (min)	Log N	Time (min)	Log N	Time (min)	Log N	Time (min)
6.23	0	6.11	0	5.4	0	6.11	0	5.4	0
5.85	0	6.40	0	5.7	0	6.04	0	5.7	0
5.74	5	5.23	5	4.54	5	4.98	5	4.7	5
5.23	5	4.54	10	4.23	5	4.98	5	4.9	5
5.2	10	4.11	10	3.7 ,	10	4.11	10	4.5	10
4.54	10	4.40	15	3.54	10	4.4	10	4.7	10
4.74	15	4.15	15	3.23	15	4.04	15	3.7	15
4.44	15	3.40	20	3.04	15	3.9	15	3.9	15
3.95	20	3.54	20	2.54	20	4.2	20	2.9	20
3.54	20	2.7	25	2.23	20	3.54	20	3.3	20
2.90	25	2.7	25	2.40	25	2.4	25	1.7	30
3.04	25	2.54	30			2.7	25		
2.23	30	2.54	30			2.3	30		
2.40	30					1.85	30		

5)

D = 7.8 7.5 7.6 8.0

N = No. of surviving spores

TABLE IV
OUTGROWTH WITH RESPECT TO TIME (IN DAYS)

Expt.	D	NaNo2		1 %				Perc	entag	e of	Tubes	Rema	ining	1	
No.	115.5°C	ppm	рН	Salt	Tubes	5	10	15	20	30	40	50	100	150	365
1	8.5	150 150 150 150	5.8 5.8 5.8	0 1.5 2.6 4.0	56 50 53 52	98 97 99 100	87 12 50 100	79 8 25 94	78 0 22 94	78 18 94	78 17 94	78 17 94	66 6 94	45 0 94	37
2	8.5	150 150 150 150	5.8 5.8 5.8 5.8	0 1.5 2.6 4.0	51 53 51 55	88 100 100 100	38 95 95 96	31 94 85 96	29 94 82 96	29 94 78 96	29 94 78 96	27 94 78 96	10 91 78 96	10 87 76 96	10 83 67 96
.3	8.5	150 150 150 150	5.8 5.8 5.8 5.8	0 1.5 2.6 4.0	53 50 52 59	100 100 100 100	100 99 100 100	100 98 100 100	100 76 100 100	98 68 100 100	98 68 100 100	98 68 100 100	81 34 89 100	69 23 54 100	52 7 23 98
4	12.9	150 150 150 150	5.8 5.8 5.8 5.8	0 1.5 2.6 4.0	25 37 48 33	100 100 100 100	80 95 100 100	60 84 100 100	53 80 100 100	40 78 100 100	24 57 98 100	0 0 90 100	77 94	76 79	50
5	12.9	150 150 150 150	5.8 5.8 5.8 5.8	0 1.5 2.6 4.0	24 43 54 48	100 100 100 100	100 100 100 100	91 100 98 100	83 100 98 100	63 100 96 100	46 100 92 100	46 100 83 100	20 93 43 100	17 91 28 100	
6	12.9	150 150 150 150	5.8 5.8 5.8	0 1.5 2.6 4.0	47 57 51 48	100 100 100 100	100 100 100 100	100 100 100 100	87 100 100 100	86 100 100 100	86 100 100 100	86 100 100 100	85 100 100 100	84 100 99 100	83 100 96 100
7	17.5	150 150 150 150	6.5 6.5 6.5	0 1.5 2.7 4.0	53 53 51 51	0 89 100 100	0 88 100	64 74	59 66	47 53	39 49	36 49	35 47	35 47	30 47
8	17.5	150 150 150 150	6.4 6.4 6.4	0 1.5 2.7 4.0	53 54 49 31	52 52 96 98	0 0 26 97	0 88	55	10	10	10	10	10	6

TABLE IV

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TABLE IV

OUTGROWTH WITH RESPECT TO TIME (IN DAYS) (cont'd)

Expt.	D	Nano	1	1 %				Perc	entage	e of '	Tubes	Rema.	ining		
No.	115.5°C	ppm	рН	Salt	Tubes	5	10	15	20	30	40	50	100	150	365
9	17.5	150 150 150 150	6.4 6.4 6.4	0 1.5 2.7 4.0	57 56 55 49	0 46 62 98	0 8 93	0 10	4	4	0				
10	8.5	150 150 150 150	6.3 6.3 6.3	0 1.5 2.7 4.0	32 46 43 31	40 63 96 98	0 0 93 97	5 97	0 97	97	97	97	97	97	97
11	8.5	150 150 150 150	6.3 6.3 6.3	0 1.5 2.7 4.0	33 50 35 36	49 100 100 100	0 0 55 91	37 76	34 75	32 75	30 75	29 75	29 75	29 75	28 75
12	8.5	150 150 150 150	6.3 6.3 6.3	0 1.5 2.7 4.0	40 31 51 42	16 100 98 100	0 19 94 90	0 89 86	85 83	82 81	80 79	80 79	80 78	79 77	78 76
13	8.5	150 150 150 150	6.5 6.5 6.5	0 1.5 2.7 4.0	52 55 48 46	0 0 78 92	46 83	16 83	4 71	4 71	4 70	4 70	4 70	4 70	3 70
14	8.5	150 150 150 150	5.8 5.8 5.8	0 1.5 2.6 4.0	56 50 30 40	70 100 100 100	17 69 97 96	12 59 84 95	12 54 82 90	12 54 82 85	5 54 82 85	4 54 82 85	4 54 77 85	2 44 72 85	0 31 67 85
15	17.5	0 50 100 200	6.5 6.1 6.1 6.1	2 2 2 2	48 43 43 62	88 100 100 100	8 100 100 100	0 100 100 94	100 100 87	98 98 69	76 78 60	67 72 38	56 63 21	56 61 19	53 57 17
16	17.5	0 50 100 200	6.4 6.4 6.5	2 2 2 2	31 36 39 52	54 100 100 100	0 38 60 76	0 3 4	3 4	3 0	3	3	0		

Expt.	D	NaNO2		%				Perc	entag	e of	Tubes	Rema	ining		
No.	115.5°C	ppm	рН	Salt	Tubes	5	10	15	20	30	40	50	100	150	365
17	17.5	0 50 100 200	6.5 6.5 6.5	2.6 2.6 2.6 2.6	50 45 54 55	100 100 100 100	78 98 96 78	59 95 96 63	50 95 96 59	43 95 96 59	35 93 96 59	27 93 96 59	22 91 93 58	22 91 93 58	1: 8: 4: 5:
18	17.5	0 100 200 400	6.4 6.4 6.4	2.6 2.6 2.6 2.6	52 36 38 50	50 97 100 100	0 36 97 100	0 42 100	10 99	8 98	8 98	7 98	6 98	5 98	8:
19	17.5	0 50 100 200	6.4 6.4 6.4	2.6 2.6 2.6 2.6	55 43 48 50	0 73 100 100	0 0 70	0							
20	17.5	0 100 200 400	6.5 6.5 6.5	2.6 2.6 2.6 2.6	49 49 41 49	62 99 99	0 0 25 82	7 13	0 5	2	2	2	2	2	
21	17.5	0 100 200 400	6.3 6.6 6.4 6.3	2.6 2.6 2.6 2.6	41 45 50 53	88 89 98 100	35 58 91 100	0 20 54 100	16 38 98	7 23 98	7 20 98	0 20 96	20 96	19	1:
22	8.5	0 50 100 200	6.3 6.3 6.3	2.6 2.6 2.6 2.6	54 43 51 53	98 97 100 100	72 73 96 96	9 90 92	0 2 78 92	0 77 87	76 87	76 85	76 84	76 83	7: 79
23	8.5	0 50 100 200	6.3 6.3 6.3	2.6 2.6 2.6 2.6	61 60 52 49	100 100 100 100	100 100 100 100	60 0 22 8	38 0 8	21	21 0	20	18	15	(
24	8.5	0 50 100 200	6.3 6.3 6.3	2.6 2.6 2.6 2.6	49 35 47 26	100 95 97 95	88 50 78 64	81 17 74 54	74 6 72 43	69 0 72 38	65 70 38	61 68 37	59 67 37	58 66 36	6:

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TABLE IV

TABLE IV

OUTGROWTH WITH RESPECT TO TIME (IN DAYS) (cont'd)

Expt.	D	Nano,		%				Pero	centac	ge of	Tubes	s Rema	ining	7	
No.	115.5°C	ppm	Hq	Salt	Tubes	5	10	15	20	30	40	50	100	150	365
25	8.5	0 50 100 200	6.4 6.4 6.4	2.6 2.6 2.6 2.6	37 44 34 33	60 50 82 80	0 0 35 51	9 38	3 38	2 38	2 38	2 38	2 38	2 38	0 36
26	8.5	0 100 200 400	5.8 5.8 5.8 5.8	2.6 2.6 2.6 2.6	52 49 53 39	100 100 100 100	94 92 100 100	88 63 88 100	86 51 78 97	84 47 72 97	84 47 72 97	84 47 72 97	81 47 71 97	81 46 66 97	81 44 61 97
27	8.5	0 100 200 400	5.8 5.8 5.8 5.8	2.6 2.6 2.6 2.6	47 55 46 56	83 100 100 100	20 100 100 100	10 100 100 98	10 98 100 98	9 98 100 98	9 98 100 98	8 98 100 98	6 98 100 98	6 98 100 98	6 96 100 98
28	8.5	0 100 200 400	5.8 5.8 5.8	2.6 2.6 2.6 2.6	52 52 45 52	91 100 100 100	82 100 100 99	67 100 98 98	53 100 98 89	43 100 98 74	40 100 98 68	32 100 98 64	26 98 98 56	21 97 98 54	19 87 98 54
29	8.5	0 100 200 400	5.8 5.8 5.8 5.8	2.6 2.6 2.6 2.6	26 44 38 29	100 100 100 100	100 100 100 100	100 100 100 100	79 100 100 100	8 98 100 100	93 100 100	4 86 100 99	4 86 98 93	4 86 97 87	4 84 95 74
30	12.9	0 100 200 400	5.8 5.8 5.8 5.8	2.6 2.6 2.6 2.6	33 40 40 48	99 100 100 100	98 100 100 100	97 100 100 100	97 100 100 100	97 99 100 100	97 98 100 100	97 98 100 100	97 97 92 100	97 95 85 100	
31	12.9	0 100 200 400	5.8 5.8 5.8 5.8	2.6 2.6 2.6 2.6	55 55 51 45	100 100 100 100	100 100 100 100	100 100 100 100	99 100 100 100	98 100 100 100	98 100 100 100	97 100 100 100	93 97 100 98	75 89 100 94	49 80 100 91
32	12.9	0 100 200 400	5.8 5.8 5.8 5.8	2.6 2.6 2.6 2.6	50 48 46 44	99 100 100 100	98 100 100 100	96 100 100 100	95 100 100 100	94 100 100 100	92 100 100 100	89 100 100 100	84 96 100 100	81 92 100 100	78 88 100 100

OUTGROWTH WITH RESPECT TO TIME (IN DAYS) (cont'd)

Expt.	D	Nano		%				Perc	entag	e of	Tubes	Rema	ining		
No.	115.5°C	ppm	рН	Salt	Tubes	5	10	15	20	30	40	50	100	150	365
33	17.5	150 150 150 150	6.3 7.1 5.7 5.1	2.6 2.6 2.6 2.6	31 33 49 50	100 37 100 100	49 0 100 100	3 100 100	3 100 100	3 100 100	3 100 100	3 100 100	0 100 100	100	100
34	17.5	150 150 150 150	6.3 7.1 5.8 5.2	2.6 2.6 2.6 2.6	41 45 59 58	100 50 100 100	0 0 100 100	100	100	100	100	100 96	100 93	100 93	100 84
35	17.5	150 150 150 150	6.3 7.1 5.1 5.7	2.6 2.6 2.6 2.6	42 38 36 37	98 100 100 100	83 58 100 100	72 30 100 100	61 17 100 100	13 8 100 100	7 0 100 97	7 100 96	7 100 88	7 100 78	7 100 0
36	8.5	150 150 150 150	4.8 5.5 6.0 6.8	2.6 2.6 2.6 2.6	32 35 39 32	97 91 94	100 94 64 33	100 92 18 0	97 91 8	97 86 6	97 86 5	97 86 5	97 86 5	97 86 4	97 83 0
37	8.5	150 150 150 150	4.9 5.5 6.1 7.2	2.6 2.6 2.6 2.6	54 50 31 49	100 100 100 0	100 100 97	98 100 91	98 100 91	98 100 91	98 100 91	98 100 91	98 99 91	98 99 91	98 96 90
38	8.5	150 150 150 150	5.0 5.5 6.3 7.2	2.6 2.6 2.6 2.6	36 39 33 36	100 100 87 0	100 97 9	100 97 4	100 97 0	99 97	99 97	99 97	98 97	97 97	97 96
39	8.5	150 150 150 150	5.0 5.7 6.2 7.3	2.6 2.6 2.6 2.6	36 46 33 60	100 100 75 0	100 97 0	100 73	100 69	100 69	100	100 69	100 69	100 69	100 69
40	8.5	150 150 150 150	6.2 6.4 6.0 5.8	2.6 2.6 2.6 2.6	33 41 42 21	100 100 100 100	100 75 100 100	88 5 98 95	84 5 98 95	80 2 90 86	77 2 88 86	74 2 88 86	70 2 88 81	70 2 88 76	70 2 86 67

OUTGROWTH WITH RESPECT TO TIME (IN DAYS) (cont'd)

Expt.	D	NaNO2		%				Per	centa	ige of	Tube	es Ren	ainir	ng	
No.	115.5°C	ppm	рН	Salt	Tubes	5	10	15	20	30	40	50	100	150	365
41	8.5	150 150 150 150	6.4 6.2 6.0 5.8	2.6 2.6 2.6 2.6	55 51 56 57	100 100 100 98	85 36 70 98	50 8 46 98	46 6 44 98	38 5 44 96	35 5 44 96	35 5 43 96	26 5 41 96	19 5 38 96	9 5 32 91
42	8.5	150 150 150 150	6.4 6.2 6.0 5.8	2.6 2.6 2.6 2.6	61 52 51 50	100 100 100 100	29 96 100 100	11 81 100 94	10 74 98 88	10 71 98 86	10 71 98 86	10 71 98 86	6 69 98 84	5 67 98 83	3 64 98 82
43	8.5	150 150 150 150	6.4 6.2 6.0 5.8	2.6 2.6 2.6 2.6	54 54 56 61	100 99 100 100	97 69 100 100	90 39 99 98	87 17 98 94	80 13 96 65	80 13 93 57	80 13 93 55	73 7 90 43	70 6 85 40	54 1 71 29
44	12.9	150 150 150 150	6.4 6.2 6.0 5.8	2.6 2.6 2.6 2.6	48 48 45 50	100 100 100 100	100 100 100 100	100 100 97 100	98 100 87 100	98 98 77 98	96 96 72 98	96 95 71 96	92 81 51 96	89 70 51 95	
45	12.9	150 150 150 150	6.4 6.2 6.0 5.8	2.6 2.6 2.6 2.6	44 49 51 35	100 100 100 100	93 100 100 100	85 97 100 98	58 94 100 94						
46	12.9	150 150	5.5	2.6	33 48	100	100	100 98	100 98	100 96	100 96	100	97 84	97 72	97 71
47	12.9	150 150 150 150	6.4 6.2 6.0 5.8	2.6 2.6 2.6 2.6	45 42 53 46	100 100 100 100	100 100 97 100	98 98 90 100	98 93 84 100	98 93 82 98	98 93 80 96	98 93 74 96	98 88 74 93	92 80 67 86	84 72 61 80

TABLE V

THE NUMBER OF TUBES REMAINING AT VARIOUS INTERVALS, i, AND SEPARATED BY PERCENTAGE REMAINING AT 50 DAYS, j

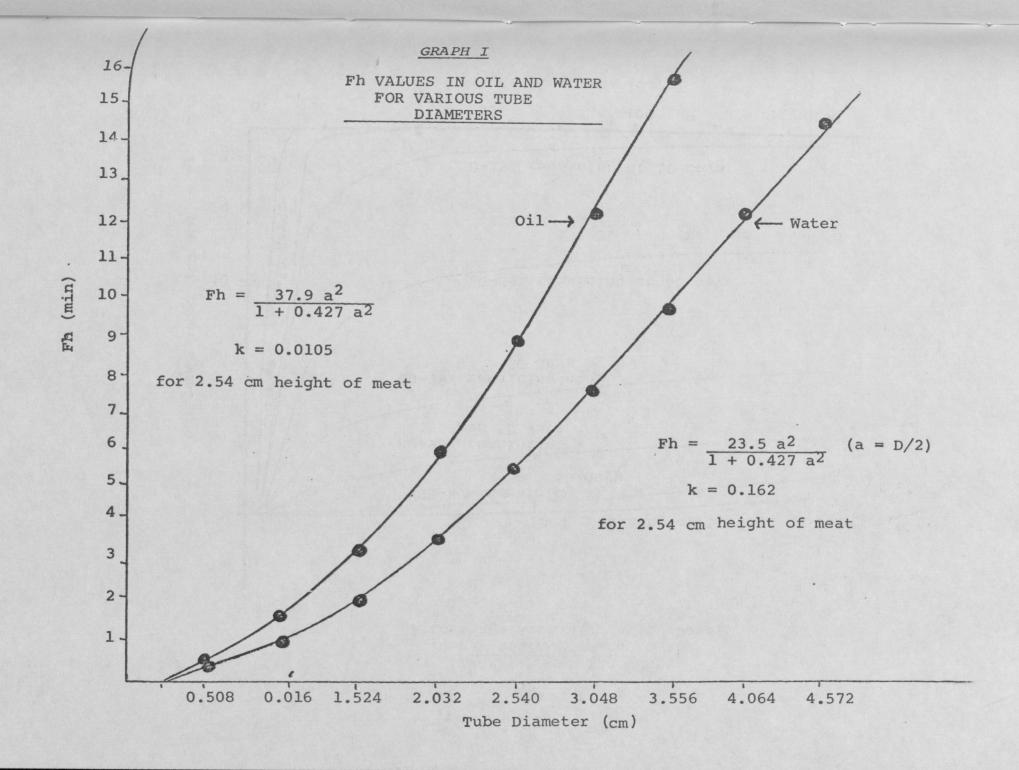
Days from	Rows				Colum	ms, j			
Start	<u>i</u>	1	2	3	4_	5	6	7_	_8_
0	1	1422	1218	998	848	888	847	977	1339
5	2	1422	1215	996	842	868	792	928	589
10	3	1422	1211	977	799	728	496	530	0
15	4	1422	1207	943	739	562	239	54	0
20	5	1422	1201	925	684	491	171	19	0
30	6	1422	1194	901	652	408	101	9	0
40	7	1422	1189	890	622	371	81	0	0
50	8	1422	1182	871	606	342	50	0	0
100	9	1394	1153	822	556	288	34	0	0
150	10	1348	1106	792	526	268	28	0	0

TABLE VI

DEFINITION OF j, THE PERCENTAGE OF TUBES REMAINING AT 50 DAYS

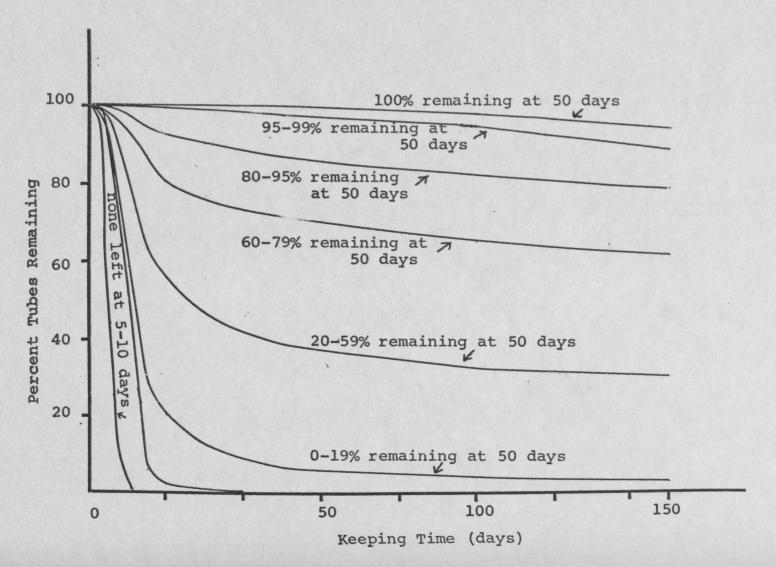
i	Definition
1	100% remaining at 50 days from start
	95-99% remaining at 50 days from start
2 3	80-94% remaining at 50 days from start
4	60-79% remaining at 50 days from start
5	20-59% remaining at 50 days from start
6	0-19% remaining at 50 days from start (but
	one more remaining at 40 days
7	None left at 15 to 40 days from start (but one more remaining at 10 days)
8	None left at 5 to 10 days from start

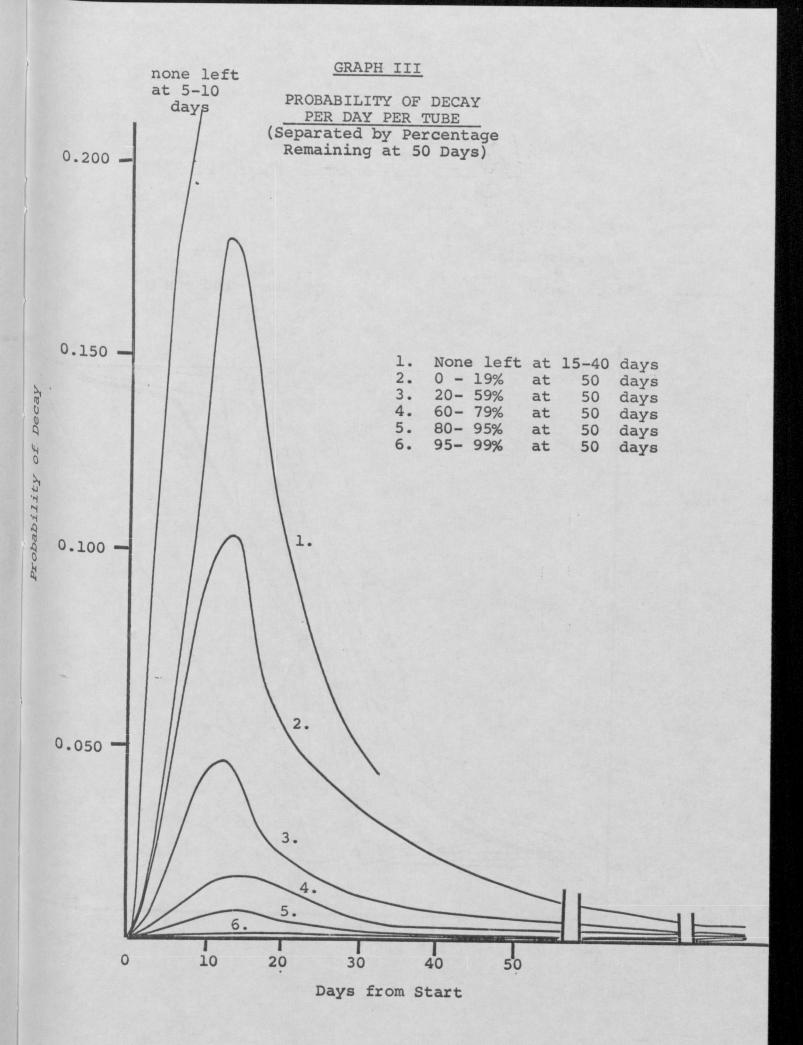




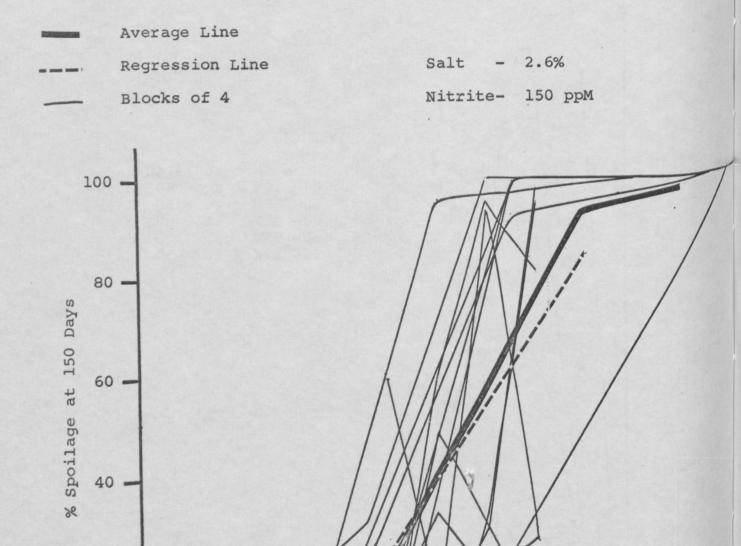
GRAPH II

PERCENT REMAINING TUBES
(Separated According to the
Percentage Remaining at 50 Days)

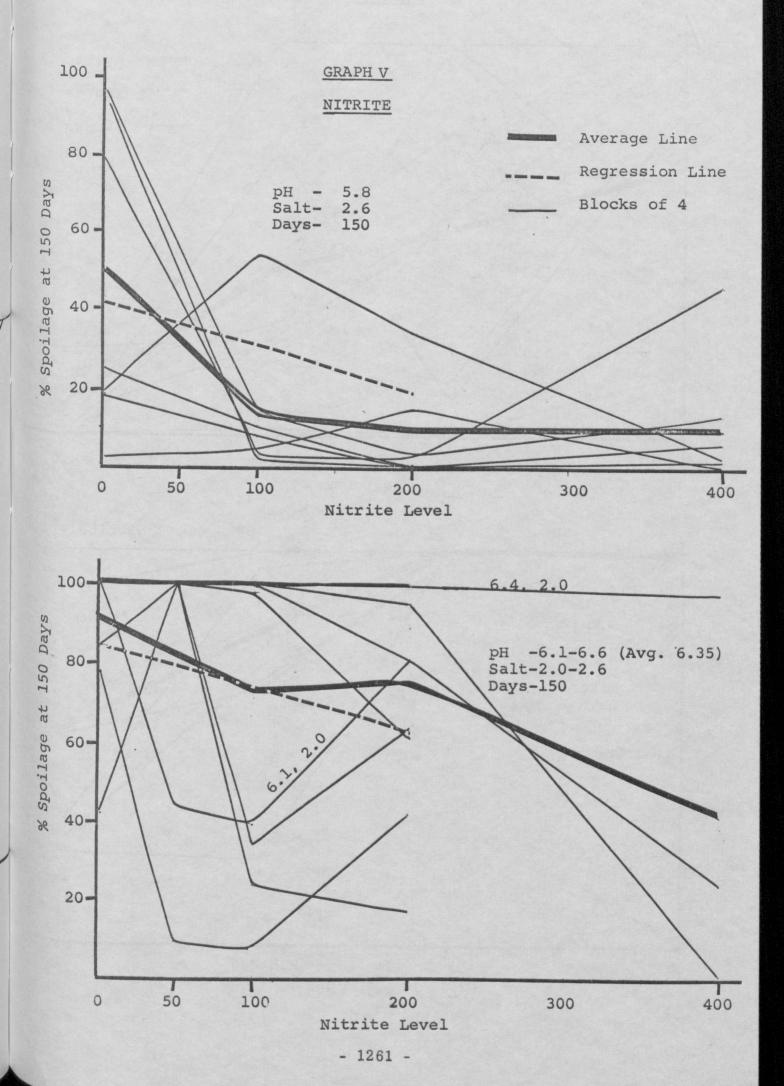




GRAPH IV 150 DAYS - % SPOILAGE - pH VARIATION



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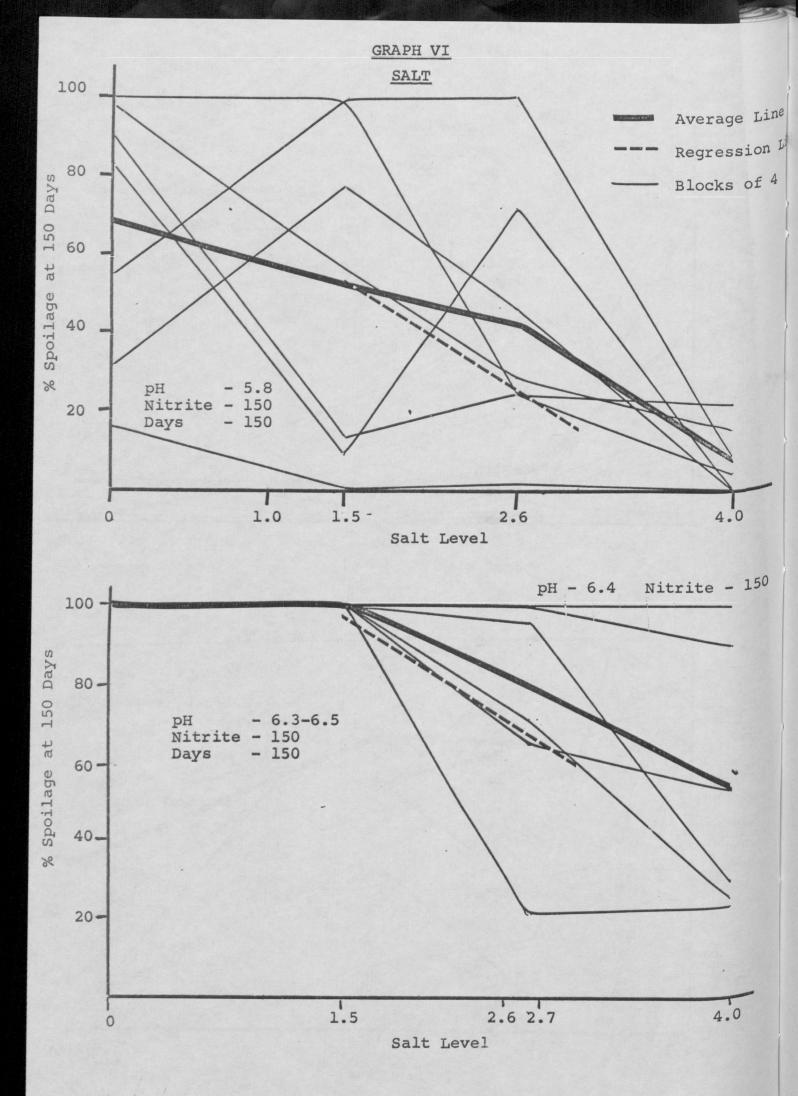


DIAGRAM I SET-UP FOR MEASURING HEAT PENETRATION

ne

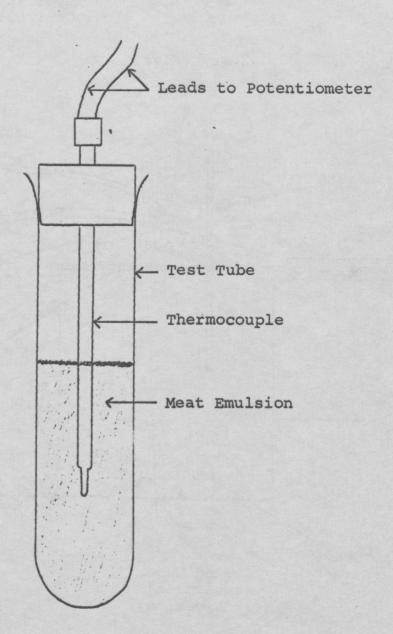


DIAGRAM II THERMAL DEATH TIME APPARATUS

