THE PASTEURISATION OF SLUDGE

FINAL REPORT

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SYNOPSIS

The aim of this research was to determine the sterilisation characteristics of a full-scale continuously operated wastewater sludge pasteuriser.

The unit was monitored over a three year period in the following ways:

1. At fixed temperatures in the range 50 - 70°C, with feeds of raw and digested sludge, over one-hour experimental periods.

2. Under routine conditions in the pre-pasteurisation mode (pasteurisation before anaerobic digestion) with target conditions of 70°C for a minimum of 30 minutes, and a feed of raw sludge plus waste activated sludge thickened in a dissolved air flotation unit.

It was found that a temperature of 53°C for 30 minutes was sufficient to inactivate Ascaris lumbricoides ova, but that 66°C was required for faecal coliforms. There was good agreement between decimal decay rates obtained in these full-scale experiments, with sludge feeds, and those in the literature for various media and conditions.

Under routine conditions, the median values for the Ascaris embryonation ratio and faecal coliform counts in the pasteurised sludge were <1% and 200/100 ml respectively. For digested sludge produced with a pasteurised feed the corresponding figures were 2% and 120 000/100 ml.

Operation in the pre-pasteurisation mode is recommended to avoid regrowth of pathogenic bacteria, and because it allows almost full heat recovery, provided suitable heat-exchangers are used and the digester heat supply is via the feed.

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Chairman:	Mr J E McGlashan	Water Research Commission
Members:	Dr J J Barnard	Department of Water Affairs
	Mr D W Osborn/Mr A R Pitman	City Health Department, Johannesburg
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1. INTRODUCTION

The treatment and disposal of various types of sludge is an important problem facing local authorities which operate sewage treatment works. Many works, which were originally situated in spacious grounds on the outskirts of cities, now find themselves surrounded by suburbs and under pressure to give up more land for housing and other purposes. Thus, the treatment and disposal of sludge by spreading it on the land within the perimeter of the works is steadily becoming a less suitable procedure. and there has for many years been a trend to the use of sludge treatment processes which require less space and which allow disposal away from the works.

Anaerobic digestion is an economical and widely used process for rendering the organic content of the sludge non-putrescible. However the resulting digested sludge, although a valuable soil conditioner, is not considered safe for unrestricted use, thereby complicating the disposal problem.

Although sludge has recently found a small-scale industrial use as a brick-making material where it acts as an internal fuel (Vail), the most beneficial use is undoubtedly in agriculture and horticulture, provided that some form of further treatment is adopted to improve its hygienic properties.

Those classes of undesirable constituents of most concern are heavy metals, pathogenic bacteria, viruses and helminths. The problems presented by heavy metals are not considered in this report but are presently best dealt with by strict control of discharges into the sewerage system and by restrictions on the amount of sludge used on any particular piece of land.

Various methods, including pasteurisation, lime-treatment and irradiation have been used for disinfecting sludge. Some stabilisation processes, such as thermophilic anaerobic digestion, composting and autothermal aerobic digestion also have disinfection capabilities. These processes can be used in various combinations with other stabilisation processes and the choice of a suitable treatment is therefore not an easy one, particularly as the associated costs and performance factors under local conditions are not always known.

To assist local authorities in dealing with such problems, the Water Research Commission has for some time been engaged in a comprehensive research programme on sludge treatment and disposal in conjuction with various other bodies. As the Cape Town City Engineer's Department was, in 1979, constructing a new wastewater treatment plant incorporating a full-scale pasteuriser, it was considered opportune to engage in a research project aimed at defining the performance of such a unit.

The aims outlined in the agreement entered into between the Commission and the Cape Town City Council were:

1. To operate a full-scale pasteurisation plant for a period of 30 months, using feeds of:

    a. mixed primary and waste activated sludge and

    b. mesophilically digested sludge.

2. To investigate the economics and the effectiveness of pasteurisation at various operating temperatures within the range 60°C to 80°C in terms of the inactivation/destruction of selected micro-organisms (Ascaris ova, E Coli).

3. To monitor suitable parameters with a view to defining the operational conditions on the plant at all stages.

4. To compile a report on the results and findings on completion of the investigation.

The programme initially also included the investigation of the economics and effectiveness of full scale thermophilic anaerobic digestion. However, the plant to be used for this purpose was subsequently found to be unsuitable and this part of the project had to be discontinued. Due to delays in commissioning the pasteuriser the completion date of the project was extended to May 1986.

2. PASTEURISATION

Pasteurisation, invented by Louis Pasteur in the 1860's, is a heat treatment process mainly used for destroying pathogenic micro-organisms in foods and beverages. For milk, the required temperature is 62°C for 30 minutes or 72°C for 15 seconds. These conditions were chosen to destroy Mycobacterium tuberculosis, one of the most heat resistant of the non-spore-forming micro-organisms capable of causing human disease. In the pasteurisation of sewage sludge the customary conditions are 70°C for 30 minutes.

Most development work on the pasteurisation of sludge has been carried out in Switzerland where the main goal has been the elimination of salmonellae (Havelaar 1984). Pasteurisation is usually carried out after conventional anaerobic mesophilic digestion but it has recently been found by the Swiss that regrowth of enterobacteria, including Salmonella, occurs readily on storage of pasteurised sludge, the numbers sometimes exceeding those in the original raw sludge (Keller 1984). The exact reasons for this effect are not yet known, but it is considered likely that the heat treatment reduces the level of competitive flora and breaks down large organic molecules to easily assimilable compounds. Regrowth only occurs with certain bacterial species and not with human or animal viruses and helminth ova (Havelaar 1984).

For this reason Swiss regulations requiring all sludge to be disinfected were suspended pending further investigations, which subsequently showed that a re-arrangement of the process sequence had a beneficial effect. It was found that pasteurisation of raw sludge followed by mesophilic anaerobic digestion gave a stable product with no regrowth of pathogens. On initial introduction of pasteurised sludge into a contaminated digester, the counts of enterobacteria were found to rapidly decrease and subsequent accidental contamination with unpasteurised sludge only resulted in temporary increases.

Pilot-plant experiments in Germany have confirmed the stability of pre-pasteurised sludge (Philipp 1981). The following levels were found:

	log coliforms/100 ml
Raw sludge	9 - 10
Pasteurised raw sludge	2 - 4
Digested pasteurised sludge	5 - 6
Stored digested pasteurised sludge	5 - 6
Mesophilically digested sludge	6 - 7
Pasteurised digested sludge	2 - 4
Stored pasteurised digested sludge	8 - 9

Artificially introduced S. senftenberg was destroyed by pasteurisation and Salmonella levels were very low during all stages of the process.

Because partial dewatering, from the secondary digesters, is practised in most plants with two-stage digestion, the volume of digested sludge is usually about half that of the raw sludge. Pre-pasteurisation thus has the disadvantage, in most installations, that twice as much sludge has to be heated as with post-pasteurisation. In the case of the

single-stage digesters used for the present study this disadvantage does not apply as dewatering is not practised. However the heated pasteurised raw sludge can be used to supply all the heat requirements of the digesters thus increasing the degree of heat recovery.

The choice of the conventional 30 min/70°C process conditions was originally an arbitrary one but it seems to be generally regarded as satisfactory (Havelaar 1984). Most pasteurisers are run on a batch system and there is not much information available on how adequate these conditions are for a full-scale continuous process.

3. PLANT DESCRIPTION

The pasteuriser system investigated forms part of the Cape Flats Wastewater Treatment Plant, situated near Muizenberg, Cape, and serving the southern suburbs of the Cape Peninsula. This activated sludge plant, of design capacity 150 Ml/d, presently treats about 100 Ml/d of raw sewage, mostly of domestic origin. Figure 1 shows the sludge treatment facilities.

Sludge from the primary clarifiers is screened and then thickened in three thickeners from where it can be pumped either to three mesophilic digesters, each of 6200 m3 capacity, operated in parallel, or to the pasteurisers. Waste activated sludge is thickened in a dissolved air flotation (DAF) unit and pumped either to the digesters or the pasteurisers. The digesters are heated by direct steam injection into the feed and mixing is by gas circulation through burper mixers. Steam is produced in two boilers which are heated by digester gas, supplemented by diesel fuel if necessary.

The pasteuriser system consists of two units, each a 7 m insulated vertical tube, 2 m in diameter with a conical bottom, with associated pipework, pumps and heat-exchangers. Figure 2 and the photographs show the general layout.

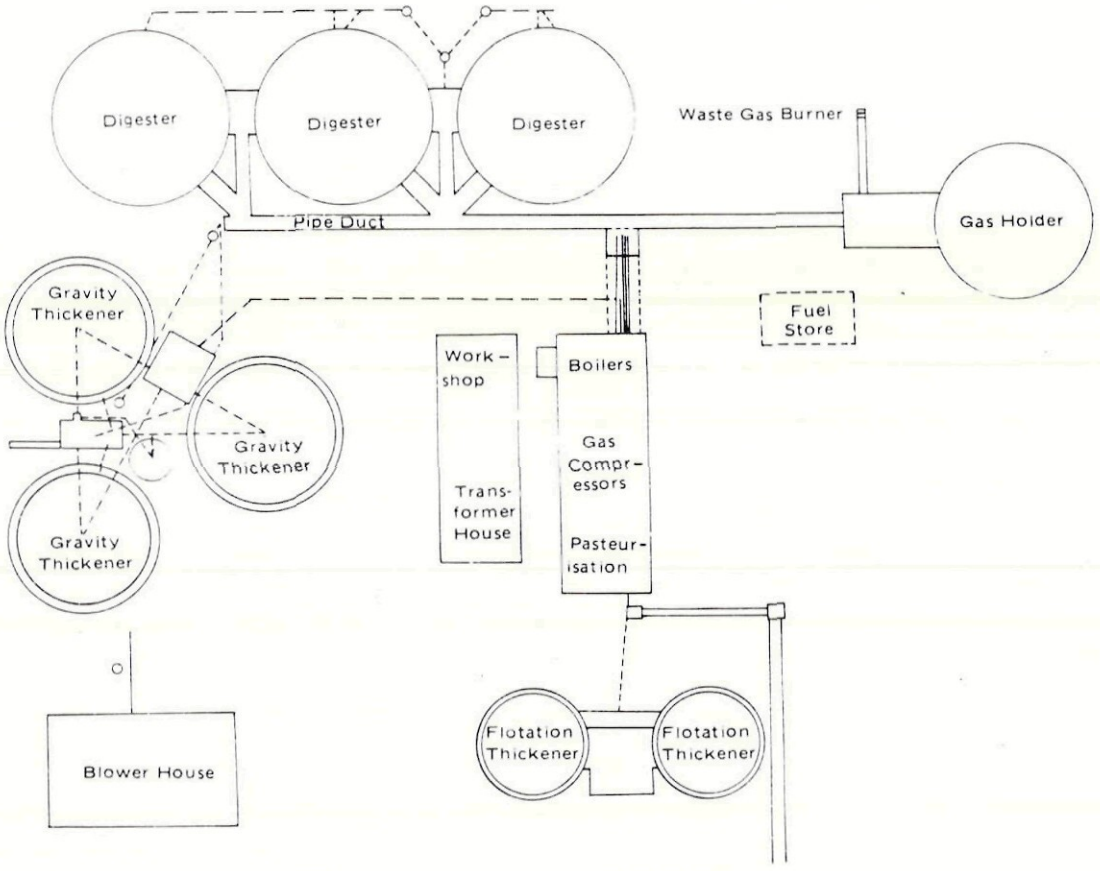
Sludge is added at the top and pumped at a controlled rate from the bottom. The outflow pumping rate, which is continuously variable, is automatically controlled to maintain the sludge level within suitable limits. The system of piping, which is fairly complicated, allows the feed to be taken either by gravity flow from the outlet of the digesters or by pumping from the primary sludge thickeners and/or dissolved air flotation thickeners for waste activated sludge.

Heating is by steam injection directly into the sludge flowing in the inlet pipe, giving a rapid and even temperature rise; a platinum resistance thermometer placed in the first 1 m of sludge at the top of the digester showed the temperature to be constant to within 0.1°C over several minutes. Overall temperature control was originally by means of a pressure-actuated on/off control valve but at the end of the experimental period this device was replaced by a proportional controller.

The design retention time and temperature are 30 minutes and 70°C respectively.

When the feed to the pasteuriser consists of raw sludge and DAF float, the pasteurised sludge is fed directly to the digesters, which require no further heating. A heat exchanger is sometimes then used to cool the sludge somewhat. The steam generation equipment is shared with the digesters.

Fig. 1 Cape Flats Sewage Treatment Works - Sludge Handling Facilities



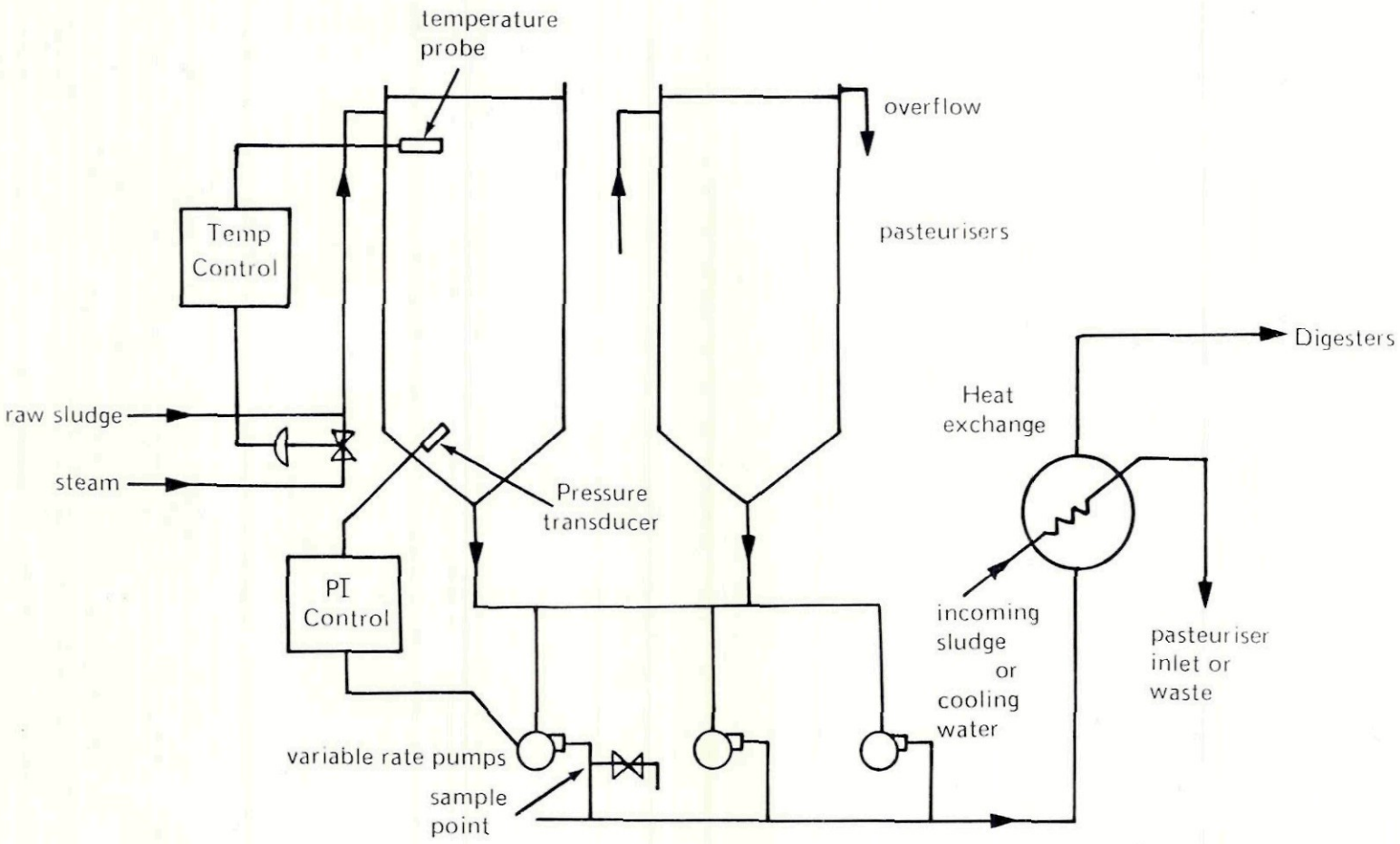
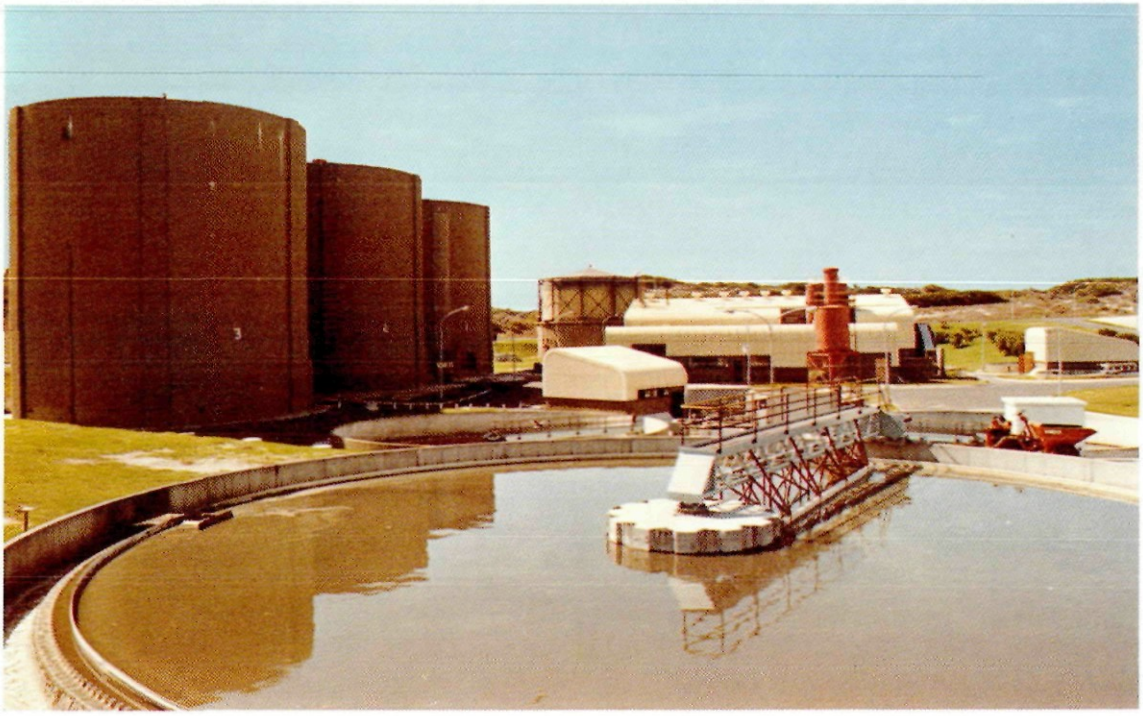
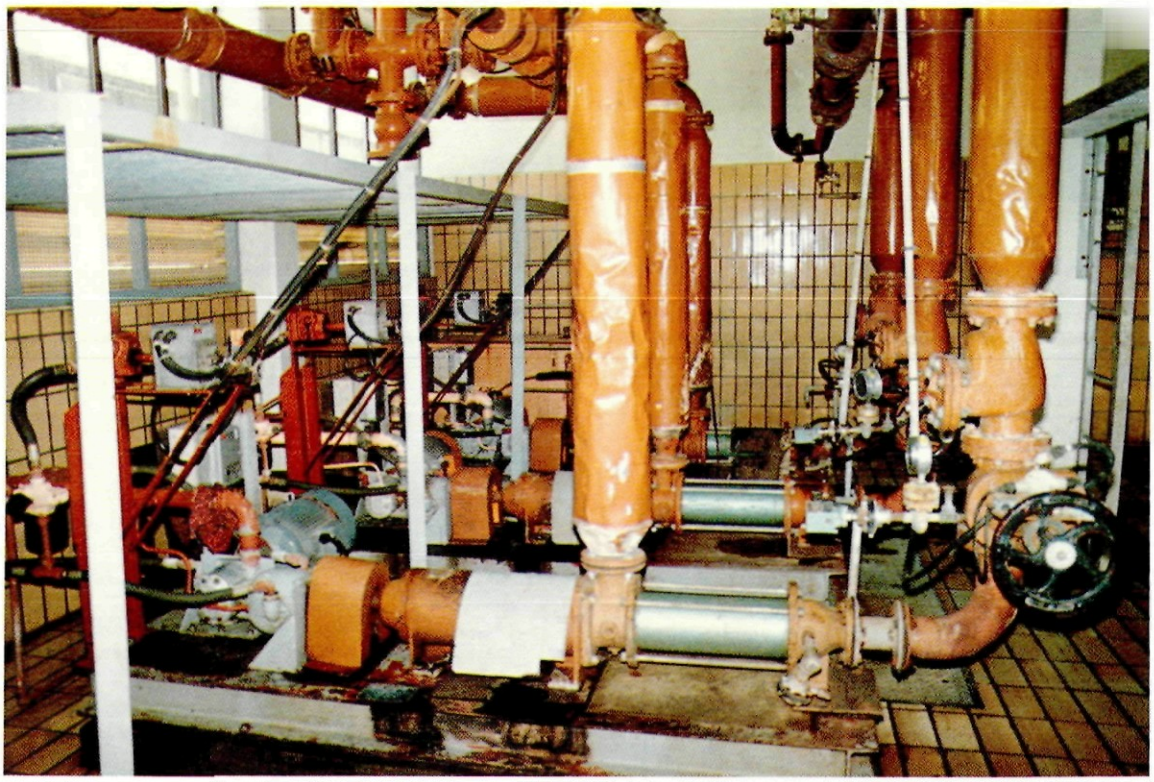


Fig. 2 Flow Sheet for Pasteurisers

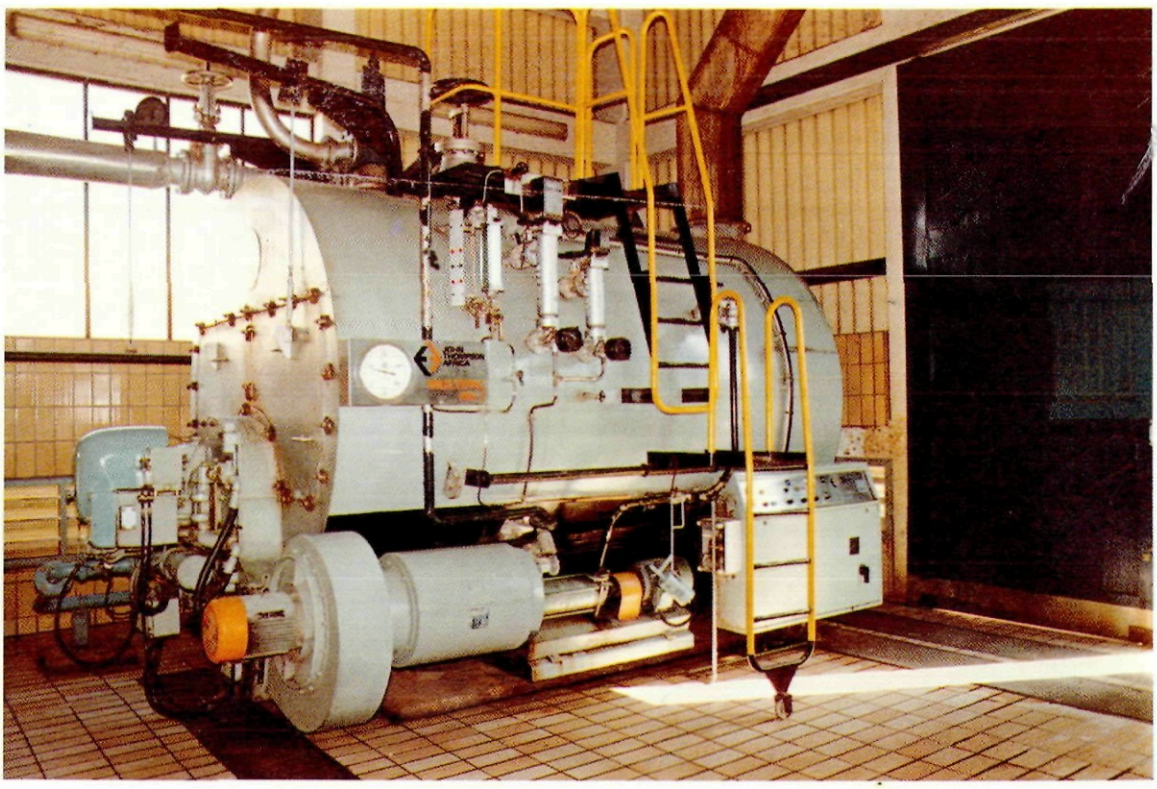


Sludge Handling Facilities

Pasteurisers



Variable Rate Sludge Pumps



Boiler House

4. EXPERIMENTAL PROGRAMME AND PROCEDURES

PROGRAMME

The experimental work for the project was divided into three phases:

1. Analytical Investigations: Delays in commissioning the pasteurisers allowed some time to be spent at the start of the project on improving the procedure for determining total Ascaris ova, a time-consuming and tedious operation which constituted a large part of the work-load for the project, and in investigating other aspects of the analytical requirements.

2. Investigation of Effect of Temperature and Time on Pasteurisation: Using feeds of 1. digested sludge and 2. thickened primary sludge plus waste activated sludge thickened in a dissolved air flotation plant, it was intended to determine the effect, on Ascaris ova and faecal coliforms, of various pasteurisation times in the range 30 - 60 min and temperatures in the range 50 - 80°C.

3. Long-Term Operation under Routine Conditions: Using the optimum conditions determined in phase 2, it was intended to monitor the operation of the plant under routine conditions. This was to involve sampling in a semi-random fashion. The feed during this phase was to be thickened raw sludge and/or DAF float and the pasteurised product was used as the sole feed for the digesters.

PROCEDURES

Runs at Fixed Time and Temperature:

The problem in these experiments was to obtain constant conditions in a pasteuriser which was not designed for experimental work and which was being used as a production unit. It was decided that it would be sufficient to maintain conditions nearly constant for two retention periods during an experimental run. During the first period, sampling would take place at the inlet and, if the requirements were met, would be continued at the outlet.

In the case of a digested sludge feed it was found that the inlet flow, ie the digester overflow, was variable because the feed to the digester was intermittent. This was handled by allowing sludge to build up in the thickeners and DAF unit for about 12 hours before an experimental run, to provide a sufficient reserve, and then feeding the digesters at a constant rate for 3 or 4 hours before the run. The pasteuriser outlet pump controllers had also to be altered from an on/off mode of operation to a proportional mode.

When the pasteuriser feed was thickened raw sludge and/or DAF float, fewer problems were encountered with variable flows because the feed was pumped directly into the pasteuriser.

Although it was intended to cover a retention time range of 30 - 60 min, it was found that in practice the time was governed by pump capacities and was therefore restricted to about 28 - 40 min. This was not a serious limitation as the design retention time of 30 min is the period of greatest interest.

Temperature control was usually manual, with the settings being kept constant as far as possible just before and during the run. The operating staff had the task of getting as near as possible to the target conditions before the run but the actual conditions achieved at the time of the run were not completely predictable. A major source of trouble was blockages in the pipes which caused many runs to be abandoned because all the requirements were not met.

The procedure for a run was as follows: Every five minutes during the run, the flow rate, sludge level and temperature gauges on the operating panel were read and samples of inlet sludge were added to separate composites for Ascaris and faecal coliform determinations, the latter in sterilised bottles. After about 25 min, the progress of the run was reviewed. The retention time was calculated to the nearest 5 min and, if the conditions were sufficiently constant, sampling was continued to the end of the calculated period, otherwise the run was abandoned. Sampling was then switched to the pasteurised sludge outlet pipe immediately downstream of the pump for a further retention period. The outlet temperature was also measured each time by placing a mercury-in-glass thermometer in the outlet stream. The thermometer was found to give readings within 0.1°C of those given by three other laboratory thermometers and a Fluke electronic thermometer.

Where desired, grab samples were also taken of other sludge streams. The temperature of the incoming pasteuriser feed was also measured with the mercury thermometer once or twice during the run.

For each run the mean and standard deviation for temperature, sludge height and a weighted mean flow value were calculated. In the latter calculation, allowance was made for the fact that the flow during any 5-minute period has a variable effect on the retention time of the sampled sludge depending on the relative position of the period in the run. The differences between the usual mean values and the weighted one were slight. From the flow value and the pasteuriser volume of 22.7 m3, the retention time for the run was calculated. Where the conditions were too variable the run was rejected.

Samples were analysed for total and viable Ascaris ova, total solids and faecal coliforms by the procedures given in Appendix 1.

Random Sampling under Routine Conditions:

The plant, operated under routine conditions with a target temperature of 70°C, was visited about once a week on week-days during office hours and occasionally more often, on a random basis. Sampling was carried out irrespective of the state of the process, provided that sludge was actually being pumped. Samples of pasteurised sludge and readings were taken over half an hour as described earlier. One or more samples of raw sludge, DAF float, pasteuriser feed or digested sludge were also taken and analysed as previously described. The temperatures and flows during these runs were rarely as constant as during the first phase.

5. PHASE 1 - DETERMINATION OF ASCARIS OVA

CHOICE OF METHOD

The method originally employed for determining Ascaris ova (Melmed) consisted of the initial separation of the ova from the bulk of the extraneous material by means of a helminth filter (Visser 1972) followed by centrifugation and decantation of the supernatant liquid, whereafter the the remaining liquid was transferred dropwise to a series of microscope slides and the ova counted. This transfer and counting procedure was a lengthy one.

Other procedures, such as flotation in a solution of controlled density followed by collection of the ova on slides by the action of surface tension, have been used for isolating the ova for counting but are time-consuming and result in low recoveries. Some investigators (Pike 1983, Arther 1981) compensate for the latter shortcoming by calibration by means of known additions of ova, a procedure also subject to error.

Some time was therefore spent searching for other means of transferring the ova from the suspension, obtained by the use of the Visser filter, to a microscope slide. The procedure finally adopted was filtration through a membrane filter which is then dried, placed on a slide or in a plastic Petri dish and rendered transparent by addition of immersion oil. Membrane filters have previously been used for this purpose with water (Legler 1950) but the high solids content of sludges renders their direct filtration impractical without the prior use of the Visser filter.

This procedure, detailed in Appendix 1, has been subjected to a number of tests and inter-laboratory comparison studies which are described in the following sections, not necessarily in the order in which they were carried out.

RETENTION OF OVA ON THE COARSE VISSER FILTER

The Visser filter consists of two concentric filters. The inner coarse mesh holds large debris and passes Ascaris ova while the outer fine mesh holds the ova and allows fine material to pass through. The filter's effectiveness depends on the accuracy with which the mesh sizes are maintained and the completeness with which the ova can be washed through the coarse mesh.

This latter point was checked as follows: A sample of sludge was placed in the coarse filter and washed for successive periods of one minute, the material passing through being separately collected and filtered through the fine filter, after which the ova were filtered out and counted in the usual manner. This procedure was carried out by an experienced person and also by one who had not used it previously and who was only given a general description of what to do. Table 1 gives the results obtained.

TABLE 1 EFFECT OF WASHING TIME

WASH CYCLE	COUNTS/2 g WET SAMPLE	
	Experienced Operator	Inexperienced Operator
1	630	604
2		19
3	9	3
4	0	0
5	0	
Total	639	626

As a further check on the retention of eggs on the coarse filter, the material retained on it was transferred to a membrane filter and examined. No ova were found, although it is possible that some remained undetected because of the debris present.

It is clear from the above evidence that the ova are easily washed through the coarse filter and that four or five minutes should be sufficient for this step. Longer periods are undesirable as more extraneous material is washed through.

RETENTION OF OVA ON THE FINE VISSER FILTER

If the fine outer filter has too large a mesh size counts will be low. This was checked by carrying out a flotation test on the effluent from the fine filter. No ova were detected, but the test cannot be considered an exhaustive one.

In addition, counts were done on a sample of sludge using three different Visser filters, one bought at a different time to the other two. Table 2 shows the essentially similar values obtained.

TABLE 2 DIFFERENCES BETWEEN VISSER FILTERS

FILTER	COUNT	MEAN
1	111	111+21
2	107 116	112+15
3	107 83	95+14

The operation of transferring ova from the fine filter to a membrane filter was checked by first doing the transfer in the usual manner and then repeating it with a fresh membrane filter. No ova were found on the second attempt.

ADDITIONAL SAMPLE CLEAN-UP

Although use of the Visser filter allows separation of most extraneous matter from the ova some still remains and, to make counting easier, it would be desirable to reduce the amount still further. Other clean-up procedures were therefore briefly investigated.

Flotation of the ova from the suspension obtained after use of the Visser filter was attempted using a solution of zinc sulphate, followed by membrane filtration of the floated material. However it was found that an appreciable proportion of the ova did not float and the method cannot be considered reliable. It is quite possible that the proportion of non-floating ova will be different for different types of sludge and also for viable and non-viable ova. To prove that this is not so would require a considerable amount of work.

It was found possible to effect a noticeable reduction in the amount of extraneous matter by acidifying the suspension from the Visser filter to 10% with hydrochloric acid for about 10 minutes before membrane filtration. The counts obtained in this manner on two samples of digested activated sludge did not differ significantly from those obtained in the normal manner. However, this somewhat drastic chemical treatment was not considered suitable as a routine method.

No other attempts at sample clean-up were made and the method as described in Appendix 1 has proved convenient in several years of use.

SAMPLE STORAGE

In interpreting the results of the interlaboratory studies, described later, the effects of sample storage need to be taken into account.

Table 3 shows the effect of storage on Ascaris counts. The differences are not statistically significant and sample storage does not seem to effect the counts per gram of wet sludge, at least over a one-week period.

TABLE 3 EFFECT OF SAMPLE STORAGE ON ASCARIS COUNTS
(Room Temperature)

SAMPLE AGE		DIGESTED SLUDGE	WASTE ACTIVATED SLUDGE	RAW SLUDGE
days		Count per 2 g of wet sample		
0		233	93	621
		247	92	562
	mean	240	93	592
7		255	109	606
		255	82	591
	mean	255	96	599

However, counts are usually expressed per unit of dry mass and the solids content also enters into the calculation. Some tests were therefore carried out on the procedure for determining solids.

Firstly, triplicate solids determinations were done on 100 g portions of two sludges with the following results:

Digested Sludge:	2.31%	2.30%	2.31% m/m
Waste Activated Sludge:	4.08%	4.08%	4.08% m/m

As expected, the results are very reproducible.

Next, various samples were analysed before and after storage. It is clear from the results in Table 4 that quite substantial changes can take place in the solids content of untreated sludges unless they are kept cool.

TABLE 4 EFFECT OF STORAGE ON SOLIDS CONTENT OF SLUDGE

DAY	RAW SLUDGE	WASTE ACTIVATED SLUDGE	PASTEURISED RAW SLUDGE	DIGESTED SLUDGE
		At room temperature, % m/m		
0	5.40	4.08	4.00	2.31
2		4.00		2.28
3	4.98		3.74	
7	4.81	3.83	3.74	2.24
14	4.49		3.54	
		At 4°C, % m/m		
0	5.40		4.00	
3	5.35		4.03	
7	5.24		4.07	

REPRODUCIBILITY AND SAMPLE UNIFORMITY

As part of an an inter-laboratory study, three sludge samples were analysed in replicate after homogenisation in a Waring blender at low speed. Table 5 shows the results obtained by an experienced analyst.

TABLE 5 REPRODUCIBILITY OF ASCARIS DETERMINATION
(Experienced analyst, ova/2 g wet sludge)

	RAW SLUDGE	WASTE ACTIVATED SLUDGE	DIGESTED SLUDGE
	621	93	233
	562	92	247
	527	72	193
	588	102	269
	740	103	230
	737	101	240
	787	106	248
	606	109	255
	591	82	255
Mean:	640	96	241
Std. Dev.:	*91*	12	*22*
Expected Std. Dev. for Poisson Distribution:	25	10	16

If the ova are randomly distributed in the sludge, then the number present in successive samples can be expected to follow a Poisson distribution, for which the standard deviation is equal to the square root of the mean count. The table shows that for the waste activated and digested sludge samples this assumption is a reasonable one. However, the results for the raw sample were much more variable than expected and it appears that, in spite of homogenisation, the sludge was not uniform.

As a consequence it would seem wise, in carrying out comparison studies, for the source laboratory to carry out duplicate determinations on the sub-samples *before they are dispatched to the other laboratories* in order to verify sample homogeneity.

The same samples were also analysed by two newly trained personnel with the results given in Table 6. Statistical analysis of these results showed once again that the raw sludge sample was non-uniform and that there was a significant between-analyst effect with the two inexperienced analysts obtaining lower counts than the experienced one. The average counts for the digested and waste activated sludge samples for analysts A, B and C were: 174, 142 and 166 respectively.

TABLE 6 REPRODUCIBILTY OF ASCARIS DETERMINATION
(Three analysts, ova/2 g wet sludge)

SLUDGE	ANALYST A (experienced)	ANALYST B	ANALYST C
Raw	588	496	595
	740	725	737
	787	623	644
Mean:	705	615	659
Waste Activated	101	90	86
	103	84	104
	101	76	91
Mean:	101	83	94
Digested	269	190	231
	230	194	251
	240	219	231
Mean:	246	201	238

INTER-LABORATORY COMPARISON STUDIES

During the course of this project four inter-laboratory studies were carried out with various experimental designs. Four laboratories took part, not all in each test, using the method for total Ascaris ova described in this report.

Comparative Counts on a Single Set of Slides:

Because Ascaris ova are durable and the immersion oil used to render membrane filters transparent dries only slowly, slides can be kept for fairly extended periods. This allows comparative counts on the same set of slides to be done at several laboratories.

This type of test was carried out on two occasions with the results shown in Tables 7 and 8. The differences between laboratories A and B are small and not statistically significant, while counts from laborarory C were about 16% lower than those from the other two.

TABLE 7 COMPARATIVE COUNTS ON SET OF SLIDES - TEST 1
(May 1982, Ascaris ova / slide)

SLIDE	OBSERVER	LAB A Digested Sludge	LAB B Raw Sludge
1	1	60	72
Digested	2	66	77
Sludge	3	74	62
2	4	262	265
Raw	5	256	272
Sludge	6	237	253
Mean:		159	167

TABLE 8 COMPARATIVE COUNTS ON SET OF SLIDES - TEST 2
(May 1985, Ascaris ova / slide)

SLIDE	LAB A	LAB B	LAB C
1	404	437	346
Raw		416	363
Sludge			
2	194	180	167
Raw+DAF		212	163
Sludge			
3	235	273	184
Digested		280	172
Sludge			
Mean:	278	277	232

Comparative Counts on Sludge Samples:

Table 9 shows the result of a comparative study between three laboratories, in which the sample homogeneity was checked as reported in an earlier section. The results obtained for the one sample which was shown to be non-uniform were rejected. Because, as noted earlier, the solids content of sludge samples can change appreciably on storage and because it was not always possible to keep the samples cool during transport, the parameter used for comparison purposes is the count per 2 g of wet sludge. Statistical analysis showed the differences between

laboratories to be non-significant in the case of the digested sludge, and significant in the case of the waste activated sludge. However, overall the agreement is considered to be satisfactory.

TABLE 9 COMPARATIVE COUNTS ON SLUDGE SAMPLES
(May 1982, ova/2 g wet sludge)

SLUDGE	LAB A	LAB B	LAB D
Digested	269 231	255	219
	194 240	208	251
	219 190		
	230 251		
	248 231		
Waste	102 86	81	110
Activated	84 101	82	128
	76 90		
	103 104		
	106 91		
Mean:	162	157	177

Table 10 gives the results of a further test in which sample homogeneity was not confirmed although care was taken with the mixing of samples. The results from laboratories A and B appear to be in satisfactory agreement while those from C are rather low. The source of the discrepancy has not been traced.

TABLE 10 COMPARATIVE COUNTS ON SLUDGE SAMPLES
(May 1985 , ova/2 g wet sludge)

SLUDGE	LAB A	LAB B	LAB C
Raw	376	320	178
		293	178
			156
			199
Raw + Activated	187	204	80
		180	89
			22
			57
Digested	212	242	108
		261	143
			56
			79
Mean:	258	250	112

VIABILITY

The viability test, detailed in Appendix 1, was that used in the Laboratory and Scientific Services Branch of the Johannesburg Health Department (Melmed) with the exception that the final examination was carried out on membrane-filters. In this procedure, the ova obtained from the Visser filter are suspended in 2% formalin for 6 weeks at 27°C, with access to air and then microscopically examined and classified according to the scheme of Murray (Murray, 1960).

The validity of the 6-week incubation period was confirmed by weekly examination of a number of suspensions, with the results shown in Table 11. It can be seen that the condition of the ova had reached a steady state after three or four weeks in the case of raw sludge and five weeks in the case of digested sludge.

TABLE 11 EFFECT OF INCUBATION PERIOD

WEEK	CONDITION OF OVA, % of total count			
	Degenerate and Indeterminate	Single Cell	Partly Developed Larvae	Larvae
		Raw Sludge		
1	5	8	9	78
3	6	11	5	78
4	2	9	4	85
5	5	8	2	85
6	4	10	4	82
8	4	7	4	86
		Digested Sludge		
0	9	87	4	1
1	13	50	37	0
2	9	23	64	4
3	12	16	53	20
5	9	8	15	68
6	7	12	16	65
8	19	4	22	55

Table 12 shows the results of an inter-laboratory study of the viability test. The agreement is considered satisfactory.

TABLE 12 INTER-LABORATORY STUDY ON VIABILITY
(May 1985, % of total count)

SLUDGE	LARVAE + PARTLY DEVELOPED LARVAE		
	Lab A	Lab B	Lab C
Raw	88	79	93
Raw+DAF	89	80	70
Digested	7	4	9

6. PHASE 2 - TEMPERATURE STUDIES

GENERAL

Appendix 2 contains the detailed results for all test runs at controlled temperatures. Figures 3 and 4 show the variations in counts for Ascaris ova and faecal coliforms in the various feeds (over the entire period of the project). There appears to have been a general decline in Ascaris levels in the raw sludge. Table 13 gives the average values obtained for the measured parameters with digested and raw sludge feeds to the pasteuriser.

TABLE 13 AVERAGE VALUES FOR ALL CONSTANT TEMPERATURE RUNS

		Raw Sludge	Waste Activated Sludge	Digested Sludge	Pasteurised Sludge
With pasteuriser feed of digested sludge: (27 Oct 83 to 3 Sep 84)					
Solids,	%m/m	2.80	3.34	1.94	1.82
Ascaris ova					
Total,	/g dry	6320	1180	7460	6690
Larvae,		89%	92%	77%	0%
Faecal coli, (median)	/100 ml	980E6	37E6	22E6	240E3
No. samples		18	18	18	18
With pasteuriser feed of raw sludge: (8 Jan 85 to 2 Apr 85)					
Solids,	%m/m	4.30		2.56	4.18
Ascaris ova					
Total,	/g dry	4360		4720	3670
Larvae,		84%		5%	0%
Faecal coli, (median)	/100 ml	2200E6		6E6	20E3
No. samples		15	0	5	18

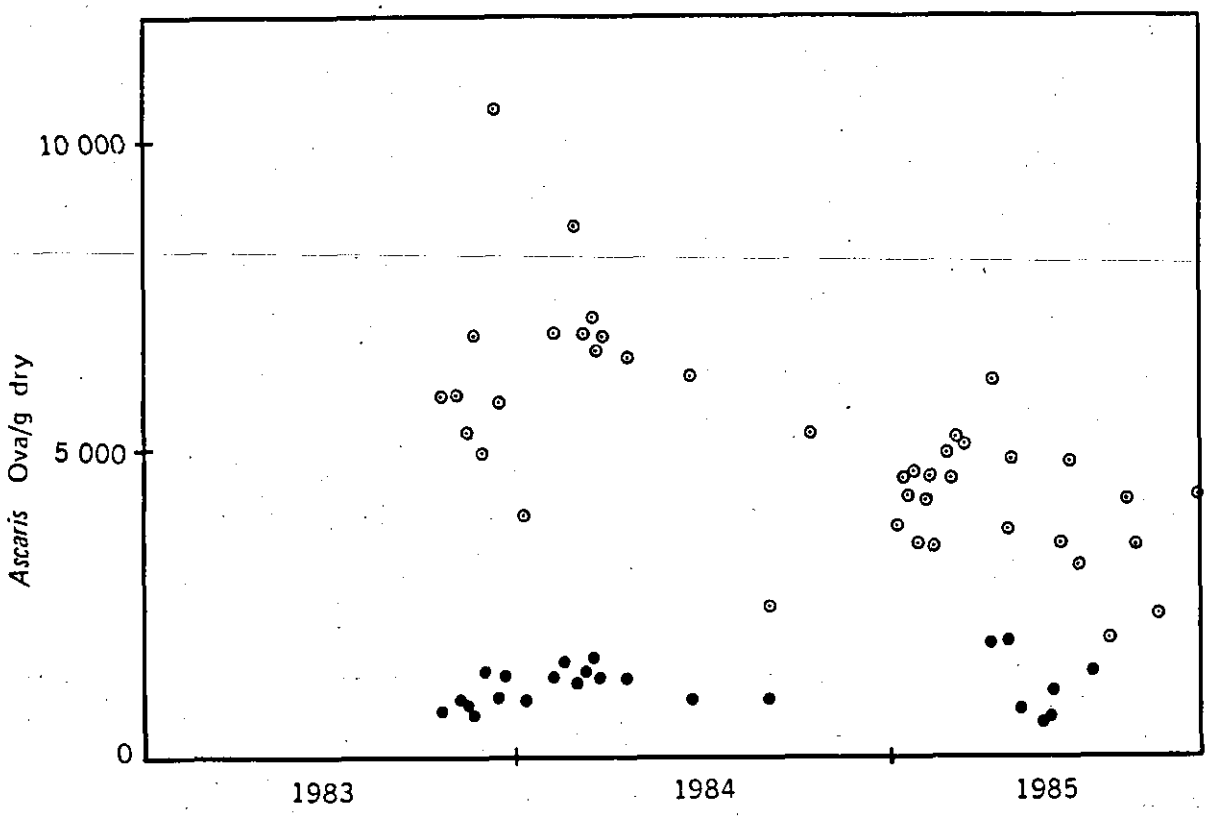


Fig. 3 Ascaris Ova in Untreated Sludge

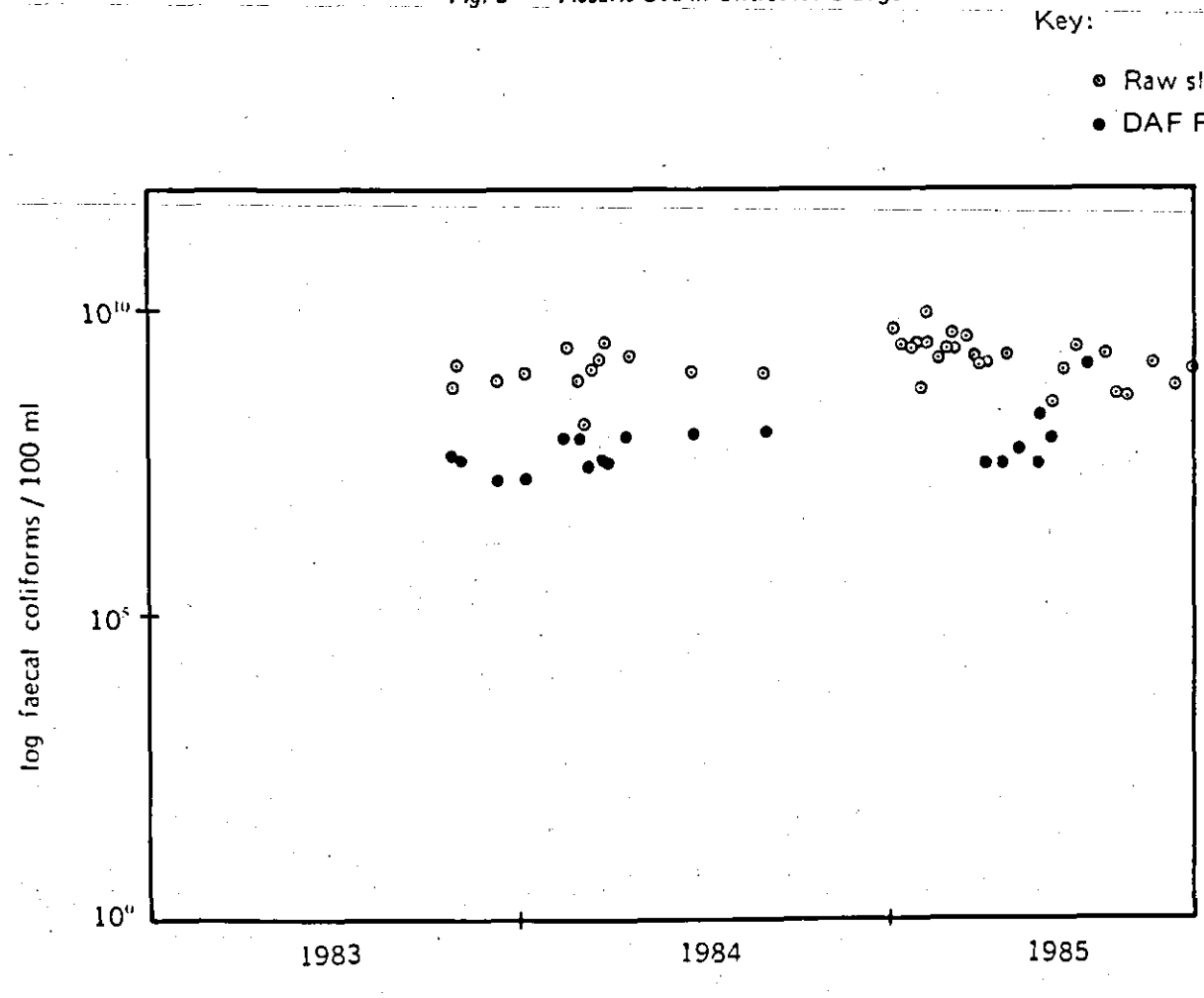


Fig. 4 Faecal Coliforms in Untreated Sludge

EFFECT OF TEMPERATURE ON ASCARIS OVA

The inactivation of Ascaris ova is governed by pasteurisation time as well as by temperature but it is only between 40 and 52°C that time effects are easily noticed as at higher temperatures inactivation is essentially complete in less than the shortest time used. For this reason and because the range of times covered in these experiments (28 - 40 min) was fairly narrow, the effect of time is for the most part ignored in the presentation that follows and the average time of 33 minutes is assumed to apply.

Figures 5 and 6 give the effect of temperature on the percentage of ova showing development to larvae, with the two types of feed. Most of the temperatures used resulted in greater than 99% inactivation. A minimum temperature of 52°C was required to produce 99% inactivation in raw sludge while about 49°C was required for digested sludge. It should be noted that only a few runs were carried out at low temperatures and these inactivation temperatures are therefore only approximate.

It would have been interesting to have used temperatures between 40 and 50°C as it is in this range that various differences might be expected to show up most clearly. This was in fact attempted, but these temperatures were so far from the normal operating range of the equipment that adequate control was not possible.

In comparing inactivation rates with those given in the literature, it is usual to calculate the so called D-values or decimal decay rates. This is the time period required to kill 90% of the organisms. The assumption is made that the die-off rate is exponential with regard to time and although this is not strictly true for all organisms, it appears to be reasonably valid for Ascaris ova (Burge 1981).

The present experiments were not designed for the determination of D-values but estimates can be obtained from the experimental data. For these calculations the actual pasteurisation times, and not the average time, were used. Figure 7 shows these values, together with some from the literature, plotted as the logarithm of the D-value against temperature. The agreement is good, particularly in the critical region between 50 and 52°C. Above 53°C the curve is not well defined but a large number of ova have to be examined to provide D-values at these high temperatures or short times have to be used. Table 14 gives the same data in tabular form.

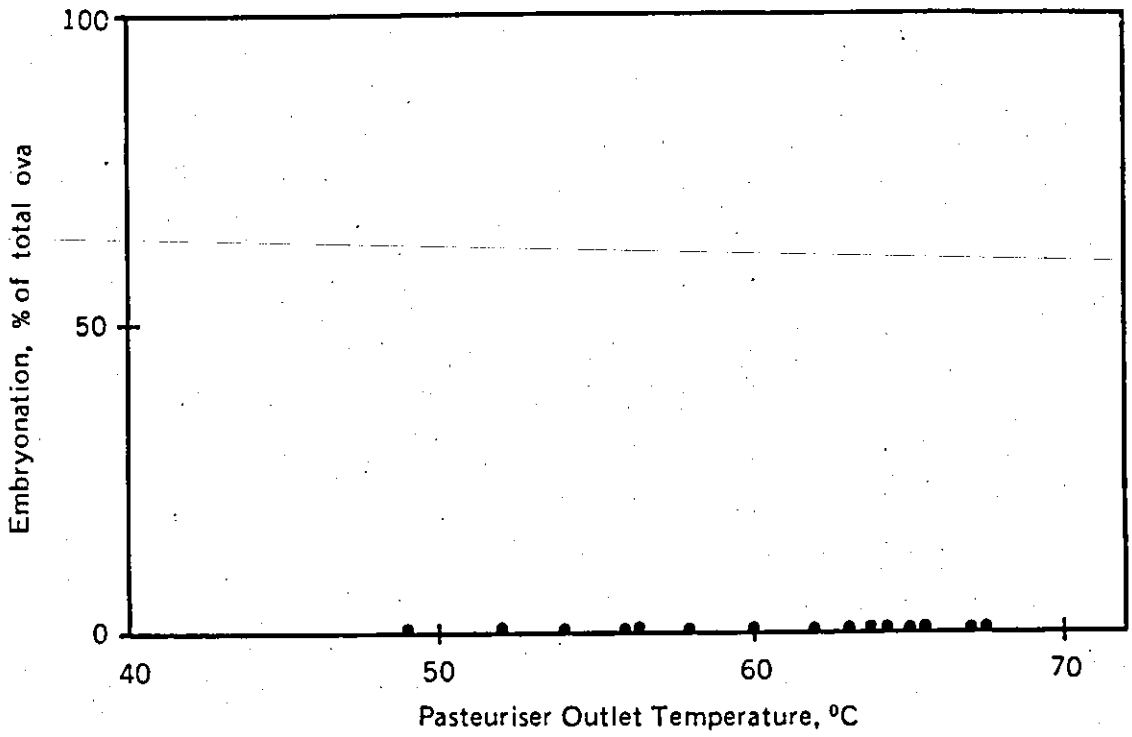


Fig. 5 Inactivation of Ascaris Ova - Digested Sludge Feed

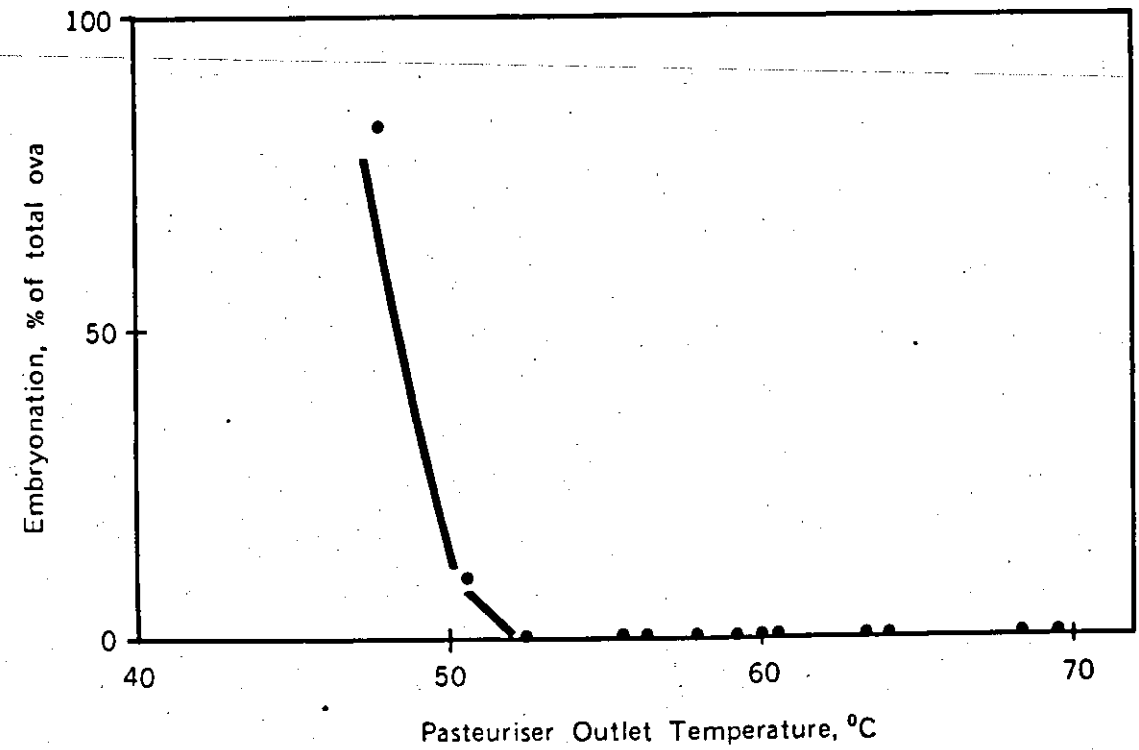


Fig. 6 Inactivation of Ascaris Ova - Raw Sludge Feed

TABLE 14 COMPARISON OF D VALUES FOR ASCARIS OVA
(Figures are for Ascaris lumbricoides)

Temp C	Medium	D Value, minutes	Reference
48	raw sludge	850	This study
51		33	"
52		17	"
56		<16	"
56		<16	"
58		<16	"
59		<16	"
60-70		<16	"
49	digested	<16	"
52	sludge	16	"
54		<16	"
56		<16	"
56		<16	"
58		<16	"
60-73		<16	"
42	raw sludge	400	Trim
42		1000	"
43		340	"
44		180	"
45		140	"
45		140	"
46		330	"
47		100	"
52		<60	"
51	water	35	Brandon
55		2	"
60		1	"
51	water	13	Sandia Labs
55	water	0.5	Krogstad
60		0.5	"
51	sludge	13	"
52	?	16	Nolf
54		2	"

These figures demonstrate the very rapid decrease in D-values that occurs over the temperature range 45 to 55°C. In 30 minutes the degree of inactivation at 52°C is about 2 orders of magnitude and at 55°C, 10 to 30 orders of magnitude.

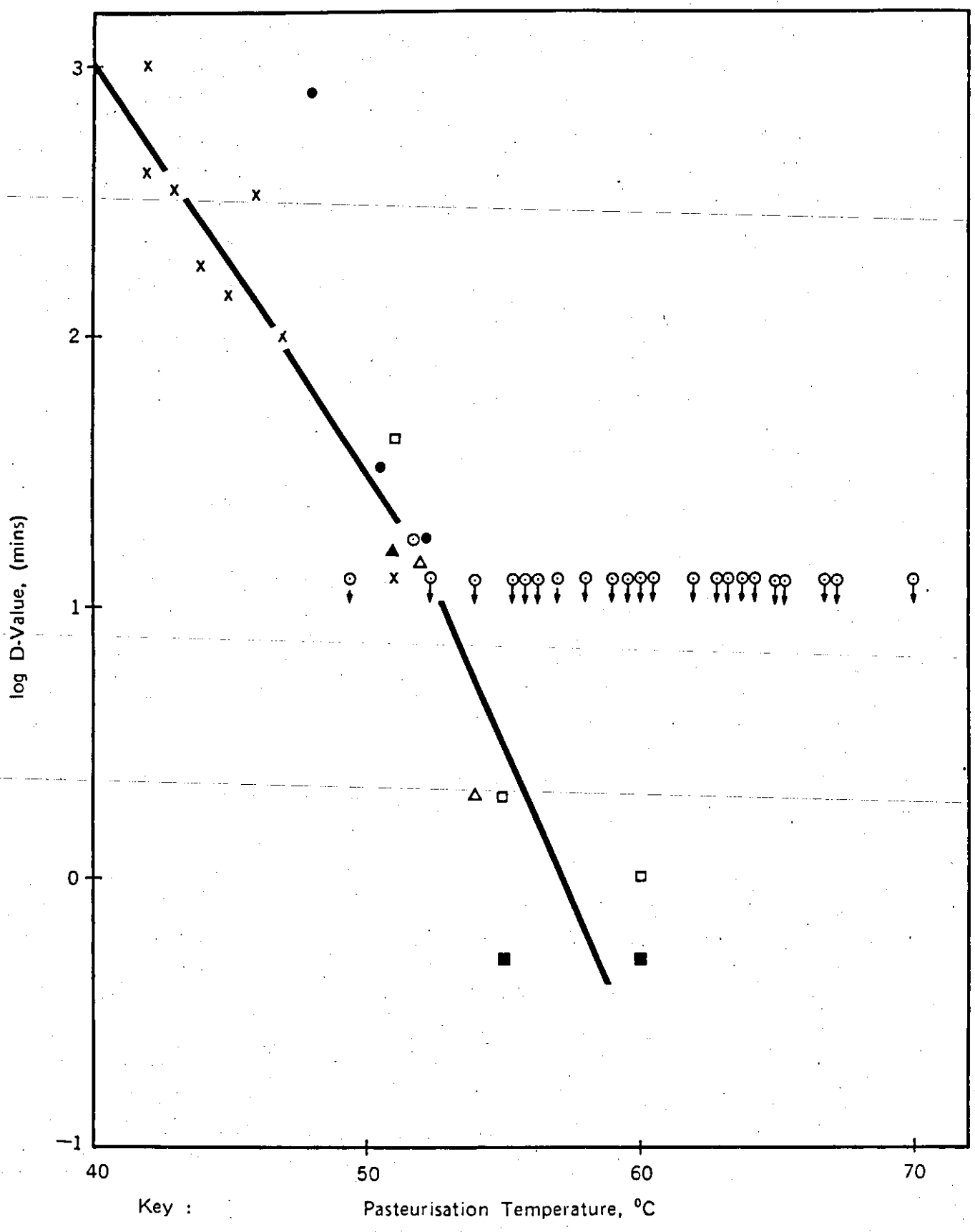


Fig. 7 D-Values for Ascaris Ova

The D-values appear to be little effected by whether the medium is raw sludge, digested sludge or water. This should not be too surprising as sludge contains about 95% water, which is a fairly good heat conductor and, providing mixing is reasonably good, all ova should rapidly be brought to the average sludge temperature. Reports in the literature concerning the protective effects of some media usually pertain to treatments like composting where convection and radiation are the main heat transmission mechanisms.

EFFECT OF TEMPERATURE ON FAECAL COLIFORMS

Figures 8 and 9 show the effect of pasteurisation temperature on faecal coliforms, for the two types of feed. Higher temperatures than in the case of Ascaris ova were required for inactivation.

Figure 10 and Table 15 show the D-values calculated from the present data over the temperature range 54 - 75°C and from the literature. The values for raw sludge are not significantly different to those for digested sludge. The decrease in D-values with increase in temperature is more gradual than in the case of Ascaris up to 64°C, after which there appears to be a very rapid drop. The agreement with values from the literature is fairly good except between 62 and 64°C where Pike reports values which are somewhat lower than those obtained here; he gives no experimental details (Pike, 1982).

TABLE 15 COMPARISON OF D-VALUES FOR FAECAL COLIFORMS

Temp °C	Medium	D Value, minutes	Reference
50	Sludge	45	This study
55		25	(from curve
60		13	of Fig 10)
65		6	
70		<2	
50	Sludge	40-45	Lund 1982
53		30-60	
55-59	?	18	Pike 1982
60-64		3	
65		0.7	

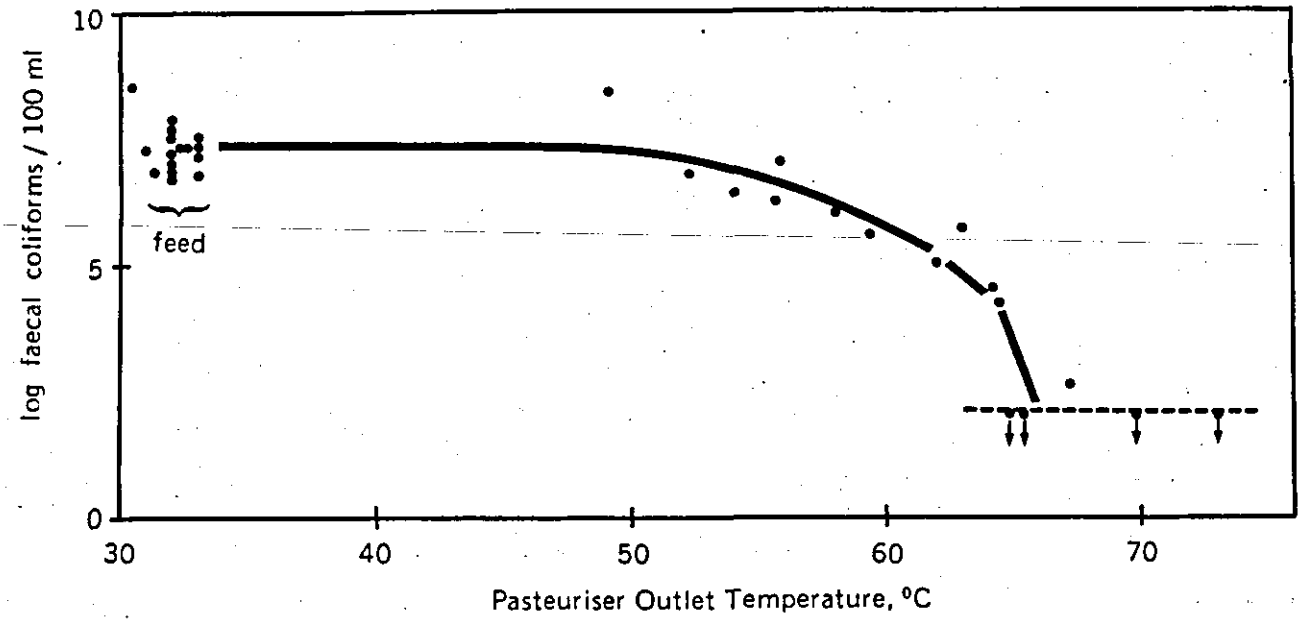


Fig. 8 Inactivation of Faecal Coliforms - Digested Sludge Feed

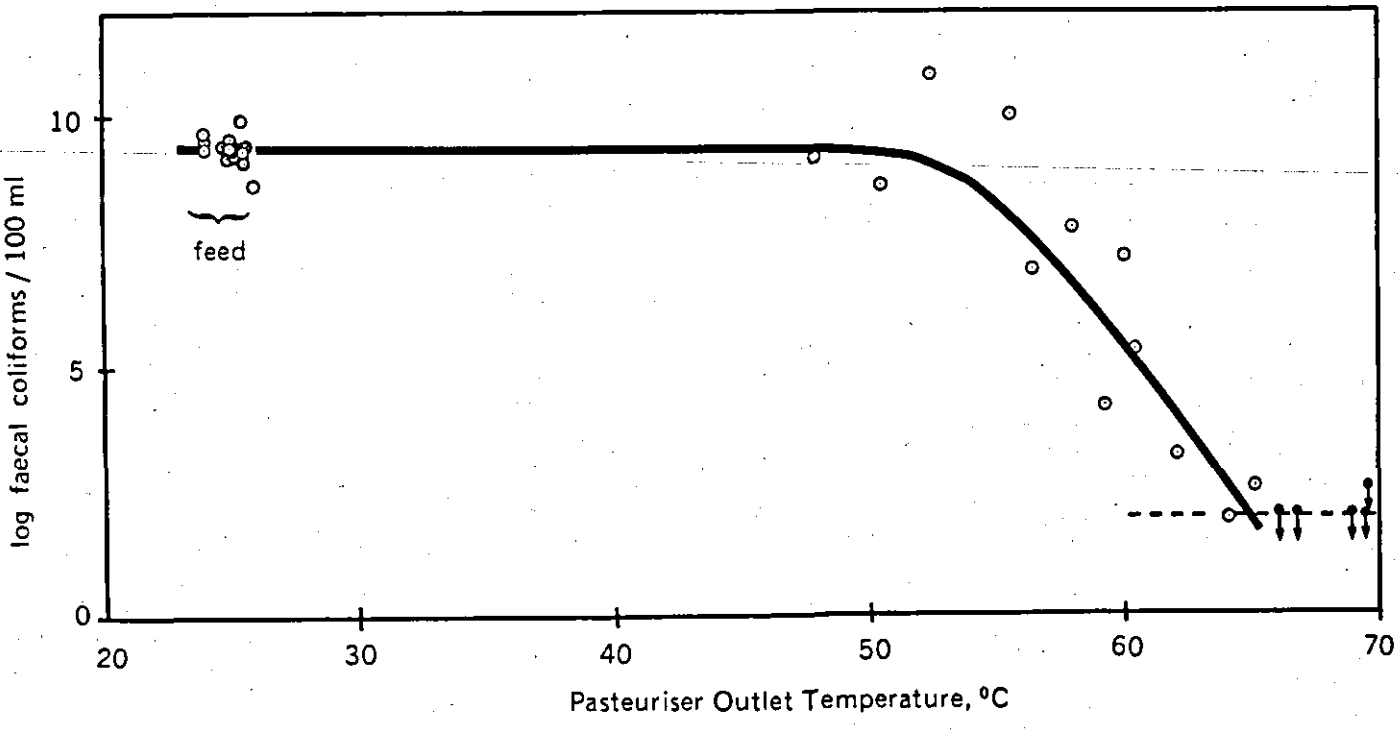
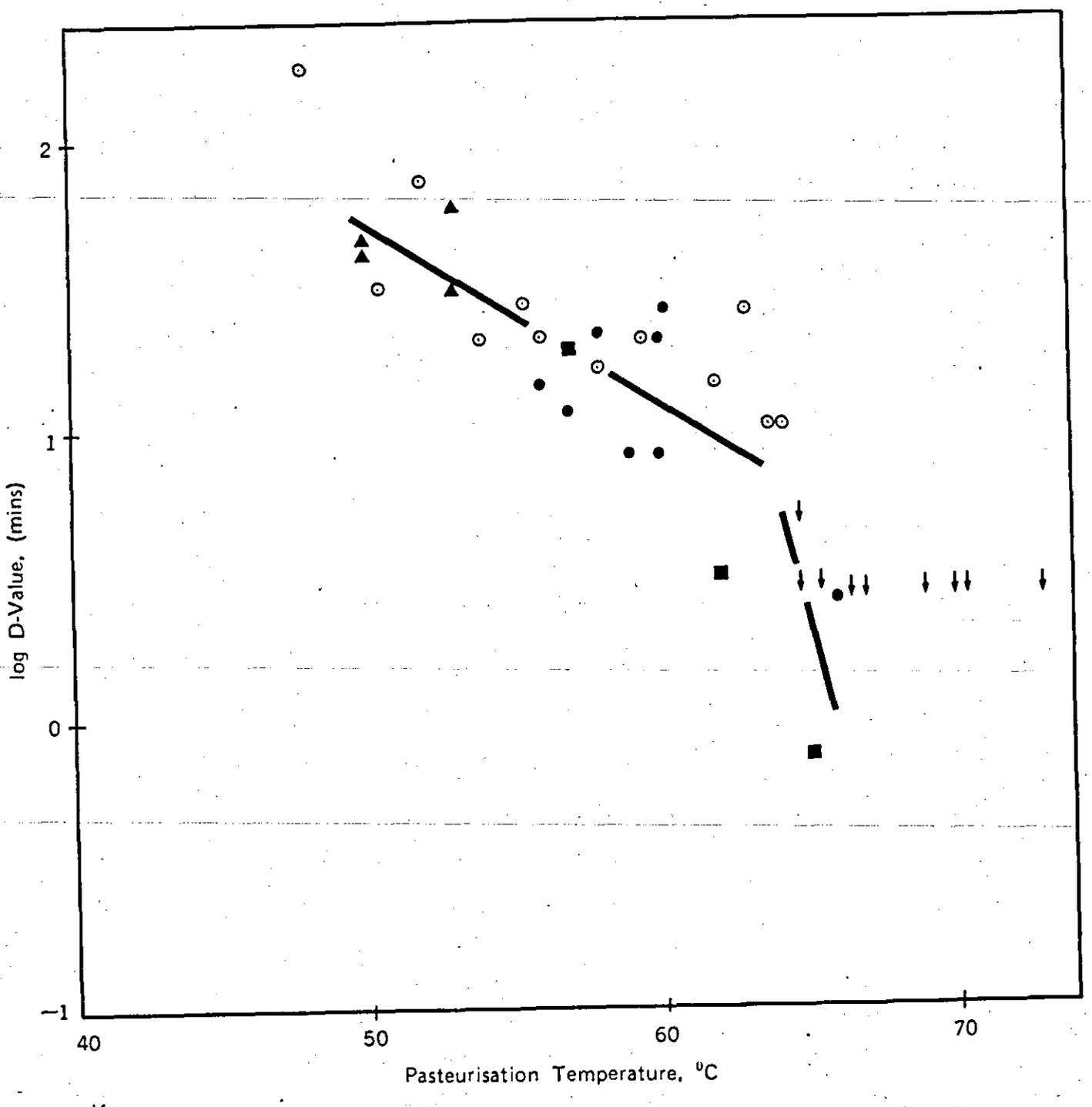


Fig. 9 Inactivation of Faecal Coliforms - Raw Sludge Feed



Key :

- ⊙ This study - digested sludge
- ● This study - raw sludge
- ▲ Lund
- ■ Pike

Fig. 10 D-Values for Faecal Coliforms

7. PHASE 3 - OPERATION UNDER ROUTINE CONDITIONS

For the third phase of the project, operation under routine conditions, it was decided to use pre-pasteurisation because of the greater potential benefit attached to this mode of operation and because the fixed temperature experimental runs described in the previous section had shown that the temperature needed for destruction of faecal coliform indicator organisms (67°C or more), was little influenced by the type of feed. The design retention period of 30 minutes and temperature of 70°C were therefore aimed at.

Table 16 contains average values for the analytical data for this phase of the work. The mean retention time was 38 minutes.

TABLE 16 SUMMARY OF DATA OBTAINED UNDER ROUTINE CONDITIONS

	Raw Sludge	Waste Activated Sludge	Pasteurised Sludge	Digested Sludge
Solids, %m/m (mean)	5.01	3.65	3.83	2.25
Ascaris ova - total/g dry				
50-percentile(median)	3550	1090	1840	3120
80-percentile	4800	1890	2900	4340
Ascaris ova - larvae, %				
50-percentile(median)	90	90	<1	2
80-percentile	93	95	<1	8
Faecal coliforms/100ml				
50-percentile(median)	1000E6	45E6	200	120E3
80-percentile	2000E6	170E6	4E6	760E3
No. samples	12	7	31	18

To measure the degree of temperature control achieved during this phase of the project, readings were taken from the pasteuriser temperature recorder chart at 8-hour intervals over a three-month period, except for a few periods when the steam supply was down. Table 17 gives some statistics for these readings.

TABLE 17 SUMMARY OF TEMPERATURE RECORDER READINGS
(Over three-month period)

	02H00	10H00	18H00
Mean,°C	75	73	73
Std Dev,°C	12	11	11
Min,°C	51	51	51
Max,°C	102	102	100
Count	83	83	83

These values may be compared with those, given in Table 18, which were obtained during sampling visits. The variability of the temperature, as measured by the standard deviation, is much the same for the two sets of results and the sampling therefore covered a representative range of conditions. The mean temperature during sampling runs was about 4°C lower than the average for general operation. When the errors of the recorder are allowed for, the average temperature reached during the three-month period summarised in Table 17 was about 2°C above the target figure of 70°C.

However, it is evident from these figures that temperature variations were greater than desirable and for a significant proportion of the time the temperature was lower than required. Subsequent inspection has revealed that the type of controller installed by the turnkey contractors was not capable of the required performance. A proportional controller installed since the completion of the experimental work has reduced the temperature variations by a factor of about 4.

TABLE 18 TEMPERATURE READINGS ON SAMPLING OCCASIONS

	Recorder °C	Laboratory Thermometer °C
Mean	69.8	68.1
Std Dev	10	10

Figure 11 shows the effect of pasteurisation on Ascaris ova. The requirements for inactivating Ascaris were met except for three occasions when steam supply problems were experienced. The situation with regard to destruction of faecal coliforms (Figure 12) was less favourable, as might be expected in view of the above-mentioned temperature variations, and 68% of samples contained detectable numbers of faecal

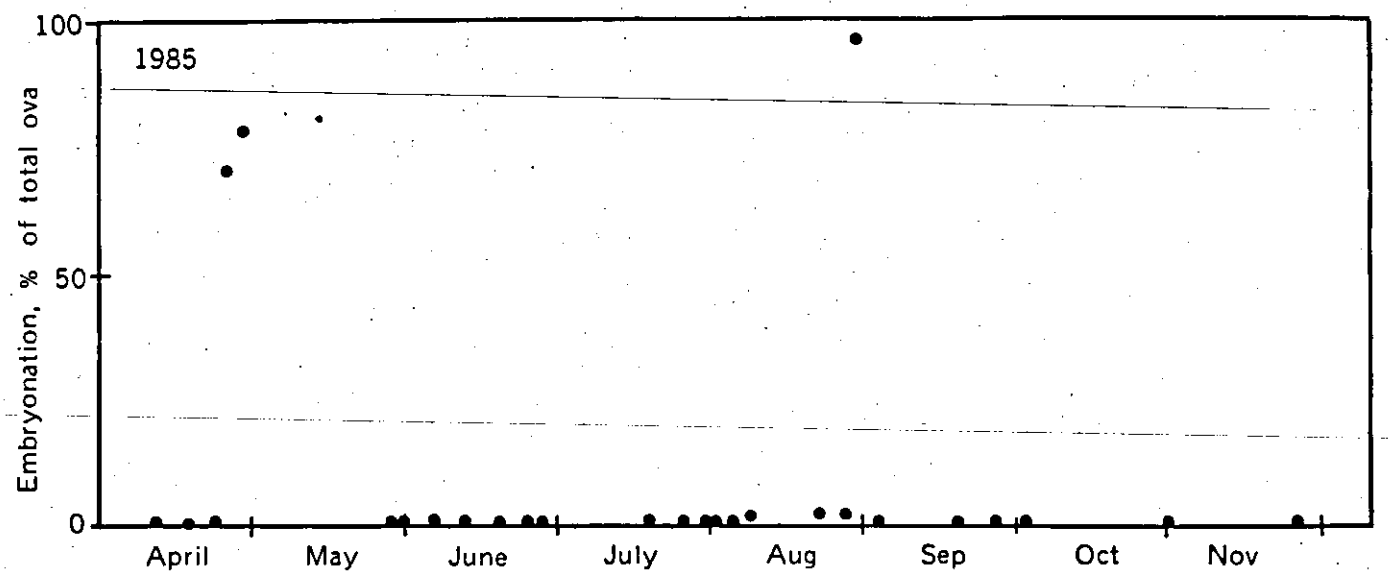


Fig. 11 Quality of Pasteurised Sludge Under Routine Operation - Ascaris Ova

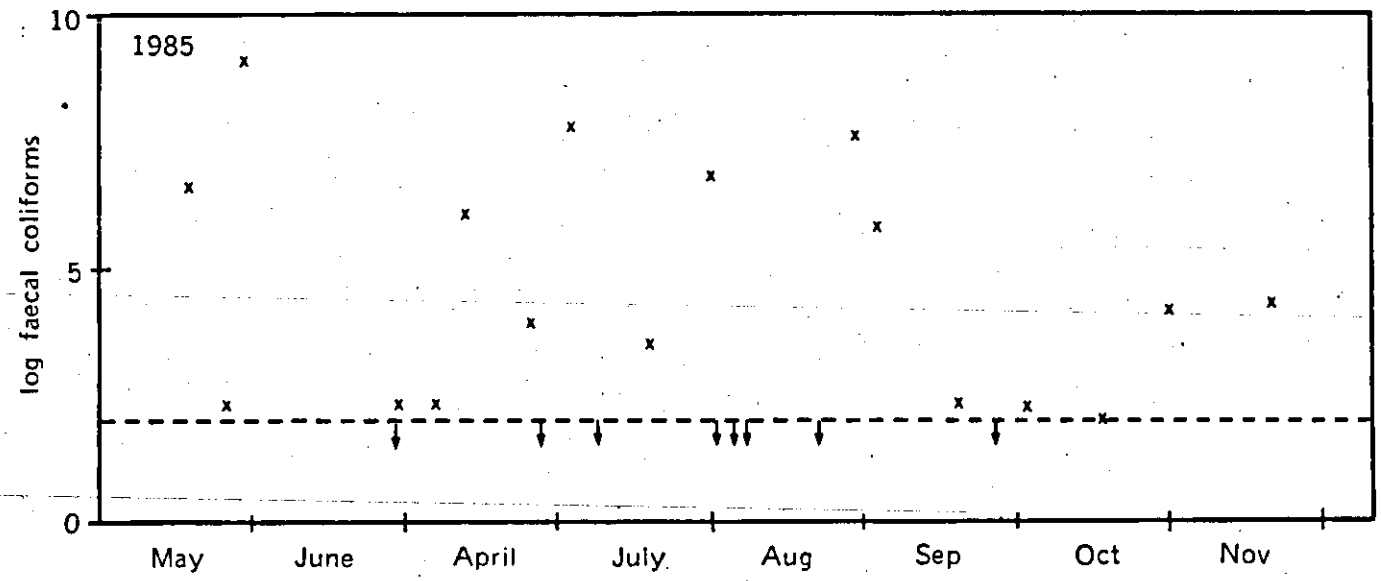


Fig. 12 Quality of Pasteurised Sludge Under Routine Operation - Faecal Coliforms

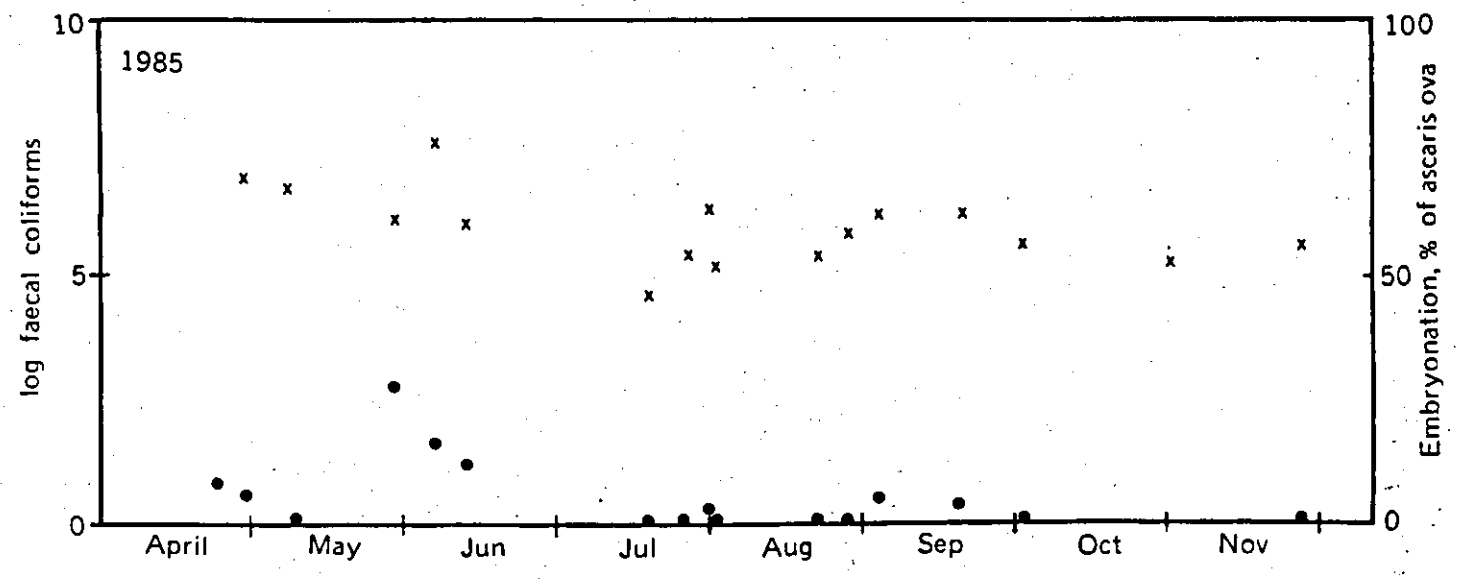


Fig. 13 Quality of Digested Sludge with Pre-Pasteurisation Mode of Operation

coliforms although the median value was only 200/100 ml.

The effect, on the digesters, of using a pasteurised feed is shown in Figure 13. In the period April - June there were a number of occasions when the steam supply was not adequate and the result can be seen in the values for percentage of larvae developing in Ascaris ova. However from July onwards matters in this regard were more satisfactory and the values dropped to a low level. These effects are also visible in the faecal coliform figures. The average value for log faecal coliforms during this latter period was about 5.5, in agreement with the values (5 to 6) given by Philipp (1981) for digested sludge with a pasteurised feed.

The dewaterability of the digested sludge was not directly measured, but observations by the operating staff show it to be essentially unchanged by operation in the pre-pasteurisation mode, being, as usual, rather poor.

To obtain an estimate of heat losses in the pasteuriser, the temperature drop across it was determined on one occasion by lowering a platinum resistance thermometer to a depth of 1 m below the inlet pipe and comparing the readings with those obtained with a laboratory thermometer at the outlet. The measured temperature drop was 1°C, in reasonable agreement with the calculated value of less than 1°C. The inlet temperatures were constant within 0.3°C during the course of the 30 minute test period, the outlet temperature was 67°C and the air temperature 24°C. Conditions were cloudless with a light breeze blowing.

8. DISCUSSION

In the period since the initiation of this work, the emphasis in regard to pasteurisation practice has changed. When cognisance is taken of recent work on the regrowth of pathogenic bacteria by reinfection of pasteurised sludge, it would seem that pasteurisation after digestion is to be regarded as an outmoded process and that future attention should focus on pre-pasteurisation. The present work shows that similar conditions are required for the destruction of faecal coliforms and Ascaris ova in both raw and digested sludge and that operation in the pre-pasteurisation mode will therefore impose no additional requirements on the process.

With pre-pasteurisation, the question of indicator organisms requires further consideration. Thus, if a faecal coliform count of 100/100 ml is used as a criterion, a pasteurised sludge might be judged as being hygienic, while the digested sludge produced from it might not be, because of the regrowth of faecal coliforms in the digester stage of the treatment. Therefore, while faecal coliforms might or might not be suitable indicators of the efficacy of the pasteurisation process, they would not be satisfactory for judging the quality of the final product. What is required is an indicator with similar regrowth characteristics to those of pathogenic bacteria.

Working Party 3 of the EEC Concerted Action Committee (Pike, 1982) has considered various candidates for the role of pasteurisation indicator organisms, and have concluded that faecal streptococci and the Enterobacteriaceae both have merit. They also considered that, where possible, pathogens should be tested for in final trials and commissioning of new plant and recommended that physical measurements (temperature, flow rate) be used for plant control and to provide an official and permanent record of plant performance. Microbiological examinations, being necessarily of a discrete nature and slow to carry out, cannot serve for day to day control but provide a final guarantee of proper quality and warn of deterioration in performance.

Table 19 shows D-values derived from the present work and the literature, for various pathogenic and indicator organisms.

TABLE 19 D-VALUES FOR PATHOGENIC AND INDICATOR ORGANISMS
(D-value is the time required to bring about a 10-fold reduction)

ORGANISM	D-VALUE at					REF
	50°C min	55°C min	60°C min	65°C min	70°C min	
Ascaris lumbricoides ova	13	0.5	0.5	-	-	Burge
	13	<13	<13	<13	<13	This study
Ascaris suum ova	430	-	<30	-	-	Carrington
Taenia saginata ova	310	174	84	-	-	Pike 1983
Faecal coliforms	-	18 (55-59°C)	3	0.7	-	Pike 1982
	44	24	13	6	<3	This study
	40-45	30-60 (53°C)	-	-	-	Lund
Faecal streptococci	-	30-70 (55-59°C)	3-20	1-2	-	Pike 1982
	-	30	-	-	-	Burge
Salmonella (121 strains)	-	0.8-2.6 (57°C)	-	-	-	Burge
Salmonella senftenberg	-	31 (57°C)	-	-	-	"
Salmonella typhi	-	5	-	-	-	"
Salmonella paratyphi	-	5	-	-	-	"
Salmonella typhimurium	50-72	30-60 (53°C)	-	-	-	Lund
Mycobacterium intracellular	-		14-75	2-4	-	Burge
Adenovirus 12	-	2.1	0.17	-	-	"
Reovirus 1	-	0.8	0.09	-	-	"
Herpes simplex	-	0.55	0.05	-	-	"
Rous sarcoma	-	3.9	0.57	-	-	"
Poliovirus 1	300-500	60	2	-	-	"
Poliovirus 2	530	-	-	-	-	"
RNA-poliovirus	50	-	22	-	-	"
Rhinovirus HGP	45	-	-	-	-	"
Bacteriophage f2	910	198	47	10	-	"

There would seem to be a lack of data for the hardier organisms at temperatures above 60°C. It is clear however that Ascaris ova are more easily inactivated than a number of other organisms and, while this is encouraging in the local context in view of the high numbers encountered in sludge, it renders them less safe as a process indicator. Faecal coliforms, the other indicator used in the present work, seem to be hardier than most of the other organisms listed with the possible exception of Taenia and Faecal streptococci. However the differences in D-values noted in the table at 60°C and above make this conclusion uncertain at present.

In deciding on safe conditions for pasteurisation the numbers of organisms in the raw sludge need to be taken into account in addition to the D-values. All the organisms likely to be present in large numbers would appear to have D-values of less than 5 minutes at 70°C, with a resultant degree of inactivation of more than 6 log units in 30 minutes. On the whole therefore the standard conditions of 70°C for 30 minutes seem to be adequate to ensure a safe sludge.

Table 19 shows that the destructive effects of heat are very markedly dependent on temperature, a drop of a few degrees often being the difference betweem success and failure of the pasteurisation proceess. To maintain an adequate margin of safety, the pasteurisation temperature of 70°C must therefore be regarded as the minimum temperature. How far above this the mean or target temperature must be placed will depend on the performance of the temperature controller. With the new controller installed at the Cape Flats plant a mean value of 76°C would be suitable.

Continuous operation, as practised in the plant investigated in the present work, places a more stringent requirement on controller performance than in the case of a batch process. On a full-scale sewage treatment plant flow changes, with resultant alterations in heat requirements, are the order of the day as a result of pump switching and blockages etc. The controller must be able to cope with this situation rapidly. Some form of feed-forward control based on the flow meter signal is indicated in addition to proportional control. It would possibly be advisable to take special steps to limit flow variations. Swiss practice seems to place great emphasis on screening and maceration of sludge to avoid some of these problems (Huber 1984).

In addition, experience on the Cape Flats plant, after the completion of the experimental work of the present study, has shown that the action of the boiler controller can also have a significant effect on the temperature control of the pasteuriser. If the two controllers are completely independent in their action, it can and sometimes does happen that an adequate amount of steam is temporarily unavailable when most needed. It would therefore seem that an overall control system, co-ordinating the action of the two local controllers and taking the sludge flow rate into account, may be needed for the best possible performance.

Operation in the pre-pasteurisation mode has the great attraction of allowing the recovery of almost all the pasteuriser heat, at least in a plant such as that tested where the heat for mesophilic digestion is supplied via the raw feed. Because the pasteurised sludge is too hot it has to be cooled down in heat-exchangers and, provided that the incoming raw sludge is used for this purpose, the only overall losses from the whole system are radiation losses from the pasteuriser, heat-exchangers and pipework. These are expected to be less than 2°C. If the digester feed has to be cooled below about 50°C, by means of cold water, there

will be additional losses but this is only expected to be necessary if exceptionally high pasteurisation temperatures are used during very hot weather.

The heat-exchangers used should be of a non-blocking design. Spiral heat-exchangers do not appear to be suitable for this purpose as blockages cause the full pressure from the pumps to be applied to the heat-exchanger, with subsequent rupture of the joints.

The variable speed pumps required at the pasteuriser outlets in order to maintain a constant sludge volume in the vessels have required a lot of maintenance. If it were possible to design the pasteurisers as sealed flow-through tubes, in line between the sludge pumps and the heat exchangers and digesters, these pumps could be eliminated. The increased pressure thereby brought about at the steam injection point would have to be taken into account.

Table 20 compares the estimated cost of the pasteurisation operation with that of the digestion process. The former figure includes the capital cost of the Cape Flats pasteurisers, heat-exchangers, sludge pumps and building (excluding the boilers and the half of the building used for them). The latter includes the capital cost of the sludge thickeners and pumps, the DAF plant, digesters, boilers, gas mixers and associated buildings. Sludge gas provides most of the fuel used for heating the sludge and fuel costs are therefore not considered. It can be seen that the pasteurisation process adds about 25% to the cost of the sludge treatment and that the major proportion of the costs consist of capital expenses.

Table 20 TREATMENT COSTS

	Pasteurisation	Digestion
Capital cost adj to Jan 1983	R 744 000	R4 513 000
Est capital cost in July 1986 (1% /month)	R1 130 000	R6 854 000
Annual charges:		
Interest and redemption (17% /yr, 15 years)	R 178 000	R1 078 000
Maintenance - spares (5% of capital cost of mech. equip.)	R 33 000	R 51 000
Labour (July 1986, including maintenance)	R 113 000	R 140 000
Electricity (July 1986)	R 21 000	R 106 000
Total annual costs	R 345 000	R1 375 000
Cost/tonne dry digested sludge:		
for 5200 tonne/yr (60% of max)	R 66/tonne	R 264/tonne
for 8700 tonne/yr (max)	R 40/tonne	R 158/tonne

9. CONCLUSIONS

As the result of several years of observation of a large (45 m3) continuous-feed plug-flow pasteurisation installation it is concluded that:

1. Ascaris lumbricoides ova, present in large numbers in sludge from South African waste water treatment works, are readily inactivated by means of pasteurisation, at temperatures of 53 C and above for 30 minutes. The data obtained in this work, under full-scale conditions with raw and digested sludges, agree sufficiently well with those found by other workers, for water and sludges, to obviate the need for further work on the effects of pasteurisation on this organism.

2. Destruction of faecal coliforms, the other process indicator tested, (and possibly some pathogens) requires a temperature of more than 66 C for 30 minutes and careful attention to temperature control. Data from the literature on conditions required to inactivate the hardier pathogens is less complete at temperatures above 60 C than for Ascaris ova.

3. The customary pasteurisation conditions of 70 C for 30 minutes appear to be be adequate for producing an hygienic sludge, but this temperature must be regarded as a minimum rather than an average or target value. Residence time is a less critical parameter than pasteurisation temperature.

4. Work done in Europe indicates that the use of pasteurisers before rather than after mesophilic digesters is desirable to avoid regrowth, in the pasteurised sludge, of pathogenic bacteria such as Salmonella.

5. Operation in the pre-pasteurisation mode provides almost complete heat recovery if the raw sludge is used to cool the pasteurised sludge via heat exchangers. Radiant heat losses in a reasonably well insulated pasteuriser are low (<1 C), at least under South African conditions. The heat exchangers used should be of a non-blocking type.

6. Treatment costs are estimated at between R40 and R66/tonne dry digested sludge (July 1986), representing an increase of 25% over the cost of the normal anaerobic digestion. When heat recovery is practised, capital and labour costs comprise the main treatment expense. The cost of supplying the steam has not been included as it is in any case required for the mesophilic digestion process.

7. To ensure a continuous supply of steam it is advisable to have three boilers, as annual inspections mean that at least one is often out of commission.

8. With pre-pasteurisation, the use of faecal coliforms as indicator suffers from the disadvantage of regrowth in the subsequent mesophilic digestion stage. Possibly faecal streptococci would be more suitable as a process indicator or, alternatively, the quality of the pasteurised sludge could be used as the criterion of acceptance rather than that of the digested sludge.

9. There is merit in using recordings of pasteuriser temperatures as the main proof of adequate pasteurisation, supplemented by calibration checks and backed up by bacteriological examinations.

10. The determination of Ascaris ova can be substantially speeded up by the use of membrane filters, in conjunction with the Visser helminth filter, to isolate them for counting.

11. Standards set by health authorities for judging the hygienic qualities of pasteurised sludge should make allowance for analytical limitations. ie the difficulties of determining small numbers of organisms in the presence of large amounts of sludge solids. The practical limit of detection for faecal coliforms is about 100 organisms/100 ml if 5 replicates are done. A level of 10 000/100 ml would be similar to that adopted by the Swiss for Enterobacteriaceae, and would also ensure that Ascaris ova were effectively inactivated.

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CITY OF CAPE TOWN

CITY ENGINEER'S DEPT — SCIENTIFIC SERVICES BRANCH

METHOD FOR TOTAL ASCARIS OVA IN SEWAGE SLUDGE

1. Mix Sample Thoroughly

2. Determine Solids Content of Sludge:

   Weigh out about 100 g sludge to decimal two places into a dried, weighed porcelain dish. This should be done on the same day as sampling or, if not possible, the sample should be stored at 4°C. (The solids content of sludge changes appreciably in one day, particularly that of raw sludge.) Dry at 105°C for 24 hours, allow to cool and reweigh. Calculate % Dry Solids.

3. Weigh out Sample:

   Weigh a suitable amount of sludge into a small beaker and transfer it quantitatively into a Visser filter. The amount required will depend on the desired precision. Because of sampling considerations, the standard deviation of the count will be approximately equal to it's square root, a count of 100 therefore having a standard deviation of 10. Masses of 1-20 g have been found suitable although at the top end of this range the amount of extraneous matter present on the membrane filter may make counting difficult and may cause the filter to curl and wrinkle.

4. Wash Visser Filter:

   Direct a jet of tap water onto the sludge and around the sides of the inner and outer filters. A 4 min wash is suitable for the inner filter after which it is removed and the outer 37 µm filter further washed to remove the bulk of the extraneous matter. Although thorough washing at this stage makes the final counting easier, the exact amount is a matter for trial and error.

5. Filter Ova onto a Membrane Filter:

   Quantitatively transfer the material remaining on the outer filter into a 1 l glass beaker and then filter through a 12 µm 50 mm membrane filter. Rinse beaker well. Switch off the suction a few seconds after the water has drained.

6. Dry Filter:

Place filter on a stainless steel support, weighting it down with a stainless steel ring of appropriate diameter to prevent curling. Drying can be done at any temperature between room temperature and about 38°C until the filter is just visibly dry. Do not heat for longer than necessary as the ova may become partly desiccated.

7. Prepare Filter for Examination:

Transfer the dried filter upside down into a 65 mm disposable plastic petri dish. The ova and other material adhere very well to the filter and it can be quite roughly handled. Apply just enough immersion oil, starting from the centre of the filter, to eventually render it transparent. Place the petri dish upside down on a 76 X 51 mm microscope slide and examine under a suitable magnification (100 or 200X) for counting.

The use of petri dishes in this feshion allows filters to be transported easily and stored safely. However the filters usually become milky after a few days and an alternative procedure is to place the filter, right side up, on a 76 X 51 mm microscope slide, and apply oil as before. If large slides are not available, the filter can be cut in half and placed on two smaller slides. Filters on glass slides do not become milky.

8. Calculate Result:

Ova/g dry sludge = (Count X 100) / (Mass Sludge X % Solids)

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METHOD FOR ASCARIS VIABLITY IN SEWAGE SLUDGE

1. Prepare a suspension of ova for embryonation:

   Weigh 20 g of well shaken sludge into a 50 ml beaker, transfer to a Visser filter and wash for about 5 min. Transfer the ova from the outer filter to a 250 ml medical flat or conical flask, add 2 ml of formaldehyde solution (40%) and make up to about 100 ml with water.

   Cap loosely or plug with cotton wool to allow access to air. Leave at 25 - 28°C for 6 weeks.

2. Prepare ova for microscopic examination:

   Shake the suspension well, filter a 20 ml portion or other suitable volume through a membrane filter and treat the filter for examination in the manner described in the method for total Ascaris ova.

3. Do a viability count:

   Examine at least 100 ova and classify as follows:

   1 - Fully developed worm inside the shell (quiet or motile).

   2 - All other ova.

4. Calculate the percentage of embryonated ova:

   Examination of 100 ova will allow results to be reported to the nearest 1%. If 0.1% precision is required, 1000 should be examined.

For some purposes the more detailed classification of Murray (J Inst Sewage Purification 1960 Vol 3 337-344) might be more suitable:

1 - Fully developed worm inside the shell (quiet or motile).

2 - Partly developed ova ie containing divided cells.

3 - Single cell ova, including infertile ova - no apparent change.

4 - Degenerated ova, with or without vacuoles.

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METHOD FOR FAECAL COLIFORMS IN SEWAGE SLUDGE

1. Shake sample well and dilute by an appropriate factor with dilution water:

   The following dilutions usually suffice.

Raw sludge:	10E7, 10E8 and 10E9
Digested sludge and DAF float:	10E4, 10E5 and 10E6
Incompletely pasteurised sludge:	100 to 10E6
Well pasteurised sludge:	10, 100

   (See 2 for the maximum amount that can be filtered).

2. Filter a suitable volume of diluted sample through a sterile 0.45 µm membrane filter:

   For all dilutions except 10 and 100, use 50 ml portions.

   For dilutions of 10 and 100 a maximum of 2 ml and 20 ml, respectively (equivalent to 0.2 ml of wet sludge), can be used without blocking the filter or blackening it too much for proper colony counting. If a limit of detection of 100/100 ml is desired, a total of 1 ml of wet sludge will have to be examined and, in such a case, five separate determinations will have to be carried out to make up the full volume.

3. Transfer filter aseptically to 60 mm petri dish containing m-FC agar.

4. Incubate at 44.5°C for 24 h.

5. Count all dark blue colonies.

6. Calculate the number of colonies per 100 ml of wet sludge.

DETAILED RESULTS FOR RUNS WITH A FEED OF DIGESTED SLUDGE

Key: Lar - larvae, PD - partly developed
SC - single cell Deg - degenerated

Sample	Date	Past Temp °C	Past Time min	Solids % m/m	f coli /100 ml	Ascaris /g dry	Lar %	PD %	SC %	Deg %
Raw	1983-10-27			3.05	530E+06	5840	89	1	5	5
DAF	1983-10-27			3.52	40E+06	800	78	0	2	20
Digested	1983-10-27			2.27	35E+06	5660	64	4	20	12
Past	1983-10-27	62	36	2.12	110000	5090	0	0	57	43
Raw	1983-11-02			3.48	1060E+06	5860	93	0	3	4
DAF	1983-11-02			3.55	34E+06	960	86	0	3	11
Digested	1983-11-02			2.18	16E+06	7160	69	1	22	8
Past	1983-11-02	63	35	2.06	590000	5170	0	0	32	68
Raw	1983-11-17			4.34		5250	92	2	2	5
DAF	1983-11-17			3.23		870	93	2	0	5
Digested	1983-11-17			2.20		5890	64	1	22	13
Past	1983-11-17	67	40	2.08	500	5000	0	0	7	93
Raw	1983-11-24			3.29		6870	87	1	6	6
DAF	1983-11-24			3.46		660	98	0	0	2
Digested	1983-11-24			2.23	12E+06	6500	61	2	13	24
Past	1983-11-24	70	35	2.09	0	5740	0	0	0	100
Raw	1983-11-30			2.98		4950	90	0	1	9
DAF	1983-11-30			3.44		1450	95	0	0	5
Digested	1983-11-30			2.26	9.2E+06	8430	65	0	10	25
Past	1983-11-30	73	31	2.10	0	7620				
Raw	1983-12-13			1.98	660E+06	10530	93	2	1	4
DAF	1983-12-13			3.33	15E+06	990	86	0	7	7
Digested	1983-12-13			1.97	22E+06	7460	75	0	2	23
Past	1983-12-13	60	39	1.86	370000	6420	0	1	57	43
Raw	1983-12-20			2.65		5770	86	0	6	8
DAF	1983-12-20			3.24		1360	98	0	0	2
Digested	1983-12-20			1.87	74E+06	8900	74	0	12	13
Past	1983-12-20	54	30	1.75	2.8E+06	7310	0	0	89	11
Raw	1984-01-12			2.96	840E+06	3920	93	0	2	5
DAF	1984-01-12			3.56	18E+06	960	100	0	0	0
Digested	1984-01-12			1.71	21E+06	7980	78	1	16	5
Past	1984-01-12	56	30	1.61	2E+06	7580	0	0	85	15

Sample	Date	Past Temp °C	Past Time min	Solids % m/m	f coli /100 ml	Ascaris /g dry	Lar %	PD %	SC %	Deg %
Raw	1984-02-09			2.38		6890	88	1	10	2
DAF	1984-02-09			3.86		1350	97	3	0	0
Digested	1984-02-09			1.79		7990	83	1	10	5
Past	1984-02-09	67	29	1.17		8070	0	0	1	99
Raw	1984-02-23			2.51	2300E+06	6850	85	0	1	14
DAF	1984-02-23			3.45	78E+06	1570	96	0	4	0
Digested	1984-02-23			1.67	6.2E+06	8800	80	1	9	11
Past	1984-02-23	65	33	1.59	0	6920	0	0	11	89
Raw	1984-03-01			1.25	620E+06	8600	90	1	2	7
DAF	1984-03-01			4.05	71E+06	1180	97	0	2	2
Digested	1984-03-01			1.63	19E+06	7640	88	1	4	8
Past	1984-03-01	52	30	1.55	7E+06	7260	1	1	82	15
Raw	1984-03-07			1.73	130E+06	6850	89	0	1	9
DAF	1984-03-07			3.76		1360	97	0	3	0
Digested	1984-03-07			1.59	310E+06	7920	84	0	2	14
Past	1984-03-07	56	30	1.50	12E+06	7630	0	2	65	33
Raw	1984-03-15			3.34	980E+06	7150				
DAF	1984-03-15			1.44	25E+06	1660	92	6	3	0
Digested	1984-03-15			1.58	47E+06	9650	64	4	4	28
Past	1984-03-15	58	28	1.47	1.16E+06	9180	0	0	73	27
Raw	1984-03-22			3.54	1600E+06	6580	85	0	1	14
DAF	1984-03-22			3.48	29E+06	1240	96	0	2	2
Digested	1984-03-22			1.67	23E+06	8683	89	1	3	8
Past	1984-03-22	64	31	1.57	18000	7360	0	1	37	62
Raw	1984-03-27			2.39	2800E+06	6800	94	0	2	5
DAF	1984-03-27			3.03	28E+06	1650	97	0	3	0
Digested	1984-03-27			1.73	33E+06	8580	90	1	2	7
Past	1984-03-27	64	28	1.63	39000	7180	0	0	27	73
Raw	1984-04-26			2.32	1780E+06	6490	85	0	0	15
DAF	1984-04-26			3.62	85E+06	1270	93	0	4	2
Digested	1984-04-26			1.68	16E+06	7770	87	0	3	10
Past	1984-04-26	65	32	1.77	0	7010	0	0	25	75
Raw	1984-06-25			2.42	890E+06	6200				
DAF	1984-06-25			2.41	88E+06	950	92	0	3	6
Digested	1984-06-25			2.42	24E+06	5600	90	0	2	8
Past	1984-06-25	49	28	2.36	260E+06	5020	0	0	53	47
Raw	1984-09-03			3.74	980E+06	2410				
DAF	1984-09-03			3.61	89E+06	970	70	0	30	0
Digested	1984-09-03			2.52	8E+06	3630	80	0	20	0
Past	1984-09-03	48	31	2.48		4820				

DETAILED RESULTS FOR RUNS WITH A FEED OF RAW SLUDGE AND DAF FLOAT

Key: Lar - larvee, PD - partly developed
SC - single cell Deg - degenerated

Sample	Date	Past Temp °C	Past Time min	Solids % m/m	f coli /100 ml	Ascaris /g dry	Lar %	PD %	SC %	Deg %
Raw	1985-01-08			3.92	4600E+06	3790	75	14	8	3
Past	1985-01-08	51	31	3.70	413E+06	3710	10	13	53	24
Raw	1985-01-15			3.54	2400E+06	4530	85	5	1	10
Past	1985-01-15	52	34	3.95	69000E+06	4260	0	1	91	8
Raw	1985-01-17			3.95	2300E+06	4220	88	2	2	9
Past	1985-01-17	48	34	4.09	1800E+06	3760	83	1	12	5
Raw	1985-01-22			5.32	2200E+06	4660	87	0	0	13
Past	1985-01-22	56	34	5.18	10000E+06	4720	0	0	73	27
Raw	1985-01-31			4.33	2040E+06	3430	85	2	1	12
Past	1985-01-31	58	34	4.15	64E+06	3750	0	0	71	29
Raw	1985-02-05			3.78	440E+06	4150	63	2	14	22
Past	1985-02-05	59	34	3.58	20000	4020	0	0	71	29
Raw	1985-02-12			4.04	2500E+06	4550	86	2	8	5
Digested	1985-02-12			2.57	29E+06	4400	16	1	59	24
Past	1985-02-12	56	34	3.92	9E+06	3920	0	0	77	23
Raw	1985-02-15			4.53	8200E+06	3390	85	0	4	11
Past	1985-02-15	60	35	4.37	220E+03	3190	0	0	52	48
Raw	1985-02-26			4.73	1400E+06	4950	89	1	2	8
Digested	1985-02-26			2.56	1.2E+06	5300	8	2	82	8
Past	1985-02-26	60	36	44	18E+06	4020	0	0	76	24
Raw	1985-03-01			4.27	2100E+06	4400	88	0	4	8
Past	1985-03-01	63	36	4.13		3630	0	0	14	86
Raw	1985-03-06			4.24	3400E+06	5160	90	0	4	6
Digested	1985-03-06			2.60	740000	4920	0	0	0	100
Past	1985-03-06	70	31	3.96	0	2470	1	0	78	22

Sample	Date	Past Temp °C	Past Time min	Solids % m/m	f coli /100 ml	Ascaris /g dry	Lar %	PD %	SC %	Deg %
Raw	1985-03-08			4.90	1900E+06	5030	91	0	1	8
Past	1985-03-08	66	32	4.62	0	2610	0	0	90	10
Past	1985-03-13	62	37		1800					
Past	1985-03-13	64	37		100					
Raw	1985-03-19				3300E+06					
Digested	1985-03-19			2.53	5.9E+06	4940	0	0	20	80
Past	1985-03-19	65	38		400					
Raw	1985-03-28				1400E+06					
Digested	1985-03-28			2.52	17E+06	4030	0	0	22	78
Past	1985-03-28	69	36		0					
Raw	1985-04-02				1100E+06					
Digested	1985-04-02			2.47	3.5E+06	3970	4	11	64	22
Past	1985-04-02	67	36		0					

DETAILED RESULTS FOR RUNS UNDER ROUTINE CONDITIONS

Key: Lar - larvae, PD - partly developed
SC - single cell Deg - degenerated

Sample	Date	Past Temp °C	Past Time min	Solids % m/m	f coli /100 ml	Ascaris /g dry	Lar %	PD %	SC %	Deg %
Raw	1985-04-11			4.12	1200E+06	6100	85	0	7	8
DAF	1985-04-11			3.85	29E+06	1830	95	0	0	5
Past	1985-04-11	56	39	3.95		4810	0	0	81	19
Digested	1985-04-16			2.26	10E+06	3940	7	1	65	28
Raw+DAF	1985-04-16			4.40		4350	95	0	0	5
Past	1985-04-16	49	44	4.10		4590	71	0	18	11
Raw+DAF	1985-04-18			3.97	2200E+06	4380	89	0	5	6
Past	1985-04-18	57	30	3.82	4E+06	3780	0	0	89	11
Raw	1985-04-23			5.77		3670	86	0	0	14
Digested	1985-04-23			2.26		3430	8	0	45	46
Past	1985-04-23	69	31	4.25		920	0	0	0	100
DAF	1985-04-25			3.14	30E+06	1890	71	0	0	29
Past	1985-04-25	72	30	2.95	200	1970				
Raw	1985-04-29			3.91	1800E+06	4800	88	0	4	9
Digested	1985-04-29			2.32	7.6E+06	4560	6	2	53	40
Raw+DAF	1985-04-29			3.26	850E+06	2870	88	0	2	9
Past	1985-04-29	47	22	3.36	1300E+06	2900	79	0	17	4
Digested	1985-05-07			2.20	5.6E+06	4570	0	0	2	98
DAF	1985-05-07			4.12	45E+06	720				
Past	1985-05-07	58	32	3.61	38E+06	2310	0	1	94	5
Digested	1985-05-28			2.36	1.2E+06	4280	28	8	38	26
DAF	1985-05-28			3.81	27E+06	520	87	0	13	0
Past	1985-05-28	90	52	3.63	0	530				
DAF	1985-05-29			3.77	170E+06	580	94	0	0	6
Past	1985-05-29	66	52	3.55	170	410	0	0	24	76

Sample	Date	Past Temp °C	Past Time min	Solids % m/m	f coli /100 ml	Ascaris /g dry	Lar %	PD %	SC %	Deg %
Digested	1985-06-06			2.13	37E+06	3120	16	2	59	23
Raw+DAF	1985-06-06			1.76	330E+06	1490	92	0	0	8
Past	1985-06-06	67	26	2.12	200	1130	0	0	24	76
Digested	1985-06-12			2.19	12E+06	3120	12	2	57	29
DAF	1985-06-12			3.11	65E+06	1090	86	0	7	7
Past	1985-06-12	61	49	5.57	1.1E+06	1480	0	1	66	33
Raw	1985-06-19			7.26	290E+06	3440	91	3	0	6
Past	1985-06-19	58	31	5.18		3510	0	0	90	10
Past	1985-06-25	75	53	3.75	7800	1480	0	0	0	100
Raw	1985-06-27			5.40	850E+06	4800	94	0	2	4
Past	1985-06-27	70	27	4.00	0	1290	0	0	0	100
Raw	1985-07-03			6.70	2100E+06	3060				
Past	1985-07-03	53		4.82	60E+06	1880				
Past	1985-07-08	80	47	3.91	0	420				
Digested	1985-07-18			2.28	45000	3060	0	0	54	46
DAF	1985-07-18			3.76	1220E+06	1380	96	0	0	4
Past	1985-07-18	63	56	3.70	3500	1240	0	1	45	55
Digested	1985-07-25			2.32	310000	2740	1	3	56	40
Raw+DAF	1985-07-25			3.40	110E+06	4040	69	26	4	0
Past	1985-07-25	62		3.37	20000	2000	0	0	60	40
Digested	1985-07-30			2.31	2.1E+06	2180	3	2	59	36
Past	1985-07-30	68	51	3.96	6.6E+06	1650	0	0	70	30
Digested	1985-08-01			2.26	150000	2170	0	0	0	100
Raw+DAF	1985-08-01			7.76	400E+06	3320	94	0	4	2
Past	1985-08-01	80	27	4.24	0	1250	0	0	0	100

Sample	Date	Past Temp °C	Past Time min	Solids % m/m	f coli /100 ml	Ascaris /g dry	Lar %	PD %	SC %	Deg %
Raw+DAF	1985-08-05			2.33	70E+06	2470				
Past	1985-08-05	71	38	3.96	0	1280	0	0	25	75
Raw	1985-08-07			4.02	1500E+06	1930	92	1	0	8
Past	1985-08-07	73	39	3.55	0	1120	1	0	0	99
Raw	1985-08-21			6.84	330E+06	4140	95	1	1	3
Digested	1985-08-21			2.25	240000	870	0	0	28	72
Past	1985-08-21	77	40	4.65	0	1230	2	0	1	96
Digested	1985-08-27			2.35	690000	2340	1	0	68	31
Past	1985-08-27	62	28	4.68	>400000	2040	2	1	80	17
Raw	1985-08-29			5.99	360E+06	3400	92	0	0	8
Past	1985-08-29	31	53	3.59	44E+06	2420	96	0	0	4
Digested	1985-09-03			2.47	1.6E+06	2920	5	0	75	20
Past	1985-09-03	58	24	4.33	640000	2490	0	0	82	18
Digested	1985-09-19			2.11	1.2E+06	4340	4	0	42	54
Past	1985-09-19	81	28	3.94	200	1970	0	0	0	100
Raw	1985-09-26			4.61	1100E+06	2280	2	0	40	58
Past	1985-09-26	75	50	3.38	0	2740	0	0	0	100
Digested	1985-10-02			2.02	440000	4640	0	0	63	37
Past	1985-10-02	70	26	2.87	200	3040	0	0	74	26
Raw	1985-10-16			0.72	510E+06	1210				
Past	1985-10-16	76	70	1.91	100	3550				
Raw	1985-10-30			4.80	1000E+06	4230	87	0	9	4
Digested	1985-10-30			2.01	180000	3600	0	0	27	73
Past	1985-10-30	64	20	5.02	16000	1274	0	0	43	57
Digested	1985-11-26			2.16	360000	2770	0	0	36	64
Past	1985-11-26	78	20	2.93	0	1840	0	0	0	100