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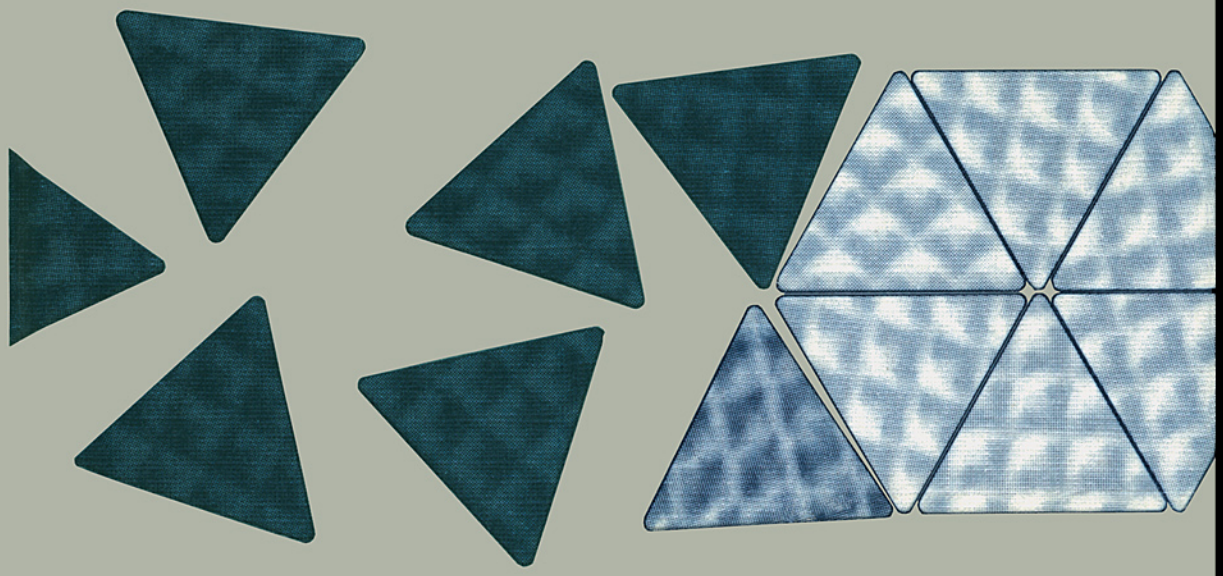
INACTIVATION OF MICROORGANISMS IN SEWAGE SLUDGE BY STABILISATION PROCESSES



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INACTIVATION OF MICROORGANISMS IN SEWAGE SLUDGE BY STABILISATION PROCESSES

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PREFACE

In the framework of the Concerted Action “Treatment and use of sewage sludge”, the Working Party 3 is responsible for “Hygienic aspects related to treatment and use of sewage sludge”. Because of the close entwinement of the hygienic problems with other problems of sewage sludge technology, hygienic aspects were discussed in the previous conferences of COST 68, in cooperation with other working parties, at Cadarache in 1979, Vienna in 1980, Zürich in 1982 and Brighton in 1983.

It became apparent that in various countries, methods for disinfecting treatment are at present being developed or tested which first of all should be discussed among the researchers involved to assess their applicability under field conditions. Among them are methods such as anaerobic thermophilic digestion, aerobic thermophilic stabilisation, utilisation of lime in sewage and sludge treatment, and dewatering of sludge in reed-covered filter beds. As far as parasites in sludge are concerned, many questions remained unanswered and therefore several papers deal with topics such as the effect of pasteurisation and various chemicals on parasite ova, a problem which is increasingly gaining in importance.

The Institute of Animal Medicine and Hygiene of the University of Hohenheim in Stuttgart was therefore asked by Working Party 3 to organise a seminar dealing with these problems and which would be restricted to scientists working in the field of sludge hygiene. To demonstrate some of the latest technological developments of sludge treatment with disinfecting effects, technical visits were organised during the seminar. Thus full-scale plants with aerobic thermophilic stabilisation of liquid raw sludge, and with bio-reactors and bio-cell-reactors for the stabilisation of dewatered sludge by composting, were visited by participants.

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CONTENTS

SESSION I

Chairmen: E.LUND
A.H.HAVELAAR

Influence of mesophilic anaerobic digestion and pasteurisation of raw and digested sludge on viruses occurring in humans and domestic animals

Influence of thermophilic anaerobic digestion (55°C) and subsequent mesophilic digestion of sludge on the survival of viruses without and with pasteurisation of the digested sludge

Aerobic thermophilic stabilization

Investigations on the hygienic effect of single stage and two-stage aerobic-thermophilic stabilization of liquid raw sludge

Sludge hygienization with different compost systems

Influence of lime treatment of raw sludge on the survival of pathogens, on the digestability of the sludge and on the production of methane—Technical experiences with high pH in the digester of a sewage treatment plant

Influence of lime treatment of raw sludge on the survival of pathogens, on the digestability of the sludge and on the production of methane—Hygienic investigations

INFLUENCE OF MESOPHILIC ANAEROBIC DIGESTION AND PASTEURISATION OF RAW AND DIGESTED SLUDGE ON VIRUSES OCCURRING IN HUMANS AND DOMESTIC ANIMALS

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Summary

The effect of the conventional mesophilic anaerobic one-step digestion process and of a two-step process with and without pre-pasteurisation on three not enveloped viruses was investigated. The experiments were performed in a pilot-plant of semi-technical scale. Reovirus (Type 1) and bovine enterovirus (ECBO-LCR-4) were completely inactivated by the one-step mesophilic anaerobic digestion process with a mean hydraulic detention time of 20 days as well as by the two-step process with a mean hydraulic detention time of two days in the anaerobic pretreatment step at 33°C or 20°C and eight days in the main digestion step at 33°C with and—with one exception—without pre-pasteurisation. None of the processes mentioned resulted in a complete inactivation of bovine parvovirus (strain Haden). Even by pasteurisation of the digested sludge this virus could not be inactivated in all cases.

1. INTRODUCTION

The experiments were performed in a pilot-plant of the Institute for Water Quality Management and Public Health Engineering of the Technical University of München in Garching. The behaviour of viruses occurring in humans and domestic animals during mesophilic anaerobic digestion of sewage sludge and pasteurisation of raw and digested sludge was investigated.

When the main degradation steps during anaerobic digestion of organic substance are proceeding in two separate reactors (= two-phase digestion), in the first reactor an acidification up to 100 % can be achieved during a very short detention time when the substrates are largely soluble. For the complete process this results also in a shortening and an intensification of the efficiency (5, 11). But with sewage sludge it is not possible to achieve such a high percentage of acidification (6, 8). The reason for that is most likely the large portion of particulate organic substance by which their hydrolysis will become the speed limiting step (6). In a two-phase anaerobic stabilisation of sewage sludge in two reactors in series connection in the first reactor the easily degradable substances are already decomposed to methane and carbon dioxide (short digestion). In the second reactor the final degradation takes place to the desired degree of stabilisation (main digestion). In this way it was possible during the experiments performed in Garching to reduce the detention time necessary for stabilisation by 25 % compared to a conventional one-phase digestion process (18). The pre-pasteurisation of raw sludge is considered to be a suitable technology to reconcile hygienic and economical aspects of sludge treatment (1, 4). The experiments in Garching showed that pre-pasteurisation of raw sludge does not increase the methane production but does im prove the degradation of organic solids as well as the dewaterability of the digested sludge (18).

2. MATERIAL AND METHODS

2.1 Viruses and cells

The following non-enveloped viruses were chosen because of their known high resistence against physical and chemical influences:

- Bovine parvovirus strain “Haden”, DNA-virus (BPV).
- Bovine enterovirus ECBO-LCR-4, RNA-virus (BEV).

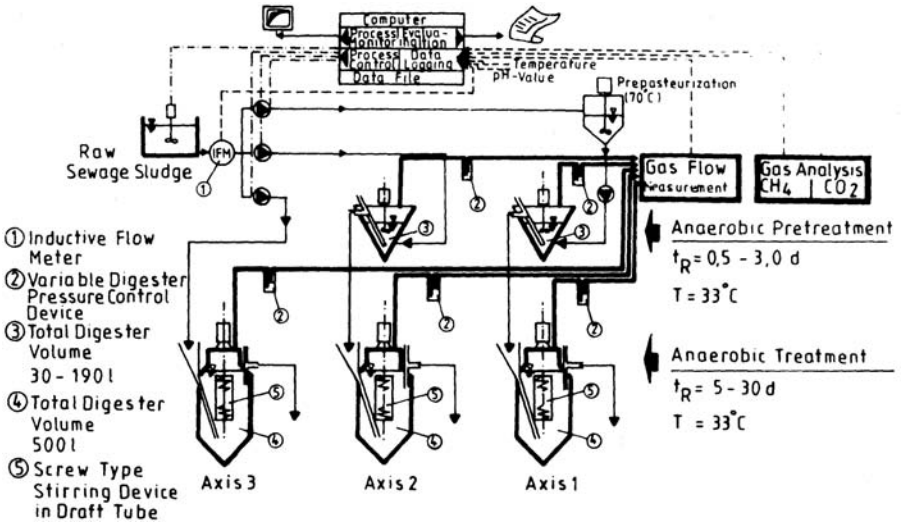

Figure 1. Flow diagram of the pilot-plant

– Reovirus type 1, RNA-virus (RV).

BPV was propagated on primary fetal lung tissue-cultures, BEV on tissue-cultures of the permanent fetal bovine kidney cell-line Au-BEK and RV on tissue-cultures of the permanent African green monkey cell-line VERO.

Virus assays were performed by using the described tissue-cultures. BPV and BEV assays were made in 24-well COSTAR-tissue-culture-plates those with RV in tissue-culture test tubes.

2.2
Pilot plant and experimental set-up

All experiments were done in a pilot plant for anaerobic sludge stabilisation which was composed of three axis (Fig. 1).

The first axis consists of a vessel for pasteurisation of raw sludge (Fig. 1—Pre-pasteurisation) which is followed by a funnel-shaped digester for anaerobic treatment (Fig. 1, No. 3) and a cylindrical digester for anaerobic treatment with a servise volume of 500 1 (Fig. 1, No. 4). The second axis consists only of the digester for anaerobic pretreatment (No. 3) and the digester for anaerobic treatment (No. 4) and the third axis only of the digester for anaerobic treatment (No. 4). The digesters No. 3 and 4 are equipped with agitators and represent, in the technical sence, ideally stirred vessels.

All three axis are fed with raw sludge from a joint vessel with a service volume of 500 1. The pilot-plant was automatically fed by pumps three times a day (8.00, 16.00, 24.00). The axis 1 and 2 receive 63 1 of raw sludge and axis 3 25 1 per day. A respective amount of digested sludge is displaced via the effluent pipes.

The pre-pasteurisation in axis 1 was performed with each automatic feeding of the system, e.g. 3 times daily. The vessel for pasteurisation has a capacity of 30 1. After the heat-up phase of approx. 4 hours the sludge was pasteurised at 70°C for 30 min.

The service volume of the vessels for anaerobic pretreatment (Fig. 1, Nos. 3) can be varied between 30 and 190 1 by a variable digester pressure control device (Fig. 1, Nos. 2). Through variation of the service volume and respective assessment of the daily feeding amount the detention time for pretreatment is determined.

The tank for raw sludge, serving all three axis, was refilled every 2—3 days. The raw sludge was infected with virus suspension, mixed with an agitator and the concentration of the respective virus was adjusted to 10_4 $TCID_{50}$/ml sludge. Each axis was supplied daily with 63 and 25 1, resp., of the virus infected sludge, divided into three portions. Infected raw sludge older than 2 days was discarded. Table I gives a survey of the different steps of treatment of the virus infected sludge.

All experiments lasted 21 days. On the day of the first addition of virus to the raw sludge 100 ml samples of raw sludge were taken before and after the infection of the sludge with virus to investigate a possible cytotoxicity or the occurrence of an unspecific cytopathic effect on the cell cultures which were used for titration of the virus containing sludge samples. Also the titers of the virus suspensions used for the experiments were controlled.

Beginning with the second day of the experiments samples were taken daily from the following positions of the pilot-plant:

– raw sludge at the inlet of the digester of axis 3,
– pretreated sludge at the outlet of the vessels for anaerobic pretreatment (Fig. 1, Nos. 3) of axis 1 and 2,

– digested sludge at the outlet of the digesters (Fig. 1, Nos. 4) of all 3 axis.

Samples from the pre-pasteurisation vessel were sporadically taken at the end of the process after 30 min at 70°C. All samples were immediately deep frozen at –30°C and later at -80°C until they were further handled in the laboratory.

In a preliminary experiment four methods or their modifications were tested for their suitability to reisolate the test-viruses from the sludge. For bovine parvovirus the elution with skim milk with subsequent organic precipitation after Goddard et al. (8) turned out as the method of choice. For ECBO- and Reovirus the elution with beef extract with adjustment of pH to 9 combined with ultra-sonic treatment after Wellings et al., modified after Koch (9) gave the best results.

3. RESULTS

3.1 Experiments with bovine parvovirus strain Haden (BPV)

3.1.1 The results of the first series of experiments with BPV as test agent are summarized in the Tables II, III and IV.

Table II: In the first series BPV could be reisolated from all 18 samples of raw sludge with losses of titer from 10^0 to $10^{1.75}$ $TCID_{50}$/ml compared to the initial titer of 10^4 $TCID_{50}$/ml in the infected raw sludge. From 18 samples of the effluent of the digester of axis 3 with mesophilic digestion and a HRT of 20 d in 6 samples virus could be isolated.

Table III: From all 16 samples taken from the effluent of the anaerobic pretreatment of axis 2 BPV could be isolated. From 14 out of 18 samples taken from the effluent of the digester of axis 2 after anaerobic pretreatment and with mesophilic digestion and HRT of 8 d BPV could be isolated.

Table IV: BPV could not be isolated from 4 samples directly taken from vessel for pre-pasteurisation of axis 1. But from 11 out of 18 samples taken from the effluent of the anaerobic pretreatment of axis 1, which was fed with pasteurised sludge, BPV could be isolated. From 4 out of 18 samples taken from the effluent of the digester of axis 2 after pre-pasteurisation and anaerobic pretreatment and anaerobic treatment, BPV could also be isolated.

Summarizing it can be said, that all 3 treatments of the first series of experiments (Conventional mesophilic digestion at 33° C with HRT of 20 d as well as the 2-phase digestion without and with pre-pasteurisation of the infected raw sludge) did not result in a complete inactivation of bovine parvovirus.

3.1.2 The results of the second series of experiments with BPV as test agent are summarized in the Tables V and VI.

Table V: In the second series BPV could be reisolated from all 21 samples of raw sludge with losses of titer from $10^{1.75}$ to $10^{3.0}$ $TCID_{50}$/ml compared to the initial titer of 10^4 $TCID_{50}$/ml in the infected raw sludge. From 5 out of 20 samples taken from the effluent of the digester of axis 3 who served as reference process with a mesophilic digestion and a HRT of 20 d BPV could be isolated. After pasteurisation (70°C—30 min) of the 5 BPV-positive samples of digested sludge, no virus could be isolated.

Table VI: BPV was isolated from 17 our of 19 samples taken from the effluent of the vessel for anaerobic pretreatment of axis 2 with a HRT of 2 d. BPV was also isolated from 7 out of 20 samples taken from the effluent of the digester of axis 2 (33° C—HRT 8 d) after this sludge was anaerobically pretreated. After pasteurisation of the effluent of the digester of axis 2 (70°C —30 min) BPV could be isolated from only 1 out of 19 samples with a titer of $10^{1.0}$ $TCID_{50}$/ml.

Summarizing it can be said that bovine parvovirus was not completely inactivated by a 2-phase digestion process with anaerobic pretreatment at 20°C with HRT of 2 d followed by anaerobic mesophilic digestion at 33°C with HRT of 8 d. Even a pasteurisation of the digested sludge at 70°C for 30 min did not in all cases result in an inactivation of bovine parvovirus. The results of the conventional mesophilic anaerobic digestion in axis 3 correspond to those described in the first series of experiments under heading 3.1.1.

3.2 Experiments with bovine enterovirus ECBO-LCR-4 (BEV)

3.2.1 The results of the first series of experiments with BEV as test agent are summarized in the Tables VII, VIII and IX.

Table VII: In the first series BEV could be reisolated from 18 out of 19 samples of raw sludge with losses of titer from 10° to $10^{2.75}$ $TCID_{50}$/ml compared to the initial titer of 10^4 $TCID_{50}$/ml in the infected raw sludge. No BEV could be isolated from all 18 samples taken from the effluent of the digester of axis 3 operated with a mesophilic anaerobic process at 33°C and a HRT of 20 d.

Table VIII: From 14 out of 19 samples taken from the effluent of the vessel for mesophilic anaerobic pretreatment (33°C—HRT 2 d) of axis 2 BEV could be reisolated. From 1 out of 19 samples taken from the effluent of the digester for mesophilic anaerobic digestion (33°C—HRT 8 d) of axis 2 after mesophilic anaerobic pretreatment BEV was isolated.

Table IX: From 1 out of 4 samples directly taken from the vessel for prepasteurisation of raw sludge of axis 1 BEV could be isolated. In 4 out of 18 samples taken from the effluent of the vessel for mesophilic anaerobic pretreatment (33°C—HRT 2 d) after pre-pasteurisation of raw sludge BEV could be demonstrated. BEV could not be isolated from all 19 samples taken from the effluent of the digester for mesophilic anaerobic digestion (33°C—HRT 8 d) of axis 1 after pre-pasteurisation of raw sludge and mesophilic aerobic pretreatment.

Summarizing it can be said that bovine enterovirus was completely inactivated by mesophilic anaerobic digestion at 33°C and HRT 20 d as well as by the 2-phase mesophilic digestion with pre-pasteurisation of the raw sludge. The virus was not completely inactivated when the 2-phase mesophilic digestion process was not preceded by pasteurisation of the raw sludge.

3.2.2 The results of the second series of experiments with BEV are summarized in the Tables X and XI.

Table X: BEV could be reisolated from 15 out of 17 samples of raw sludge with losses of titer from $10^{0.25}$ to $10^{3.0}$ $TCID_{50}$/ml compared to the initial titer of 10^4 $TCID_{50}$/ml in the infected raw sludge. (The sample taken 30 min after the infection of the raw sludge was not considered because it was taken, in the contrary to all other samples, directly from the vessel for raw sludge and it is very likely that at this time a complete mixture of sludge and virus suspension had not yet taken place.).

No BEV could be demonstrated in all 18 samples which were taken from the effluent of the digester of axis 3 who served as reference process with mesophilic anaerobic digestion (33°C—HRT 20 d). Since BEV in the samples of the digested sludge was already completely inactivated the effect of a pasteurisation (70°C—30 min) of the digested sludge could not be evaluated.

Table XI: BEV was isolated from 9 out of 17 samples taken from the effluent of the vessel for anaerobic pretreatment (20°C—HRT 2 d) of axis 2. BEV could not be demonstrated in all 18 samples taken from the effluent of the digester of axis 2 (33°C—HRT 8 d) after this sludge was anaerobically pretreated. Since BEV in the samples of the digested sludge was also already completely inactivated (as in Table X) the effect of a pasteurisation (70°C—30 min) of the digested sludge could not be evaluated.

Summarizing it can be said that bovine enterovirus was completely inactivated by a 2-phase digestion process with anaerobic pretreatment (20°C—HRT 2 d) with subsequent mesophilic anaerobic digestion (33°C— HRT 8 d). The results of the conventional mesophilic anaerobic digestion (33°C—HRT 20 d) correspond to those described for the first series of experiments under heading 3.2.1. The effect of pasteurisation of the digested sludge on the virus could not be evaluated because the virus was already inactivated in the effluent of the digesters.

3.3 Experiments with Reovirus Type 1 (RV)

3.3.1 The results of the first series of experiments with RV are summarized in the Tables XII, XIII and XIV.

Table XII: In the first series RV could be reisolated from 10 out of 19 samples of raw sludge with losses of titer from $10^{1.5}$ to $10^{3.0}$ $TCID_{50}$/ml compared to the initial titer of 10^4 $TCID_{50}$/ml in the infected raw sludge. No RV could be isolated from all 18 samples taken from the effluent of the digester of axis 3 operated with a mesophilic anaerobic digestion process at 33°C and a HRT of 20 d.

Table XIII: From 7 out of 18 taken from the effluent of the vessel for mesophilic anaerobic pretreatment (33°C–HRT 2 d) of axis 2 RV could be reisolated. RV could not be reisolated from all 17 samples taken from the effluent of the digester for mesophilic anaerobic digestion (33°C–HRT 8 d) of axis 2 after mesophilic anaerobic pretreatment.

Table XIV: All 3 samples directly taken from the vessel for pre-pasteurisation of raw sludge of axis 1 were negative for RV. All 18 samples taken from the effluent of the vessel for mesophilic anaerobic pretreatment (33°C–HRT 2 d) of axis 1 after pre-pasteurisation of raw sludge were free of RV as well as all 17 samples of the effluent of the digester for mesophilic anaerobic digestion (33°C–HRT 8 d) of axis 1 after prepasteurisation of raw sludge and mesophilic anaerobic pretreatment.

Summarizing it can be said that reovirus type 1 was completely inactivated by mesophilic anaerobic digestion at 33°C and HRT 20 d as well as by the 2-phase mesophilic digestion with or without prepasteurisation of the raw sludge.

3.3.2 The results of the second series of experiments with RV are summarized in the Tables XV and XVI.

Table XV: RV could be reisolated from 12 out of 15 samples of raw sludge with losses of titer from $10^{1.75}$ to $10^{3.0}$ $TCID_{50}$/ml compared to the initial titer of 10^4 $TCID_{50}$/ml in the infected raw sludge.

No RV could be demonstrated in the effluent of the digester of axis 3 who served as reference process with mesophilic anaerobic digestion (33°C –HRT 20 d). Since RV in the samples of the digested sludge was already completely inactivated the effect of a pasteurisation (70°C–30 min) of the digested sludge could not be evaluated.

Table XVI: RV was isolated from 7 out of 14 samples taken from the effluent of the vessel for anaerobic pretreatment (20°C–HRT 2 d) of axis 2. RV could not be demonstrated in all 14 samples taken from the effluent of the digester of axis 2 (33°C–

HRT 8 d) after this sludge was anaerobically pretreated. Since RV in the samples of the digested sludge was also completely inactivated (as in Table XV) the effect of a pasteurisation (70°C–30 min) of the digested sludge could not be evaluated.

Summarizing it can be said that reovirus type 1 was completely inacti vated by a 2-phase digestion process with anaerobic pretreatment (20°C– HRT 2 d) with subsequent mesophilic anaerobic digestion (33°C–HRT 8 d). The results of the conventional mesophilic anaerobic digestion (33°C– HRT 20 d) correspond to those described for the first serie of experiments under heading 3.3.1. The effect of a pasteurisation of the digested sludge on the virus could not be evaluated because the virus was already inactivated in the effluent of the digesters.

4. DISCUSSION

In the literature we could not find any publications dealing with corresponding experiments and the same viruses which we used for our experiments. Most of other authors used digesters without continuous or semi-continuous feeding with raw sludge. Therefore from such experiments statements are possible about the inactivation of viruses in the course of a digestion process in dependence of the time (2, 3, 7). The pilot-plant which we used does not allow such an assessment because in the course of each feeding of a digester sludge is also drained with particles which were exposed to the influences of the digestion process for a shorter period of time than the calculated mean hydraulic detention time may indicate. Hydraulic short-circuits may be the reason for such events.

Some authors have isolated viruses in anaerobically stabilised sludge which were not artificially added but originate from excretions of human or animal carriers (13, 14, 19). Nielsen and Lydholm (13) isolated Coxsackie-B5-virus which also belongs to the group of enteroviruses. Our test enterovirus ECBO-LCR-4 was completely inactivated by the digestion process. Merely bovine parvovirus with its known high tenacity and thermostability up to 80°C (12) could be reisolated in our experiments from digested and even pasteurised sludge.

Only few investigations with reovirus in digested sludge are published. Ward and Ashley (15, 16, 17) found that ionic detergents in sewage sludge decrease the thermostability of reoviruses. The inactivation of reovirus type 1, which are known to possess a relatively high thermostability, was possibly caused by such detergents in the sludge used for our experiments.

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Table I: Outline of the various treatmeahvayunts of virus in infected raw sludge in the course of the investigations

Treatment step	First course of experiments			Second course of experiment	
	Axis 1	Axis 2	Axis 3	Axis 2	Axis 3
Pre-pasteurisation	70°C 30 min	–	–	–	–
Anaerobic pretreatment	33°C 2 d HRT	33°C 2 d HRT	–	20°C 2 d HRT	–
Anaerobic treatment	33°C 8 d HRT	33°C 8 d HRT	33°C 20 d HRT	33°C 8 d HRT	22°C 20 d HRT
Pasteurisation after digestion	–	–	–	70°C 30 min	70°C 30 min

– = Treatment step not done
HRT=Mean hydraulic retention time

Legend to all tables:
Titres in $\log_{10}$ $TCID_{50}$/ml

x	= Days of refilling of the raw sludge tank
n.d.	= not done
–	= no virus recovered
AD_1, AD_2, AD_3	= samples taken from the effluent of anaerobic digesters 1, 2, or 3
PT_2, PT_2	= samples taken from the effluent of anaerobic pretreatment digesters 1 or 2
PP	= sample taken out of the prepasteurisation tank
$AD_{2/P}$, $AD_{3/P}$	= AD_2 or AD_3 samples pasteurised after digestion

Table II. Virus titer in the course of conventional mesophilic anaerobic digestion (33°C) at mean hydraulic retention time of 20 d; test agent bovine parvovirus (strain Haden)– First course of experiments, axis 3

Time after first addition of the virus		Raw sludge	AD_3
×	30 min	6.25	n.d.
×	1 d	4.0	–
	2 d	n.d.	–
	3 d	3.5	–
	5 d	4.0	–
×	6 d	2.75	1.25
	7 d	4.0	2.5
×	8 d	2.5	1.75
	9 d	2.5	1.25
×	10 d	3.25	–
	12 d	2.75	–
×	13 d	2.5	–
×	14 d	2.75	1.25

Time after first addition of the virus		Raw sludge	AD_3
	16 d	3.25	–
×	17 d	2.25	–
	19 d	4.0	2.25
×	20 d	3.25	–
	21 d	3.0	–
	22 d	3.5	–

Table III. Virus titer in the course of two-step mesophilic anaerobic digestion (33°C) at mean hydraulic retention time of 2 d in anaerobic pretreatment and 8 d in the following digester;
test agent bovine parvovirus (strain Haden)—
First course of experiments, axis 2

Time after first addition of the virus		Raw sludge	PT_2	AD_2
×	30 min	6.25	n.d.	n.d.
×	1 d	4.0	3.75	–
	2 d	n.d.	1.5	–
×	3 d	3.5	n.d.	–
	5 d	4.0	3.0	2.25
×	6 d	2.75	2.25	1.75
	7 d	4.0	3.25	1.5
×	8 d	2.5	2.75	1.25
	9 d	2.5	n.d.	2.0
×	10 d	3.25	2.75	1.5
	12 d	2.75	3.5	1.0
×	13 d	2.5	2.5	1.5
×	14 d	2.75	2.5	1.25
	16 d	3.25	3.5	2.5
×	17 d	2.25	2.0	–
	19 d	4.0	3.5	2.25
×	20 d	3.25	2.5	1.5
	21 d	3.0	2.25	2.5
	22 d	3.5	2.75	2.75

Table IV. Virus titer in the course of two-step mesophilic anaerobic digestion (33°C) at mean hydraulic retention time of 2 d in anaerobic pretreatment and 8 d in the following digester with prepasteurisation of the raw sludge; test agent bovine parvovirus (strain Haden)—First course of experiments, axis 1

Time after first addition of the virus		Raw sludge	PP	PT_1	AD_1
x	30 min	6.25	n.d.	n.d.	n.d.
x	1 d	4.0	n.d.	1.75	1.0
	2 d	n.d.	n.d.	–	–
x	3 d	3.5	n.d.	–	–
	5 d	4.0	n.d.	–	–
x	6 d	2.75	n.d.	–	–
	7 d	4.0	n.d.	2.25	–
x	8 d	2.5	–	2.25	–
	9 d	2.5	n.d.	–	–
x	10 d	3.25	n.d.	2.25	2.75
	12 d	2.75	n.d.	1.5	–

Time after first addition of the virus		Raw sludge	PP	PT_1	AD_1
x	13 d	2.5	n.d.	2.25	3.5
x	14 d	2.75	n.d.	1.75	–
	16 d	3.25	n.d.	–	–
x	17 d	2.25	–	–	–
	19 d	4.0	n.d.	2.75	1.75
x	20 d	3.25	n.d.	2.5	–
	21 d	3.0	–	1.5	–
	22 d	3.5	n.d.	2.25	–
	24 d	n.d.	–	n.d.	n.d.

Table V. Virus titer in the course of conventional mesophilic anaerobic digestion (33°C) at mean hydraulic retention time of 20 d with pasteurisation after digestion; test agent bovine parvovirus (strain Haden)—Second course of experiments, axis 3

Time after first addition of the virus		Raw sludge	AD_3	$AD^{3/p}$	Difference in titers AD_3–$AD_{3/p}$
x	30 min	2.0	n.d.	n.d.	0
	1 d	2.25	–	–	
x	2 d	2.25	–	–	
	3 d	1.75	–	–	
x	4 d	1.75	–	–	
	5 d	1.5	–	–	
	6 d	1.5	1.25	–	1.25
x	7 d	1.25	–	–	0
	8 d	2.5	–	–	
x	9 d	2.0	–	–	
	10 d	1.75	–	–	
x	11 d	1.5	–	–	
	12 d	2.25	–	–	
	13 d	1.0	–	–	
x	14 d	2.0	–	–	
	15 d	1.75	1.0	–	1.0
x	16 d	2.25	1.0	–	1.0
	17 d	2.25	1.5	–	1.5
x	18 d	1.5	–	–	0
	19 d	1.5	–	–	0
	20 d	1.5	1.0	–	1.0

Table VI. Virus titer in the course of two-step mesophilic anaerobic digestion (33°C) at mean hydraulic retention time of 2 d in anaerobic pretreatment and 8 d in the following digester with pasteurisation after digestion; test agent bovine parvovirus (strain Haden)— Second course of experiments, axis 2

Time after first addition of the virus		Raw sludge	PT_2	AD_2	$AD_{2/p}$	Difference in titers AD_2–$AD_{2/p}$
x	30 min	2.0	n.d.	n.d.	n.d.	n.d.
	1 d	2.25	–	1.25	1.0	0,25
x	2 d	2.25	3.25	–	–	
	3 d	1.75	2.5	–	–	0
x	4 d	1.75	2.25	–	–	
	5 d	1.5	1.5	–	–	n.d.
	6 d	1.5	1.0	–	–	0
x	7 d	1.25	2.5	–	–	0
	8 d	2.5	n.d.	1.0	–	1.0
x	9 d	2.0	1.25	–	–	0
	10 d	1.75	–	–	–	0
x	11 d	1.5	1.5	1.25	–	1.25
	12 d	2.25	1.5	1.25	–	1.25
	13 d	1.0	2.0	1.0	–	1.0
x	14 d	2.0	1.0	1.25	–	1.25
	15 d	1.75	1.5	1.5	n.d.	n.d.
x	16 d	2.25	1.5	–	–	
	17 d	2.25	1.75	–	–	
x	18 d	1.5	1.5	–	–	
	19 d	1.5	1.5	–	–	0
	20 d	1.5	2.25	–	–	

Table VII. Virus titer in the course of conventional mesophilic anaerobic digestion (33°C) at mean hydraulic retention time of 20 d; test agent bovine enterovirus (ECBO-LCR-4)— First course of experiments, axis 3

Time after first addition of the virus		Raw sludge	AD_3
x	30 min	3.5	n.d.
	1 d	3.5	–
x	2 d	3.5	–
	3 d	2.25	–
x	4 d	4.0	–
	5 d	3.25	–
x	7 d	4.0	–
	8 d	n.d.	–
x	9 d	3.25	–
	10 d	3.0	–
x	11 d	–	–
	12 d	2.75	–
	13 d	3.25	–
x	14 d	3.25	–
	15 d	3.0	–

Time after first addition of the virus		Raw sludge	AD_3
x	16 d	3.75	–
	17 d	3.25	–
×	18 d	3.5	n.d.
	20 d	2.0	–
	21 d	1.25	–

Table VIII. Virus titer in the course of two-step mesophilic anaerobic digestion (33°C) at mean hydraulic retention time of 2 d in anaerobic pretreatment and 8 d in the following digester; test agent bovine enterovirus (ECBO-LCR-4)— First course of experiments, axis 2

Time after first addition of the virus		Raw sludge	PT_2	AD_2
x	30 min	3.5	n.d.	n.d.
	1 d	3.5	1.75	–
x	2 d	3.5	1.75	–
	3 d	2.25	2.25	–
x	4 d	4.0	–	–
	6 d	3.25	1.5	–
x	7 d	4.0	1.75	–
	8 d	n.d.	–	–
x	9 d	3.25	–	–
	10 d	3.0	1.25	1.0
x	11 d	–	2.25	–
	12 d	2.75	–	–
	13 d	3.25	1.5	–
x	14 d	3.25	1.25	–
	15 d	3.0	–	–
x	16 d	3.75	1.75	–
	17 d	3.25	1.25	–
x	18 d	3.5	2.25	–
	20 d	2.0	1.5	–
	21 d	1.25	1.0	–

Table IX. Virus titer in the course of two-step mesophilic anaerobic digestion (33°C) at mean hydraulic retention time of 2 d in anaerobic pretreatment and 8 d in the following digester with prepasteurisation of the raw sludge; test agent bovine enterovirus (ECBO-LCR-4)— First course of experiments, axis 1

Time after first addition of the virus		Raw sludge	PP	PT_1	AD_1
x	30 min	3.5	n.d.	n.d.	n.d.
	1 d	3.5	n.d.	–	–
x	2 d	3.5	n.d.	–	–
	3 d	2.25	–	1.0	–
x	4 d	4.0	n.d.	–	–
	6 d	3.25	n.d.	–	–
x	7 d	4.0	n.d.	–	–
	8 d	n.d.	n.d.	–	–
x	9 d	3.25	n.d.	–	–
	10 d	3.0	–	–	–
x	11 d	n.d.	1.25	–	–
	12 d	2.75	n.d.	–	–
	13 d	3.25	n.d.	–	–
x	14 d	3.25	n.d.	–	–
	15 d	3.0	n.d.	1.0	–
x	16 d	3.75	–	–	–

Time after first addition of the virus		Raw sludge	PP	PT_1	AD_1
	17 d	3.25	n.d.	–	–
x	18 d	3.5	n.d.	1.0	–
	20 d	2.0	1.25	n.d.	–
	21 d	1.25	n.d.	–	–

Table X. Virus titer in the course of conventional mesophilic anaerobic digestion (33°C) at mean hydraulic retention time of 20 d with pasteurisation after digestion; test agent bovine enterovirus (ECBO-LCR-4)—Second course of experiments, axis 3

Time after first addition of the virus		Raw sludge	AD_3	$AD_{3/p}$	Difference in titers AD_3–$AD_{3/p}$
x	30 min	4.25	n.d.	n.d.	n.d.
	1 d	1.25	–	–	
x	2 d	3.75	–	–	
	3 d	1.75	–	–	
x	7 d	1.25	–	–	
	8 d	3.5	–	–	
x	9 d	–	–	–	
	10 d	1.25	–	–	
x	11 d	2.25	–	–	
	12 d	3.5	–	–	
	13 d	2.0	–	–	0
x	14 d	1.25	–	–	
	15 d	n.d.	–	–	
x	16 d	1.5	–	–	
	17 d	–	–	–	
x	18 d	1.0	–	–	
	19 d	n.d.	–	–	
	20 d	2.5	–	–	
	21 d	1.0	–	–	

Table XI. Virus titer in the course of two-step mesophilic anaerobic digestion (33°C) at mean hydraulic retention time of 2 d in anaerobic pretreatment and 8 d in the following digester with pasteurisation after digestion; test agent bovine enterovirus (ECBO-LCR-4) –Second course of experiments, axis 2

Time after first addition of the virus		Raw sludge	PT_2	AD_2	$AD_{2/P}$	Difference in titers AD_2–$AD_{2/p}$
x	30 min	4.25	n.d.	n.d.	n.d.	n.d.

Time after first addition of the virus		Raw sludge	PT_2	AD_2	$AD_{2/P}$	Difference in titers AD_2–$AD_{2/p}$
	1 d	1.25	3.75	–	–	
x	2 d	3.75	1.0	–	–	
	3 d	1.75	2.25	–	–	
x	7 d	1.25	–	–	–	
	8 d	3.5	2.5	–	–	
x	9 d	–	1.0	–	–	
	10 d	1.25	–	–	–	
x	11 d	2.25	–	–	–	
	12 d	3.5	–	–	–	
	13 d	2.0	–	–	–	0
x	14 d	1.25	–	–	–	
	15 d	n.d.	1.0	–	–	
x	16 d	1.5	1.0	–	–	
	17 d	–	2.0	–	–	
x	18 d	1.0	–	–	–	
	19 d	n.d.	n.d.	–	–	
	20 d	2.5	–	–	–	
	21 d	1.0	1.5	–	–	

Table XII. Virus titer in the course of conventional mesophilic anaerobic digestion (33°C) at mean hydraulic retention time of 20 d; test agent reovirus (type 1)—First course of experiment, axis 3

Time after first addition of the virus		Raw sludge	AD^3
x	30 min	1.25	n.d.
	1 d	1,5	–
x	2 d	–	–
	3 d	1.25	–
x	4 d	1.0	–
x	7 d	2.0	–
	8 d	–	–
x	9 d	–	–
x	10 d	1.75	–
x	11 d	1.25	–
x	14 d	–	–

Time after first addition of the virus		Raw sludge	AD[3]
	15 d	1.75	–
x	16 d	–	–
x	18 d	2.5	–
	19 d	–	–
	20 d	–	–
x	21 d	1.5	–
	22 d	–	–
	23 d	–	–

Table XIII. Virus titer in the course of two-step mesophilic anaerobic digestion (33°C) at mean hydraulic retention time of 2 d in anaerobic pretreatment and 8 d in the following digester;
test agent reovirus (type 1)—First course of experiments, axis 2

Time after first addition of the virus		Raw sludge	PT_2	AD_2
x	30 min	1.25	n.d.	n.d.
	1 d	1.5	–	–
x	2 d	–	–	–
	3 d	1.25	1.75	–
x	4 d	1.0	–	–
x	7 d	2.0	–	–
	8 d	–	1.25	–
x	9 d	–	1.5	–
	10 d	1.75	–	–
x	11 d	1.25	1.0	–
x	14 d	–	–	–
	15 d	1.75	–	–
x	16 d	–	1.5	–
x	18 d	2.5	1.0	–
	19 d	–	–	–
	20 d	–	–	–
x	21 d	1.5	1.25	n.d.
	22 d	–	–	–
	23 d	–	–	–

Table XIV. Virus titer in the course of two-step mesophilic anaerobic digestion (33°C) at mean hydraulic retention time of 2 d in anaerobic pretreatment and 8 d in the following digester with prepasteurisation of the raw sludge;
test agent reovirus (type 1)—First course of experiments, axis 1

Time after first addition of the virus		Raw sludge	PP	PT	AD
x	30 min	1.25	n.d.	n.d.	n.d.
	1 d	1.5	n.d.	–	–
x	2 d	–	–	–	–
	3 d	1.25	n.d.	–	–
x	4 d	1.0	n.d.	–	–
x	7 d	2.0	n.d.	–	–
	8 d	–	n.d.	–	–
x	9 d	–	n.d.	–	–
	10 d	1.75	n.d.	–	–
x	11 d	1.25	–	–	–
	14 d	–	n.d.	–	–
	15 d	1.75	n.d.	–	–
x	16 d	–	n.d.	–	–

Time after first addition of the virus		Raw sludge	PP	PT	AD
x	18 d	2.5	–	–	–
	19 d	–	n.d.	–	n.d.
	20 d	–	n.d.	–	–
x	21 d	1.5	n.d.	–	–
	22 d	–	n.d.	–	–
	23 d	–	n.d.	–	–

Table XV. Virus titer in the course of conventional mesophilic anaerobic digestion (33°C) at mean hydraulic retention time of 20 d with pasteurisation after digestion;
test agent reovirus (type 1)—Second course of experiments, axis 3

Time after first addition of the virus		Raw sludge	AD_3	$AD_{3/p}$	Difference in titers AD_3–$AD_{3/p}$
x	30 min	–	n.d.	n.d.	n.d.
	1 d	1.25	–	–	0
x	2 d	1.75	–	–	
x	5 d	1.0	n.d.	–	n.d.
	6 d	2.25	–	–	0
x	7 d	1.0	–	–	
	8 d	–	–	–	
x	9 d	1.5	–	–	
	11 d	2.0	–	–	
x	12 d	–	–	–	
	13 d	1.5	–	–	
x	14 d	2.0	–	–	
	15 d	1.75	–	–	
x	16 d	1.75	–	–	
	19 d	2.25	–	–	

Table XVI. Virus titer in the course of two-step mesophilic anaerobic digestion (33°C) at mean hydraulic retention time of 2 d in anaerobic pretreatment and 8 d in the following digester with pasteurisation after digestion;
test agent reovirus (type 1)—Second course of experiments, axis 2

Time after first addition of the virus		Raw sludge	PT_2	AD_2	$AD_{2/P}$	Difference in titers AD_2–$AD_{2/p}$
x	30 min	–	n.d.	n.d.	n.d.	n.d.

Time after first addition of the virus		Raw sludge	PT_2	AD_2	$AD_{2/P}$	Difference in titers AD_2–$AD_{2/p}$
	1 d	1.25	2.25	–	–	0
x	2 d	1.75	–	–	–	
x	5 d	1.0	–	–	–	
	6 d	2.25	–	–	–	
x	7 d	1.0	–	–	–	
	8 d	–	2.25	–	–	
x	9 d	1.5	2.0	–	–	
	11 d	2.0	–	–	–	
x	12 d	–	–	–	–	
	13 d	1.5	1.75	–	–	
x	14 d	2.0	–	–	–	
	15 d	1.75	2.75	–	–	
x	16 d	1.75	1.75	–	–	
	19 d	2.25	1.25	–	–	

INFLUENCE OF THERMOPHILIC ANAEROBIC DIGESTION (55°C) AND SUBSEQUENT MESOPHILIC DIGESTION OF SLUDGE ON THE SURVIVAL OF VIRUSES WITHOUT AND WITH PASTEURISATION OF THE DIGESTED SLUDGE

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Summary

The influence of thermophilic anaerobic pretreatment of sewage sludge with subsequent mesophilic digestion with and without pasteurisation on the activity of viruses was investigated. In a reference process the experiments were performed and the results were compared with the two-step treatment of sludge.

The viruses investigated could be isolated after the thermophilic pre treatment, but the number of positive samples varied depending on the type of virus. After thermophilic pretreatment (3d—55°C) with subsequent mesophilic digestion (12 d—33°C) bovine parvovirus still could be isolated as well as from the reference process (20 d—33° C) The differences in the elimination of parvovirus between the two systems investigated were very insignificant.

The reference process as well as the two-step process clearly show in their results the problems of hydraulic short-circuits caused by the continuous feeding of the digesters. After pasteurisation of the infected sludge no viruses could be isolated in both systems.

1. INTRODUCTION

The experiments were performed in a pilot-plant of the Institute for Water Quality Management and Public Health Engineering of the Technical University of München in Garching. As test agents the following viruses were chosen because of their resistence against environmental influences: 1. Bovine parvovirus (strain Haden); 2. ECBO-virus LCR-4; Reovirus Type 1.

2. MATERIAL AND METHODS

2.1 Pilot Plant

In Fig. 1 the schematic built-up of the pilot-plant is shown. The plant is operated in three parallel axis. All of the axis are supplied with raw sludge from a common tank with a capacity of 500 1. The feeding of the system with raw sludge is automatically controlled by an inductive flow meter every eight hours (8.00–16.00–24.00 h). The two vessels of the first step for anaerobic pretreatment are cone-shaped with a service volume from 30 to 190 1. Thus a change of the hydraulic detention time from step one to step two of 1:17 to 1:2.6 can be achieved. For mixing of the contents an agitator with 150 rpm is used. The heating is done by filaments in the wall of the vessel. The temperature can be ad justed via the temperature of the sludge or of the wall. Feeding and draining are based on the displacement principle.

Three constructionally identical digesters with a service volume of 500 1 each constitute the second step in axis 1 and axis 2 or function as reference vessel in axis 3. Axis No. 2 has not been used in the experiments discussed here. The contents of the digesters are steadily mixed by a screw shaped agitator with 75 rpm in the draft tube through which also the heating occurs (Fig. 1, No. 5). Feeding and draining of the digesters are also based on the displacement principle as in the first step.

The tank for raw sludge is emptied three times per week during the whole duration of the experiment (21 day for each virus investigated), refilled and infected with the respective test virus. Thus a daily feeding of the two axis with virus-contaminated sludge is ensured. The titer of the virus in raw sludge is 10^4 $TCID_{50}$/ml. To achieve an even distribution of the virus suspension the raw sludge is agitated with a stirrer (75 rpm) for 20 minutes.

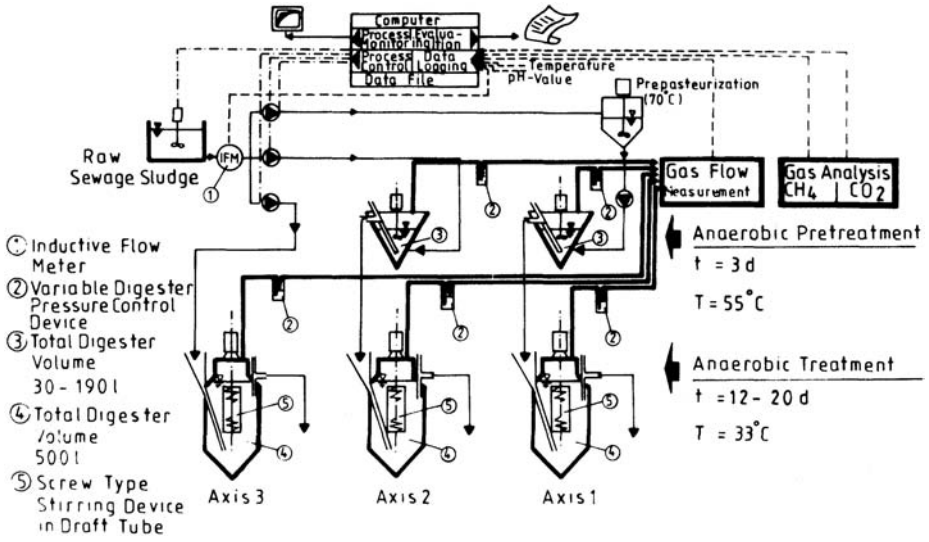

Figure 1. Flow diagram of the pilot-plant

On the day of the first addition of virus to the raw sludge 100 ml samples of sludge are taken before and after the addition of the virus to investigate a possible cytotoxicity or the occurrence of an unspecific cytopathic effect (cpe) on the cell cultures which are used for titration of the virus containing sludge samples.

2.2 Sampling

Samples are taken daily at the following points: raw sludge at the inlet of the digester (Fig, 1, Axis 3, No. 4), pretreated sludge at the outlet of the vessel for anaerobic pretreatment (Fig. 1, Axis 1, No. 3), digested sludge at the outlet of the digesters (Fig. 1, Axis 1 and Axis 3, No. 4). The samples of the anaerobic pretreatment (Axis 1, No. 3) have a mean hydraulic detention time of 3 days at 55°C, those of the digester (Axis 1, No. 4) of 12 days at 33°C. The samples of the digester of the reference process (Axis 3, No. 4) have a mean hydraulic detention of 20 days at 33°C.

From the digesters of axis 1 and axis 3 (Fig. 1, No. 4) two samples daily are taken, one of which is pasteurized in a water-bath at 70°C for 30 minutes. All samples are immediately deep-frozen and stored at –80°C until they are finally investigated.

2.3 Virus and cells

Parvovirus strain “Haden” is propagated using fetal bovine lung tissue cultures (PBFK). The virus assay is obtained by using PBFK tissue cultures in COSTAR 24 wells-tissue-culture-plates in order to get tissue culture infectious doses (TCID50). Cytopathic effects (cpe’s) are evaluated six days p.i.; growth medium is changed twice during this period.

ECBO-virus strain “LCR–4” is propagated on tissue-cultures of the permanent fetal bovine kidney cell line Au-BEK. The virus assay is also done on these cells. Cpe's are evaluated two days p.i..

Reovirus type 1 is propagated on tissue-cultures of the permanent African green monkey kidney cell line VERO. The cpe's are evaluated 6–7 days p.i.; the growth medium is changed twice during this period.

2.4 Isolation methods

Isolation of sludge suspended parvovirus is carried out by eluation with skim milk and following organical flocculation according to the method of Goddards and Bates (1). Sludge suspended ECBO- and Reovirus are isolated beefextract-eluation with rise of pH to 9 according to the method of Wellings et al., modified by Koch (2, 3).

3. RESULTS

Table I shows the course of the titer of Reovirus Type 1 and the pH values during the two-step digestion with a mean hydraulic detention time in the anaerobic pretreatment of 3 days at 55°C and a subsequent digestion time of 12 days at 33°C compared with the reference process of an only digestion for 20 days at 33°C.

It can be seen that the amount of virus of 10^4 $TCID_{50}$ adjusted in the raw sludge in none of the samples could be reisolated. In the sample taken 30 minutes after the addition of the virus to the sludge no virus was detectable. The reason can be an insufficient agitation of the contents of the vessel with raw sludge thus causing an insufficient distribution of the virus suspension.

From 12 out of 14 samples of raw sludge virus could be reisolated and the titers were between $10^{1.0}$ and $10^{2.25}$ TCIDso/ml. The values of pH in the raw sludge were between 5.6 and 6.12. After the anaerobic pretreatment (3 days, 55°C) 4 samples are virus-positive, one of them already after the first day of the experiment. Therefore it can be assumed that in this case a hydraulic short-circuit occurred. It can be further assumed that the reason for the survival of the virus in the 3 other samp les was a too short detention time because Reovirus in general is inactivated in the thermophilic range within minutes or hours, resp. (4). A further indication is the fact that the thermostability of Reovirus is decreased by detergents which are contained in sewage sludges (5). The pH-values of the pretreated sludge are higher than those of the raw sludge and range between 6.32 and 7.08.

Neither after the 2-step digestion nor after the digestion process in the reference digester Reovirus could be isolated. The mean pH-values for these two processes varied between 7.24 and 7.35.

Table II shows the course of the titer of ECBO LCR-4 virus and the pH-values during the two-step digestion with a mean hydraulic detention time in the anaerobic pretreatment of 3 days at 55°C and a subsequent digestion time of 12 days at 33°C compared with the reference process of an only digestion for 20 days at 33°C.

In the sample taken 30 minutes after addition of the virus to the raw sludge ECBO-virus could be isolated with a titer of $10^{4.25}$ $TCID_{50}$/ml. This value is $10^{0.25}$ $TCID_{50}$/ml above the initial titer in the raw sludge and can be explained with concentration of the virus caused by the adsorption to particles of sludge (2). From 14 out of 17 samples of raw sludge ECBO-virus could be isolated. The titers vary between 101.0 and $10^{3.75}$ $TCID_{50}$/ml and thus they are partly above those of Reovirus, shown in Table I. The pH-values of the raw sludge were determined between 5.45 and 6.12.

After the pretreatment only in two samples virus could be detected with titers of $10^{1.25}$ and $10^{1.75}$ $TCID_{50}$/ml, resp.. It is uncertain if the reason for that result is a too short detention time in the pretreatment or the influence of detergents in the sludge which increase the heat-resistence of enterovirus (5). The pH-values range between 6.44 and 7.08 and correspond to those shown in Table I.

Neither after the 2-step digestion nor after the digestion process in the reference digester ECBO-virus could be isolated. The pH-values also correspond to those in Table I with 7.21 and 7.28 as a mean.

Table III shows the course of the titer of bovine Parvovirus and the pH-values during the two-step digestion with a mean hydraulic detention time in the anaerobic pretreatment of 3 days at 55°C and a subsequent digestion time of 12 days at 33°C compared with the reference process of an only digestion for 20 days at 33°C.

In the sample taken 30 minutes after addition of the virus to the raw sludge Parvovirus could be isolated with a titer of $10^{2.0}$ $TCID_{50}$/ml. In all 20 samples of raw sludge which were investigated Parvovirus could be detected. The titers range between $10^{1.0}$ and $10^{2.25}$ $TCID_{50}$/ml which corresponds to the values shown for Reovirus in Table I. After the pretreatment from 5 out of 18 samples Parvovirus could be isolated with titers between $10^{1.5}$ and $10^{2.0}$ $TCID_{50}$/ml. The pH ranges between 6.7 and 7. 18

After the 2-step digestion in 5 out of 18 samples Parvovirus could be found with titers between $10^{1.25}$ and $10^{1.75}$ $TCID_{50}$/ml. The mean hydraulic detention time of that complete process is 15 days. Since already within 15 days from 4 samples Parvovirus could be isolated it can be assumed that the detention time of the respective sample in the complate process was too short. After pasteurisation no virus could be isolated from all samples investigated.

In the reference process in 5 samples Parvovirus could be detected over the complete treatment process of 21 days. The reason for that seem also to be the too short detention times. In the pasteurized samples no virus could be detected.

Figure 2 shows a comparison of the percentage of virus-positive samples for Parvovirus after the 2-step digestion and the reference process, with and without pasteurisation. The differences in the elimination of Parvovirus through both treatments are only very low: 27.7% or 25%, resp.. The anaerobic thermophilic pretreatment of sludge with Parvovirus does not produce better hygienic results than the usual anaerobic mesophilic digestion. Only after pasteurisation of the digested sludge at 70°C for 30 minutes the Parvovirus was inactivated.

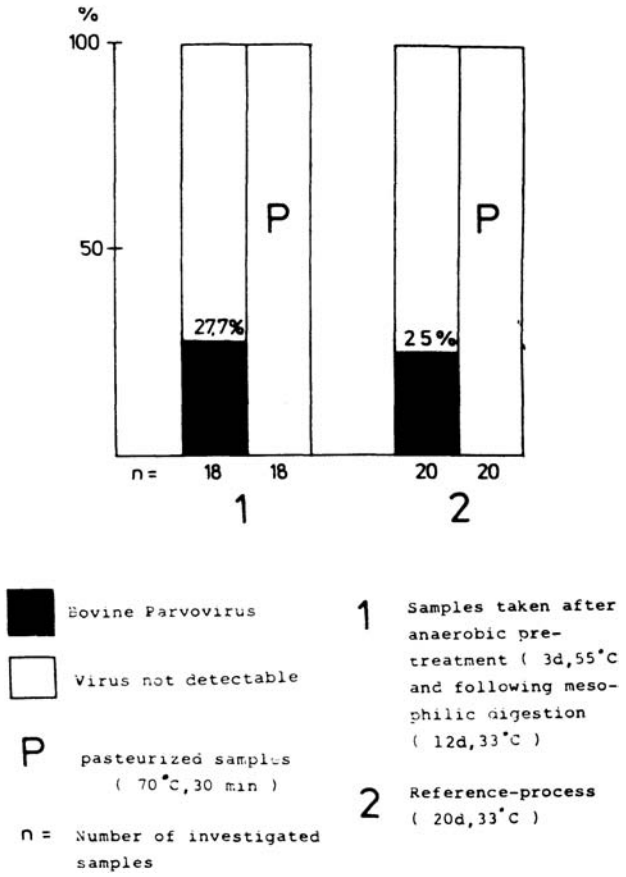

Figure 2. Effect of various treatments on parvovirus

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Table I. Titer of Reovirus Type 1 and pH-values within the 2-step digestion and in the reference-process

Time after first virusa ddition (d)	Titer in $\log_{10}$ $TCID_{50}$/ml in raw sewage sludge	pH	Titer in $\log_{10}$ $TCID_{50}$/ml after pretreatment	pH	Titer in $\log_{10}$ $TCID_{50}$/ml after digestion without	with pasteurisation	pH	Titer in $\log_{10}$ $TCID_{50}$/ml in the reference-process without	with pasteurisation	pH
⋆30 min	–	5.58	n.d.	6.62	n.d.	n.d.	7.22	n.d.	n.d.	7.32
1	1.25	5.90	1.25	6.62	n.d.	n.d.	7.22	n.d.	n.d.	7.32
⋆ 2	1.75	5.95	–	6.66	–	–	7.24	–	–	7.36

Time after first virusa ddition (d)	Titer in log_{10} $TCID_{50}$/ml in raw sewage sludge	pH	Titer in log_{10} $TCID_{50}$/ml after pretreatment	pH	Titer in log_{10} $TCID_{50}$/ml after digestion without with pasteurisation		pH	Titer in log_{10} $TCID_{50}$/ml in the reference-process without with pasteurisation		pH
3	n.d.	/	n.d.	/	n.d.	n.d.	/	n.d.	n.d.	/
⋆ 4	n.d.	/	n.d.	/	n.d.	n.d.	/	–	–	/
5	1.0	6.00	–	6.80	–	–	7.39	–	–	7.42
6	2.25	5.75	–	6.32	–	–	6.86	–	–	7.02
⋆ 7	1.0	5.60	–	6.36	–	–	7.39	–	–	7.46
8	–	/	1.0	/	–	–	/	–	–	/
⋆ 9	1.5	6.10	–	6.75	–	–	7.32	–	–	7.32
10	n.d.	/	n.d.	/	n.d.	n.d.	/	n.d.	n.d.	/
⋆11	2.0	/	–	/	–	–	/	–	–	/
12	–	5.20	–	6.90	–	–	7.32	–	–	7.40
13	1.5	5.75	1.5	6.72	–	–	7.22	–	–	7.30
⋆14	2.0	5.90	–	6.90	–	–	7.34	–	–	7.50
15	1.75	6.12	1.5	6.89	–	–	7.12	–	–	7.24
⋆16	1.75	6.05	–	7.02	–	–	7.45	–	–	7.52
17	n.d.	/	n.d.	/	/	n.d.	/	n.d.	n.d.	/
⋆18	n.d.	/	n.d.	/	/	n.d.	/	n.d.	n.d.	/
19	2.25	5.60	–	7.08	–	–	7.26	–	–	7.40

– = no virus detectable
n.d. = not done
/ = no result
⋆=raw sewage sludge charged with virus

Table II. Titer of ECBO-LCR-4 virus and pH-values within the 2-step digestion and in the reference-process

Time after first virusa ddition (d)	Titer in log_{10} $TCID_{50}$/ml in raw sewage sludge	pH	Titer in log_{10} $TCID_{50}$/ml after pre treatment	pH	Titer in log_{10} $TCID_{50}$/ml after digestion without with pasteurisation		pH	Titer in log_{10} $TCID_{50}$/ml in thereference-process without with pasteurisation		pH
⋆30 min	4.25	5.60	n.d.	/	n.d.	n.d.	/	n.d.	n.d.	/
1	1.25	5.70	–	6.90	–	–	7.22	–	–	7.26
⋆ 2	3.75	5.80	–	6.98	–	–	7.20	–	–	7.34
3	1.75	5.50	–	7.16	–	–	7.10	–	–	7.30
⋆ 4	n.d.	/	n.d.	/	n.d.	n.d.	/	n.d.	n.d.	/
5	n.d.	/	n.d.	/	n.d.	n.d.	/	n.d.	n.d.	7
6	n.d.	/	n.d.	/	n.d.	n.d.	/	n.d.	n.d.	/
⋆ 7	1.25	5.45	1.25	6.76	–	–	7.08	–	–	7.20
8	3.5	5.75	–	7.08	–	–	7.22	–	–	7.34
⋆ 9	–	5.47	–	6.93	–	–	7.41	–	–	7.47
10	1.25	6.12	–	7.04	–	–	7.16	–	–	7.21
⋆11	2.25	6.05	–	6.92	–	–	7.20	–	–	7.18
12	3.5	/	–	/	–	–	/	–	–	/
13	2.0	/	–	/	–	–	/	–	–	/
⋆14	1.25	5.35	–	6.98	–	–	7.20	–	–	7.29
15	–	5.53	–	7.03	–	–	7.24	–	–	7.28
⋆16	1.5	5.50	–	7.03	–	–	7.38	–	–	7.42
17	–	5.80	1.75	6.44	–	–	7.22	–	–	7.17
⋆18	1.0	5.90	–	7.72	–	–	7.20	–	–	7.18
19	n.d.	/	–	/	–	–	/	–	–	/
20	2.5	/	–	/	–	–	/	–	–	/
21	1.0	/	–	/	–	–	/	–	–	/

Time after first virusa ddition (d)	Titer in log_{10} $TCID_{50}$/ml in raw sewage sludge	pH	Titer in log_{10} $TCID_{50}$/ml after pre treatment	pH	Titer in log_{10} $TCID_{50}$/ml after digestion without with pasteurisation		pH	Titer in log_{10} $TCID_{50}$/ml in thereference-process without with pasteurisation		pH

–=no virus detectable
n.d.=not done
/=no result
*=raw sewage sludge charged with virus

Table III. Titer of bovine Parvovirus and pH-values within the 2-step digestion and in the reference-process

Time after first virusa ddition (d)	Titer in log_{10} $TCID_{50}$/ml in raw sewage sludge	pH	Titer in log_{10} $TCID_{50}$/ml after pre treatment	pH	Titer in log_{10} $TCID_{50}$/ml after digestion without with pasteurisation		pH	Titer in log_{10} $TCID_{50}$/ml in the reference-proces: without with pasteurisation		pH
*30 min	2.0	5.60	n.d.	/		n.d.	/	n.d.	n.d.	/
1	2.25	5.90	n.d.	/		n.d.	/	–	–	7.22
* 2	2.25	5.76	n.d.	/	n.d.	n.d.	/	–	–	7.20
3	1.75	5.80	–	6.88	–	–	7.18	–	–	7.14
* 4	1.75	5.85	2.0	6.77	–	–	7.16	–	–	7.12
5	1.5	/	1.5	/	–	–	/	–	–	/
6	1.5	/	–	/	1.75	–	/	1.25	–	/
* 7	1.25	5.40	–	6.94	1.5	–	7.26	–	7.10	7.10
8	2.5	5.60	1.5	7.00	–	–	7.13	–	–	7.16
* 9	2.0	5.60	–	6.98	–	–	7.06	–	–	7.00
10	1.75	5.80	–	7.10	1.75	–	7.27	–	–	7.23
* 11	1.5	5.65	–	6.95	–	–	7.18	–	–	7.14
12	2.25	/	1.5	/	–	–	/	–	–	/
13	1.0	/	–	/	–	–	/	–	–	/
* 14	2.0	5.35	–	7.08	–	–	7.20	–	–	7.14
15	1.75	5.80	–	7.10	–	–	7.18	1.0	–	7.18
* 16	2.25	5.65	–	7.18	–	–	7.40	1.0	–	7.23
17	2.25	5.90	–	7.00	1.5	–	7.20	1.5	–	7.12
* 18	1.5	/	–	/	1.25	–	/	–	–	/
19	1.5	/	1.5	/	–	–	/	–	–	/
20	1.5	/	/	–	–	/	1.5	–	/	/

–=no virus detectable
n.d.=not done
/=no result
*=raw sewage sludge charged with virus

AEROBIC THERMOPHILIC STABILIZATION

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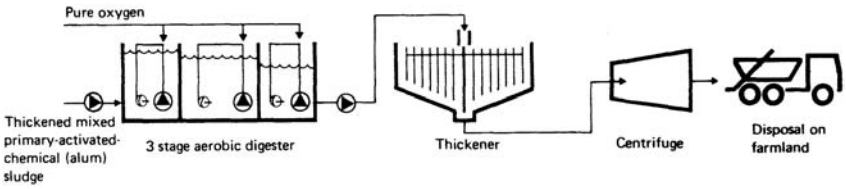

Figure 1 Full scale aerobic thermophilic stabilization of sewage sludge.

Summary

Thickened sewage sludge was stabilized in a full scale unit with three separate chambers by an aerobic thermophilic process using pure oxygen. Samples of sludge were examined weekly for the concentration of indicator organisms (faecal coliforms, faecal streptococci, and spores of Clostridium perfringens), a pathogenic bacteria (Salmonella), and bacteriophages (coliphage MS 2). Detention times of 5, 7, and 10 days with mean temperatures of 60–65 °C in the third chamber were sufficient to inactivate faecal coliform and Salmonella bacteria. In Norway this sludge could be used on land etc. (except sport grounds) without any restrictions because of parasitological or microbiological aspects.

1. INTRODUCTION

Most of the sewage sludge production in Norway comes from chemical precipitation plants and septic tanks. Concern about pathogens in sewage sludge limits disposal alternatives in many municipalities. The State Pollution Control Agency and The Ministry of Health advise against using possible pathogen-containing sludge on land used for production of food for humans and animals, on lawns, sport grounds, etc.

Many investigations have shown that untreated sewage sludge regularly contains pathogenic viruses, bacteria, protozoa, and parasites. By different treatment processes it is, however, possible to reduce the concentration, or totally destroy, most types of pathogens occuring in sewage sludge (1, 2, 3, 4, 5, 6, 7).

Destruction of pathogens and stabilization of sewage sludge by an aerobic thermophilic process using pure oxygen is a new composting method. With conventional aerobic stabilization about 3–6 m^3 air is added to the sludge pr. m^3 sludge and pr. hour. The purpose of the air addition is both supplying of oxygen and stirring. By supplying the oxygen in pure form, the need of gas is reduced to approximately 1 % of the volume mentioned above. The heat loss is greatly reduced because use of pure oxygen does not remove heat by warming the air and evaporating water. Results from pilot scale studies with thermophilic stabilization of sewage sludge using pure oxygen have recently been published (8). The present paper will discuss the fate of indicator organisms and Salmonella bacteria in a full scale plant using aerobic thermophilic stabilization (composting) with pure oxygen. This research project has been a co-operation between Norwegian Institute for Water Research, Norgas A/S, and Norwegian College of Veterinary Medicine. The project time was from May 1983 to March 1984 (9).

2. STABILIZATION USING PURE OXYGEN

The investigation was carried out in a primary biological-chemical sewage treatment plant (HIAS) treating waste water from approximately 50 000 people. An existing concrete tank was divided into three chambers and pumping facilities were installed in each chamber. See Figure 1.

After a batch of stabilized (composted) sludge was pumped out of the last chamber (eight times a day), an equal volume of thickened mixed primary-activated-chemical (alum) sludge was pumped into the first chamber, and equal volumes of sludge were consequently transported from the first to the second, and from the second to the third chamber. The net volumes in each chamber were 140 m^3, 80 m^3, and 60 m^3 respectively. The dry matter content in the raw sludge varied between 2% and 6%.

In each chamber the sludge was continuously pumped through a high pressure pipeline, and pure oxygen was injected into this sidestream. The average detention times were 3, 5, 7, and 10 days. At the end of the project a heat exchanger was used between untreated (cold) and treated (warm) sludge in order to preheat the raw sludge, the detention time was 3 days.

The consumption of oxygen varied from approximately 10 kg $0_2/m^3$ (3 days detention) to approximately 25 kg $0_2/m^3$ (10 days detention). This corresponds to 2.5–3.3 kg 0^2/day and m^3.

3. TEST MICROBES AND MICROBIOLOGICAL METHODS

Weekly samples of raw sludge, stabilized sludge, and intermediate samples from the first and second chambers were examined for the concentration of faecal coliforms, faecal streptococci, spores of Clostridium perfringens, and Salmonella bacteria. In the last test period (when the heat exchanger was used) bacteriophages (coliphage MS 2 ATCC 15597-B) were added to the raw sludge, and the concentration was analyzed in samples from the three chambers.

Faecal coliforms, faecal streptococci and spores of Clostridium perfringens are normally present in human and animal faeces. They are normally non-pathogenic. Salmonella bacteria are enteric bacteria causing disease in man and animal.

It is difficult to find good indicators of the behaviour of pathogenic viruses in sewage sludge. Coliphage MS 2 was used because of the morphological likeness to hepatitis-A-virus.

Faecal coliforms were detected using a most probable number (MPN) method with 10-fold dilutions and five tubes with MacConkey Broth (Difco) for each dilution. After 48 hours incubation at 37 °C, bacteria were seeded out from tubes with both gas and acid production to Brilliant Green Bile Broth (Difco). Cultures producing gas in this broth after 48 hours at 44.0 °C were registered as faecal coliforms.

Faecal streptococci were detected using the pour plate method with m-Enterococcus Agar (Difco) and 48 hours incubation at 44°C.

Spores of Clostridium perfringens were detected by first killing the vegetative cells in the dilutions by a 75 °C heat treatment for 20 minutes. After rapid cooling the samples were examined on SFP-Agar (Difco) to which 0.04% D-cycloserine had been added. The plates were incubated anaerobically at 44 °C for 16–24 hours before examination.

Salmonella bacteria were enumerated using a MPN-method with 10-fold dilutions and three tubes of Selenite Broth (Difco) at each dilution. The broth was incubated at 41.5 °C and plated onto Brilliant Green Agar (Difco) after 2 and 3 days incubation. Lactose- and saccharose-negative colonies resembling Salmonella bacteria were plated onto Brilliant Green Agar (Difco) to which 2 % sorbitol had beed added. Sorbitol-positive colonies were subjected to further characterization by the use of Enterotube II (F.Hoffmann-La Roche & Co) or a three tube method (10), and to polyvalent Salmonella test sera (Behring). One Salmonella culture from each positive sample was verified and typed at The National Institute of Public Health, Oslo.

Bacteriophages (coliphage MS 2 ATCC 15597-B) were enumerated using the agar-layer method with Escherichia coli (ATCC 15597) in MacConcey Agar (Difco).

4. RESULTS AND DISCUSSION

4.1 Chemical properties

Figure 2 shows the average reduction of dry matter, organic matter, chemical oxygen demand (COD), and biological oxygen demand (BOD_7) in the sludge after detention times of 3, 5, 7, and 10 days (9).

4.2 Temperatures

With 5, 7, and 10 days average detention time, and with 3 days detention and heat exchanger, the temperatures in the sludge in the second and third chamber were 55–60°C. With 3 days detention time and without the heat exchanger, it was difficult to exceed 50°C.

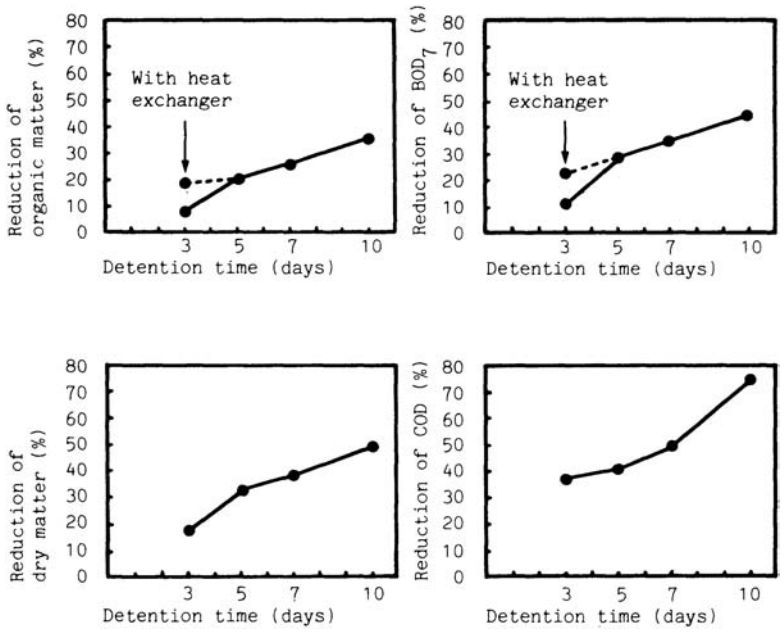

Figure 2 Reduction of dry matter, organic matter, chemical oxygen demand (COD), and biological oxygen demand (BOD_7) in the sewage sludge.

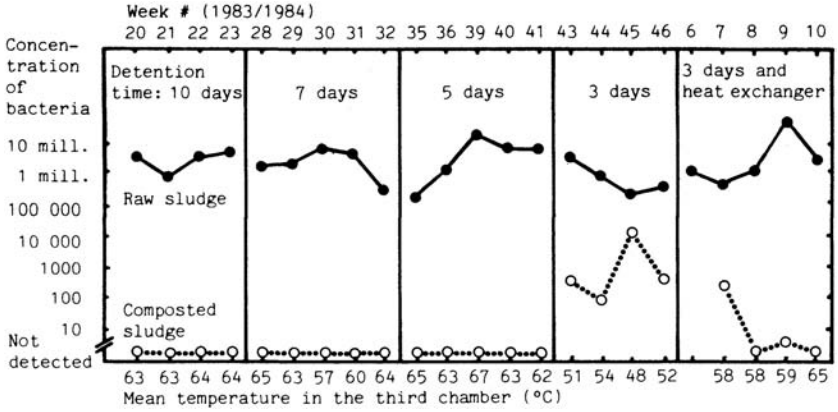

Figure 3 Concentration of faecal coliforms in raw and composted sewage sludge.

The correspondence was good between the content of dry and organic matter in the sludge—and the temperature. When the concentration of dry and organic matter decreased, the temperature also decreased—and vice versa (9).

4.3 Microbiological aspects

The raw sludge always contained faecal coliforms, faecal streptococci, spores of Clostridium perfringens, and Salmonella bacteria. The mean concentration of Salmonella bacteria in raw sludge was 3200 pr. gram dry matter (min. 320 max. 10 000). The domination of Salmonella oranienburg in the samples can be explained by the fact that there was an epidemic of salmonellosis caused by this serotype at that time. (The source was pepper sold from cooperative food stores.)

The survival/decimation of indicator bacteria (e.g. faecal coliforms, faecal streptococci, and spores of Clostridium perfringens), bacteriophages (coliphage MS 2), and Salmonella bacteria during the composting process can give an indication of the survival/decimation not only of Salmonella bacteria, but also of other pathogens. Figure 3–7 show the concentrations of the test organisms in raw and in composted sludge. Table I shows all the results of the microbiological analyses of the sludge samples and the mean temperatuers.

With 60–65 °C in the third chamber and 5, 7 or 10 days average detention time it has to be assumed that virus and most species of pathogenic bacteria (except species in the family Bacillaceae) would be totally destroyed.

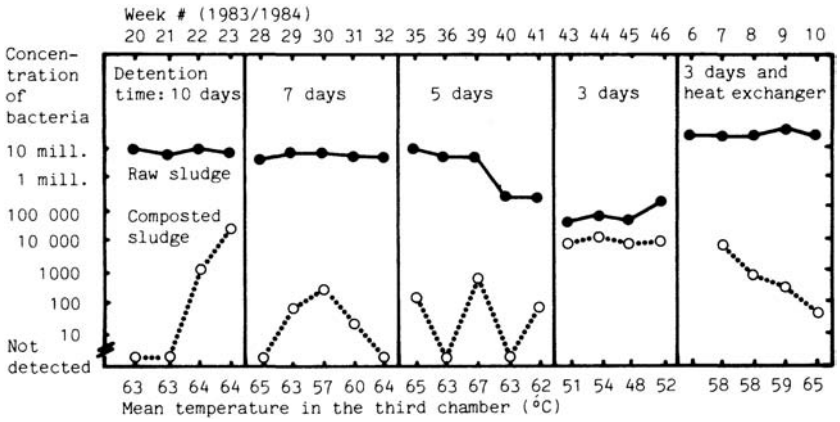

Figure 4 Concentration of faecal streptococci in raw and composted sewage sludge.

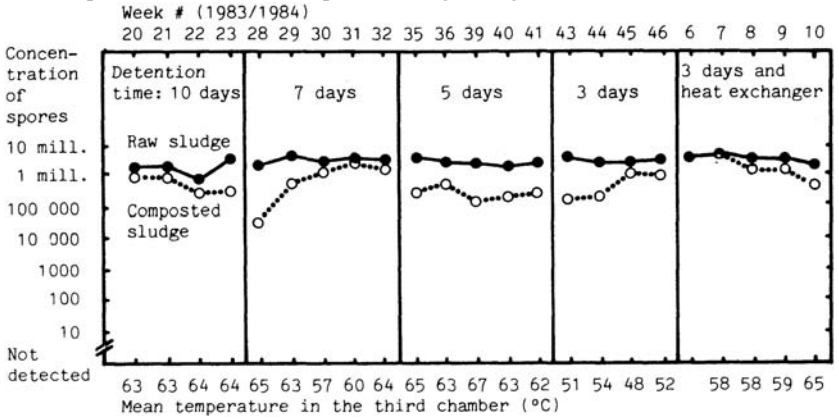

Figure 5 Concentration of spores of Clostridium perfringens in raw and composted sewage sludge.

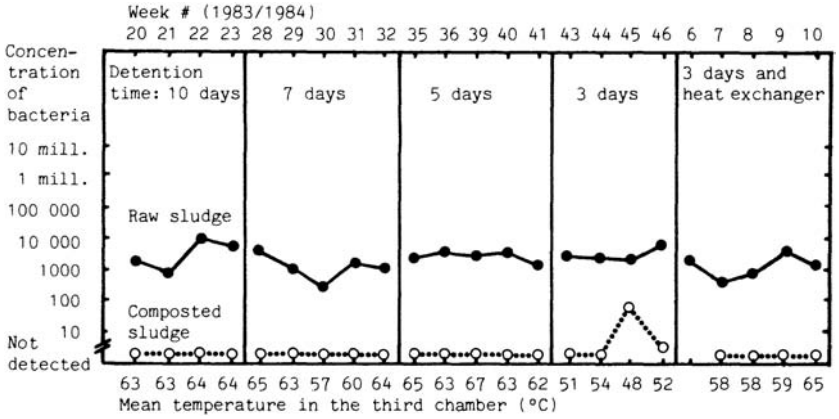

Figure 6 Concentration of Salmonella bacteria in raw and composted sewage sludge.

With an average of 3 days composting and the use of heat exchanger the test organisms were considerably inactivated if the temperature in both the second and the third chamber were 60–65°C. Sometimes the temperature was too low, howewer, and some faecal coli, and a greater fraction of the faecal streptococci survived.

From the results listed in Table I it can be seen that three chambers were necessary to provide a satisfactory decimation of the microorganisms.

The results (Figure 3–7) show good correlation between decimation of the test organisms (except spores of Clostridium perfringens) on the one hand—and both detention time and the temperature on the other.

In publications concerning the fate of pathogens during composting of sewage sludge (using air instead of pure oxygen) temperatures equal to or above 50–60 °C are mentioned as necessary to achieve total destruction of parasites, patho-genic viruses, and pathogenic vegetative bacteria (1, 5, 11, 12, 13, 14). In the present study the criteria for the temperature was higher, possibly because of the relatively short detention times.

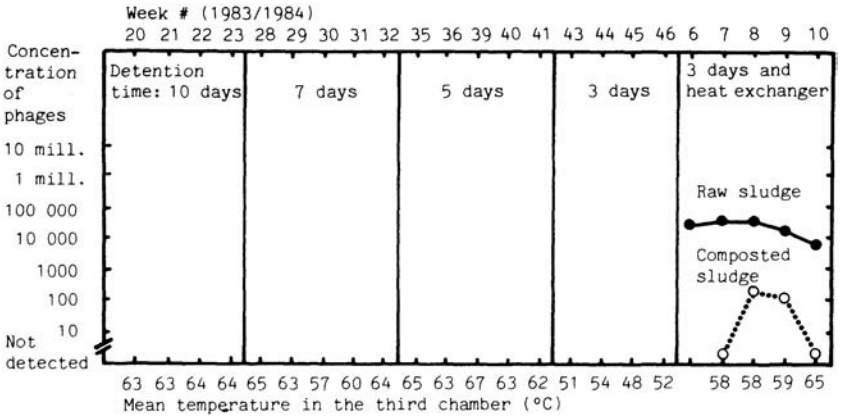

Figure 7 Concentration of added bacteriophages (coliphage MS 2) in raw and composted sewage sludge.

Strictly speaking both time and temperature must be considered when discussing the destruction of pathogens, both generally and during treatment of sewage sludge (5, 7).

A general problem with composting of sewage sludge and other material is the fact that material close to the surface does not reach the same high temperature as the rest of the material. In the present study this was no problem because of the continuous stirring caused by the pumping of the sludge in the side stream.

With three days average detention time and without the use of the heat exchanger the temperature in the second and third chambers did not exceed 54 °C and the hygienic effect of the composting was limited. In one such sample of unsatis-factory composted sludge the concentration of Salmonella bacteria was as much as 100 pr. gram dry matter.

The composting had minimal decimating effect on the spores of Clostridium perfringens (Figure 5). These organisms can be used as indicators for the survival of spores of the tetanus bacteria (Clostridium tetani) which normally occur in human faeces.

5. CONCLUSIONS

In the process described, composting times of 5, 7, and 10 days with mean temperatures of 60–65 °C in the third chamber, faecal coliforms and Salmonella bacteria were not detected in composted sludge. The concentration of faecal streptococci was considerably reduced.

With 3 days of composting and a pre-heating of the raw sludge by means of a heat exchanger, the effect on faecal coliforms, Salmonella bacteria, and bacteriophages was considerable when the temperature both in the second and the third chamber was 60–65 °C.

Three days composting without pre-heating of the raw sludge did not give sufficent decimation of the test organisms in order to use the sludge on land without further treatment or storage.

The effect of the composting (aerobic thermophilic stabilization) on the test organisms used (except spores of Clostridium perfringens) was correlated both to temperature, and to time.

The results show that the process should be carried out in at least three chambers in series.

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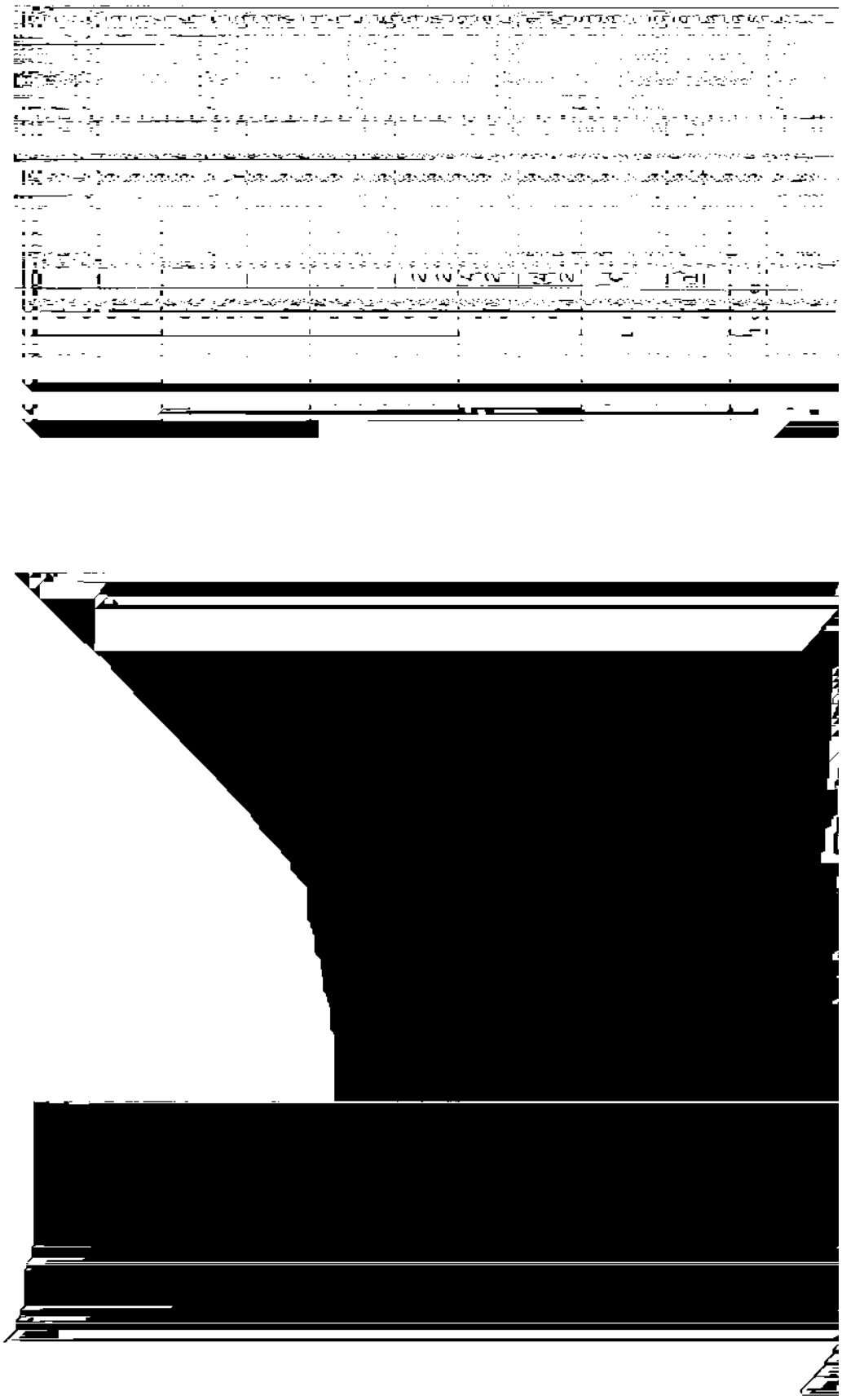

Table I Mean temperatures and logarithmic concentrations of the test bacteria and added bacteriophages in samples of sewage sludge from the composting unit. -: Not detected, FC: faecal coliforms, FS: faecal streptococci, SCP: spores of Clostridium perfringens, SALM: Salmonella bacteria, BP: bacteriophages (coliphage MS 2), °C : tempera-ture ($_0$C) .

INVESTIGATIONS ON THE HYGIENIC EFFECT OF SINGLE STAGE AND TWO-STAGE AEROBIC-THERMOPHILIC STABILISATION OF LIQUID RAW SLUDGE

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Summary

With single-stage aerobic-thermophilic stabilisation an inactivation of pathogens in sewage sludge can be achieved under certain conditions and observance of certain parameters. But it seems nearly impossible in the daily practical operation to constantly maintain these necessary parameters and control them. Moreover hydraulic short-circuits followed by still infectious effluent cannot be excluded. Even in the two-stage aerobic-thermophilic stabilisation process one has to reckon with a breakthrough of pathogens in reactor 1 for similar reasons as in the single-stage process which can be counterbalanced by the biochemical activities in reactor 2. Therefore aerobic-thermophilic stabilisation of sludge for the purpose of eliminating pathogens basically should only be operated as a two-stage system. The results of the studies also show that coliforms in parts are earlier inactivated than salmonellas. These observations are casting doubt upon the role of coliforms as indicator organisms for the occurrence of salmonellas in disinfected sludge.

1. INTRODUCTION

Aerobic-thermophilic treatment and stabilisation (ATS) of highly concentrated organic liquids is in Germany derived from agriculture where that type of aeration process was originally used for deodoration of pig slurry (3, 8, 9, 10, 12). The observation that by the aeration also thermophilic processes are initiated was at first of secondary importance. Only when it was found that by the thermophilic effects stabilisation of the substrate and possibly disinfection could be achieved the technology became interesting for sewage sludge engineering. Before the term “aerobic-thermophilic stabilisation” was introduced other terms like “liquid composting”, “hot aeration” and “rotating or circulating aeration” were used. To date the system developed by Hubert Fuchs is the one on which most of the published technical and hygienic investigations have concentrated.

In first hygienic investigations with sludge in a pilot plant working on a batch basis the minimum parameters for destruction of salmonellas were found to be 48 h aeration at pH 8.7 and 48°C or 10 h aeration at pH 8.5 and 45°C (13, 14). For the destruction of eggs of Ascaris suum the parameters were 57 h aeration at 49°C—54°C; the pH seems to be of no decisive importance for the destruction of parasite eggs (14). Also the inactivation of Picornavirus was investigated (in pig slurry); the parameters were 30 h aeration at pH 6.6 and 48°C or 22 h aeration at pH 9.2 and 30°C (11). Other investigators recorded an inactivation of indigenous salmonellas in sludge in an aeration period of 3–11 d at temperatures between 40°C—65°C and pH-values between 7.0 and 7.9 combined with a reduction of coliforms from 2–5 $\log_{10}$. At 55°C no active virus could be detected and eggs of Ascaris spp. were reduced in number (5) . Salmonellas were also destroyed in 24 h aeration at 45°C and in a few hours at 60°C (2) Besides the described parameters duration of aeration, temperature and pH the antagonistic effects of the microflora of the sludge during aerobic-thermophilic stabilisation on pathogens are stressed (6). This author found also that aerobic-thermophilically stabilised sludge is not reinfected during storage in a sewage treatment plant.

In the meantime several systems for aerobic-thermophilic sludge stabilisation are on the market. Since this system of stabilisation is considered to be a potentially practical and economical alternative to pasteurisation for the disinfection of sewage sludge (7) it is necessary to investigate hygienic problems which may arise in the course of technological changes of this system of stabilisation.

It is known from the anaerobic digestion of sludge that the occurrence of pathogens in the effluent of the digesters is to a certain extent caused by hydraulic short-circuits within the digester. Therefore it is a thought that suggests itself to suppose that similar phenomenons might occur in a single stage reactor for aerobic-thermophilic stabilisation. A comparison of single stage and two-stage systems therefore was also of hygienic interest.

2. MATERIAL AND METHODS

2.1 Single stage system

The "THIEME-recal-system" for aerobic-thermophilic stabilisation is concentrating a mixture of primary and secondary surplus sludge in a drum screen with addition of organic flocculants from originally 3 % DM to ca. 12% DM. Thus only one third to one fourth of the original sludge volume has to be stabilised by ATS. The retention time of the sludge in the reactor is between 3 and 4 days. The volume of sludge in the reactor was ca. 18 m^3. On Monday and Thursday ca. 6m^3 of ATS-sludge were drained and 6 m^3 raw sludge added.

2.1.1 Preparation and investigation of samples

Salmonella cultures (S.senftenberg W 775) were adsorbed to pieces of leather as germ carriers dried, and packed into metal containers similar to tea balls with large holes. Tissue cultures of parvovirus (strain Haden) were filled into ampules. Eggs of Ascaris suum were filled into silk-gauze bags and also given into the containers together with the germ carriers for salmonellas and the ampules with virus. The containers were fastened to a weighted steel chain which was lowered into the reactor. Thus the samples were located in three different heights in the sludge of the ATS-reactor (2.1 m, 1.4 m, 0.7 m). The samples stayed in the sludge either from Thursday to Monday or from Monday to Thursday.

Besides the test agents in the samples the following parameters were investigated: total aerobic germ count, coliforms and enterobacteriaceae of raw sludge, of ATS-sludge of the reactor and of ATS-sludge in the storage silo.

Salmonellas were reisolated from the germ carriers by pre-enrichment in buffered peptone water (24 h—37°C), then inoculated into enrichment medium tetrathionate MÜLLER-KAUFFMANN (24 h and 48 h—43°C) , then streaked on brome-phenolred-lactose-agar and brome-thymolblue-lactose-agar (24 h— 37°C). Total aerobic germ count was made on Standard-I-agar (24 h—37°C), coliforms on Endo-agar (24 h—37°C) and enterobacteriaceae on Mc-Conkeyandbiolet-red-bile-agar (48 h —30°C).

Bovine parvovirus was propagated on primary fetal lung tissue cultures Virus assays were performed by using these tissue cultures in 24-wells COSTAR-tissue culture-plates.

Ascaris suum eggs were incubated at 29°C for 4 weeks. The development of larva II in the egg was controlled weekly. Assessment of the damage of eggs or larvae was based on morphological criteria. The infectivity of developed larve was tested in mice: oral administration of material, after 24 h investigation of slides with squeezed liver of the mice; if larva III was found it was considered positive.

2.1.2 Results

Salmonellas could not be reisolated (Table I) from the germ carriers even in experiment no. 3 where the temperatures were not above 55°C. From the raw sludge indigenous salmonellas were isolate twice before ATS.

The values of the counts of total aerobic germs, coliforms and enterobacteriaceae are shown in Table II. Coliforms and enterobacteriaceae were never found in the ATS-sludge. A regrowth of enterobacteriaceae was registered in the stored sludge twice but only in few number.

Parvovirus in ampules (Table III) was reisolated several times. It has to be pointed out that the virus had no direct contact with the sludge and because of the ampules was not exposed to any adverse effects of the milieu of the sludge except the temperature.

Ascaris suum eggs were completely degenerated by the ATS-process (Table IV).

These results indicate that the contents of the ATS-reactor are thoroughly mixed. But, as already mentioned, in experiment No. 3 the temperatures never rose above 55°C. Several monthsafter our experiments were performed and we had certified that the ATS-system in the respective sewage treatment plant produces a hygienically safe sludge we heard by chance that the same ATS-plant had a lot of difficulties in reaching temperatures above 50°C.

2.2 Two-stage system

The FUCHS-system of ATS was investigated in a sewage treatment plant with an oxidation ditch for biological treatment. The surplus sludge is thickened to 5 % DM (Figure 2) and pumped into reactor I of the ATS-system with a service volume of

24 $m^{3.}$ During the semi-continuous operation a certain amount of ATS-sludge was drained once a day from reactor II into the pre-pit of the storage silo. The contents of the two reactors was then equalized and the respective daily amount of sludge pumped into reactor I: The daily amount of sludge produced was 8 $m^{3.}$ The minimum retention time therefore was 1 day which was also necessary for hygienic reasons. A comprehensive technical description of the whole system has already been published alsewhere (1) and will again be given tomorrow during our excursion.

Table I. Reisolation of test salmonellas from sludge after one-stage aerobic-thermophilic stabilisation (3 parallel samples per experiment)

No. Of experiment	Days of ATS	Height of carriersgerms the reactor			Sludge of reactor	Sludge of storage silo	Temperature of reactor	pH	Indigenous salmonellas in raw sludge
		0.7m	1.4m	2.1m					
1.	4	–	–	–	–	–	48 h >55°C after 24 h 62°–64°C 33 h >60°C	8.3	–
2.	3	–	–	–	–	–	60 h >55°C after 24 h 61 °C 44 h >60° C	8.5	–
3.	4	–	–	–	–	–	at least 24 h around 55°C	8.4	+
4.	3	–	–	–	–	–	48 h >55°C at least 24 h 60°C	7.2– 8.4	+
5.	4	–	–	–	–	–	55 h >53°C ca. 20 h at 60°C	8.8	–

–=saimonellas detecable
+=salmonellas isolated

Table II. Bacteriological counts of raw, ATS and stored sludges per milliliter

Experiment No.	Days of ATS	Bact. parameter	Raw sludge	ATS sludge	Stored sludge
1.	4	TGC	1.5×10^{8}	1×10^{6}	5.7×10^{6}
		CF	5.0×10^{6}	–	–
		EB	1.2×10^{7}	–	–
2.	3	TGC	4.0×10^{7}	3.1×10^{5}	1.2×10^{7}
		CF	2.3×10^{6}	–	–
		EB	7.0c10^{6}	–	–
3.	4	TGC	3.2×10^{8}	4.0×10^{5}	3.3×10^{6}
		CF	5.0×10^{6}	–	–
		EB	1.8×10^{7}	–	8.0×10^{1}
4.	3	TGC	1.7×10^{8}	3.9×10^{5}	1.6×10^{7}
		CF	7.0×10^{6}	–	–
		EB	3.2×10^{7}	–	1.0×10^{1}
5.	4	TGC	1.9×10^{8}	9.0×10^{5}	6.2×10^{6}
		CF	5.0×10^{6}	–	–
		EB	3.2×10^{7}	–	–

ATS=Aerobic-thermophilic stabilisation –=No CF or EB isolated
TGC=Total aerobic germ count
CF=Coliforms
EB=Enterobacteriaceae

Table III. Isolation of residual virus in ampules after ATS in /ml (2 parallel samples per experiment)
Testvirus: bovine parvovirus, strain Haden

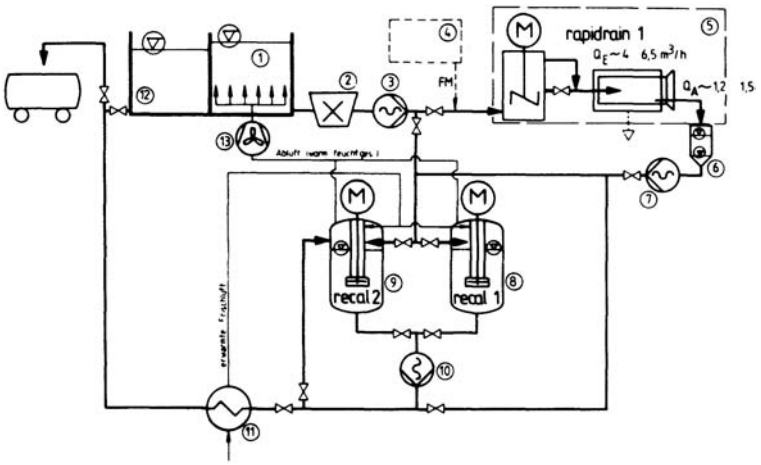

Figure 1. Flow chart of a two-stage aerobic-thermophilic stabilisation system (“recal” Fa. Thieme) which was operated during the experiments as a single-stage unit

Initial titer: $10^{6.25}$

		Temperature of sludge							
		>60°C		<60°C		>60°C		∿60°C	
Height of sample in the reactor	Days of ATS	3		4		3		4	
2.1 m		–	–	–	–	$10^{2.25}$	–	$10^{1.25}$	–
1.4 m		–	–	–	$10^{1.25}$	–	–	–	–
0.7 m		–	$10^{1.25}$	–	$10^{1.25}$	–	–	–	–

TCID=Tissue culture infective dosis
−=No virus isolated

Table IV. Mean parasitological results of 2×100 analyzed eggs of Ascaris suum

Days of ATS	Height of germ carriers in the reactor	Egg development in %		
not developed	2–8 morula stadium	degenerated		
4	2.1 m	3	0	97
	1.4 m	0,5	0	99.5
	0.7 m	0.5	0	99.5
3	2.1 m	1	0	99
	1.4	2	0	98
	0.7 m	1	0	99
4	2.1 m	1.5	0	98.5
	1.4 m	1	0	99
	0.7 m	0	0	100
3	2.1 m	0	0	100
	1.4 m	0,5	0	99.5
	0.7 m	0	0	100
4	2.1 m	0	0	100
	1.4 m	0	0	100
	0.7 m	1	0	99

1. Storage tank for raw sludge with ring aeration by waste air from the reactors
2. Sludge dosing pump with comminutor
3. Pump for liquid raw sludge
4. Station for flocculant

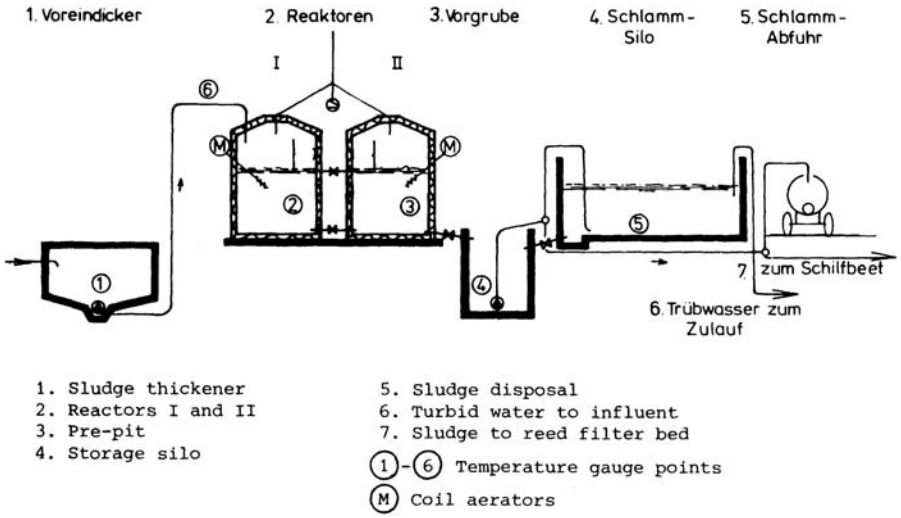

Figure 2. Flow diagram of the two-stage aerobic-thermophilic sludge treatment (Rüprich 83012)

5. Dewatering unit “rapidrain” Type 1
6. Tank for thickened raw sludge
7. Pump for thickened raw sludge
8. Reactor 1
9. Reactor 2
10. Pump for ATS-sludge
11. Heat exchanger
12. Storage tank for ATS-sludge
13. Air blower

2.2.1
Preparation and investigation of samples

The preparation and reisolation of the test salmonellas was done similarly as described in 2.1.1. In the course of the experiments it was found that the tea-ball-like containers for the germ carriers were covered with hairs and coarse material that the medium sludge came only with difficulties into contact with the test germs (Salmonella senftenberg W 775 and S.typhimurium). Therefore for the last three experiments we used pieces of fur-velour-leather instead of silk-gauze as germ carriers which were fastened in different heights to a thin steel rope and directly lowered into the reactor with a weight.

2.2.2
Results

A total of 17 series of experiments was performed which are published and discussed in detail elsewhere (1, 4). In the following only a selection of characteristic results is shown and discussed.

Figure 3 shows that in this experiment the temperature in reactor I did not reach 40°C and in reactor II not 50°C. As a result the salmonellas were not destroyed in both reactors. In the experiment shown in Figure 4 the salmonellas survived in reactor I at temperatures between 45°C and 55°C but were finally inactivated at constant temperatures of about 55°C. During the experiment of Figure 5 the salmonellas were already eliminated in reactor I under very similar conditions as in the experiment of Figure 4

The last three experiments (Figure 6, 7, 8) were made as batch experiments only in reactor II which was filled in the first two runs with sludge from reactor I (Fig. 6, 7) and in the last run with raw sludge from the thickener. The results show that in one case (Fig. 6) the test salmonellas were not inactivated after 24 h of aeration but were eliminated after 48 h. In another run (Fig. 7) the salmonellas were not demonstrable after 10 h of aeration but could again be isolated after 24 h under similar temperature conditions but different pH-conditions. Apparently in this case the salmonellas were enveloped in protective substances from the sludge and reanimation occurred under favorable reisolation conditions. This is a fact which stresses the demand that ATS-systems as a rule should be operated as two-stage systems to prevent such a hygienic setback.

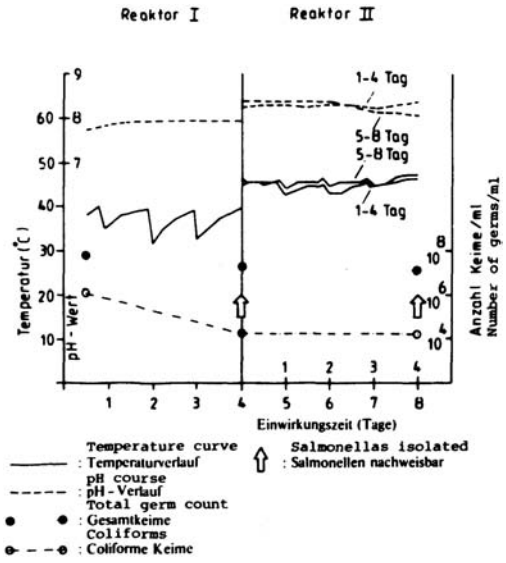

Figure 3. Results of an experiment operated as two-stage ATS

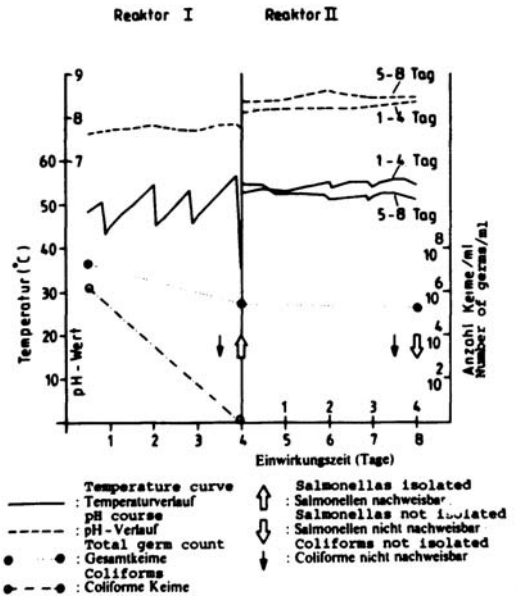

Figure 4. Results of an experiment operated as two-stage ATS

When raw sludge with an initial temperature of 12°C was used the salmonellas were inactivated only after 9 days and coliforms already after 8 days (Fig. 8).

Of great importance for the policy of evaluating hygienic measures for disinfection of infectious sewage sludge is the observation during these experiments that coliforms were not demonstrable at a time when salmonellas still could be isolated. This can be seen in the Figures 4, 5, 7 and 8.

3. DISCUSSION

The results of the experiments in a full scale single stage ATS with salmonellas, virus and parasite eggs have shown that, in principle, a disinfection of sewage sludge can be achieved. But the obligatory substantiated claims to the prerequisites which have to be met during the operation of an ATS-system are very high. The daily experiences under practical conditions show that it is extremely difficult to comply with the various parameters which have to be observed. Even in our experiments (No. 3) under routine conditions the temperatures were fulfilling only the minimum requirements. And, as already mentioned, we received the information that later on the temperatures dropped below values which have to be demanded from the hygienic point of view.

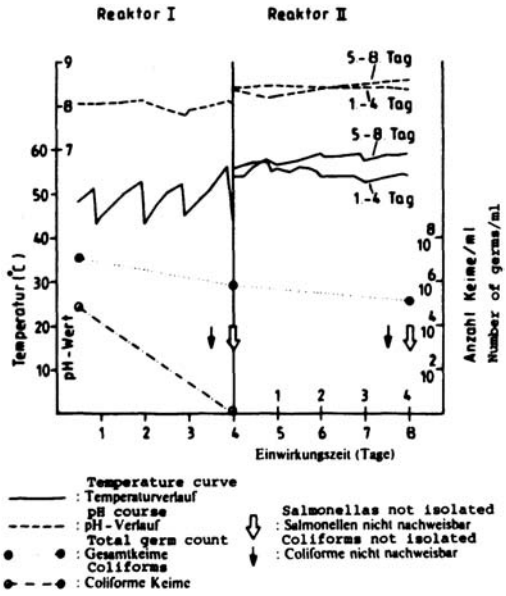

Figure 5. Results of an experiment operated as two-stage ATS

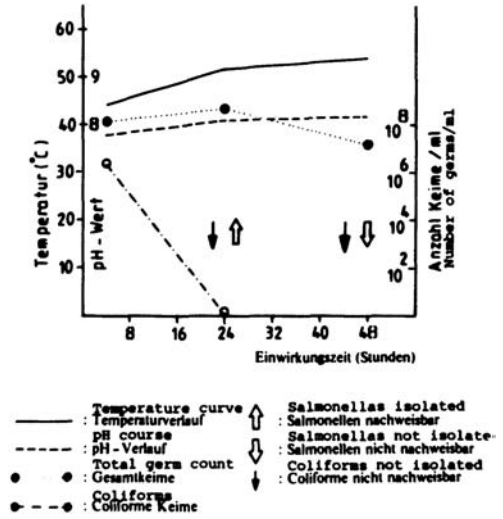

Figure 6. Results of an experiment operated in reacter II as single-stage ATS but with sludge which had already passed reactor I

Another point are the hydraulic conditions in the vessels for ATS. As it was already shown this morning in the second paper for single stage anaerobic digesters it can never be excluded with certainty that hydraulic short-circuits result in the drainage of particles from such vessels that did not fulfill the requirements for the retention time. Also human failure in the operation and supervision of ATS-systemshave to be taken into account.

All these reasons together lead us to the conviction that single stage ATS-systems should not be used for the disinfection of sewage sludge which is utilized in agriculture without an additional disinfecting treatment. It has to be mentioned here that we are not competent to judge the method which is used in Switzerland (6) where an aeration of sludge at relatively low temperatures is practised followed by an anaerobic digestion. The exponents of this technology claim that infected sewage sludge is disinfected by this type of treatment. We had no opportunity yet to scrutinize this technology thoroughly under practical conditions.

The results of our experiments in the two-stage ATS-plant have shown that a disinfection of sludge can be achieved. The necessary parameters are for

continuous operation:	48 h aeration above 50°C and pH about 8, or
	24 h aeration when 58°C are reached and pH 8.2.
batch operation:	(with initial temperatures of 10°C—15°C)
	9 days retention time, or

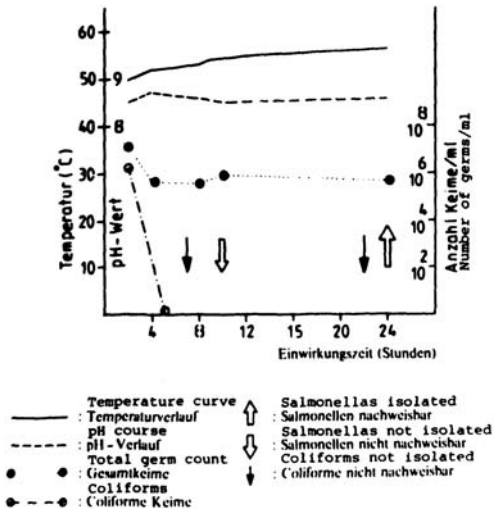

Figure 7. Results of an experiment operated in reacter II as single-stage ATS but with sludge which had already passed reacter I

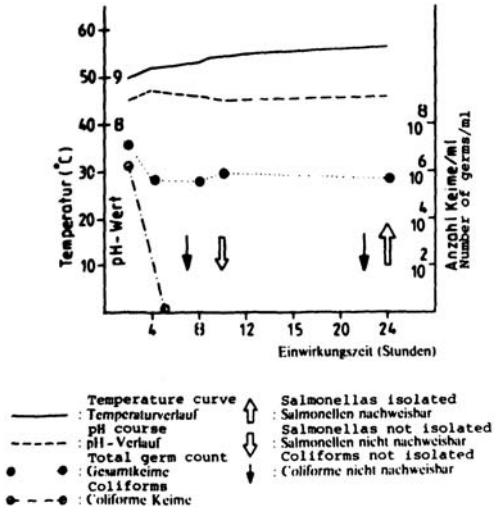

Figure 8. Results of a batch experiment in reacter II started with cold raw sludge

48 h aeration ofter temperatures of at least
50°C and pH about 8 have been reached.

But some of our results show also that even the two-stage ATS is a very sensible biological system which by manifold imponderables can be thrown off-balance. For these cases the second reactor is at disposal to cushion the strain of temporary difficulties and compensate dysfunctions of the first reactor. This is a safety factor which should not be abandoned without necessity.

Another observation should finally be discussed. In Switzerland a sludge is considered to be hygienized when not more than 100 enterobacteriaceae per gram can be isolated. In the Federal Republic of Germany the legislature expects proposals from the hygienists within the next three years for a definition of the term in the present ordinance on sludge utilisation “hygienically safe”. In thinking of borrowing the Swiss regulation with 100 enterobacteriaceae into our legislation we are puzzled by our already mentioned observation that in some of our experiments the samples were already free of coliforms whereas salmonellas still could be isolated. These findings were not only made during ATS sewage sludge but in earlier investigations also during aerobic-thermophilic treatment of slurries from domestic animals in agriculture. These observation again raise the question how reliable the Swiss method really is related to the interrelations of enterobacteriaceae and salmonellas. We think that further investigations in this respect are necessary. We must discuss the same problem to-morrow during the session of our working group because a part of to-morrows session is a discussion about the definition of the term “disinfection” in the context of sludge treatment. It should be decided whether this term is tenable at all or should be replaced by another wording.

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SLUDGE HYGIENIZATION WITH DIFFERENT COMPOST SYSTEMS

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Summary

Different systems of sludge composting have been evaluated for the efficiency in controlling pathogenic bacteria. The differences in composting methods include open and closed systems, systems of aeration, air flow direction and bulking agents. The following microorganism groups of pathogens were determined before and after composting processes: Salmonella sp, Fecal coliforms and Fecal streptococci. Some considerations on the effectiveness of the different composting systems in controlling pathogens are reported.

1. INTRODUCTION

Waste water treatment in Europe has led to a marked production of sewage sludge, a production which is still growing at present.

Because incineration and landfill are becoming increasingly less popular for ecological and economical reasons, agricultural utilization of sludge is spreading in many European countries.

Agricultural land in Europe have been intensively farmed for many centuries and need to recover the organic matter depleted by several crops in some way in order to mantain their biological fertility and their physical structure. It must be realised however that sewage sludge contains a great variety of pathogenic microorganisms in relatively high numbers and that the use of sewage sludge on land can intensify infection transmission cycles.

Therefore measures should be taken to effect a substantial reduction in the concentration of pathogens in sewage sludge before it is allowed to come into contact with crops such as fresh vegetables and fruits that are brought into the kitchen raw (18, 25).

Even if technologically it is possible to hygienize sludge, economically and practically a no-risk level cannot be obtained (19).

Of all the different processes proposed for sludge hygienization, composting, which gives rise to a thermophilic stage, seems to give the best guarantees at a low cost (4, 5, 11).

Because legal specifications which define compost or composting processes are still lacking, today a vast assortment of "organic fertilizers" exists on the market. These products, obtained through many processes of sludge treatment, differ from each other not only in chemical composition and biological stabilization, but also in pathogenic microorganism content.

In this paper several different systems of sludge composting are evaluated in order to verify their efficiency in reducing pathogens.

2. METHODS

2.1. Composting systems

The sludge composting processes with open systems were carried out at the experimental plant of the Istituto di Microbiologia Agraria, University of Pisa (Fig. 1). Piles consisting of approximately 20 metric tons each were made, using aerobically stabilized primary sludge from municipal waste-water treatment mixed with different bulking agents: (a), the biodegradable organic fraction of solid urban waste; (b), rice hull; (c), cork saw dust; (d), wheat straw; (e), inert plastic spheres of 50 mm in diameter (Biofill 50); (f), wood chips. Composting processes were carried out in 30 days. In the turned system a twice-weekly turning cycle was used. In the vacuum-induced ventilation system (Suction), the ventilator was controlled by a timer with

cycles of 40 seconds suction every 13 min. In the pile aerated with forced-pressure ventilation the same equipment was used, but with inverted flow (Blowing). In all static systems, piles were blanketed with a layer of mature sanitized compost for insulation.

Sludge composted with closed systems (reactors) came from different industrial plants: vertical reactors: Ennepetal, Germany "Weiss system"; Senigallia, Italy "BAV system; horizontal reactors: Yokohama, Japan; Columbus, Ohio, USA" the Paygro system.

2.2. Microbiological analyses

For isolation and identification of Salmonella sp, fecal coliforms and fecal streptococci, standard methods reported in literature have been used (2, 3, 14, 22, 23). All numbers reported in Tables represent a colony forming unit (C.F.U.) per g d.w. of sludge.

3. RESULTS

3.1. Practical systems of composting

There is still much confusion today over the meaning of the word compost. Composting is a microbial reaction of mineralization and partial humification of organic substances which under optimum conditions take place within a month. It is very difficult to decrease this time and it is not possible for composting to take place in a few days as many assert. Composting time depends on the biological cycles of the microorganisms involved. Their replication time is conditioned by environmental factors and genetical constitution of the microorganisms. Although environmental factors may be improved, genetic limits remain (6).

In nature, organic matter spontaneously undergoes several processes of microbial transformation according to the composition of the substrate and the chemical and physical environment which the microorganisms inhabit. The end-products are completely different depending on whether conditions are aerobic or anaerobic. Composting requires that the process be mostly aerobic so that organic matter is partially mineralized and humified (7).

Spontaneous composting occurs in nature, for instance the transformation of plant litter and manure; however these processes are slow, discontinuous and heterogeneous. To make composting suitable for the waste disposal industry requires three fundamental points to be met: (1°), brevity of the process and low energy consumption; (2°), a guarantee of standard end-products not only safe for agriculture use but also of satisfactory fertilizing value; (3°), hygienic safety of plants and end-products.

For these three requirements to be satisfied simultaneously, composting cannot be spontaneous. It must be controlled in order to guarantee hygienized and high quality end-product. This means that several different kinds of practical composting systems have been devised: these are reported in Table I.

Table I. Summary of composting systems for sludge.

OPEN SYSTEMS	
Turned pile	
Static pile:	– air suction – air blowing – alternating ventilation – air blowing in conjunction with temperature control
CLOSED SYSTEMS	
Vertical reactors	– continuous – discontinuous
Horizontal reactors	– static – with movement of material

The aim of all these systems is basically that of creating the best conditions for the process. These conditions directly influence the growth and metabolism of microorganisms which carry out the process. Therefore the factors which directly influence the process of composting are those which condition microbial metabolism (5, 6, 7, 8, 9, 10, 11).

In composting the main factor that can be most influenced by technology, around which system designs are developed, is the availability of oxygen. With respect to design, the equipment for providing aeration ranges from the relatively simple to the very complex. This range leads to the generalized classification of compost technology described earlier as open (windrow, pile) and closed (mechanical, in a vessel or container) (13).

The main features of open and closed systems are reported below.

3.2. Windrow

A windrowed mass of composting material can be aerated by either or both of two methods namely turning and forced aeration.

3.2.1. Turned pile

Pile turning, though widely used because it is simple, has its limits. In the first place the pile is oxygenated only periodically by turning. Since the optimal level of oxygen must be kept constant to enhance biological oxidations, interrupted aeration may be unsuitable (6, 11, 12).

Periodic turning for pathogen control is not completely satisfactory (11, 17). It must be added that, during the final stages of composting, when the material is nearly dry, dust containing a lot of Aspergillus fumigatus spores is released into the air (20, 21).

In Table II, data regarding the persistence of some pathogenic bacteria with turned pile composting system, using two different bulking agents, are reported. This system does not seem to be very efficient in controlling fecal coliforms and streptococci. This may be due to the non homogeneous diffusion of high temperatures when composting material is periodically turned.

3.2.2. Static pile

The idea of aerating a static composting pile by forced air is perhaps the best, because it allows exact amounts of oxygen to be given and enables the control of other important parameters such as moisture and temperature. Ventilation of piles can be done in several ways (see Table I).

The suction system was devised at Beltsville by the U.S.D.A. and is widely adopted particularly in the U.S.A. (15, 26).

The process devised at Rutgers University in New Jersey is based on blowing and temperature control (16). This system has two main advantages in comparison to the preceding: blowing enhances evaporation giving a low moisture end- product, guaranteeing high stability. Automatic temperature control avoids long periods of high temperatures. Since most microorganisms involved in composting do not survive at temperatures above 60°C, it is advisable that this temperature should not be reached in composting so that microbial activity is at maximum efficiency.

Table II. Effect of sewage sludge composting (30 d) with turned pile using two different bulking agents, the biodegradable organic fraction of solid urban waste (RSU) and rice hull on the recovery of some pathogenic bacteria.

MICROORGANISMS	SLUDGE+RSU		SLUDGE+RICE HULL	
	sludge	compost	sludge	compost
Salmonella sp	2.4×10^{1}	absent	absent	absent
Fecal coliforms	4.8×10^{5}	5.6×10^{4}	5×10^{6}	3×10^{5}
Fecal streptococci	2.4×10^{5}	2.0×10^{5}	1.8×10^{5}	3×10^{4}

Table III. Effect of sludge composting with static pile aerated by vacuum induced pressure on the recovery of some pathogenic bacteria. Three different bulking agents have been used: biodegradable organic fraction of solid urban waste (RSU), wood chips and inert plastic spheres.

MICROORGANISMS	SLUDGE+RSU		SLUDGE+WOOD CHIPS		SLUDGE+INERTS	
	sludge	compost	sludge	compost	sludge	compost
Salmonella sp	2.4×10^{1}	absent	1×10^{5}	absent	5×10	absent
F.coliforms	4.8×10^{5}	4.1×10^{2}	2×10^{7}	5×10^{1}	7×10^{7}	1×10^{1}

MICROORGANISMS	SLUDGE+RSU		SLUDGE+WOOD CHIPS		SLUDGE+INERTS	
	sludge	compost	sludge	compost	sludge	compost
F.streptococci	2.4×10^5	7.0×10^2	7×10^6	3.5×10^1	7×10^5	1.1×10^1

High temperatures are highly selective towards the microflora; very few sporigenous bacteria survive above 70°C. This means composting is arrested until temperature falls and the microorganisms can reinvade the mass.

Although high temperatures inhibit microbial population, they have, however, a positive effect in reducing pathogens. For this reason several processes include an initial phase of suction composting which permits the temperature to rise for a few days; the air stream is then inverted and blowing in conjunction with temperature control continues the process.

In Table III, data regarding the persistence of some pathogenic bacteria during static pile composting, aerated by vacuum-induced ventilation (suction), are reported. Three different bulking agents were used: 1, the organic fraction of solid urban waste; 2, wood-chips; 3, inert plastic spheres. These composting processes seem to give satisfactory reduction of pathogens, when they are well-conducted. The height of the pile is an important factor controlling the process and therefore for obtaining a good hygienized end-product. Indeed, piles that are too high (> 3 m) do not permit a homogeneous oxygenation of the mass; anaerobic zones will be present in the mass with low temperatures and consequently with scarce reduction of pathogens.

When the static pile is oxigenated by forced ventilation (blowing) the hygienization of the mass seems to be satisfactory (Table IV). In three independent experiments using sludge mixed with different bulking agents (organic fraction of S.U.W., cork sawdust and wheat straw) a drastic reduction of pathogenic bacteria was observed. In composted sludge Salmonella was always absent.

3.3. Closed Systems

3.3.1. Vertical reactors

These reactors, generally over 4 m high, can be continuous— composting material in one large mass—or discontinuous—mass arranged on different floors. Discontinuous vertical reactors contain, on different levels, piles no higher than 2 or 3 m and therefore have no disadvantages apart from the high cost of the plant and maintenance.

In continuous vertical reactor, which may contain one single mass up to 9 m high, the process is extremely difficult to control, because uniform oxygen cannot be obtained even when blowing from beneath is used. Since volume of air per unit surface area must be proportional to the height of the pile, the lower part of the mass is over-ventilated with excessive cooling and drying and the upper layers are insufficiently aerated, because as air passes through the composting mass, it changes composition losing oxygen and acquiring carbon dioxide.

In the experimental plant at Ennepetal, sludge mixed with the organic fraction of solid urban waste was treated in a closed vertical reactor (Weiss) of 100 cubic meters for 14 days. Analyses carried out in two independent experiments of pathogenic microorganisms present in the

Table IV. Effect of sludge composting with static pile aerated by forced pressure ventilation (blowing) on the recovery of some pathogenic bacteria. Three different bulking agents have been used: the organic fraction of solid urban waste (RSU), cork saw dust and wheat straw.

MICROORGANISMS	SLUDGE+RSU		SLUDGE+CORK		SLUDGE+STRAW	
	sludge	compost	sludge	compost	sludge	compost
Salmonella sp	2.4×10^1	absent	3.1×10^2	absent	1.2×10^2	absent
F. coliforms	4.8×10^5	1.2×10^3	9.3×10^5	5.9×10^2	8.5w10	7.8×10^2
F. streptococci	2.4×10^5	8.2×10^2	6.0×10^6	8.1w10^2	4.8×10^6	3.0×10^3

Table V. Effect on recovery of some pathogenic bacteria in sludge after 14 day permanence in vertical reactor Weiss, Sludge was mixed with the organic fraction of solid urban waste.

MICROORGANISMS	STARTING MATERIAL	AFTER REACTOR PERMANENCE
Salmonella sp	absent	absent
F.coliforms	1.4×10^7	2.5×10^6
F.streptococci	3.4×10^5	9.0×10^6

starting and in the treated material are reported in Tables V and VI. After 2 week treatment the resulting material was not hygienized. Control of the process inside such big continuous reactor seems to be really difficult, even when air is blown from the bottom. Indeed anaerobic zones are nearly always present and low temperatures prevent the destruction of pathogens. This partial anaerobic mesophilic process may explain the high content of streptococci in the end-product.

A further experiment was made in another vertical reactor (BAV, Senigallia) and the relative data on the pathogens present are reported in Table VII. Sludge is mixed with hardwood sawdust and with composted sludge in this reactor, with a retention time of 15 days. The survival of F. coliforms and of streptococci, in this reactor too, has to be attributed to a poor control of the process, giving anaerobic zones and too-low temperatures caused by a deficient oxygenation of the material during the course of the experiment.

3.3.2. Horizontal reactors

Horizontal reactors are generally used for composting sludge mixed with bulking agents in static or periodically turned piles no higher than 2.5 m. In these reactors air is nearly always blown from the bottom. If this equipment is used correctly it combines the advantages of both open and closed systems, giving better control over the process, pathogens and odours.

For this research materials coming from two different horizontal reactors were tested: Yokohama (Japan) and Columbus (Ohio, USA).

In Yokohama, sludge mixed with composted sludge is treated for 10 days in a horizontal reactor in which material is turned daily. Although 10 day retention does not seem to be enough to obtain a mature compost, hygienization does occur quite well during the process (Table VIII).

At Columbus, sludge is composted in a Paygro horizontal reactor; bark and sawdust are added as bulking agents. Low-pressure blowers supply air to a plenum located at the base of the reactor tanks, with 14 day retention time, during which the complete mass undergoing composting exceeds a temperature of 55°C for a minimum of three days. This guarantees a good hygienization of the treated material (Table IX).

4. CONCLUSIONS

Several factors affect pathogen survival during treatment. In composting, heat is one of the primary factors contributing to pathogen inactivation. Most pathogenic microbes are destroyed by heating for several hours to temperatures above 50°C. Microbial competition is an other important factor in controlling pathogens diffusion during composting. A variety of saprophytic microorganisms partecipates in the composting process; these microorganisms might be considered the indigenous or natural microflora of the compost system. Municipal sludge contains a second microbial population, the pathogens, which represent a numerically

Table VI. Effect on recovery of some pathogenic bacteria in sludge after 21 day permanence in vertical reactor Weiss. Sludge was mixed with the organic fraction of solid urban waste.

MICROORGANISMS	STARTING MATERIAL	AFTER REACTOR PERMANENCE
Salmonella sp	absent	absent
F.coliforms	2.2×10^{8}	7.8×10^{3}
F.streptococci	8.5×10^{6}	1.9×10^{9}

Table VII. Effect on recovery of some pathogenic bacteria in sludge after 15 day permanence in vertical reactor BAV. Sludge was mixed with composted sludge and saw dust.

MICROORGANISMS	STARTING MATERIAL	AFTER REACTOR PERMANENCE
Salmonella sp	absent	absent
F.coliforms	3.1×10^{8}	2.5×10^{5}
F.streptococci	4.7×10^{7}	7.6×10^{6}

TableVIII. Reduction of pathogenic microorganisms in sludge after 10 day permanence in horizontal reactor (Yokohama, Japan). Sludge was mixed with composted sludge.

MICROORGANISMS	STARTING MATERIAL	AFTER REACTOR PERMANENCE
Salmonella sp	1.3×10^{2}	absent

MICROORGANISMS	STARTING MATERIAL	AFTER REACTOR PERMANENCE
F.coliforms	5.7×10^{7}	2.5×10^{2}
F.streptococci	8.0×10^{6}	1.7×10^{2}

Table IX. Reduction of pathogenic microorganisms in sludge after 14 day permanence in horizontal reactor Paygro (Columbus, Ohio, USA) Sludge was mixed with sawdust and composted sludge.

MICROORGANISMS	STARTING MATERIAL	AFTER REACTOR PERM.
Salmonella sp	8.1×10^{2}	absent
F.coliforms	7.9×10^{8}	9.2×10^{1}
F.streptococci	4.5×10^{6}	1.9×10^{2}

insignificant fraction of the total microbial population. Hence competition comes into play when the community is heterogeneous and the population density is high relative to the supply of any limiting feature of the environment. The indigenous saprophytic population has a highly distinct competitive advantage over the other population; composting material is not the natural environment for pathogen microorganisms, therefore in this ecosystem competition will tend to result in the elimination of the less fit rival (1).

The choice of the most suitable composting process depends on differing local situations and conditions; nevertheless only processes which guarantee good quality and hygienized end-products should be chosen.

All the experimental data reported in this paper indicate that static composting systems provide a better control over pathogens than does turning.

In the closed systems, horizontal reactors seem to guarantee better control over pathogens than vertical continuous reactors. This may be mainly due to the fact that masses over three meters high are difficult to control during the composting process. In particular oxygenation of the mass is not homogeneous, giving anaerobic zones with lower temperatures (6, 12, 24).

Since the term “composting” encompasses numerous processes which may create physiologically disparate physico-chemical environments for pathogens in the sludge being composted, it can not be tacitly assumed that composting renders municipal sludge pathogen-free. However, well-conducted processes with prolonged periods of high temperatures do seem to provide a sanitized product.

Finally, there is a need for a better definition of the term “sanitized compost”. At present, there is no legal specification of what should be a disinfected sludge.

For this reason, and because it is essential for compost to have specifications for pathogens too, it is of primary importance to lay down not only which microorganisms are to be monitored, but also their maximum permitted level in a sanitized product.

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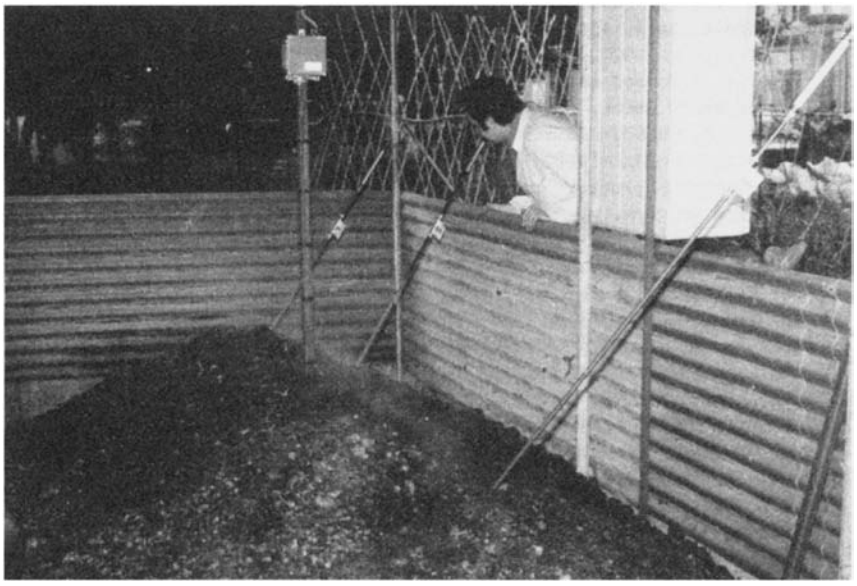

Fig. 1. Experimental plant of sludge composting of the Istituto di Microbiologia Agraria, University of Pisa.

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INFLUENCE OF LIME TREATMENT OF RAW SLUDGE ON THE SURVIVAL OF PATHOGENS, ON THE DIGESTABILITY OF THE SLUDGE AND ON THE PRODUCTION OF METHANE — TECHNICAL EXPERIENCES WITH HIGH pH IN THE DIGESTER OF A SEWAGE TREATMENT PLANT—

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Summary

A description of the technical parameters of the sewage works of the City of Lindau at Lake Constance is given with special reference to phosphate precipitation, the amount of sludge produced and the experiences with liming of raw sludge for disinfecting purposes.

1. INTRODUCTION

The old former imperial town of Lindau is located at the eastern shore of Lake Constance. Already in the early fifties the citizens and the city council perceived the necessity of a proper drinking water and sewage management. The water of the available springs will not suffice for the drinking water supply in the future.

The importance of Lake Constance as service reservoir for drinking water for millions of people at the lake and in more distant conurbations is increasing from year to year. Lake Constance with its water volume of 50 billions m^3 is a closed biological system which is, by way of calculation, renewed only every 4.5 years via the inlets.

The realization that the protection of Lake Constance is only possible by close cooperation of the riparian states resulted in 1959 in the foundation of the International Commission for the Prevention of Pollution of Lake Constance. Thus the prerequisites were created that it was possible to counteract the pollution of the lake by means of statutory regulations and laws unhampered by state and national borders.

As a most important source of pollution the excessive inflow of the plant nutrients phosphate and nitrogen is considered which are contained in sewage and wash-out of fertilizers.

In 1972 the Bavarian Authorities for Water Management demanded the introduction of the phosphate precipitation in the sewage works of Lindau. With this demand a development was put in action which even to-day after 12 years is not yet concluded.

2. SEWAGE WORKS OF LINDAU

2.1 Development and drainage area

The construction of the sewage works began in 1952. Besides the piping work for the sewers the sewage works were expanded in further stages until to-day. Besides the City of Lindau five Bavarian municipalities at Lake Constance and, frontier-crossing, one municipality of the Land Baden-Württemberg are connected to the sewage works. The technical and economical central handling is done by the City of Lindau (Fig. 1).

2.2 Short description of the sewage works

The sewage works are ountlined for the connection of 85 000 population equivalents. The daily dry-weather influent is 12 000–14 000 m^3 sewage in the average. The mean organic loads are between 3000–4500 kg BOD_5/day, peak loads of 6000–7000 kg BOD_5/day can occur in the autumn of each year. They are caused during the campaign of a fruit-processing plant.

The sewage is collected in a pumping station and after a coarse purification by screens and a circular sand-trap elevated into the feed channel via pressure-pipeline. After inductive measurement of the sewage flow and passage of a fine screen the first

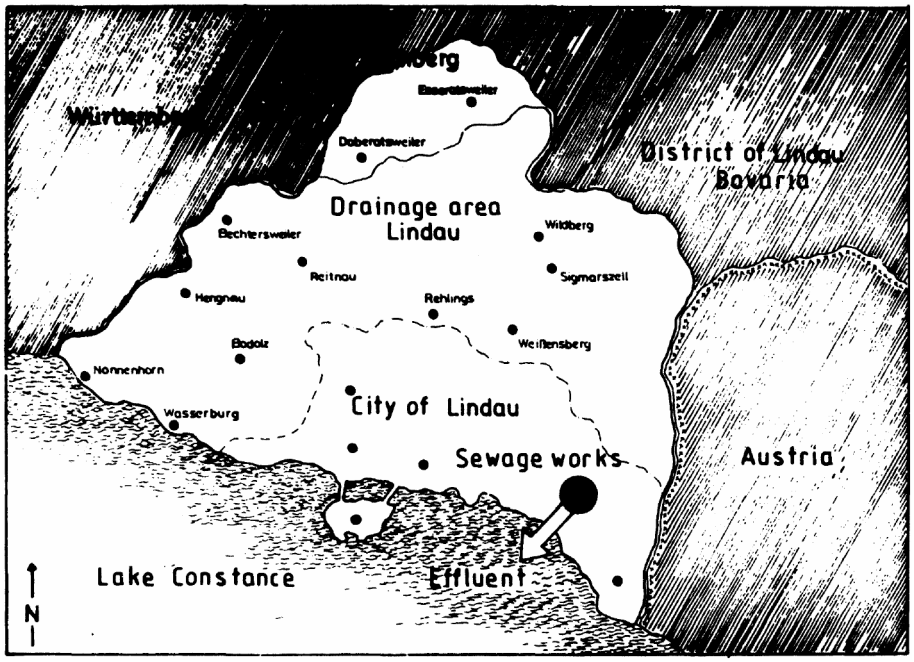

Figure 1. Layout plan of the sewage works of the city of Lindau

flocculant is given into the inflowing sewage. As flocculant for the pre-flocculation lime hydrate (Ca(OH)2) is used as lime milk (Fig. 2).

By a pH-measuring and -adjusting device in the feed channel a preselective increase of pH of the sewage can be adjusted. According to our experiences over a long time the degradation values and operating costs in Lindau are most favorable in the pH-range of 9.0–9.5. The amount of lime used in the average of a year is 100–120 g of Ca(OH)2 per m^3 of sewage.

After the rise of pH in the feed channel the influent flows into 4 parallel primary settling tanks with a total volume of 1720 m^3 and a surface of 852 m^2. The residence time is 2–3 hours at dry-weather flow.

The following biological step consists of an activated sludge plant according to the Swedish Inka-system with middle-bubble aeration. The retention time in the 6 aeration basins with a total volume of 2610 m^3 is 2.5–4 hours.

The sizing of the plant is based on the following operating data:

Space loading	=	1.4 kg BOD5/m^3 d
Sludge loading ratio	=	0.46 kg BOD_5/kg
Dry weight of sludge	=	3.0 kg DM/m^3
Sludge volume index	=	60–80 ml/g DM

Into the influent to the activated sludge plant 5 liters of iron-II-salts in saturated solution are injected per minute for further phosphorus elimination.

After the activated sludge plant the sewage flows into 2 series—connected secondary sedimentation tanks with a volume of 2350 m^3 and a surface of 1100 m^2 each. The purified effluent is discharged via a pipeline into the lake 12 m below low water level.

Legend to Figure 2

1 Coarse screen
2 Sand trap
3 Main pumping station
4 Fine screen
5 Vessel for mixing lime milk
6 Lime milk into influent of primary settling tank
7 pH measurement and lime milk dosing
8 Primary settling tank
9 Activated sludge plant (Inka)
10 Final settling tank 1

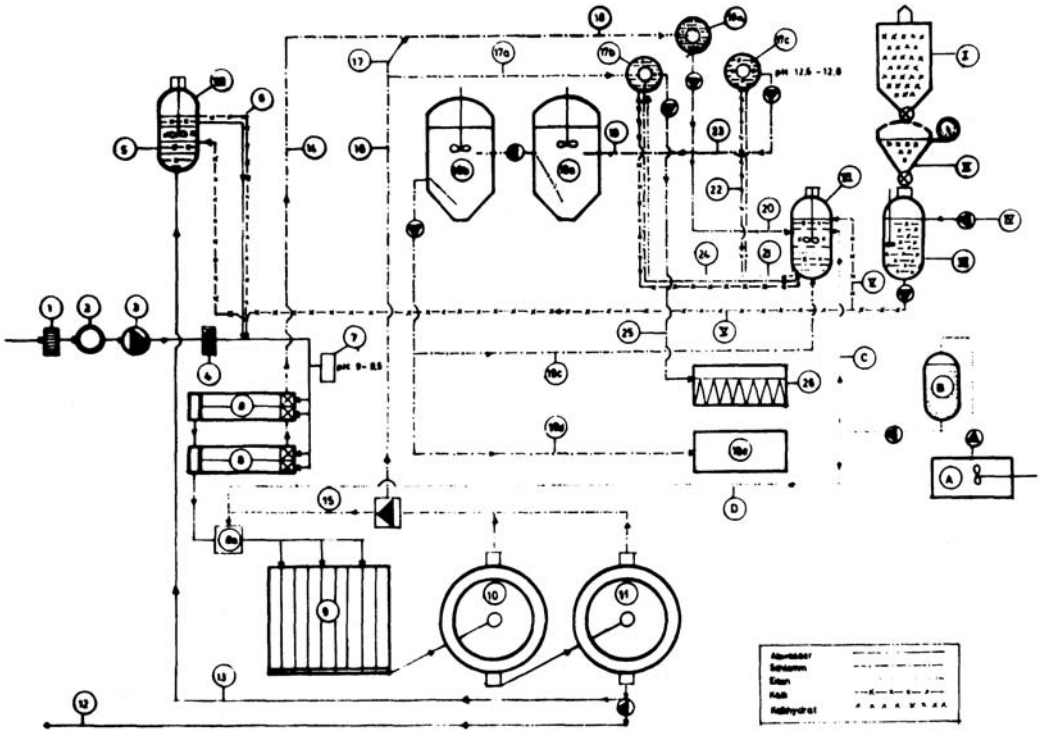

Figure 2. Flow diagram of the sewage works of the city of Lindau

11 Final settling tank 2
12 Effluent into Lake Constance
13 Industrial water for lime milk
14 Raw sludge from primary sedimentation via pipeline 18 to pre-sludge thickener
15 Return-sludge from final settling tanks 1+2 to influent of activated sludge plant
16 Outlet for excess sludge via pipelines 16, 17, 18 to pre-thickener (18a), also for separated thickening in reaction tank (17b/17c) via pipelines 16 and 17
17 Sludge pipelines
+1
7a
17b Reaction thickener
+1
7c
18 Sludge pipeline
18a Pre-sludge thickener
19 Sludge pipeline from pre-thickener to digester 1
19a Digester 1
19b Digester 2
19c Pipeline for digested sludge to reaction mixing tank (VI)
19d Digested sludge to interim storage tank (19e)
19e Storage basin for digested sludge
20 See I—VII
21 Sludge conditioned with lime/iron from reaction mixing tank (VI) via pipelines 21+24 to reaction thickener (17b) for dewatering
22 See I—VII
+2
3
24 Sludge pipeline
25 Sludge pipeline to filter press (26)
26 Filter press

I Silo for lime hydrate ($Ca(OH)_2$)
II Lime scales
III Lime mixing tank (suspension)
IV Industrial water from a well
V Lime milk pipelines to reaction mixer (VI) and lime milk dissolving tank (VII)
VI Reaction mixer
VII Lime milk dissolving tank

A Dissolving tank for iron-II-sulfate
B Storage tank for iron-II-sulfate
C Pipeline for iron-II-sulfate to reaction mixer (VI)
D Pipeline for iron-II-sulfate to simultaneous precipitation influent aeration

_____ Sewage
-.-.-.-.-.-.-.-.-.-.-. Sludge
..... Iron
-x-x-x-x-x-x-x-x Lime

2.3
Phosphate precipitation and amount of sludge produced

Before the combined flocculation with lime and iron-II-sulfate, which is used today, was introduced extensive tests in technical scale with other flocculants were made between 1972 and 1974.

As a result great problems emerged in Lindau as far as sludge was concerned. The sludge volume index fell into its normal swing with values between 250 and 300 ml/g DM. Odor nuisances, poor dewaterability, dropping pH-values in the digesters during this period required a massive application of lime again and again. Therefore it was a thought that suggested itself to utilize lime for the elimination of phosphorus in the pre-flocculation.

Against all expectations the daily amount of raw sludge produced was not increased by the amounts of lime utilized in Lindau in comparison with the former practised mechanic-biological mode of operation.

The daily amount of sludge produced amounts to 70–80 m^3 with a solids content between 8–12%. In the subsequent treatment of sludge the primary and secondary sludges can be thickened either together or separately. During a high production of excess sludge the thickening is done separately.

In 2 heated digesters with a volume of 900 m^3 each and 35°C the sludge is digested. After a digestion time of ca. 20 days the sludge should be treated in the existing pasteurisation plant at temperatures of about 70°C for 30 minutes to be transferred into a hygienically unobjectionable state. Since the hygienic requirements were not attainable by the pasteurisation of digested sludge the plant was temporarily shutdown.

Nearly 70–80 % of the sludges produced in the sewage works of Lindau are utilized in agriculture until today in liquid state on forage land around the whole year. The rest of 20–30 % is conditioned with ferric chloride and lime, dewatered in filter press and composted or in the autumn as filtered sludge distributed on arable land used for growing com (maize).

Due to a twofold application of lime (pre-flocculation and sludge conditioning) the necessary amounts of conditioners are considerably reduced. The consumption of lime hydrate (Ca(OH)2) amounts to 10–12 kg/m^3 and 1— 3 liters of iron-III-chloride/m^3. The time for dewatering sludge in the filter press is reduced for 1 hour/batch.

3.
DISINFECTION OF RAW SLUDGE

By the reorganization of the agricultural utilization of sewage sludge by the new ordinance in the Federal Republic of Germany the persons responsible in the civil engineering department in Lindau were confronted with not easily solvable tasks and problems. Therefore we were all too ready to follow a suggestion of Prof. Strauch to perform a trial in technical scale for the disinfection of raw sludge in our sewage works. The scientific close observation was the duty of the University of Hohenheim, the technical realization that of the sewage works.

The hygienic results are presented in the following paper. Our initial objections against such a massive addition of lime to the raw sludge and the subsequent digestion process became groundless in the operational course of the experiment in our sewage works.

During the test period one of the staff members was responsible for the implementation and control.

The loading rate of the limed raw sludges was limited to 40 m^3/day, the rest was dewatered in the filter press. The mixed primary and secondary sludges were stored in the pre-thickener and limed once a day in the available reaction mixer of the dewatering installations. During a contact time of 3 hours the sludges were stored in a sedimentation vessel, mixed and constantly controlled on their pH.

The originally high consumption of lime in the first 2 weeks of the experiment amounting to 20 kg of lime hydrate/m^3 raw sludge could be reduced in the following time to 12–15 kg. After the contact time the batches of about 6 m^3 of limed raw sludge were pumped into digester Nr. 1. The 2 available digesters were operated in series connection. Digester No. 1 is designed for pre-digestion and digester No. 2 for secondary digestion. Operational differences or influences on methane formation were not observed during the whole period of the experiment. Operating troubles with pumps and other installations were in the ordinary course of operation.

It is important to follow the advice that for a liming of raw sludge all pipelines used must be constituted from a material which is resistant against acidifiant clearance. Since the application of lime in the sewage works once a year extensive inspections of structures, the pipeline network and other installations are conducted.

After 10 years of operation we cannot imagine the treatment of sewage and sludge in our works in Lindau without the application of lime. The experiences and knowledges with the lime treatment of raw sludge will be realized by a supplementary constructional measure. Then all sludges produced shall be limed to a pH of 12.6–12.8 before they are digested.

INFLUENCE OF LIME TREATMENT OF RAW SLUDGE ON THE SURVIVAL OF PATHOGENS, ON THE DIGESTABILITY OF THE SLUDGE AND ON THE PRODUCTION OF METHANE
—HYGIENIC INVESTIGATIONS—

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Summary

The hygienic effect of lime added to raw sewage sludge was investigated with special consideration of the influence on the following digestion process. In preliminary investigations in laboratory scale the necessary pH-value and contact time of the sludge/lime mixture for a safe inactivation of salmonellas as test microorganisms were determined. In a further laboratory experiment the effect of the high alkalinity of the limed raw sludge on the following digestion process was investigated for a mean hydraulic retention time of 20 days. No adverse effects could be recorded. Based on these results a large-scale experiment was made in the sewage works of the City of Lindau at Lake Constance with 80.000 population equivalents. Salmonella senftenberg as test microorganism was inactivated by a pH of 12.8 within 3 hours in the preliminary laboratory experiments and in the large-scale experiment in the sewage treatment plant as well. No adverse effects on the digestion process nor the gas quality were observed.

1. INTRODUCTION

It is well known that by the use of lime as quicklime (CaO) or Calcium hydroxide ($Ca(OH)_2$) the concentration of bacteria and viruses is reduced with raising pH by the antimicrobial effect of the alkalinity. According to Tullander (2) three main principles for the technical treatment of sewage sludge are observed: 1. before thickening, 2. before dewatering and 3. after dewatering.

In earlier investigations in a municipal sewage treatment plant which continuously is liming the influent with lime hydrate we have observed that the raw sludge of the primary sedimentation in this plant was always free of salmonellas because an enrichment of lime in this thickened sludge led to pH-values of 12.2–13.5 (1). From there it seemed possible to feed the digesters with a sludge which is already free of pathogenic bacteria and viruses. Based on these results we began the experiments described in this paper to further study the effect of high pH-values on the digestion process and the hygienic quality of the raw primary sludge.

2. MATERIAL AND METHODS

As test microorganism Salmonella senftenberg was used. The germs were added in the laboratory experiments to the raw sludge as suspension. The density was ca. 108 salmonellas/ml of sludge. For reisolation of the salmonellas the usual method of pre-enrichment and enrichment was used. One part of the infected limed sludge was neutralized to pH 7.0–7.5 before inoculation of the pre-enrichment media the other part was inoculated into the pre-enrichment without neutralization. Neutralization was made because in a digester a limed sludge will also be reduced in its pH by the carbon dioxide.

In the preliminary experiments in the laboratory raw sludges were adjusted to different pH-values by addition of a 10 % lime milk and thoroughly mixed with a magnet agitator. In certain intervals samples were taken and—as described—neutralized and not neutralized bacteriologically investigated. The total number of aerobic bacteria was determined by plate count after Koch and the coliforms were counted in dilution rows on fuchsine-lactose-agar.

3. RESULTS

3.1 Preliminary experiments on inactivation of salmonellas in dependence of pH and contact time with lime

Tables I–III show the course of pH in raw sludge limed up to pH's of 12.8–12.5, the results of inactivation of salmonellas, total germ count and coliform count. The preliminary experiments had the results that it is necessary to lime a raw sludge up to a pH of 12.8 with a contact time of three hours to achieve a safe inactivation of salmonellas. During the time of agitation the pH may decrease for 0.5 pH. When the initial pH in the raw sludge was only 12.5 the pH decreased considerably and salmonellas could be reisolated over the whole contact and stirring time of 5 days. The results show further that one will have false negative results of salmonella isolation when the limed sludge samples are not neutralized before the enrichment media are inoculated.

3.2 Effects of limed raw sludge on a following digestion process in laboratory scale

The experiments were made in two identical laboratory scale pilot-plants in four steps.

Step 1: During the first 20 days of experiment untreated raw sludge is fed into the digesters. The process is adjusted to a mean hydraulic retention time of 20 days. The plants are tested for gas tightness.

Step 2: The raw sludge for digester No. 1 is treated with 10 % lime milk, digester No. 2 is fed with untreated raw sludge.

Step 3: The raw sludges for both digesters are inoculated with salmonella suspension and the sludge for digester Nr. 1 is further treated with lime.

Step 4: The raw sludges for both digesters are inoculated with salmonella suspension. Sludge for digester No. 1 is treated with decreasing amounts of lime, sludge for digester No. 2 is also treated with lime.

Table I. Bacteriological effect of different amounts of lime in raw sludge
Dry solids of raw sludge: 3.63% (by weight)
Loss on ignition of raw sludge: 1.83% (by weight)
pH-value of raw sludge without lime: 6.3
Total bacterial count of raw sludge without lime: 10^0/ml
Coliform count of raw sludge without lime: 106/ml
Added lime milk (10%): 22 ml
Test salmonellas: S. senftenberg;
+=s.s. isolated; –=s.s. not isolated

Duration of agitation	pH-value	Isolation of salmonellas from neutralized sludge incubation		Isolation of Salmonellas from alkaline sludge incubation		Total bacterial count	Coliform count
min/days		24 h	48 h	24 h	48 h		
2	12.80	+	+	–	–	1.0×10^8	1.1×10^6
15	12.80	+	+	–	–	5.1×10^6	7.1×10^3
30	12.82	+	+	–	–	8.1×10^5	1.0×10^2
45	12.80	+	+	–	–	2.8×10^5	–
60	12.69	+	+	–	–	1.2×10^5	–
80	12.55	+	+	–	–	1.0×10^5	–
100	12.45	+	+	–	–	$1.5\times10^{.5}$	–
120	12.35	+	+	–	–	2.0×10^5	–
140	12.35	+	+	–	–	1.8×10^5	–
160	12.30	–	–	–	–	1.0×10^5	–
180	12.25	–	–	–	–	1.2×10^5	–
1	12.20	–	–	–	–	2.3×10^5	–
2	12.10	–	–	–	–	1.3×10^5	–
3	12.00	–	–	–	–	1.3×10^5	–
4	12.00	–	–	–	–	1.1×10^5	–
5	12.00	–	–	–	–	1.8×10^5	–

Table II. Bacteriological effect of different amounts of lime in raw sludge
Dry solids of raw sludge: 3.63% (by weight)
Loss on ignition of raw sludge: 1.83% (by weight)
pH-value of raw sludge without lime: 6.2
Total bacterial count of raw sludge without lime: 10^0/ml
Coliform count of raw sludge without lime: 10^6/ml
Added lime milk (10%): 19 ml
Test salmonellas: S. senftenberg;
+=s.s. isolated; –=s.s. not isolated

Duration of agitation	pH-value	Isolation of salmonellas from neutralized sludge incubation		Isolation of Salmonellas from alkaline sludge incubation		Total bacterial count	Coliform count
min/days		24 h	48 h	24 h	48 h		
3	12.68	+	+	–	–	1.2×10^8	3.3×10^6
15	12.67	+	+	–	–	3.6×10^7	7.2×10^5
30	12.57	+	+	–	–	6.1×10^6	2.4×10^4
45	12.48	+	+	–	–	4.0×10^6	1.0×10^4
60	12.39	+	+	–	–	2.1×10^6	–
80	12.25	+	+	–	–	2.0×10^6	–
100	12.14	+	+	–	–	1.1×10^6	–
120	12.02	+	+	–	–	1.8×10^6	–
140	11.90	–	–	–	–	2.3×10^6	–
160	11.78	+	+	–	–	4.9×10^5	–
180	11.70	–	–	–	–	1.0×10^6	–
1	11.20	+	+	–	–	1.9×10^6	–
2	10.90	+	+	–	+	2.3×106	–
3	10.90	+	+	+	+	1.1×10^6	–
4	10.80	–	–	–	+	1.6×10^6	–
5	10.70	+	+	+	+	1.0×10^6	–

Table III. Bacteriological effect of different amounts of lime in raw sludge
Dry solids of raw sludge: 5.18% (by weight)
Loss on ignition of raw sludge: 3.52% (by weight)
pH-value of raw sludge without lime: 6.2
Total bacterial count of raw sludge without lime: 10/ml
Coliform count of raw sludge without lime: 107/ml
Added lime milk (10%): 17.5 ml
Test salmonellas: S. senftenberg;
+=s.s. isolated; –=s.s. not isolated

Duration of agitation	pH-value	Isolation of salmonellas from neutralized sludge incubation		Isolation of Salmonellas from alkaline sludge incubation		Total bacterial count	Coliform count
min/days		24 h	48 h	24 h	48 h		
2	12.52	+	+	–	–	1.0×10^9	2.3×10^6
15	12.25	+	+	–	–	3.6×10^7	3.9×10^5
30	12.04	+	+	–	–	2.7×10^6	1.5×10^3
45	11.85	+	+	–	–	4.1×10^6	2.0×10^1
60	11.67	+	+	–	–	3.1×10^6	–
80	11.47	+	+	–	–	1.0×10^6	–
100	11.28	+	+	–	–	2.0×10^6	–
120	11.00	+	+	–	–	2.9×10^6	–
140	10.70	+	+	–	–	4.5×10^6	–
160	10.40	+	+	–	–	4.5×10^6	–
180	10.20	+	+	–	–	7.0×10^6	–
1	9.31	+	+	–	–	4.1×10^6	–
2	9.00	+	+	–	–	1.0×10^7	–

Duration of agitation	pH-value	Isolation of salmonellas from neutralized sludge incubation		Isolation of Salmonellas from alkaline sludge incubation		Total bacterial count	Coliform count
min/days		24 h	48 h	24 h	48 h		
3	9.00	+	+	+	+	1.1×10^7	–
4	8.71	+	+	+	+	6.6×10^6	–
5	8.80	+	+	+	+	1.1×10^7	–

3.2.1
Results of step 1 (day 1–20)

The total bacterial count of raw sludge and digested sludge was 10– 10^8/ml. The coliform count did not show any differences and was $10^5/10^6$/ml in raw and digested sludge. The gas quantity and quality are shown in Figure 1 and Table VIII.

3.2.2
Results of step 2 (day 21–39)

The pH of digester No. 1 was adjusted from 6.2 to 12.8 with 10% lime milk. During three hours of agitating the pH decreased for 0.5 units. The total germ count in the raw sludge was 10^8/ml and the coliforms 10^6/ml. After three hours of agitating of sludge with lime the total germ count was reduced for 3–4 log10, no coliforms could be isolated. At the end of step 2 in the digested sludge No. 1 the total germs were reduced for 2 logic, coliforms were near zero. The bacteriological data of the raw sludge and the digested sludge No. 2 were the same as in step 1. The gas quantity and quality are also shown in Figure 1 and Table VIII.

3.2.3
Results of step 3 (day 40–50)

The total germ count and the coliforms in the agitated raw sludge with lime was reduced for 4 log10 or to zero, respectively. Salmonellas were only isolated from inoculated raw sludge No. 2 (Table IV). During the days 40–50 from the digested sludge No. 1 salmonellas were never isolated, the total germ count was 106/ml and coliforms could also not be isolated. In the digested sludge No. 2 the numbers of the total germs and coliforms did not change compared with the raw sludge. Salmonellas were isolated from each sample (Table V). The gas quantity and quality are also shown in Fig. 1 and Table VIII.

3.2.4
Results of step 4 (day 51–60)

The total germs increased in the raw sludge for digester No. 1, coliforms appeared on the 54th day and also at the same day salmonellas were reisolated after 3 hours of agitating. Already one day later (55 th day) the salmonellas could also be detected in the digested sludge (Table VI and VII).

The total germs in the raw sludge for digester No. 2 which was for the first time also treated with lime, was reduced after agitation of 3 hours for 3 log10 to 105/ml. Coliforms and the salmonellas were not isolated. Since digester No. 2 during this step only received raw sludge with low number of total germs, no coliforms and no salmonellas these parameters also changed in the digested sludge. The total germs were reduced from 108 to 106/ml, the coliforms from 10^6 to 10^4/ml. Salmonellas were isolated in each samples but the member of their colonies on the agar plates decreased obviously and if the experiment had gone a few days more it can be assumed that the digested sludge were free of salmonellas according to the experiences in step 3 (Table VII). The gas quantity and quality are also shown in Fig. 1 and Table VIII.

Table IV. Hygienic parameters of raw sludge after addition of lime (No. 1) and without lime (No. 2)

Day of the experiment	Raw slud No. 1					Raw sludge No. 2				
	pH-A	pH-B	TGC	CF	Salmonella	pH-A	pH-B	TGC	CF	Salmonella
40	12.9	12.3	10^4	–	–	6.2	6.2	10^8	10^6	+
41	12.9	12.3	10^4	–	–	6.2	6.2	10^8	10^6	+
42	12.8	12.3	10^4	–	–	6.2	6.3	10^8	10^5	+
43	12.9	12.4	10^4	–	–	6.1	6.2	10^8	10^6	+

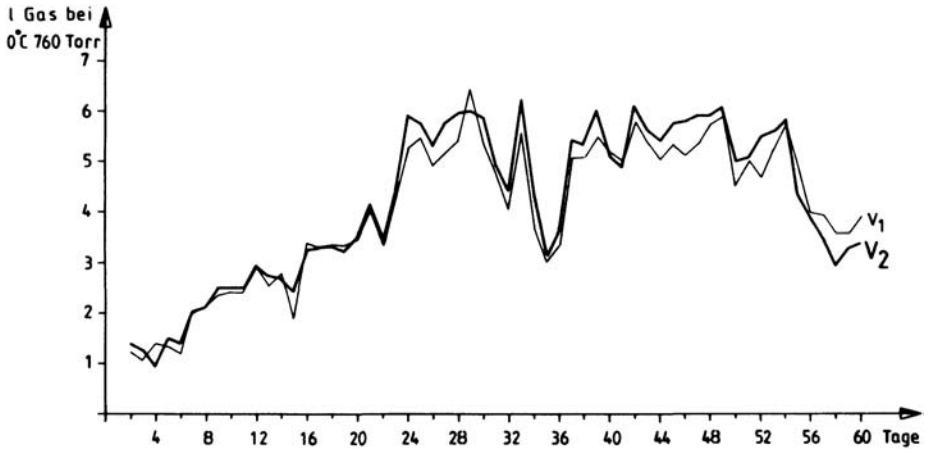

Figure 1. Cource of gas production in experimental reacter v_1 and v_2

Day of the experiment	Raw slud No. 1					Raw sludge No. 2				
	pH-A	pH-B	TGC	CF	Salmonella	pH-A	pH-B	TGC	CF	Salmonella
44	12.9	12.3	10^4	–	–	6.0	6.0	10^8	10^6	+
45	12.9	12.3	10^4	–	–	6.1	6.0	10^8	10^5	+
46	13.0	12.2	10^4	–	–	6.1	6.1	10^8	10^5	+
47	12.9	12.2	10^4	–	–	6.1	6.1	10^8	10^6	+
48	12.8	12.2	10^4	–	–	6.1	6.2	10^8	10^6	+
49	12.7	12.3	10^4	–	–	6.1	6.1	10^8	10^6	+
50	12.9	12.3	10^4	–	–	6.3	6.3	10^8	10^6	+

pH-A=pH at the beginning of agitation with/without lime
pH-B=pH at the end of 3 hours of agitation
TGC=Total germ count
CF=Number of coliforms
+=Isolation of salmonellas
–=No isolation of Salmonellas

Table V. Hygienic parameters of digested sludge DG1 and DG2

Day of the experiment	Digested sludge DG1				Digested sludge DG2			
	PH	TGC	CF	Salmonella	PH	TGC	CF	Salmonella
40	7.3	10^6	–	–	7.2	10^8	10^6	–
41	7.3	10^6	10^2	–	7.2	10^8	10^6	+
42	7.3	10^6	–	–	7.2	10^8	10^5	+
43	7.2	10^6	–	–	7.2	10^8	10^5	+
44	7.3	10^6	–	–	7.2	10^7	10^6	+
45	7.2	10^5	–	–	7.1	10^7	10^6	+
46	7.3	10^5	–	–	7.2	10^8	10^6	+
47	7.3	10^6	–	–	7.2	10^7	10^5	+
48	7.3	10^6	–	–	7.2	10^8	10^6	+
49	7.2	10^6	–	–	7.2	10^8	10^6	+
50	7.3	10^6	–	–	7.2	10^8	10^6	+

TGC=Total germ count
CF=Number of coliforms
+=Isolation of salmonellas
–=No isolation of salmonellas

Table VI. Hygienic parameters of raw sludge after reducing the lime content of raw sludge No. 1 and liming of raw sludge No. 2

Day of the experiment	Raw sludge No. 1					Raw sludge No. 2				
	pH-A	pH-B	TGC	CF	salmo-nellas	pH-A	pH-B	TGC	CF	Salmo-nellas
51	12.9	12.2	10^4	–	–	12.8	12.3	10^5	–	–
52	12.8	12.2	10^4	–	–	12.8	12.3	10^5	–	–
53	12.7	12.2	10^5	–	–	12.8	12.3	10^5	–	–
54	12.5	11.2	10^4	10^3	+	12.8	12.3	10^5	–	–
55	12.7	12.2	10^4	–	+	12.9	12.3	10^4	–	–
56	12.7	12.0	10^5	10^3	+	12.9	12.2	10^4	–	–
57	12.6	12.0	10^5	–	+	12.8	12.2	10^5	–	–
58	12.5	11.9	10^6	10^3	+	12.9	12.3	10^5	–	–
59	12.2	11.6	10^6	10^3	+	12.8	12.3	10^5	–	–
60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

pH-A=pH at the beginning of agitation with/without lime
pH-B=pH at the end of 3 hours of agitation
TGC=Total germ count
CF=Number of coliforms
+=Isolation of salmonellas
–=No isolation of salmonellas

Table VII. Hygienic parameters of digested sludge after reducing the lime content of raw sludge No. 1 and liming of raw sludge No. 2

Day of the experiment	Digested sludge No. 1				Digested sludge No. 2			
	PH	TGC	CF	Salmonella	pH	TGC	CF	Salmonella
51	7.3	10^6	–	–	7.2	10^8	10^6	+
52	7.3	10^6	–	–	7.2	10^8	10^6	+
53	7.3	10^6	–	–	7.2	10^7	10^6	+
54	7.3	10^6	–	–	7.2	10^7	10^4	+
55	7.3	10^6	–	+	7.3	10^7	10^5	+
56	7.3	10^6	–	+	7.3	10^5	10^5	+
57	7.2	10^6	10^3	+	7.2	10^6	10^4	+
58	7.3	10^6	10^3	+	7.3	10^6	10^5	+
59	7.2	10^6	10^4	+	7.3	10^6	10^4	+
60	7.2	10^6	10^4	+	7.3	10^6	10^4	+

TGC=Total germ count
CF=Number of coliforms
+=Isolation of salmonellas
–=No isolation of salmonellas

Table VIII. Gaschromatographic analysis of the gas of digester No. 1 (DG1) and No. 2 (DG2)

Day of the experiment	CO_2 in Vol.%		O_2 in Vol.%		N_2 in Vol.%		CH_4 in Vol.%	
	DG1	DG2	DGl	DG2	DGl	DG2	DG1	DG2
10	16.51	n.d.	6.15	n.d.	41.61	n.d.	32.95	n.d.
14	19.80	23.55	4.57	3.6	35.01	30.05	39.04	42.25
18	22.72	21.91	2.84	4.8	24.91	29.72	47.22	41.53
26	23.05	31.59	1.19	0.97	12.18	8.86	63.23	58.60
32	22.75	21.43	2.36	7.24	16.33	32.46	57.86	36.85
38	21.43	31.21	2.22	1.29	17.09	10.84	57.78	55.65
44	21.60	31.50	1.24	0.53	11.30	8.30	62.75	56.88
50	21.70	32.20	1.36	0.27	12.50	6.5	59.62	58.53
60	22.10	17.60	1.56	2.44	18.70	28.5	54.82	49.31

n.d.=not done

4. DISCUSSION

The preliminary experiments had shown that by lime treatment salmonellas in raw sludge can be inactivated within 3 hours when the initial pH is about 12.8. It seems to be possible to transfer these results also to raw sludges which are to be digested after the lime has been added. This is important because according to the German Ordinance for Utilization of Sewage Sludge it is not allowed to deliver raw sludge for any purpose.

The method described here was tested in the sewage treatment plant of the City of Lindau at Lake Constance with the same results as they were achieved in the experiments in laboratory scale.

Early fears that a digestion process could be damaged by very high pH-values of raw sludge did not come true. The amounts of gas were reduced by the lime treatment but the percentage of methane was increased. By the lime treatment the raw and the digested sludge as well could be kept free of salmonellas and coliform bacteria. It is important to maintain the high initial pH of the raw sludge because otherwise a recontamination of the contents of the digester will occur. On the other hand it is also possible to begin with a lime treatment of raw sludge prior to digestion and thus achieve a disinfection also of formerly infected digested sludge in a time of about 2 weeks. According to own results with viruses and those of other authors it can be assumed that the majority of pathogenic viruses in sludge can also be inactivated by this kind of lime treatment. This method of disinfection of infectious sewage sludge in its bacteriological and virological effect therefore can be compared with the prepasteurisation of raw sludge which is recommended for instance by the authorities in Switzerland. As far as parasites are concerned the discussion of the papers dealing with parasites later this day will show whether these aspects of lime treatment also apply to parasites. Further details will be published in the thesis of Pfuderer (3).

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SESSION II

Chairmen: J.HANNAN
E.B.PIKE

Influence of sewage sludge with and without lime treatment on the development of ascaris suum eggs

Effect of lime on spores of bac. anthracis in the sludge of a treatment plant connected with some tanneries

Pasteurisation—Effects upon ascaris eggs

The effects of pasteurisation and stabilisation of sludge on taenia saginata eggs

Microwave treatment as an alternative pasteurisation process for the disinfection of sewage sludge — Experiences with the treatment of liquid manure

The effect of chemical disinfectants on taenia eggs

Recovery of helminth eggs in compost in the course of composting

Bacteriological and parasitological investigations on the influence of filter beds covered with reed on the survival of salmonellas and ascaris eggs

INFLUENCE OF SEWAGE SLUDGE WITH AND WITHOUT LIME TREATMENT ON THE DEVELOPMENT OF ASCARIS SUUM EGGS

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Summary

Sludge samples from 58 randomly selected sewage treatment plants of the Land Baden-Württemberg in the Federal Republic of Germany were investigated for the occurrence of helminth ova with three different methods. In about one-third of the samples parasite stages were found, but generally in very low numbers. The spectrum included thinshelled eggs of nematodes, predominantly eggs of trichostrongylids and one egg of trichuris.

The investigations on the effect of digested sludge without and with lime on eggs of Ascaris suum showed that digested sludge without lime has already a certain inhibitory effect on the development of these eggs. A limed digested sludge with an initial pH of 12.5 is damaging the eggs considerably within two months of contact time. Therefore it can be assumed that by liming of sludges in the mentioned pH-range and a minimum storage time of two months the possible epidemiological risk caused by eggs of Ascaris suum when sludge is utilized in agriculture can be minimized to an acceptable risk.

1. INTRODUCTION

In the so-called Sewage Sludge Ordinance which is in force in the Federal Republic of Germany since 1 April 1983 it is enacted that sewage which is considered to be hygienically unsafe is not allowed to be utilized on pasture and forage land beginning with 1 January of 1987. But the legislator did not give an exact definition of the conditions under which a sludge is considered to be hygienically safe like it was done in Switzerland where a sludge is considered to be hygienically safe when it has less than 100 enterobacteriaceae in 1 gram and no infectious parasitic stages. In the German Sludge Ordinance it is only said that a sludge is safe which was by chemical or thermal conditioning, thermal drying, heating, composting, chemical stabilization or another treatment rendered free of pathogens. No definition is given how the term “rendered free of pathogens” must be proved in the daily practice. Therefore each institute in this country which is involved in the hygienic control of sewage sludges is using its own methods and definitions. But the Upper House of Parliament demanded that this loop-hole should be filled within 5 years after the validity of the ordinance. Therefore our Ministry of the Interior will appoint an ad-hoc committee in the very near future to work on a proposal to fill this gap in the ordinance. There is no doubt that it will be no problem to come to an agreement for a respective definition as far as bacteria and viruses are concerned with all the expertise which is assembled in national and international committees, especially in our Working Party 3.

But we doubted that we would achieve an agreement concerning parasitic stages with the knowledge and experiences of today. For the Federal Republic of Germany we had no recent data about the potential hygienic importance of parasite ova in sewage sludge. In case that there were an epidemiological risk caused by parasites in sewage sludge we need a choice of methods to destroy them prior to the utilization of sludges in agriculture. As far as the influence of heat on parasitic ova is concerned we believe that sufficient data are available to draw conclusions from them for compulsory recommendations. We felt, however, that this was not the case for the various scopes of lime application in wastewater and sludge technology.

Therefore we made a survey in sewage works of the Land Baden-Württemberg of the occurrence of parasitic ova in sewage sludges. Based on the fact that lime is a readily available and ecologically beneficial alkali which is widely used in the treatment of sewage and sludge and which will probably be required to meet exacting standards of the future we conducted some investigations on the efficacy of lime in sewage sludge on eggs of Ascaris suum.

2. LITERATURE

It is a well established fact that parasites and their ova get into sewage as well as bacteria and viruses via the excretions of humans and animals. The frequency of infestations with parasites of native and foreign patients was studied in the university

clinics of Heidelberg (14). The natives had an infestation frequency of 0.8%, the foreigners 16.3% in com parison. When these figures were converted to the entire German population ca. 450.000 citizens were carriers of parasites whereas completed by some 800.000 foreigners living in this country. Besides parasites excreted by humans considerable numbers are excreted by animals which also may get to a certain extent via different ways into the sewers and the sewage (12). For Central Europe the number of parasites drained daily in the sewers of a large city was estimated at one milliard (10). Others found an average of 2200 parasite eggs in 1 liter of sewage sludge in Germany (15). In this country eggs of the following parasites were found in sewage and sludge Ascaris, Toxocara, Trichuris, Capillaria, Oxyuris, Strongylus, Ancylostoma, Nematodes, Cestodes, Taenia and Trematodes (18). In the digested sludge of 11 sewage works in Switzerland eggs of ascaris were found in 8 works and of Toxascaris and Capillaria in one works each. Also in the UK a broad variety of parasite stages were found in sewage sludge (2, 8) as well as in a lot of other countries and continents (18). In the majority of investigations ova of Ascaris, Ancylostoma, Trichuris, Hymenolepis and Taenia were identified.

In Central Europe one starts out that only 2 parasites, namely Ascaris and Taenia play a role under hygienic aspects for the agricultural utilization of sewage sludge (20). The reason is the great resistence especially of ascaris eggs against environmental factors like cold, heat, dryness, radiation, chemicals and others which are described in great detail elsewhere (18). The safest method to inactivate ascaris eggs is the use of temperatures above 50°C.

For our experiments we used lime because it is an interesting chemical from the economic point of view for smaller sewage treatment plants because no expensive equipment is needed (17) and it is used as flocculant and for odor removal (4). Other authors consider liming as an effective and easy to handle method which has not much effect on the fertilizing value of sludge and, what is more, the availability of most toxic metals for plants is decreased. Because of its easy handling and low price the lime is considered to be the disinfectant of the future (16). An elimination of pathogenic parasitic stages should be demanded for the agricultural utilization of sewage sludges not least because of the high tenacity of the eggs of ascaris and taenia (18).

Liming of sludge to a pH of 11.5 for 24 hours is said to have little influence on taenia eggs and even after 6 months the oncospheres are still living (4). Others stated that lime has no effect on ascaris eggs (1) or only an insignificant ovicidal effect (16) or they have the opinion that the risk of infection with taenia is reduced because the hatching of their larvae is retarded (17). Limed sludge should have a pH of at least 11.0 but not all parasitic stages are inactived at this value. At pH 9.0 the same numbers of ascaris eggs were found in the limed sludge as in the control whereas at pH 11.2 after 3 days less eggs were counted (7). After 2–4 months storage of lime stabilized sludge a reduction of 78 % of viable ascaris eggs was observed (19). By liming of sludge to pH 11.5 the development of larvae in ascaris eggs after 24 and 48 hours, resp., was reduced down to 1 % compared to 61 % after 24 hours and 63 % after 48 hours when the eggs were kept in water at the same pH as those in sludge (13). It may become possible by lime treatment of sludge to influence the eggs of Ascaris suum and Taenia saginata in such a way that they do not longer play a role for the epidemiology of these parasitic diseases (20).

3. MATERIAL AND METHODS

3.1 Investigation on parasite eggs in sludges of sewage works in Baden-Württemberg

100 randomly selected sewage treatment plants (STP) were asked to send us 2 samples of sewage sludge: 1 sample of raw sludge (A) and 1 sample of sludge as it is leaving the plant (B) for any purpose (utilization in agriculture, gardening, forestry, landfill or other disposal). From 58 sewage works we received 55 samples A und 54 sampes B. For the isolation of helminth eggs from the sludge samples we used the 3 methods described in Annex I.

3.2 Experiments with limed digested sludge

The ascarids needed for that study were collected in the slaughterhouse of Stuttgart and the eggs collected from female worms in the laboratory (Annex I). 700–800,000 eggs were packed in one nylon bag. 4 glass flasks were filled with 16 of these bags each plus 1.8 1 of digested sludge (6 % DM) with different pH adjusted by the addition of different amounts of lime hydrate ($Ca(OH)_2$). As a control one additional flask with 16 bags was filled only with physiological saline. Another 4 glass flasks with 16 bags with ascaris eggs were filled with physiological saline whose pH was adjusted with NaOH to the same values as that of the sludge: 7.7, 10.6, 11.6, 12.5 (Table II). All flasks were kept at room temperature for 16 weeks. The pH was continuously measured in all flasks for 16 weeks but not readjusted when it had changed. Every week one bag with eggs was taken from each sample and the development stage of the eggs microscipically investigated. Then the eggs were incubated for

4 weeks at 29°C and once in a week their development was controlled microscopically. After 4 weeks the final result was determined (Table III) according to the following system:

1-cell form	= no development
2-cell to pre-larval form	= partial development
larval form	= full development

degenerated.

For testing the infectivity of developed eggs partially mice were fed with eggs and the larvae in their liver controlled.

4. RESULTS

4.1 Results of the investigations in sewage treatment plants (Table I)

In 12 out of 55 samples (A) of raw sludge thin-shelled eggs of nematodes, predeominantly of trichostrongylids, were found (22%). In 13 out 54 samples (B) of somehow treated sludge also eggs of trichostrongylids and other thin-shelled nematode eggs were found, in 1 sample 1 egg of trichuris The number of eggs was relatively small, with a few exceptions (Table I). In the sample A of No. 10 we counted 43 eggs/10 ml raw sludge, in sample B of the same plant more than 300 eggs/10 ml sludge. In STP No. 17 in sample A 96 eggs and in sample B 9 eggs/10 ml sludge were counted (Table I). In the samples of 38 STP (65,5%) neither in sample A nor in sample B eggs of parasites were found, that is only in ca. one-third of 58 randomly selected STP in Baden-Württemberg eggs of helminths could be found with 3 different isolation methods. Eggs of ascaris and cestodes were not detected.

4.2 Results of the experiments with limed digested sludge (Table III)

The pH in the control varied between 7.2 and 7.8 and in sludge sample 1 without lime between 7.5 and 7.9. In sample 2 the pH dropped relatively even during the investigation period from 10.6 to 8.0, in sample 3 from 11.6 to 8.1. In sample 4 the pH declined slowly from 12.5 to 11.4 during the first 9 weeks of the experiment, at the end a pH of 8.7 was measured.

The complete results of this study are published elsewhere (18). In this paper only the results of egg development after 4 weeks of incubation at 29°C are analyzed for each week of contact of the eggs with lime in the sludge (Table III).

In the first 5 weeks of the experiments the numbers of larvae were relatively close together in all samples. After that in sample 4 the number declined, increased again in the 12th week, dropped and increased drastically in the 15th week. Sample 3 had the lowest value in the 9th and 10th week, increased again and dropped after the 13th week. In samples 1 and 2 very similar numbers of larvae were found without a tendency in dependence of the duration of the experiment. Larvae developed from about 50 % of the eggs. The mean value of developed larvae of sample 3 was 39%, of sample 4 it was 20.8%. When separated mean values for sample 4 are calculated for weeks 1–8 and 9–16 these are at 34.8% and 6.8%, resp.. This clearly

TABLE I. Number of parasite eggs counted in 10 ml of sewage sludge of 58 different sewage works in Baden-Württemberg (mean values of 3 different isolation methods)

STP No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Sample A	0	0	0	1.0	0.3	0	0	2.0	0	43.0	0	0	0	0	0	0.7	96.0	0.3	0.7	0
Sample B	0	0	0	/	0	0	0	0	0	306.0	0	0	0	0	/	0.7	9.0	0.3	1.0	0
STP No.	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Sample A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.0	/	/	/	0	0
Sample B	0.3	0	0	0	0	0	0	0	0	0	0	1.0	0	0.3	0	0.3	0	0	0	0
STP No.	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58		
Sample A	0	0	0	0	0	0	2.0	0	0	0	0	0	0	0	0.3	0	0	0.3		
Sample B	0	0.3	0	0	0	/	/	0	0	0.7	0	1.0	0	0	0	0	0	0.3		

STP=Sewage treatment plant
/=No sample received

TABLE II. Set-up of the experiments with eggs of Ascaris suum in limed digested sludge and in physiological saline with sodium hydroxide

Sample No.	Digested sludge	Lime solution	Initial-pH-value
1	1.8 l	–	7.7
2	1.8 l	39.2 ml	10.6
3	1.8 l	42.2 ml	11.6
4	1.8 l	53.3 ml	12.5
	Physiol. NaCl	32 % NaOH	
1a	1.0 l	–	7.6
1b	1.0 l	1.37 ml	10.6
1c	1.0 l	1.8 ml	11.7
1d	1.0 l	4.3 ml	12.5

TABLE III. Efficacy of limed digested sludge on the development of eggs of Ascaris suum *

Duration of expt. in weeks	Control	Sample 1 Initial pH 7.7	Sample 2 Initial pH 10.6	Sample 3 Initial pH 11.6	Sample 4 Initial pH 12.5
1	69.0	41.0	39.5	49.5	39.5
2	75.5	47.5	51.0	47.0	40.5
3	76.0	49.5	44.0	40.0	39.0
4	75.0	48.5	44.5	38.5	43.0
5	60.0	54.0	44.0	37.0	38.0
6	76.0	58.0	51.0	56.5	31.5
7	74.5	46.0	41.5	39.5	29.0
8	71.5	43.5	45.0	40.5	17.0
9	57.5	48.0	44.0	24.0	3.5
10	71.5	42.0	37.5	23.5	7.5
11	72.5	42.5	47.5	42.0	2.5
12	80.0	57.5	51.0	41.0	11.5
13	64.5	51.5	57.0	49.5	4.5
14	69.0	44.5	54.5	40.0	1.5
15	74.5	49.0	58.5	31.0	22.0
16	70.5	55.5	48.0	22.5	1.5

*Percentage of eggs developed to the larval form after 4 weeks of incu bation at 29°C.
Control=Eggs kept in physiological saline
Samples 1–4 are identical with samples 1–4 of Table II.

shows a decrease of the number of developed ascaris eggs in dependence of the contact time in highly limed sludge with an initial pH of 12.5.

5. DISCUSSION

5.1 Survey in sewage treatment plants in Baden-Württemberg

It was not possible with the 3 methods used to obtain exact figures of the occurrence of helminth ova in the sludge samples of 58 sewage treatment plants in Baden-Württemberg. Only certain orders of magnitude were found. The amount of 30 ml of sludge from all three isolation methods used is relatively small so that possibly a sample which was negative may in reality have been infested with parasites. If the results which were found from another research group in the Federal Republic of Germany with an average of 2,200 eggs in 1 liter of sludge and one-sixth of ascaris among them are considered to be representative (15), in our investigations with a total count of more than 1,500 eggs at least 250 eggs should have been from ascaris. But we found no ascaris eggs at all. In 1954 in an investigation 86 % of all eggs in sewage sludge were from ascaris (9). The conclusion is that the infestation of the human and animal population in our country has enormously deminished and

may only regionally be differently pronounced. It is also possible that the eggs are attached to larger sludge particles by their protein membrane and thus are not detected during an investigation

The majority of the strongylid eggs were empty shells; the larvae had already left the egg and thus are suspended in the sludge. It *is* said that trichostrongylid larvae do not survive the sewage purification processes (6) but other authors found living larvae of nematodes in the sludge (10, 15). Since trichostrongylides can hibernate (3) this question is of importance. Not disinfected sewage sludge spread on pasture in late autumn could have an epidemiological importance for the spread of trichostrongylosis.

5.2
Experiments with limed digested sludge

The method which we used for the experiments with the limed sludge seems to be suitable for that purpose. A certain disadvantage is the centri fugation of the eggs for preparing the filling of the nylon bags because they lose the protein membrane. Thus their resistivity might be diminished. The changes of pH during the experiments were not adjusted because it was too difficult to achieve an even distribution of the lime in the flasks as long the nylon bags were in the sludge. The relatively low numbers of developed eggs in the control of Table III with a mean value of 71 % is attributed to the fact that these samples as all others were not aerated during incubation. Thus there were no optimal conditions for the development of these eggs. The research group in Liverpool (4) found that the development of ascaris eggs in limed sludge with pH 11.5 is stopped completely at the pre-larval stage. But also sludge without lime is already inhibiting the development of ascaris eggs (Table III, sample 1). It is uncertain whether toxic substances in the sludge are responsible for that phenomenon. The researchers found further that in limed sludge with pH 11.5 already after 24 hours a reduction of thedevelopment of larvae down to 1 % was observed (13). This finding could not be confirmed in our investigations. Only after 11 weeks of experiment with an initial pH of 12.5 we observed for the first time a reduction of the larval development of 2.5% (Table III, sample 4).

The percentages of 11.5 after 12 weeks of experiment and 22.0 after 15 weeks (Table III, sample 4) are considered to be runaways. A possible reason could be that the several hundred thousands of eggs in one of the nylon bags were not evenly distributed but conglomerated and those eggs in the inner part of that clump had no intensive contact with the limed sludge. After resuspension of the eggs in the Petri-dishes for incubation those eggs were set free and thus faked the results.

As a result of these experiments with limed sludge it can be stated that ascaris eggs are especially seriously damaged at an initial pH of 12.5 provided they are long enough exposed to that environment—at least 9 weeks. Similar values are described from a study group in the USA which observed a 78 % reduction of viable ascaris eggs in lime-stabilized sludge between the 2nd and 4th month of storage. Lime at pH 12.5 seems to be superior to sodium hydroxide in the same pH-range (Table III) as far as the damage of ascaris eggs is concerned. This was found in another part of that study whose results are published elsewhere in more detail (18).

From our results one tendency is clearly discernible: the more alkaline the initial pH in the sludge is the less eggs with larvae can be found .This is true not only for limed sludge but to a certain extent also for saline with NaOH. There is also a distinct inter-dependence between pH and contact time recognizable as far as the reduction of developed ascaris larvae is concerned (Table III, sample 4). In the pH-range of 11.7 (Table III, sample 3) this influence is hardly to observe.

6.
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ANNEX I
Parasitological investigation methods used

1. Flotation

   Medium: 262 g NaCl and 275 g $ZnCl_2$ solved in 1 liter of water. 10 ml of sludge are given into a beaker+100 ml medium, mix thoroughly, screen into another beaker. The whole contents of that beaker is filled into test tubes until the upper meniscus and covered with a coverslip. After 30 min the coverslip is removed and examined microscopically.
2. Combination of flotation and sedimentation 10 ml sludge are given into a beaker together with 100–150 ml of water mix thoroughly, screen contents into another beaker, leave for 15 min, decant supernatant, resuspend the sediment and proceed with flotation as described in 1, but without screening.
3. Filtration

   With the improved helminth filter from Visser (21) after Visser and Pitchford (22) which was originally developed for the enrichment of helminth ova in human feces. It consists of 2 filters which fit into one another. The inner one has 95 µm and the outer one 50 µm diameter of pores. Thus the outer filter holds back all particles between 50 and 95 µ (Figure 1).

   The filter is fastened at a stand over a 5 1 flask to catch the filtered water of the sewage sludge. The shutoff cock is closed, 10 ml sludge are given into the inner filter, rinse with 1.5–3 1 water. When all water but that in the funnel-shaped part of the filter is drained out of the outer filter, the rest in the funnel is poured into a test tube. 0.3 1 are pipetted onto 2 slides and examined microscopically without a coverslip. 2×200 eggs were counted, evaluated and the mean value calculated.
4. Removal of eggs from ascarids

   Female ascaris worms are cut crosswise shortly behind the bifurcation. By pressure with closed scissors or another blunt object the uterus is removed and the first 2 cm are cut off to obtain as much fertile eggs as possible. The uterus is fixed with tweezers and the eggs pressed out with a blunt object into a Petri-dish filled with water. The water with eggs is poured into a centrifuge tube and centrifugeed at 2,500 rpm for 5 min. The number of eggs/ml is determined by dilution rows.

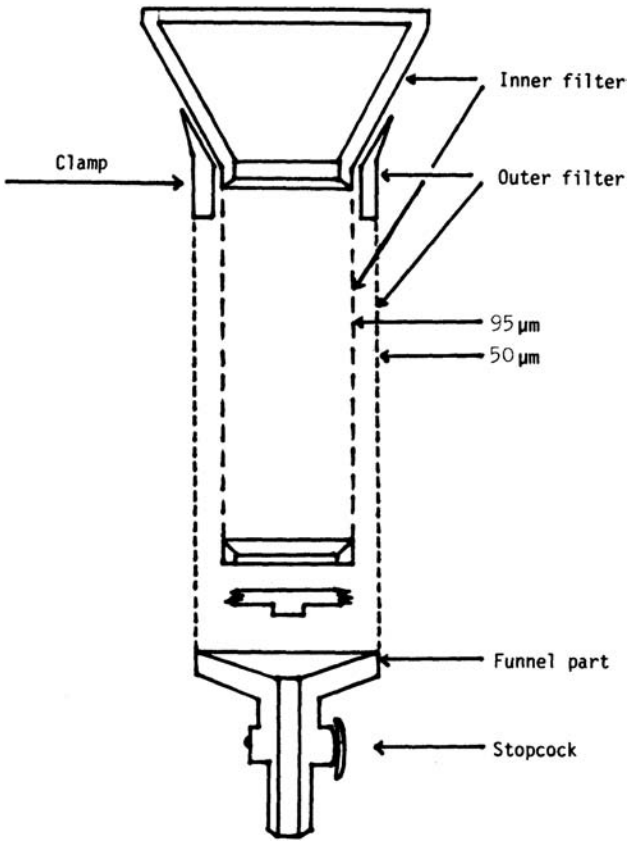

Figure 1. Helminth filter after Visser (21, 22)

EFFECT OF LIME ON SPORES OF BAC. ANTHRACIS IN THE SLUDGE OF A TREATMENT PLANT CONNECTED WITH SOME TANNERIES

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Summary

The question is investigated, if slurry contaminated with spores of Bacillus anthracis can be disinfected by lime-treatment. Germ-carriers contaminated with 10^6CFU of anthrax-spores were put into slurry treated with 10 or 20 kg $Ca(OH)_2/m^3$ and 10 or 20 kg CaO/m^3 respectively and dehydrated in a compartment type filter press for 2 h. The filter cakes were stored under practical conditions on a controlled tipping for 23 weeks. A high number of spores survived the pressing as well as the deposition in all cases.

1. INTRODUCTION

Anthrax spores are very rare to find in municipal waste-water, but if they occur, it will cause a lot of difficulties. The reason why this investigations were carried out was the occurrence of a special problem in a town near Stuttgart. Since there are a lot of tanneries and factories working with hides, the community got the order by the government in the early seventys to burn the sludge from their wastewater treatment plant. The new built sludge incinerator never worked satisfactory and was finally shut down last year, because of the high cromium emission. So the question was, if the sludge could be be brought to the local controlled tipping after a treatment with lime. According to Strauch et al., 1978, lime is a cheap chemical for disinfecting municipal sludge from Salmonella, as well as from other pathogenic microorganisms and a common admixture to sludge for the purpose of neutralisation, desodoration, flocculation, precipitation, sedimentation and dehydration (3,5). No informations were available concerning the effect of lime, especially CaO to anthrax spores in connection with a pressing procedure followed by deposition. Therefore the following experiments were carried out under practical conditions in the above mentioned waste-water treatment plant'and the controlled tipping belonging to the same community.

2. DESCRIPTION OF THE WASTE-WATER TREATMENT PLANT

If spores of Bacillus anthracis occur in the effluents of tanneries, nearly 100 % of them could be found in the sludge (4). The sludge investigated here, originated to one part from the households and to five parts from the tanneries. It was treated in the following steps in the wastewater treatment plant as can be seen in the flow diagram (Fig. 1) too:

a) The preliminary clarification was done by screen, sand trap, regulation-and primary sedimentation tank. The sludge then came into a thickening tank.
b) The main clarification was done using the"Katox"-method. In two streets (A and B) a three step procedure was applied innitiating an oxidation process by giving oxigen and activated carbon to the waste-water.
c) The final clarification starts with a flocculation step using ferrous sulphate and calziumhydroxide as chemicals, followed by a neutralisation with ferrous chloride. A polyelectrolyte serves as coagulant aid. The treated waste-water goes to the receiving water, while the sludge from the flocculation tank is pumped into the sludge thickener.
d) The concentrated sludge receives a chemical conditioning with 14% calziumhydroxide and 4% ferrous chloride

The dehydration down to 35–40% of dry matter is done in three com¬ partment type filter presses after addition of another 10 kg calziumhydroxide/m^3 of sludge.

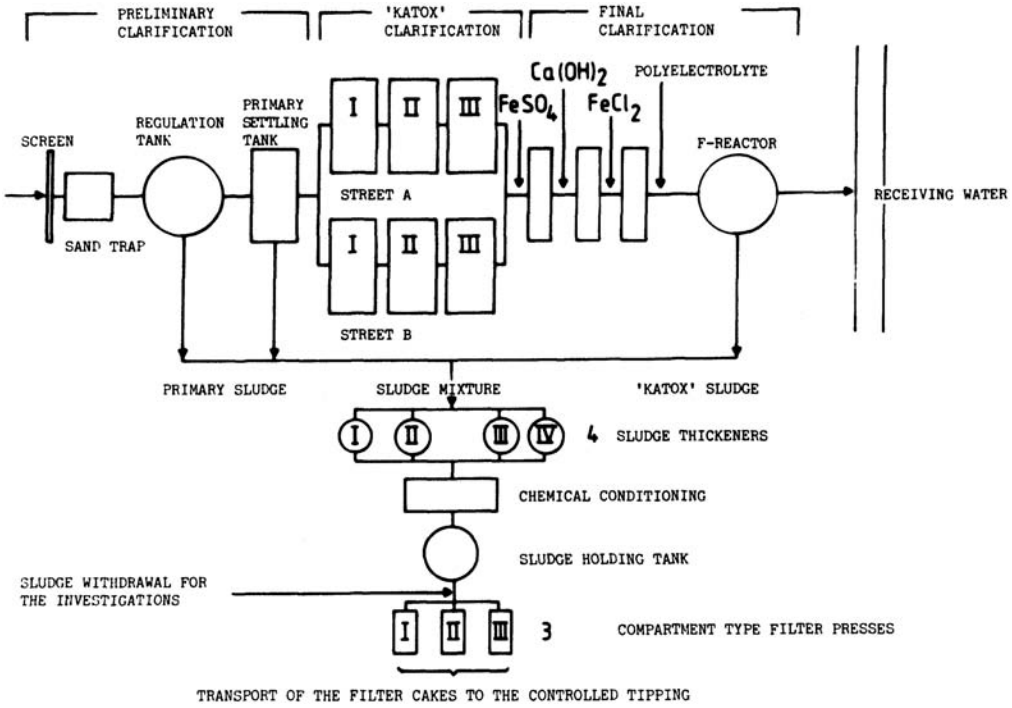

Fig. 1. Plan of the sewage treatment plant producing the sludge used in the experiments.

3. MATERIAL AND METHODS

Dormant spores of Bacillus anthracis strain 15 from the instituts culture collection were prepared as described by Böhm and Dietz, 1979, and adjusted to 107 CFU/ml in the use dilution (1).

Fresh undehydrated sludge from the above described treatment plant was used in the experiments. Since the reisolation is much more easy, germ carriers were used in this survival study. 0.1 ml of the spore suspension were dried to 3×5 cm pieces of nylon gossamer for 24 h. Ten of those germ-carriers were put on one nylon string for each filter chamber. Those strings were fixed with adhesive tape to the inside of the filtering cloth, in a way that the germ carriers will be pressed into the sludge cake during the operation. A laboratory type of press was used in the experiments, producing filter cakes of 3×30×30 cm.

For the dehydration the sludge was prepared in four ways:

- 10 kg $Ca(OH)_2/m^3$ sludge
- 20 kg $Ca(OH)_2/m^3$ sludge
- 10 kg CaO/m3 sludge
- 20 kg CaO/m^3 sludge

Each 50 1 portion of the mixtures was stirred for ten minutes. Pure sludge served as a controll. The operation time of the compartment type filter press was 2 h. The filter cakes were put together to pyramides (40×60×60 cm) on the controlled tipping of the community and stored under open air conditions for 23 weeks.

Samples were taken immediately after pressing and one day later. For 5 weeks every week one sample was taken, from then up to 23 weeks germ carriers were controlled every two weeks for surviving spores.

The reisolation technique for the anthrax spores was as follows:

a) Germ carriers were put in 50 ml of sterile distilled water in an 100 ml screw capped bottle and shaked at 8°C for 24 h with 800 rpm.
b) The liquid was filtered through five layers of gauze which was subsequently rinsed with additional 50 ml of sterile distilled water.
c) The filtered liquid was centrifugated for 20 min at 10°C with 3000 rpm and the sediment was resuspended in 10 ml of distilled water.
d) The suspension was heated for 30 min to 80°C for killing the vegetative contaminants.

e) After this step the plate count was performed using blood agar plates containing 0,08 ml of a trimethoprim - sulfametoxazol-solution (Wirtschaftsgemeinschaft deutscher Tierärzte e.G., D-3000 Hannover 1) per liter.
f) Plates were read after an incubation at 37°C for 24 h. The identification of the colonies was done by looking for the characteristic growth of Bac. anthracis, and if necessary by phage-test with the -phage (2).

4. RESULTS

Before the results are described in details, some remarques must be done to make their interpretation more easy. The sensitivity of the applied quantitative method is not very high. The detection limit is 102 CFU of spores on the germ carrier. That means if about 106 microorganisms are dried to the nylon gossamer, the germ count found with that reisolation procedure is only 104. So the real number of viable spores is probably about 102 CFU higher than that determined in this experiments.

The results of the control experiments indicate that the spores survived an storage time of 23 weeks. The spore count decreased from 104 to 10^3 CFU/ml in the first 4 weeks, and afterwards to 10^2 until the end of the experiments (Fig. 2).

In the experiments with Ca(OH)2 it was found that the spores survived the pressing and a storage time of 23 weeks. The mixture containing 10 kg Ca(OH)2/m^3 contained about 10^4 CFU of spores per ml immediately after the pressing. The germ count dropped to about 102/ml in the 9th week, and stayed at this level until the 23rd week (Fig. 3). Nearly the same results could be obtained with 20 kg Ca(OH)2/m^3 exept that the number of viable spores was between 10^4 and 10^3/ml immediately after the pressing and the dropping in the germ count to 102 CFU/ml was found in the 4th week (Fig. 4).

In all experiments with CaO as admixture the spores survived the pressing and a storage of the filter cake over 23 weeks too. No significant differences could be found between the experiments with 10 kg CaO/m^3 and 20 kg CaO/m^3 (Fig. 5 and 6). Immediately after the pressing the spore count was between 104 and 103 CFU/ml. The number of viable spores decreased to between 103–102 CFU/ml with 10 kg CaO/m^3 in the 7th week and with 20 kg CaO/m^3 in the 4th week. In both cases it stayed at this level until the end of the experiments, exept in the 11th week where a unexplicable drop to below 102 CFU/ml could be found.

Finally it can be stated, that the lime treatment of sludge failed to inactivate spores of Bacillus anthracis. In the case of accidental pollution of the waste-water with such spores the above described treatment of the sludge is not an alternative method for burning it in a fluid bed reactor.

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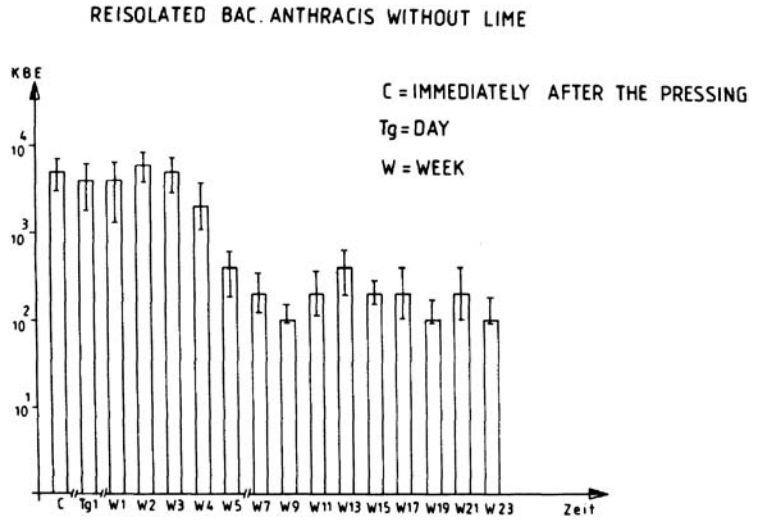

Fig. 2. Survival of spores in sludge without lime after pressing

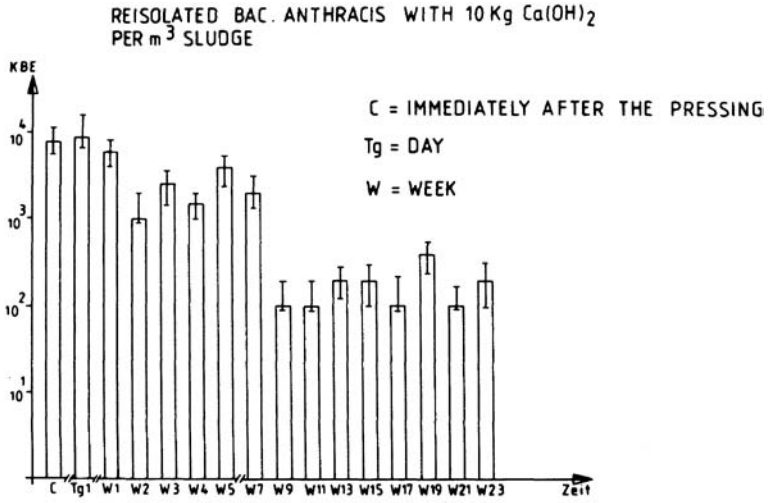

Fig. 3. Survival of spores in sludge with 10 kg $Ca(OH)_2/m^3$ after pressing and deposition

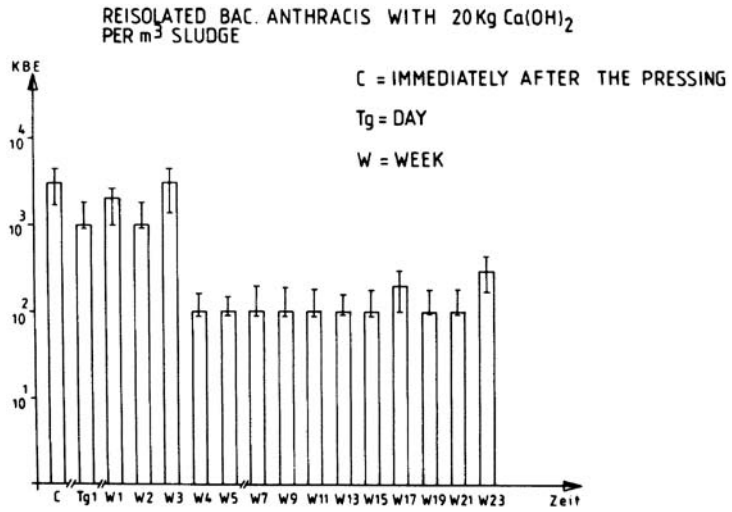

Fig. 4. Survival of spores in sludge with 20 kg $Ca(OH)_2/m^3$ after pressing and deposition

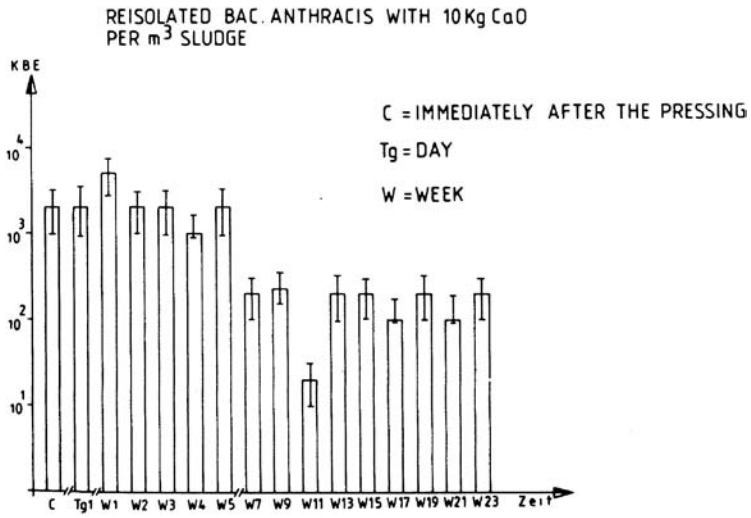

Fig. 5. Survival of spores in sludge with 10 kg CaO/m^3 after pressing and deposition

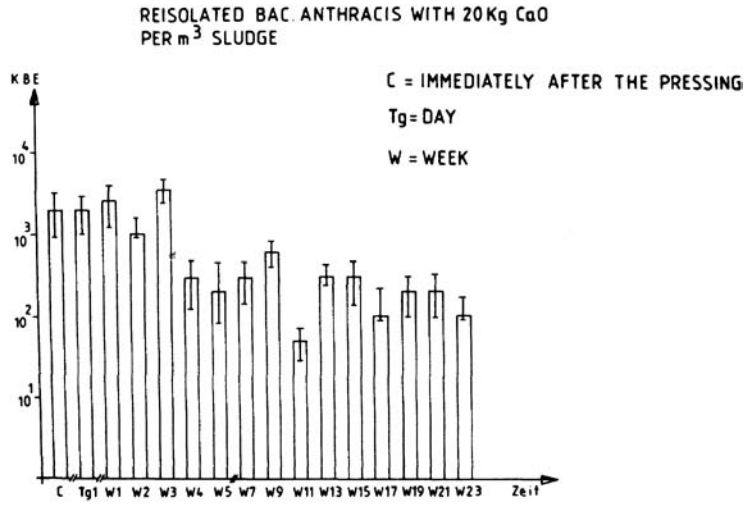

Fig. 6. Survival of spores in sludge with 20 kg CaO/m^3 after pressing and deposition

PASTEURISATION; EFFECTS UPON ASCARIS EGGS

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Summary

The option to be able to utilise sewage sludge on land is important to farmers and to the UK water industry. Although Ascaris is not an important parasite in Europe its ova are very heat resistant and therefore are a useful and convenient indicator of the efficiency of heat treatment processes. A study has been made over a range of temperatures and times which show that Ascaris ova added to sewage sludge are not viable after exposureto temperatures in excess of 55°C for periods of about 2 hours. Calculations have shown that if the pasteurised sludge is subsequently digested sufficient methane can be produced to provide fuel to heat the pasteurising plant. The design of a plant would have to be such as to prevent contamination of treated sludge by untreated sludge.

1. INTRODUCTION

The option to be able to utilise sewage sludge on agricultural land is one which is beneficial to farmers and the water industry. However there is a requirement to ensure that humans, animals, plants and the environment are safeguarded against any harmful effects(1). One of the ways to ensure that disease is not spread by this means is to pasteurise the sludge before application to land. Where pasteurisation has followed sludge digestion re-infection of the sludge has been a problem(2), but other workers have shown that pasteurisation before digestion is satisfactory(3).

Pasteurisation is usually thought of as heating the material at 70 C for a few minutes but similar effects can be achieved more economically by heating at a lower temperature for a longer time.

Infection with Ascaris worms is usually relatively harmless although as much as 10% of the host's protein intake may be absorbed by the worms(4) Infection rates in temperate regions are low, about 1300 cases per year in Britain but in tropical regions the infection rate may be as high as 50% of the population.

As well as being pathogenic in their own right the ova of Ascaris serve as an indicator of the destruction of Taenia ova during sludge treatment and the use of Ascaris ova in experimental procedures have several advantages over. the use of Taenia .o.va. Ascaris ova have similar resistance to heat as Taenia ova(4, 5). Ascaris ova can readily be obtained from pig slaughter houses whereas the supply of Taenia saginata ova is very limited. Because of their ready availability Ascaris ova can be added experimentally in large numbers to sludges, vastly outnumbering any indigenous ova. The recovery of Ascaris ova from sludges and their viability testing is easier than the recovery and testing of Taenia ova.

The author has carried out studies to determine the efficiency of pasteurisation prior to digestion for rendering sludge free of viable eggs of Ascaris and therefore other pathogens and to examine, in terms of energy input, the feasibility of a combined pasteurisation and digestion plant.

2. METHODS

The ova used during these studies were obtained by dissecting the ova from the lower uteri of mature female Ascaris suum worms obtained from a local abattoir. Their viability was assessed by incubating a suspension in water at 22°C for 21 days and then microscopically examining the ova and classifying them as (i) 'undeveloped', having not divided or not divided to more than 8 cells (ii) 'multicelled' having divided into many cells but not differentiated into larvae or (iii) containing developed larvae.

The ova were recovered from the sludge using a method modified from that of Meyer, Miller and Kaneshiro(6). One hundred cm^3 of dilute sodium hypochlorite was added to 75 cm^3 of sludge. After 10 minutes the volume was made up to 225 cm^3 with dilute sodium hypochlorite in a centrifuge bottle. After one hour the scum was removed by aspiration and the solids sedimented by centrifugation at 800 g for 2 min. The deposit was further washed once in dilute anionic detergent and twice in

distilled water. The ova were floated by centrifugation in zinc sulphate solution (specific gravity 1.2) and recovered by negative filtration onto a membrane filter (average pore size 8 μm. The ova were scraped from the membrane into a petri dish containing water and a small amount of 0.1N sulphuric acid before incubation at 22°C. After 21 days each dish was systematically examined using a 40×microscope and the development of each ovum recorded.

Pasteurisation was carried out by stirring sludge, to which a known number of ova had been added, in a beaker immersed in a water bath. By using time switches to control heating and cooling units the bath could be raised to and maintained at a pre-determined temperature for the required time and then rapidly cooled.

Digestion was carried out in a batch fed digester of 4-litre capacity maintained in a water bath at 35C. A mean retention period of 13.3 days was maintained by adding daily to the test or control digester 300 cm^3 of pasteurised or unpasteurised sludge respectively, after the removal of a similar volume of digested sludge. The digesters, which were not fed on Sundays but had a feed of double volume on Saturdays were allowed to stabilise, judged by the pH of the withdrawn sludge and the volume of gas produced over a period of two weeks. Over the next 8 days the level of Ascaris ova in the digester was allowed to build up by adding ova at double the final concentration to the feed sludge. Ova were recovered and their viability examined from 15 samplings of treated sludge taken over the following three weeks.

During the sampling period the dry weight of the withdrawn sludge was estimated and the quantity and quality, in terms of oxygen, nitrogen, carbon dioxide and methane was measured.

The initial effects of pasteurisation were established by exposing ova in water or with sludge to temperatures of 45, 50 or 55°C for periods up to 3.5 hours and comparing their viability with that of unheated controls.

Using the data from the first experiments as a guide the effects of pasteurisation prior to digestion were examined by comparing the viability of ova in sludge which had either been pasteurised at one of the following conditions; 45°C for 3 hours, 47°C for 3 hours, 49°C for 3 hours, 50°C for 2 hours, 51°C for 1 hour or 53°C for 1 hour, with sludge that had been pasteurised and then digested at 35°C and with a mean retention period of 13.3 days or and with sludge that had been digested only.

3. RESULTS

Pasteurisation of ova suspended in water at 45°C for up to 3.5 hours had no significant effect upon their development, but no larvae developed after exposure at 50°C for periods in excess of 2.5 hours. After exposure at 55°C no larvae developed from the first sample, taken after 15 minutes, or from any subsequent sample. The presence of sludge had a mildly protective effect. At 50°C the period of time to achieve the same degree of kill as that required by the water suspension was approximately double. Above and below this temperature the effect was not so marked.

Table 1 shows the results from the pasteurisation and digestion experiments. It shows the proportion of the recovered ova which developed to larvae and the proportion which did not fully develop, together with the results obtained with the control ova which were only exposed to water.

Table 1. The final development of ova recovered from each set of conditions examined

Pasteurisation conditions (temperature and time)				
Sludge treatment	Proportion of ova showing development:			
not to larvae	to larvae			
45°C	3 h	Pasteurisation and digestion	0.39	0.61
45°C	3 h	Pasteurisation only	0.43	0.57
–	–	Digestion only	0.39	0.61
–	–	Water*	0.41	0.59
47°C	3 h	Pasteurisation and digestion	0.57	0.43
47°C	3 h	Pasteurisation only	0.44	0.56
–	–	Digestion only	0.48	0.52
–	–	Water*	0.41	0.59
50°C	2 h	Pasteurisation and digestion	0.99	0.01
50°C	2 h	Pasteurisation only	0.69	0.31
–	–	Digestion only	0.52	0.48
–	–	Water*	0.41	0.59
51°C	1 h	Pasteurisation and digestion	0.87	0.13

Pasteurisation conditions (temperature and time)				
Sludge treatment	Proportion of ova showing development:			
not to larvae	to larvae			
51°C	1 h	Pasteurisation only	0.88	0.12
–	–	Digestion only	0.49	0.59
–	–	Water*	0.08	0.92
53°C	1 h	Pasteurisation and digestion	0.99	0.01
53°C	1 h	Pasteurisation only	0.99	0.01
–	–	Digestion only	0.09	0.91
–	–	Water*	0.08	0.92

* Control-developed in water, not exposed to sludge.

Pasteurisation for 3 hours at temperatures below 47°C did not affect the viability of Ascaris ova. Pasteurisation between 47°C for 3 hours and 50°C for 2 hours had no detectable effect upon viability, but after subsequent digestion, the ova showed a decrease in viability related to the pasteurisation temperature. Pasteurisation at 50°C caused a decrease in viability which was enhanced by subsequent digestion. At temperatures between 51 °C and 53°C pasteurisation had an adverse effect upon viability, increasing with temperature, which was not enhanced by subsequent digestion. Digestion alone, at 35°C with a nominal retention period of 13.3 days did not affect the viability of Ascaris ova added to the feed sludge.

Pasteurisation at temperatures up to 53°C did not affect the quantity or quality in terms of methane production of the gas produced by subsequent digestion of the sludge, or affect the reduction of total solids compared with unpasteurised sludge.

4. DISCUSSION

The results of this work show that, if sludge containing Ascaris ova is heated to 55°C and maintained at this temperature for 2 hours the treated material will be free of viable Ascaris ova. Because of the resistance of Ascaris ova to heat it is likely that such treated sludge will also be free of other pathogens.

Digestion of a typical sludge having a dry solids content of 4.5% will produce an amount of methane—about 250 $cm^3\ g^{-1}$ of sludge solids, equivalent to 9000J g^{-1} , which on combustion, will raise the temperature of the sludge using a gas boiler and hot-water heat-exchanger system (60% efficiency), through as much as 58°C. This rise is in addition to the ambient temperature of the sludge (5–15°C depending upon the season). A digester with a 13 day retention period and average heat losses could be maintained at 35°C by cooling the pasteurised sludge to about 40°C before using it as feed for the digester.

Efficient killing requires adequate mixing within the batch of sludge so that heat penetration is rapid and evenly spread throughout the sludge. Continuously fed pasteurisers, however, must have plug flow characteristics since any mixing of treated and untreated would seriously reduce the amount of disinfection obtained. Likewise the engineering arrangements of a pasteuriser must be such that there is no mixing between treated and untreated sludges either during filling and emptying or during storage between pasteurisation and digestion.

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THE EFFECTS OF PASTEURISATION AND STABILISATION OF SLUDGE ON TAENIA SAGINATA EGGS

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Summary

In an initial experiment to assess viability of normal ova, 7 calves were infected when 3–4 weeks old with 5,000) viable T.saginata ova. At post mortem examination all calves were infected with an average of 368 (range 29–832) cysticerci distributed throughout the muscles and viscera with at least 20% present in the heart. Approximately 7% of viable ova administered were recovered as cysticerci from the calves. Groups of three calves per treatment were thereafter used to assess the effect of the various treatments on infectivity. In vitro viability tests of the ova were also carried out with the same batches of ova before and after treatment. Sludge treatment to 55°C for 3 hours reduced infectivity by 99%. Anaerobic digestion for 10 days at 35°C followed by lagooning for 15 days completely destroyed viability and infectivity. If sludge was treated with lime (pH 12), digested aerobically for 16 days or lagooned for 28 days, infectivity was reduced to 96%, 82% and 99% respectively. In vitro viability tests did not correlate with infectivity. The results obtained from these small scale experiments indicate effective treatments to kill T.saginata ova and suggest the probable future value of some of these to small sewage treatment plants.

1. INTRODUCTION

Several authors have examined the problems associated with sewage disposal when it contains both pathogens and parasites capable of infecting man and his domestic animals. In a recent review it was pointed out that in the U.K. alone 1.25 million tonnes of sludge dry solids are produced annually. Approximately half is disposed of on land and some 187,500 tonnes of this onto grazing land. (1) Disposal on land, apart from being one of the cheapest methods of disposal is valuable to farmers as fertiliser due to the contained nitrogen and phosphates in the sludge.

T.saginata is not a serious disease of man in the U.K., there being approximately 80 cases reported annually, but the incidence is likely to be higher. In cattle, the incidence of infection (0.042%) does not make it a serious disease threat either, but the annual cost is approximately £¾ million in down graded or condemned beef. (2) At present, U.K. national guidelines recommend that, when raw or secondary sludges are applied to grassland, there should be no grazing by cattle or pigs for a period of 6 months following application. This restriction is also applied to sludges treated by aerobic digestion or lime since the effect of these treatments on infectivity of tapeworm ova was uncertain. These two treatments are, however, often used at rural works. Approximately half the sludge used in agriculture (1975) was digested anaerobically. Since Regional Water Authorities are concerned to protect the health of animals and man while minimizing costs of sludge treatment and disposal, it becomes important to demonstrate the extent to which existing practices or new, low-cost methods of disinfection, such as submerged combustion (2) destroy infectivity of tapeworm ova.

This paper describes initial experiments designed to assess the effect of temperature and various treatments on the viability and infectivity of T.saginata ova. It will be appreciated that although tapeworm eggs may hatch in vitro, this does not necessarily mean they will be infective to calves. Both in vitro viability and in vivo infectivity trials after treatment were therefore carried out and the results examined to see if any correlations were obvious. The effects of different temperatures on tapeworm eggs kept in saline have been reported by previous workers and will not be dealt with here in detail. It has been found that a temperature of 55°C produces a decrease in viability of 90% in 2.9 hours and a temperature of 60°C the same effect in 1.4 hours, with 100% kill of ova after 3 hours. (2)

2. METHODS

2.1 Life cycle of T. saginata

The beef tapeworm T. saginata has a complex life cycle in which the ova-producing adult worm, lives in man and the cysticercus stage lives in cattle. Cattle are infected by the ingestion of viable eggs which after hatching in the small intestine liberate hexacanth embryos which migrate via the blood vessels to the muscle blocks around the body. In this situation the cystic stage grows for approximately 3 months when it is infective to man. Man is usually infected by eating undercooked or raw infected beef.

2.2 Isolation of Taenia ova

Eggs were obtained from gravid proglottides evacuated naturally with the faeces or following medical treatment of infected individuals. Ova were stripped from the proglottides, washed by repeated centrifugation in physiological saline (0.85%) and stored at 4°C with added antibiotics (benzyl penicillin, 1 g/1; streptomycin sulphate, 1 g/1).

2.3 In vitro studies

Hatching (liberation of the oncosphere from the embryophore) and activation (liberation of the embryo from the oncosphere) were assessed using an enzymic outgrowth method based on that of Silverman (3) with the addition of fresh cow bile. Ova able to be activated were presumed to be viable. Activated ova were examined microscopically, counted using a McMaster worm egg counting slide and scored in categories representing their level of maturity, hatching and activation.

2.4 Containment and retrieval of ova after treatment schedules

Studies of laboratory or plant-scale treatment processes were conducted by enclosing ova samples in 20 ml perspex containment cells bound on two sides by nylon bolting cloth (20 μm mesh aperture). The cell was provided with a threaded attachment to allow a cable or rod to be fixed to it for retrieval of the chamber. Using this technique subsamples of ova could be retrieved at regular intervals without the need to separate ova from large volumes of sludge.

2.5 Infectivity testing in calves

Young male calves (Friesian or Ayrshire cross), 3–4 weeks old and from a known T.saginata free environment were used. All animals were infected orally with a known dose of ova suspended in approximately 20 ml saline. Animals receiving ova from different treatments were housed separately from each other and care was taken to avoid any cross-contamination. All bedding litter was removed and burnt for the first three days after dosing. Animals were given food and water ad libitum. To assess the numbers of cysticerci present in the calves, the animals were killed and viscera removed and the carcasses boned out. The majority of animals were killed 12 or more weeks after infection when the cysts were easily recognised. In an initial experiment 2 calves were killed earlier to assess the ease of counting the younger cysticerci. To assess infection, the following regions were examined: the diaphragm, heart, lungs, trachea, liver, kidney, oesophagus, mesentery and tongue. All muscle blocks including the masseters were dissected off the bone and every muscle and organ sliced at approximately 0.5 cm intervals to detect cysticerci. Any cysts found were counted and a note made of their appearance.

2.6 Experimental protocols and different treatments examined

Expt. 1. Infectivity of T.saginata ova to calves

In this initial experiment seven calves were infected with a nominal 5,000) viable eggs. The in vitro viability of the eggs was approximately 20%. Two calves were killed at 5 and 8 weeks and the remaining five at 12 and 13 weeks after infection.

Expt. 2. The effect of anaerobic digestion, lagooning and pasteurisation on infectivity

Nineteen calves divided into six groups were used. Group 1 had 4 calves and Groups 2–6 had 3 calves per group.

Group 1. Control (no treatment of ova)., Animals infected with ova at start of experiment.
Group 2. Treatment—Anaerobic digestion at 35°C for 10 days followed by lagooning for 15 days (plant scale).
Group 3. Treatment—Anaerobic digestion at 35°C for 24 hours (plant scale).

Ova were exposed to treatment conditions using the perspex containment chambers previously described. Exposure to anaerobic digestion was achieved by suspending the chamber in the outlet bellmouth of the digester which was operated continuously at a mean temperature of 35.6°C during the treatment period. Transfer to the lagoon was accomplished by containing the chamber in 10 1 of digested sludge, cooling overnight and resuspending, on a weighted line, in the lagoon. Temperature readings taken from the lagoon (6°C) and chamber during the cooling phase (34–6°C) indicated that this procedure provided a close approximation to plant conditions.

Group 4. Pasteurisation—3 hours in crude sludge at 55°C.
Group 5. Pasteurisation—3 hours in crude sludge at 55°C followed by 1 days anaerobic digestion at 35°C.

At the time of these tests the submerged combustion plant was not operational and it was therefore necessary to simulate its operation in the laboratory. This was done by heating a 10 1 vessel to 55°C in a closed water jacketed incubator, unfortunately it was not possible to maintain temperatures at 55° throughout these tests. The temperature of exposure (Table 1) deviated little from the expected values for treatment 4 but for submerged combustion followed by digestion (treatment 5) the error was considerable.

Table I Temperature of exposure of T. saginata during simulated submerged combustion

	Temperature °C	
	Treatment	Treatment
Time (hrs)	4	5
	0	54.5	51.0
	0.5	55.0	51.0
	1.0	55.0	51.0
	1.5	55.0	52.0
	2.0	55.0	53.0
	2.5	55.0	53.0
	3.0	55.0	55.0
Average		55.0	52.3

Group 6. Bench control (no treatment of ova).
Eggs were kept under optimal conditions on the bench for the duration of treatments to assess any normal decline in infectivity.

Expt. 3. The effect of lime treatment, aerobic digestion and lagooning on infectivity

Five groups of three calves were used as follows:
Group 1. Control (no treatment)

Group 2. Treatment—Lime with Ca 0 to pH 12.0 (Laboratory scale) in a closed vessel for 24 hours. The pH dropped to 11. 5 in this period.
Group 3. Treatment—Aerobic sludge digestion (plant scale) for 16 days.
Group 4. Treatment—Lagooning in raw sludge (plant scale) for 28 days.
Group 5. Bench control (no treatment of ova) kept under optimal bench conditions to assess normal decline in infectivity.

Note The number of ova placed in the perspex chambers was standardised for each separate experiment.

3. RESULTS

Expt. 1. Infectivity of T.saginata ova to calves

The results are given in Table II. Calf No. 1 was found to have the majority of cysts fibrotic and some were dead, calf No. 2 had a few fibrotic cysts also. In the other 5 calves the cysts were all alive. The masseter muscles and heart were heavily infected and at least 20% of the cysts found in each animal occurred in heart muscle.

The results demonstrate that 100% of calves could be infected by our techniques and that an average of 368 cysts were found although the range varied from 29–832. The in vitro viability was 20% and as the dose of 5,000 viable ova gave an average cyst count of 368, only 7.4% of these viable eggs were recovered as cysts or 1.6% of the total eggs administered.

Table II Number of cysticerci found in calves after oral dosing with a nominal 5,000 viable eggs of T.saginata/calf

Calf No.	Infection age at PM (weeks)	No. eggs given	No. cysticerci
1	5	18,690	203
2	8	19,809	219
3	12	24,710	423
4	12	27,266	149
5	12	22,539	29
6	13	24,780	832
7	13	22,260	720

Expt. 2. The effect of anaerobic digestion, lagooning and pasteurisation on infectivity

The results of the experiment are given in Table III. There was no significant difference between Groups 1, 3 and 6 representing the control, anaerobic digestion at 35°C for 24 hours and the bench control. The other three treatment groups were significantly different from the controls, but not from each other. Anaerobic digestion for 10 days at 35°C, followed by lagooning for 15 days completely destroyed viability and infectivity. Treating ova in sludge to 55°C for 3 hours reduced infectivity by 99.4%.

Table III The effect of anaerobic digestion, lagooning and pasteurisation on infectivity

	Treatment	Ova given (mean viability)		Viable ova given	Cysts found (mean)		Degenerate cysts found	% take of ova (mean)		% take of viable ova (mean)	
1	Control	38066	(21%)	7917	1623	(1660)	150	4.26	(4.30)	20.5	(20.7)
36066	7501	2357	0	6.53	31.4						
39466	8209	1438	457	3.60	17.5						
43333	9013	1228	79	2.82	13.6						
2	Digest, at 35°C 10 days+15 days lagooning	10733	(0%)	0	0	(0)	0	0	(0)	0	(0)
10000	0	1?	1?	0	0						
10533	0	0	0	0	0						
3	Digest, at 35°C for 24 hours	24400	(9.9%)	2416	849	(713)	24	3.47	(2.91)	35.1	(29.3)
27600	2732	645	3	2.34	23.6						
22133	2191	645	6	2.91	29.4						
4	3 hours in crude sludge at 55°C	14733	(2.4%)	354	7	(5)	0	<0.1	(<0.1)	2.8	(1.6)

	Treatment	Ova given (mean viability)		Viable ova given	Cysts found (mean)		Degenerate cysts found	% take of ova (mean)		% take of viable ova (mean)	
14200	340	6	3	<0.1	2.0						
12000	288	3	0	<0.1	1.0						
5	3 hours in crude sludge at 52°C (av.) +1 day digest, at 35°C	27200	(9.0%)	2448	68	(23)	32	0.25	<0.1	2.8	(0.1)
11600	1044	1	0	(<0.1)	0.1						
20400	1836	1?	1?	<0.1	<0.1						
6	Bench control	32266	(32.7%)	10567	663	(872)	63	2.05	(2.90)	6.3	(8.9)
29400	9628	1262	158	4.29	13.1						
29400	9628	692	35	2.35	7.2						

Table IV The effect of lime treatment, aerobic digestion and lagooning on infectivity

	Treatment	Ova given (mean viability)		Viable ova given	Cysts found (mean)		Degenerate cysts found	% take of all ova (mean)		% take of viable ova (mean)	
1	Control	20685	(28.1%)	5833	262	(198)	2	1.27	(0.96)	4.5	(3.4)
20685	5833	197	3	0.95	3.4						
20685	5833	134	2	0.65	2.3						
2	Lime treatment 24 hours in raw sludge, pH 12	18000	(2.2%)	396	1	(5)	0	<0.1	(<0.1)	0.3	(1.3)
18400	404	15	1	<0.1	3.7						
17150	377	0	0	0	0						
3	Aerobic digestion 16 days	21100	(10.1%)	2131	24	(25)	0	0.11	(0.10)	1.1	(1.2)
21100	2131	41	0	0.19	1.9						
21100	2131	11	0	<0.1	0.5						
4	Lagooning 28 days	24800	(21.3%)	5282	0	(0.3)	0	0	(0)	0	(0)
24800	5282	1	0	<0.1	<0.1						
24800	5282	0	0	0	0						
5	Bench control	18900	(37.6%)	7106	138	(137)	4	0.73	(0.72)	1.9	(1.9)
18900	7106	193	0	1.02	2.7						
18900	7106	79	0	0.42	1.1						

Expt. 3. The effect of lime treatment, aerobic digestion and lagooning on infectivity

The results of this experiment are given in Table IV. No significant difference was found in Groups 1 and 5 (control and bench control). Groups 2, 3 and 4 treatment groups were significantly different for the controls, but not each other. Reduction in infectivity of ova compared with those of the untreated eggs of the same age were:-

Group 2 Lime treated sludge at pH 12–96.5%

Group 3 Aerobic digestion in inactivated sludge plant for 16 days—82%
Group 4 Sludge lagoon, 28 days—99.9%

3.2
Correlation of in vitro viability and infectivity

An examination of Tables III and IV with respect to % viability and infectivity shows that there appears to be little correlation. For example in Table III Groups 1 and 6 (controls) show that on storage viability appears to increase and yet with such increased viability there was an apparent decrease in infectivity albeit not significant. When in vitro viability was found to be zero, no calves were subsequently found to be infected with ova from the same batch.

4.
DISCUSSION

The results of the first experiment on infectivity of untreated ova to calves show the wide variation in susceptibility of individual animals of the same age. In one animal, the majority of the cysts found were fibrosed and many were dead. The reasons for this are not known, but it is possible that immune factors are involved. Previous authors have reported degenerate cysts as early as 20 days after experimental infections and that cysts die off at different rates in individual animals, especially if infected with different numbers of ova.(4), (5) The overall 'take' of 7% of viable ova given which we found, agrees well with previous authors. It was considered that at least three calves/group would be necessary to indicate any effects of various treatments on tapeworm egg infectivity. The cysts were mainly found in the usual predilection sites of tongue, masseters and heart, with the latter organ being the best indicator of infection in our experiments. However, in low grade infections the heart was not always infected. There did not appear to be a correlation between % viability and infectivity to calves. In both treatment experiments (Expt. 2 and 3) there was an increase in viability as the ova aged (control and bench control), but this was not reflected in infectivity which appeared to fall. In an additional (unpublished) experiment using older eggs which had a 24% viability, no infection was detectable in the 3 calves given eggs from the same batch.

The results obtained after the various treatments indicate that anaerobic mesophilic digestion is very effective and this is in agreement with the findings of Silverman and Guiver. (6) The other stabilisation treatments which are often used in the smaller works also result in a significant reduction in infectivity and indicate that pasteurisation conditions used in Europe (70°C, 30 mins) offer a considerable safety margin. The costs of different disinfection systems for sludge has recently been examined by the Water Research Centres Processes Directorate and when this data is available it should have important economic implications relevant to possible future plant usage.

5.
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6.
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MICROWAVE TREATMENT AS AN ALTERNATIVE PASTEURISATION PROCESS FOR THE DISINFECTION OF SEWAGE SLUDGE—EXPERIENCES WITH THE TREATMENT OF LIQUID MANURE

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Summary

Microwave inactivation of suspensions from four different bacteria species in drinking water was studied. A 1 KW/2450 MHz microwave flow heater was used in this study. The initial inoculum of test organisms was 10–10 germs/ml in all experiments. At temperatures between 67 C and 69 C and an average holding time in the UHF field of 7,03–7,54 seconds all species were inactivated. Salmonella senftenberg 775 W was the most resistant test organism and therefore selected for further experiments with liquid manure. In liquid manure 10 CFU of Salmonella senftenberg were inactivated after microwave treatment, if temperatures between 67 C and 69°C and an average holding time of 6,81–7,04 seconds were reached.

1. INTRODUCTION

A spontaneous generation of heat that could cause the destruction of pathogenic bacteria will not occur in liquid manure either in summer nor in winter, therefore a self-disinfection is not to expect. Transmission of infection after spreading of slurry to pastures has been observed. Thus an additional treatment of infected liquid manure, leading to its decontamination, is necessary.

Disinfection is possible in a biological way by aeration in a specially designed equipment by which an aerobic-thermophilic process is achieved.

The application of chemical disinfectants, for example sodium hydroxide, is also possible. But this will lead to a manure, unsuitable agricultural use because it will be toxic to plants and soil microorganisms (7). Moreover any distribution of chemicals in the environment should be avoided.

A separation of the solid and the liquid phases of the manure can be used. Thereby the solids are compostable and the liquid phase needs less chemicals for disinfection if the solids are separated. But the environmental problem still exists. Therefore a physical method seems to be favorable.

Microwave treatment offers the possibility to achieve high temperatures in liquid manure.

The microwave treatment of food in industrial production or in the household is becoming more and more important. The main advantages of this method are the extremely rapid heating of water containing material, which acts as a dielectric medium in a high-frequency electric field, and the possibility of designing relatively light and mobile devices for microwave-pasteurisation of liquids in a flow heater (6).

Effects of microwaves on microorganisms in foods are influenced by intrinsic characteristics of foods (i.e. pH, moisture level, chemical composition,…) and extrinsic characteristics (i.e. temperature, frequency and intensity of radiation etc.). Furthermore, the physical and chemical composition of the microorganisms being irradiated, their biological stage and their numbers present are also important factors (2).

The effects of microwaves on higher plants and phytopathogenic organisms were investigated too. There was observed, that 650 W applied for 120 seconds to soil samples containing cysts of the sugar beet nematode “Heterodera schachtii” completely suppressed the formation of new cysts in a greenhouse test (3).

The inactivation of virus material of different species suspended in water and liquid manure was also reported (4) (5). In emergency cases such mobile microwave treatment units could be very useful for the rapid decontamination of liquid manure containing highly infectious pathogenic bacteria or viruses. The following study was carried out to investigate the inactivation of bacteria suspended in drinking water and liquid manure using a specially designed flow microwave heater.

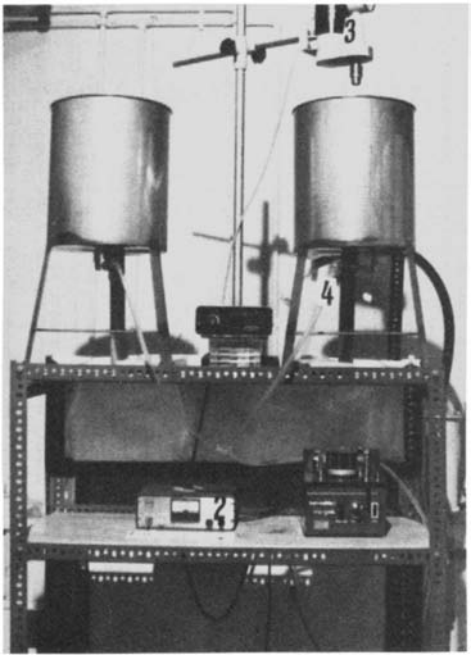


Figure 1 – Microwave heating unit. 1. Peristaltic pump. 2. Control unit (microwave generator). 3. Electric Themmcouple (starting temperature).

2. MATERIAL AND METHODS

In the experiments a 1 kW/2450 MHz microwave flow heater was used for the heating of the liquid material.

Figure 1 and Figure 2 show the microwave unit with its several components (peristaltic pump, control unit, electric stirrer, thermocouple, UHF generator/power supply, circulator element, electric thermometer).

Two 20 litre reservoirs-containing contaminated drinking water or liquid manure under steady stirring-were connected with the energy transmitter by a two-way valve and silicon tubes.

The flow rate was dependent on the amount of matter in the liquid phase, on the elasticity of the silicon tube and the number of revolutions.

The temperature was measured continously before, and directly after, the energy transmitter using glass thermocouples which were connected with an electric thermometer.

The liquid manure used in all experiments originated from the experimental station of the University and was a mixture of bovine and swine slurry.

The species Escherichia coli 0:86, Salmonella senftenberg 775 W which is very resistant to heat treatment, Erysipelothrix rhusiopathiae (NCTC Nr. 8163) and Yersinia eneterocolitica 0:3 were used as test organisms.

The flow rate was measured and adjusted before each experiment. The average holding time of water or liquid manure in the UHF-field (in seconds) was calculated. The relationship between temperature (°C) and average holding time (sec) is shown in Figure 3.

3. SURVIVAL EXPERIMENTS WITH TEST ORGANISMS FREE SUSPENDED IN DRIN-KING WATER

The initial inoculum of all test bacteria in the suspension in the reservoirs was 10^6–10^7 germs/ml. Immediately after passing the energy transmitter, 40 ml samples of the treated suspension were taken at various temperatures between 19°C and 74°C. The samples were dispensed into screw-capped glass vials and subsequently cooled down to =4°C.

The total bacterial count was determined with the MPN-technique (Most Probable Number), a statistical method for quantitative evaluation as follows: Starting with 1 ml of the sample a 6–8 step serial dilution was made. Then three tubes with nutrient broth were inoculated with 1 ml from dilution-step.

After 18–24 h incubation a loop-full from each tube was streaked on the selective media. The incubation temperature was 37°C for Escherichia coli, Salmonella senftenberg and Erysipelothrix rhusiopathiae, but 28°C for Yersinia enterocolitica. The identification of suspicious colonies was carried out by testing the biochemical reactions, slide agglutination and Grams stain. The result was obtained by using the MPN-table of Mc. Crady.

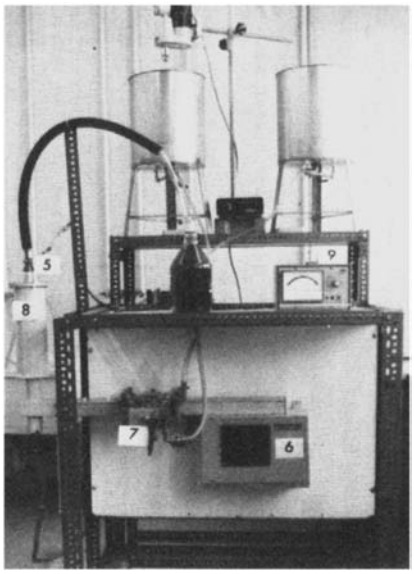


Figure 2 – Microwave heating unit. 5. memocouple (final terqerature) . 6. UHF generator/power supply. stirrer. 4. 7. Circulator element. 8. mergy transmitter elemnt. 9. Electric thermmeter.

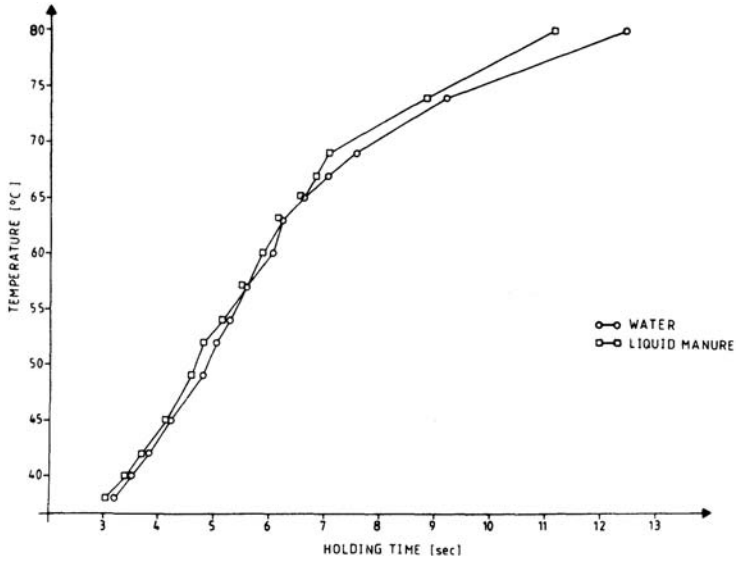


Figure 3 – Relationship between temperature (drinking water, liquid manure) and average holding time in the UHF field.

All experiments were carried out twice and repeated six times with each species to investigate their inactivation rate. First the experiments were carried out with drinking water, in order to select the most resistant species for the main experiences with slurry.

The mean germ-counts at different temperatures are summarized in Table I. Figure 4 represents the inactivation kinetics of Escherichia coli, Salmonella senftenberg, Erysipelothrix rhusiopathiae and Yersinia enterocolitica.

Escherichia coli was no longer detectable in the suspension if a temperature of 67°C with an average holding time in the UHF field of 7,03 seconds was reached. The reduction of the germ-count started at temperatures between 52°C and 54°C. If the average holding time of 7,54 seconds and a temperature of 69°C were reached, Salmonella senftenberg could no longer be detected in the samples. The germ-count of Erysipelothrix rhusiopathiae was nearly constant up to 49°C-52°C. Subsequently the number of surviving bacteria decreased. At the tempera ture of 60°C and an average holding time of 6,03 seconds it could no longer be detected. The temperature of 65 C and an average holding time in the UHF field of 6,6 seconds were necessary to inactivate all species of the free suspended Yersinia enterocolitica.

These results indicate, that Salmonella senftenberg was the most resistant test organism to a microwave treatment. Erysipelothrix rhusiopathiae was the most sensible one.

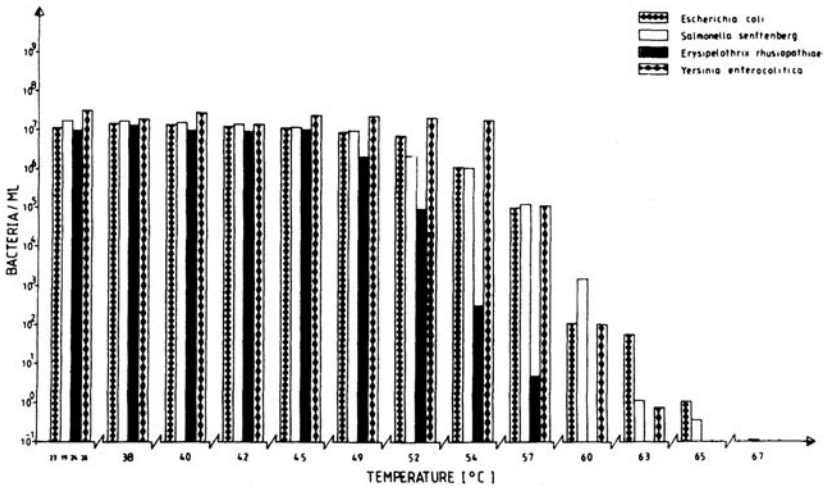


Figure 4 – Reduction of germ-count of Escheria coli, Salmonella senftenberg Erysipelothrix rhusiopathiae and Yersinia enterocolitica suspended in drinking water

Table I: Results of the experiments with test organisms suspended in drinking water (mean values of every six experiments)

temperature (°c)	cells/ml			
E. coli	S. senft.	E. rhus.	Y. ent.	
19–26	$1,5\times10^{7}$	3×10^{7}	$9,8\times10^{6}$	$5,5\times10^{7}$
38	$2,5\times10^{7}$	$3,1\times10^{7}$	$1,1\times10^{7}$	$3,6\times10^{7}$
40	$2,3\times10^{7}$	$2,8\times10^{7}$	1×10	$5,2\times10^{7}$
42	2×10^{7}	$2,5\times10^{7}$	$1,2\times10^{7}$	$2,6\times10^{7}$
45	$1,6\times10^{7}$	$1,7\times10^{7}$	$1,3\times10^{7}$	$4,6\times10^{7}$
49	$9,8\times10^{6}$	$1,2\times1^{0}7$	$4,2\times10^{6}$	$4,4\times10^{7}$
52	9×10^{6}	$4,4\times10^{6}$	1×10^{5}	$4,2\times10^{7}$
54	$1,7\times10^{6}$	$1,6\times10^{6}$	$5,6\times10^{2}$	$3,7\times10^{7}$
57	$1,3\times10^{5}$	$2,2\times10^{5}$	7,2	$1,7\times10^{5}$
60	$1,5\times10^{2}$	$2,7\times10^{3}$	0	$1,2\times10^{2}$
63	$7,8\times10^{1}$	1,5	0	0,9
65	1,3	0,6	0	0
67	0	0,03	0	0
69	0	0	0	0

4. SURVIVAL EXPERIMENTS WITH SALMONELLA SENFTENBERG SUSPENDED IN LIQUID MANURE

Following investigations concerned with the effect of microwave treatment on free suspended bacteria in liquid manure. The experiments were carried out with Salmonella senftenberg 775 W, which proved to be the most heat resistant test organism in the above described experiments. Therefore the results obtained by the microwave treatment of Salmonella senftenberg suspended in liquid manure will be representative for the other three species, Escherichia coli, Erysipelothrix rhusiopathiae and Yersinia enterocolitica.

The starting germ-count of Salmonella senftenberg suspended in liquid manure was 10 germs/ml. The untreated, not aerated liquid manure was stored at +4 C no longer than five days, it had a pH value between 6,99 and 7,56 and contained 1, 21%–1,98% dry matter.

Before each experiment the flow rate had to be adjusted by the peristaltic pump. Immediately after passing the energy transmitter, 40 ml samples of the treated liquid phase were taken at various temperatures up to 74 C.

Regard to the practical application of this treatment the samples were not put into ice water, thus the temperature could affect for a prolonged period of time.

The MPN-technique in combination with the pre-enrichment method of EDEL and KAMPELMACHER was used for quantitative determination of surviving Salmonella (1). The pre-enrichment in buffered peptone water with consecutive incubation at 43°C in Na-tetrathionate (MÜLLER-KAUFMANN) was carried out in duplicate. The presence or absence of growth was controlled by inoculating a petri dish with a loopfull of every tube. The identity of the grown colonies was controlled by slide agglutination. The obtained results were used for the MPN-estimate.

The experiments were repeated eight times with Salmonella senftenberg.

In Table II the results of each experiment and in Table III the mean values and standard deviations of all eight experiments are summarized. Figure 5 shows the inacvtivation kinetics of Salmonella senftenberg suspended in liquid manure after microwave treatment (mean values of eight experiments).

The average values indicate that no reduction of the germ-count occurred between 38°C and 57°C. Then the number of viable Salmonella rapidly decreased at temperatures between 57°C and 69°C from 4,5×10 to zero. The germs could no longer be detected in treated manure if the average holding time in the UHF field was 7,04 seconds and a temperature of 69°C was reached.

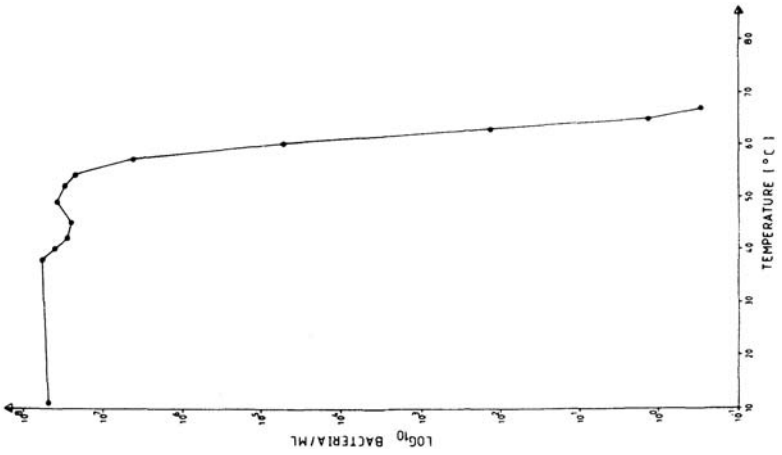
Table II: Results of the experiments with Salmonella senftenberg 775 W suspended in liquid manure

temperature (°C)	cells/ml							
exp. 1	exp. 2	exp. 3	exp. 4	exp. 5	exp. 6	exp. 7	exp. 8	
8–15	6×10^{7}	6×10^{6}	6×10^{7}	6×10^{7}	6×10^{7}	$2{,}5\times10^{7}$	6×10^{7}	6×10^{7}
38	6×10^{7}	$2{,}5\times10^{7}$	6×10^{7}	6×10^{7}	6×10^{7}	$1{,}3\times10^{8}$	9×10^{7}	$2{,}5\times10^{7}$
40	$2{,}5\times10$	6×10^{7}	$2{,}5\times10^{7}$	6×10^{7}	6×10^{7}	6×10	6×10^{6}	6×10^{7}
42	$2{,}5\times10^{7}$	6×10^{6}	6×10^{7}	$2{,}5\times10^{7}$	$2{,}5\times10^{7}$	6×10^{7}	$2{,}5\times10^{7}$	$2{,}5\times10^{7}$
45	$2{,}5\times10^{7}$	6×10^{7}	$2{,}5\times10^{7}$	$2{,}5\times10^{7}$	$2{,}5\times10^{7}$	$1{,}3\times10^{7}$	$2{,}5\times10^{7}$	$2{,}5\times10^{7}$
49	6×10^{7}	$2{,}5\times10^{7}$	$2{,}5\times10^{7}$	6×10^{7}	6×10^{7}	$2{,}5\times10^{7}$	$2{,}5\times10^{7}$	6×10^{7}
52	$2{,}5\times10^{7}$	$1{,}3\times10^{7}$	6×10^{7}	$2{,}5\times10^{7}$	6×10^{7}	$1{,}3\times10^{7}$	$1{,}3\times10^{7}$	6×10^{7}
54	6×10^{6}	$1{,}3\times10^{7}$	$2{,}5\times10^{7}$	$2{,}5\times10^{7}$	7×10^{7}	$2{,}5\times10^{7}$	$1{,}2\times10^{7}$	$2{,}5\times10^{7}$
57	$2{,}5\times10^{6}$	6×10^{6}	$2{,}5\times10^{6}$	6×10^{6}	$2{,}5\times10^{6}$	$2{,}5\times10^{6}$	$1{,}3\times10^{7}$	6×10^{5}
60	$2{,}5\times10^{4}$	6×10^{4}	7×10^{4}	6×10^{2}	$2{,}5\times10^{3}$	$2{,}5\times10^{4}$	$2{,}5\times10^{5}$	$2{,}5\times10^{4}$
63	6×10^{1}	6×10^{2}	5×10^{1}	6×10^{1}	6	$2{,}5\times10^{1}$	$2{,}5\times10^{2}$	6×10^{1}
65	2,5	6×10^{1}	6	$2{,}5\times10^{1}$	6	2,5	6	2,5
67	0	0	0	2,5	0	0	0	0
69	0	0	0	0	0	0	0	0

exp.=experiment

Table III: Results of the experiments with Salmonella senftenberg suspended in liquid manure (mean values of the eight experiments)

temperature (oC)	mean value (cells/ml)	standard deviation
11	$4{,}9\times10^{7}$	$2{,}1\times10^{7}$
38	$6{,}4\times10^{7}$	$3{,}4\times10^{7}$
40	$4{,}5\times10^{7}$	$2{,}2\times10^{7}$
42	$3{,}1\times10^{7}$	$1{,}9\times10^{7}$
45	$2{,}8\times10^{7}$	$1{,}4\times10^{7}$
49	$4{,}3\times10^{7}$	$1{,}9\times10^{7}$
52	$3{,}4\times10^{7}$	$2{,}2\times10^{7}$
54	$2{,}5\times10^{7}$	$1{,}9\times10^{7}$
57	$2{,}5\times10^{6}$	$3{,}9\times10^{6}$
60	$5{,}7\times10^{4}$	$8{,}2\times10^{4}$
63	$1{,}4\times10^{2}$	2×10^{2}
65	$1{,}4\times10^{1}$	$2{,}1\times10^{1}$
67	0,3	0,9
69	0	0



Figue 5 – Inactivation kinetics of Salmnella senftenberg suspended in liquid manure (meanvalues of eight experiments).

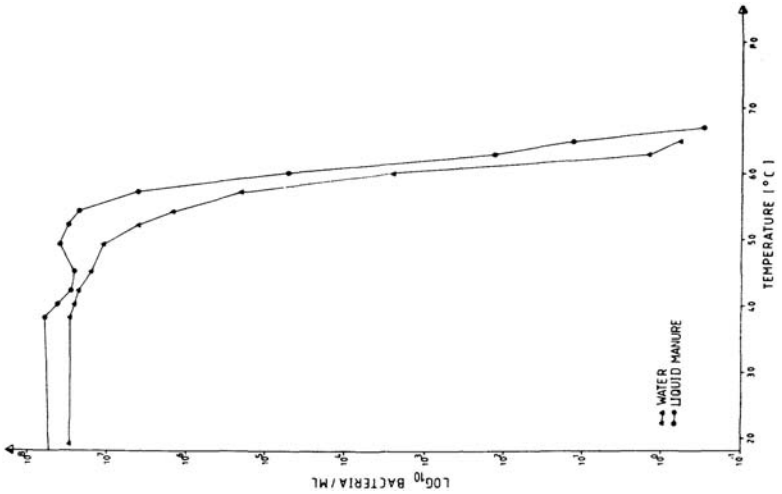


Figure 6 – Comparative representation of the inactivation kinetics of Salmnella senftmberg suspended in drinking water and liquidmanure after microwave treatment

5. DISCUSSION

Figure 6 shows a comparitive representation of the inactivation kinetics of Salmonella senftenberg suspended in drinking water and liquid manure after microwave treatment.

At 54°C a significant reduction of the germ-count of Salmonella senftenberg suspended in liquid manure occurred. Between 67°C and 69°C and an average holding time of 6,81–7,04 seconds all Salmonella were inactivated. In contrast to that inactivation of Salmonella senftenberg in drinking water samples set in at temperatures between 49 C and 52°C. At temperatures between 67°C and 69°C with an average hol ding time of 7,03 and 7,54 seconds the inactivation was complete.

Since both curves run nearly parallel, the differences are caused by the different germ-counts in the beginning of the experiments ($\times 10^7$ in water, $\times 10^7$ in liquid manure), because the inactivation kinetics is strongly influenced by the number of bacteria.

Same to Escherichia coli, Erysipelothrix rhusiopathiae and Yersinia enterocolitica, because they had previously shown to be more sensitive to the microwave treatment.

The results indicate, that there exists no significant reduction of the thermo-effect of the microwaves in the presence of anorganic and organic contaminations (i.e. proteins). The observetions of MAHNEL and STETTMUND von BRODOROTTI confirm this statement referring to a thermal virus decontamination of fluids and materials by microwaves (5) .

With respect to the problem of chemical residuals, microwave treatment offers an effective alternative to chemical disinfection. Furthermore heat inactivation of bacteria in liquid manure with a microwave flow heater can be achieved with extremely short holding times in the UHF field. The energy consumption could be lowered and the flow rate elevated by using a heat exchange system.

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THE EFFECT OF CHEMICAL DISINFECTANTS ON TAENIA EGGS

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Summary

The disposal of sewage sludge to land is one of the several ways in which helminth infections could be spread. Various chemical compounds and mixtures have been tested to determine whether they have any effect on eggs of Taenia species; but only a small number have shown potential as agents for the disinfection of sewage sludges.

Some chemicals, particularly the lime and formalin-based mixtures, affect the embryophore and prevent the egg from hatching in artificial digestive juice—but the embryo inside the egg appears to be unharmed. Other chemicals, such as the bis-biguanides and quaternary ammonium salts, are able to penetrate the embryophore and reduce the viability of the contained embryo. Finally, some copper-containing compounds show considerable ovicidal action, but the mechanism by which these compounds affect the embryo is not known.

Work is required to find cheaper and more potent chemicals. Certainly very few of the compounds and mixtures tested would at present be cost-effective within the normal working practice of the British Water Industry. However, where economic considerations are different or where longer contact times are possible, chemical disinfection of sewage sludge may prove to be of value in the future.

1. INTRODUCTION

A considerable number of protozoan and helminth parasites of man now occur outside the tropics, and many of them could theoretically be transmitted through sewage. In fact, however, it seems rare for sewage-borne transmission to take place in developed countries. The beef tapeworm, Taenia saginata, is probably the only helminth parasite of man which is regularly transmitted through sewage in the United Kingdom. This is probably because it has an indirect life cycle, with transmission through cattle which serve as intermediate hosts, and so it is not necessary for the sewage-borne stage (the egg) to be ingested by man.

It is probable that the transmission of the eggs of T.saginata from human faeces to susceptible cattle in the U.K. is governed by a complex interplay of several factors, one of which is the application of sewage sludges on grazing land. This practice provides an economic and efficient method of disposing of sewage sludge, and is commonly adopted by Water Authorities where there is no suitable coastal discharge. Such direct contamination of the pasture is not the only, or necessarily the most important, route by which eggs may be transmitted; birds, invertebrate animals, streams and surface water all play a part in carrying eggs to pastures. Nevertheless, the Department of the Environment in London has sponsored a series of investigations on the treatment of sewage sludges prior to disposal on land, and much of the work described in this paper has been supported by the D.o.E.

It seems unlikely that the commonly-used methods of sewage treatment will prevent transmission of Taenia (or any other helminth) by killing the eggs. Novel methods of sewage treatment have therefore been investigated by several British workers. The use of ionizing radiation, submerged combustion, heated digestion, heat treatment and other such techniques may involve a considerable outlay in plant, equipment and money, and for these reasons they are not highly attractive to the Water Industry. Chemical treatment possibly offers the best and most attractive prospect at present for the disinfection of sewage sludges before they are applied to agricultural land. For this reason we have tested a wide range of chemicals against Taenia and Ascaris eggs in water or sludge liquor (1). Some of these chemicals show promise as ovicides, but only few have so far been tested against helminth eggs in sewage sludge under normal field conditions.

Much of the work has been carried out with eggs of Ascaris, mainly A.suum, but it is proposed in this paper to concentrate on the work with Taenia. One of the reasons for this decision is that it is difficult, and often confusing, to compare the effects of chemicals on Taenia eggs with those on Ascaris eggs. In general, Ascaris eggs have for a long time been considered more resistant to adverse conditions and to chemical treatment than Taenia eggs, but this now appears to be an over-simplification

of the true position. The two eggs are very different in their morphology and biochemical structure, and so react differently. Some chemicals appear to be more effective against Ascaris than against Taenia, and vice versa.

Since our work on chemical treatment of sewage began, most of the compounds which we have tested as sludge "disinfectants" have been, and in the main continue to be, randomly provided by the manufacturers. Many, although not all, of these compounds are already available, having been originally developed as deodorants or detergents with bactericidal properties. As might be expected in a screening system such as ours, many of the compounds which have been tested have proved to be of little value. Some groups of chemicals show promise as ovicides for use with sewage, but only a small minority of compounds appear to have a marked effect on eggs of T.saginata. Of the compounds which have been found to possess ovicidal properties, very few could be used at cost-effective concentrations or over contact times that would fit in with normal working practice within the Water Industry in Britain. However, where economic constraints differ or where contact times may be extended, it may well be worth testing a greater range of compounds.

Much of the research work has been based on in vitro assessment of the viability of the treated eggs. It is obvious that before one can investigate the survival of eggs under various conditions, it is necessary to have an accurate indication of whether or not the eggs have survived exposure to those conditions. With Ascaris eggs this can easily be ascertained by keeping the eggs in clean water and allowing them to develop; but Taenia eggs do not develop in a way that can be checked by simple observation, so other techniques are necessary. The normally-used method is to hatch the eggs and then activate the embryo in artificial digestive juices—acid pepsin followed by alkaline trypsin and bile. We use the term "hatching" to mean the removal of the embryophore in the early stages of the pepsin-trypsin-bile digestion and the term "activation" to mean the emergence of the embryo from its surrounding membrane in the final stages of the pepsin-trypsin-bile digestion.

Once the eggs have been hatched and the embryos freed from their membranes, it has traditionally been the practice to use vital staining to determine whether the embryos are alive. We now use tetrazolium salts to distinguish between "live" and "dead" embryos. The tetrazolium reaction relies on the ability of living organisms to reduce tetrazolium salts to their formazan dye bases, and since this reduction requires an active enzyme system there is no doubt that an embryo which has reduced the tetrazolium salt and so become stained is alive.

2. MATERIALS AND METHODS

To determine the viability of eggs of Taenia it is necessary to remove the embryophore and then to stimulate the embryo into emerging from its surrounding membrane. This is accomplished by using a combined "hatching" and "activation" technique. Once the embryo has been stimulated into emerging or actively attempting to emerge, its viability can be determined by seeing whether the embryo will become stained when exposed to tetrazolium salts.

a) Hatching and activation. Two solutions are required. The first, the acid-pepsin solution, is prepared by dissolving 1.5 g of pepsin powder in 100 ml of a 1% solution of concentrated hydrochloric acid in 0.85% (physiological) saline. The second solution, the trypsin-bicarbonate-bile solution, is prepared by dissolving 0.05 g of trypsin (Sigma T8253; Type III, 10–13,000 BAEE units/mg) in 100 ml of a 1.18% solution of sodium hydrogen carbonate. 2 ml of this solution is then mixed with 1 ml of freshly obtained (or deep frozen) whole cattle bile.

The eggs to be tested are washed in water and transferred to 5 ml of the acid-pepsin solution in capped centrifuge tubes in a water bath kept at 38°C. The tubes must be shaken vigorously every 10–15 minutes, and a total of 90 minutes in the water bath is optimal. After this period the eggs are sedimented by centrifuging, and all but 0.5 ml of the acid-pepsin solution is discarded. The 2:1 mixture of trypsin-bicarbonate and whole bile is then prepared, and 2 ml of this mixture is added to the centrifuge tube containing the eggs. The cap is placed on the tube immediately and the tube is shaken vigorously for several seconds. Finally the tube is replaced in the water bath and left for 45–60 minutes, being vigorously shaken every 10 minutes.

After this time a sample of fluid is drawn off from the tube, and the relative numbers of active and inactive embryos are determined. Increasing the activation time up to 4 hours does not yield larger proportions of activated embryos, so activation for 1 hour appears to be sufficient. While the embryos are being examined microscopically it is advisable to support the coverslip (for example, on small fragments of broken coverslip) to avoid damaging the embryos by pressure.

b) Viability determination. Following activation in the trypsinbicarbonate-bile mixture, the eggs and embryos are washed a minimum of three times in physiological saline to remove all traces of the mixture. The washed material is then placed in a 0. 025% solution of a tetrazolium salt (MTT or NBT) in physiological saline, and incubated overnight in a water bath at 38°C. It is essential that the water bath is covered to exclude all light.

After incubation the embryos are examined microscopically. The viable embryos will contain deposited dark formazan dye, while the non-viable embryos will be colourless. Differentiation is simple because the background remains pale yellow.

c) Liberation. Following treatment with certain chemicals, the embryophore of the Taenia egg is chemically changed and consequently the egg will not "hatch" when exposed to pepsin-trypsin digestion. A method was therefore devised to remove

the embryophore and "liberate" the enclosed embryo, so that the viability of the embryo could be determined. Liberation is achieved by exposing the egg to a solution of sodium hypochlorite containing 5% available chlorine.

The treated eggs are washed in water, then placed in a 15 ml centrifuge tube containing 1 ml of 0.85% (physiological) saline. 1 ml of the sodium hypochlorite solution is added, and the tube is immediately capped and shaken gently for 3 minutes. After 3 minutes the tube is filled with physiological saline to stop the reaction, the eggs are sedimented by centrifugation, and the supernatant fluid is discarded. The washing in saline is repeated at least three times, and the eggs are then hatched and activated as described above. The acid-pepsin pre-treatment is not strictly necessary, but if it is used it creates more normal conditions (in pressure of carbon dioxide) in the tube.

d) Storage of solutions and eggs. The acid-pepsin medium keeps well at 4°C and may be stored in bulk without deterioration. The trypsinbicarbonate medium must be kept frozen, and to avoid repeated freezing and thawing it should be stored in small quantities each sufficient for a single test. It is advisable to prepare a large volume and store it as many single-use aliquots, so that the same batch of medium is used for each hatching and activation. Bile may also be kept in a frozen state for a considerable period of time (over 12 months), but it should be stored in small volumes that are thawed and used for a single test. Serious deterioration results from repeated freezing and thawing.

The tetrazolium salts may be prepared as stock solutions and kept at 4°C in the dark. A convenient method is to store the solutions ready for use in 15 ml centrifuge tubes kept in closed cardboard boxes.

Sodium hypochlorite solutions do not store well. Unless it is inten-ded to assay the chlorine level before the solution is used, reliable results can only be obtained if freshly-prepared solutions are employed.

Taenia eggs may be preserved at 4 C in physiological saline. If the saline is changed frequently it is not necessary to add antibiotics, which may influence the viability of the eggs. Eggs of T.saginata, T.ovis, T. pisiformis and T. hydatigena remain viable for several months under these conditions.

3. RESULTS

Taeniid eggs are well known for their apparent resistance to the effects of a wide variety of chemical treatments (2, 3), although sodium hypochlorite has been shown to dissolve the embryophore of Echinococcus granulosus (4), and to dissolve both the embryophore and the hexacanth embryo of T. saginata (5). The results of our investigations into the effects of other chemicals are summarised below.

a) Compounds which inhibit hatching. Taenia eggs which have been treated with certain chemicals, particularly strong lime or formalin solutions, will not hatch in artificial digestive juices. However, the embryophore can be removed from these eggs by exposing them to a solution of sodium hypochlorite containing 5% available chlorine. We use the term "liberation" to mean the release of the embryo in this way, as opposed to normal "hatching" after exposure to pepsin-trypsin-bicarbonate-bile media. After liberation, if the released embryo is thoroughly washed in saline and then exposed to artificial digestive juices, it will be activated in the normal way to become an apparently viable embryo.

a, i) Lime. Lime is frequently applied to sludges destined for disposal to land because it acts as a flocculant and a deodorant. Sufficient lime is usually added to raise the pH to 11.5. At this concentration lime has little effect on Taenia eggs which are exposed to it for 24 hours in sludge (although it is much more effective against eggs in lime water, as shown in Table I). If eggs are exposed to lime in sludge liquor at a pH of 11.5 for more than 48 hours they will not hatch in artificial digestive juices, but the embryos within the eggs are not obviously affected and they are apparently viable six months later. Lime may therefore be said to act partly as a preservative for the eggs.

a, ii) "DF 955" (Odex). This is an alkaline formalin-based mixture, and like formalin alone it inhibits hatching of the eggs in the same way as lime (Table I).

TABLE I
Effects of lime and DF 955 on eggs of T.saginata

Compound	pH or concentration	Exposure time	Percentage of eggs hatched	Percantage of embryos viable
Lime in water	10.0	4 hrs.	100.0	6.0
11.0	4 hrs.	3.0	0	
11.5	4 hrs.	0	–	
Control			95.0	10.0
Lime in sludge	11.5	6 hrs.	85.0	74.0
11.5	12 hrs.	95.0	57.0	
11.5	24 hrs.	90.0	44.0	
Control			98.0	73.0

Compound	pH or concentration	Exposure time	Percentage of eggs hatched	Percantage of embryos viable
DF 955	1.0%	3 hrs.	71.0	28.0
1.0%	24 hrs.	0	-	
Control			91.0	42.0
Formalin	5.0%	7 days	83.0*	9.0
0.5%	7 days	100.0*	25.0	
Control			100.0*	29.0

* "Liberated" with sodium hypochlorite to determine viability

a, iii) "R-1" (Redoxan). This is a formalin-quaternary ammonium salt mixture which has recently been tested. At a concentration of 1400 ppm in water it causes total inhibition of hatching of Taenia eggs, and trials with this mixture are still in progress.

It is clearly important that lime and formaldehyde mixtures should be studied further, and animal trials carried out to determine whether the inability of the treated eggs to hatch in vitro means that the eggs will not hatch if they are ingested by a suitable animal. In one small trial, lime-treated hatch-inhibited eggs of T.hydatigena were fed to young lambs (6). Although not conclusive, the results of this trial suggest that such eggs will not develop in vivo. There is, therefore, a possibility that lime and the formalin-based mixtures could prove useful for control of the transmission of Taenia through sewage sludges.

b) Compounds of variable activity. Two compounds, "SX-1" and "Proxitane 4002" fall into this category. In each case there appear to be distinct differences in the action of the compound at different concentrations.

b, i) "SX-1" (I.C.I.). This is another formalin-based solution, but it is highly acidic. Because it was tested primarily against eggs of Ascaris suum and showed considerable activity, and because acidification of formalin for use as a histological fixative greatly enhances its action, it is surprising that SX-1 showed so little activity against eggs of Taenia saginata. At low concentrations (4.5% v/v) it appears to penetrate the embryophore and affect the contained embryo, but at concentrations of 10% v/v or higher it acts like other formalin mixtures. This may be because, while the chemical can penetrate the embryophore at low concentrations, at high concentrations the formalin renders the embryophore impermeable and so prevents penetration of the chemical (and at the same time prevents hatching of the egg).

b, ii) "Proxitane 4002" (Interox). This is a formulation of peracetic acid which shows variability in its action both as a hatch-inhibitor and as a penetrating ovicide over a range of concentrations and exposure times. At concentrations below 1000 ppm in water, rapid inhibition of hatching occurs—although an increased hatching rate was observed after 1 day's exposure of eggs to a concentration of 500 ppm and 7 days' exposure to a concentration of 100 ppm. Ovicidal activity was observed following exposure of eggs to a concentration of 500 ppm for 7 days or to a concentration of 1000 ppm for 1 day. In both digested and raw sludges, inhibition of hatching was observed at a concentration of 500 ppm—but again there was a contradictory result in that an increased hatching rate was observed after exposure to a concentration of 100 ppm (Table II).

The indications of an increased hatching rate at low concentrations resemble certain of the results obtained with lime treatment, but clearly much more work remains to be done before the value of peracetic acid can be determined.

c) Compounds showing penetrative ovicidal activity. Some compounds appear able to penetrate the embryophores of eggs of T. saginata and to affect the embryos directly. The effects, however, seem to be relatively slight (Table III).

c, i) "VAN-1", "VAN-2" and "VAN-3" (I.C.I.). These compounds are bis-biguanides and are said to affect cell membranes. Initial tests show that after exposure times of 1 hour they are able to penetrate the embryophore of the Taenia egg and reduce the viability of the contained embryo.

c, ii) "V-CL" (I.C.I.). This is a quaternary ammonium compound and, like the bis-biguanides, appears to penetrate the embryophore of the egg and affect the viability of the embryo.

d) Other compounds.

d, i) "MB 724", "MB 725" and "MB 725 modified" (Water Management Chemicals). This is a series of copper-based compounds of varying natures, MB 724 contains 1500 ppm of copper, MB 725 contains 500 ppm of copper, MB 725 Mod 1 is the copper-free base and MB 725 Mod 2 contains 1500 ppm of copper. These compounds have shown considerable ovicidal activity at very low levels of exposure over a period of 48 hours. It seems probable that the copper is the active agent in the MB compounds, because an increase in the concentration in the compounds leads to a lowering of the dose at

which ovicidal activity becomes apparent (Table IV).

TABLE II
Effects of "Proxitane 4002" on eggs of T.saginata

Concentration in water	Exposure time	Percentage of eggs hatched	Percentage of embryos viable
100 ppm	30 min.	0	–

Concentration in water	Exposure time	Percentage of eggs hatched	Percentage of embryos viable
24 hrs.	0	–	
7 days	100.0	78.0	
500 ppm	30 min.	0	–
24 hrs.	100.0	85.0	
7 days	100.0	25.0	
1000 ppm	30 min.	2.0	–
24 hrs.	100.0	58.0	
7 days	100.0	33.0	
Control		98.5	74.6

—viability of embryos could not be calculated

TABLE III
Effects of bis-biguanides and "V-CL" on eggs of T.saginata

Compound	Concentration	Exposure time	Percentage of eggs hatched	Percentage of embryos viable
In water				
VAN-1	20%	1 hour	94.0	13.0
VAN-2	20%	1 hour	92.0	13.0
VAN- 3	20%	1 hour	91.0	29.0
V-CL	10%	1 hour	97.0	27.0
Control			100.0	31.5
In sludge liquor				
VAN-1	20%	1 hour	98.0	17.0
VAN-2	20%	1 hour	98.0	22.0
V-CL	20%	1 hour	100.0	15.0
Control			100.0	29.5

TABLE IV
Effects of copper-based compounds on eggs of T.saginata

Compound	Concentration	Exposure time	Percentage of eggs hatched	Percentage of embryos viable
MB 724	500 ppm	48 hrs	0	–
250 ppm	48 hrs	24.2	17.4	
125 ppm	48 hrs	76.5	19.4	
62.5 ppm	48 hrs	95.1	25.6	
Control			87.9	30.0
MB 725 (Mod 1)	1000 ppm	48 hrs	69.0	32.0
500 ppm	48 hrs	92.7	56.7	
250 ppm	48 hrs	97.1	46.5	
100 ppm	48 hrs	97–7	46.5	
50 ppm	48 hrs	94.7	49.5	
Control			100.0	62.4
MB 725	1000 ppm	48 hrs	18.4	11.1
500 ppm	48 hrs	35.3	20.6	
250 ppm	48 hrs	71.5	33.6	
100 ppm	48 hrs	96.6	42.1	
50 ppm	48 hrs	98.1	47.3	
Control			100.0	62.4
MB 725 (Mod 2)	1000 ppm	48 hrs	8.1	5.4
500 ppm	48 hrs	6.2	4.3	
250 ppm	48 hrs	16.7	11.3	
100 ppm	48 hrs	33.2	20.3	

Compound	Concentration	Exposure time	Percentage of eggs hatched	Percentage of embryos viable
50 ppm	48 hrs	87.6	59.9	
Control			95.6	65.9

4. DISCUSSION

There appear to be two ways in which chemicals can prevent the development of the Taenia egg, either by penetrating the outer covering of the egg and affecting the enclosed embryo, or by affecting the outer covering and preventing hatching. We know very little of the means by which the various chemicals which we have tested produce any of these effects, although there has been some work on the effects of chemicals on the embryophore of the taeniid egg.

Inhibition of hatching of Taenia eggs has been shown to occur in vitro (and in vivo in the lamb-T.hydatigena model) after the eggs have been treated with lime or with formalin-based compounds such as DF 955 or R-1. Penetrative and ovicidal activity follows exposure of the eggs of T.saginata to bis-biguanides (the VAN compounds), quaternary ammonium salts (V-CL), peracetic acid (Proxitane 4002) and copper-based compounds (the MB compounds). Copper-based compounds have previously been investigated and shown to exert marked effects on Taenia eggs. Parnell (7) found that exposure to a 1% solution of copper carbonate not only inhibited hatching but also caused disintegration of the eggs. In our work we noticed that MB 724 in aqueous solutions below 100 ppm caused complete lysis of eggs of T.saginata.

Strongly alkaline conditions, and "fixative" chemicals such as formaldehyde, almost certainly produce their effect on the eggs of Taenia by changing the protein—or the protein-lipid complex—of the embryophore. They appear in this way to prevent the normal disruption of the embryo by acid-pepsin digestion followed by alkaline trypsin-bile activation in vitro. Inhibition of hatching in this way may prove to be a method of preventing infection of cattle by eggs of T.saginata, but it would be effective only if natural hatching of the eggs in the intestine of cattle relies exclusively on pepsin and trypsin and does not occur through the agency of some other chemical or enzyme which removes the embryophore in the same way that sodium hypochlorite does.

The growing interest in "chemical disinfection" of sewage sludge is indicated by the range and number of compounds and mixtures submitted by the manufacturers for testing, and also from the publicity given recently to field trials with Proxitane 4002 (8). Interest in possibly ovicidal chemicals is also being shown by the World Health Organization,

Although sewage-borne parasitic infections may not at present pose any serious public health problem in Europe, there is nevertheless a need to develop an economical means of disinfecting sewage sludge which is to be applied to land because there is some risk of outbreaks of sewage-borne human or animal disease. In addition, it should be remembered that parasitic infections are common in developing countries and that many of them are carried in sewage, which is an important source of fertilizer and is widely spread on land which is used for growing crops. A cheap and effective way of sterilizing this sewage could well lead to a marked improvement of health in these countries.

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RECOVERY OF HELMINTH EGGS IN COMPOST IN THE COURSE OF COMPOSTING

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Summary

In various sorts of composts with sludge from wastewater treatment plant and garbage Nematodes (Ascaris spp, Trichuris spp, Toxocara spp) and Cestodes (Hymenolepis spp) eggs were recovered but no Trematodes eggs was found. In 60% final samples obtained after 120 days of compostage, Helminth eggs were recovered. This contamination rate is important but study about viability of eggs are needed before the real importance of these composts as a transmitting medium for parasitological diseases can be adequately assessed (13).

1 Introduction

The use of sludge from water treatment plant for agricultural purposes has been widely studied from the point of view of improvment products (10) as well as the point of view of the sanitary aspect (2, 3, 4, 6, 12).

Within that frame, a mixed composting experiment was made at The Lavandou on the french mediterranean coast using sludge on one side and garbage on the other. It as seem interesting to study the parasitological contamination not only of sludge and garbage but also of composts all through the composting processes. We have tried to reveal the presence of helminth eggs in our samples.

2 Materials and Methods

2.1 Sampling

Analyses have been made on raw materials and on several composts in which the proportion of raw materials was variable.

2.1.1 Raw materials

Two raw materials have been analysed:
Crushed garbage and sludge

2.1.1.1 Crushed garbage

It was taken from the garbage station of The Lavandou which processed 10000 tons of garbage in 1983. The distribution over one years shows an increased production during the summer with 60 to 75 tons per day whereas in the rest of the year only 17 to 30 tons per day are gathered. The sampling was made by random selection on the moving belt of the mixer during the crushing processes.

2.1.1.2
Sludge

Sludge were provided by sewage treatment plant at Bormes. The station included two treatment systems.

- one using activated sludge is in service all the time,
- and there is also a physicochemical one in service only during the

2 or 3 months of the summer.

When the 2 systems are in operation, the number of population equivalents is 73000.

Some activated sludge (BB) are taken after déhydratation (BBS). Samples understudied constituted with a mixture of several random samplings. The physicochemical sludge are conditionned with several chemicals to obtain 2 types of mixtures.

Terbnary misture with lime (BCA)

$Ca(OH)_2$:	200 g/m^3
$FeC1_3$:	100 g/m^3
Polymer F A 57 H:	1.17 g/m^3

After this treatment, the pH of the sludge is 8.2.

Binary mixture with lime (BCB)

$FeC1_3$:	125 g/m^3
Polymer FA 57 H:	1.05 g/m^3

After this treatment, the pH of the sludge is 6.7 .

Sampling of each of this mixture are made after dehydratation so that two types of samples are obtained: physicochemical dried sludge with lime (BCAS) and physicochemical dried sludge without lime (BCBS) .

2.1.2
The composts

Three experimental mixtures of variable but well defined composition have been brought together in order to make the compost. In all of them there were 65 % of garbage and 35 % of dried sludge. According to the nature of the sludge, the 3 mixtures had the following composition and denomination:

OM-BB	:	garbage	+	activated sludge
OM-BCA	:	garbage	+	physicochemical sludge with lime
OM-BCB	:	garbage	+	physicochemical sludge without lime

After the beginning of the composting, the temperature inside the experimental mixtures rose to 70°C in 10 days. When the sampling was done, it fell to 55°—60°C in 2 or 3 days. That temperature remained constant for 120 days.

When the compost was made, the temperature of some of experimental mixtures was measured during 3 weeks and it was found to remain around 60°C.

Each windrow weight 40 tons and was 2 meters high. After 120 days of maturing, the windrow weight 20 tons. Sampling were made by drilling vertical hole in its middle. At the end of the composting processes, the samples were taken by quartering the terminal product according to AFNOR norm (NFU 44–101).

The sanitary control was carried out through parasitological analysis

- on the raw materials
- on mixture at the beginning of the composting
- in the windrow after 10, 25, 65 days and at the end of the composting process (120 days).

2.2 Methods

On arrival at the laboratory, the samples were pretreated and enriched then they were submitted to microscopic study.

2.2.1 Pretreatmert of samples

The solid samples were added with 1.5 1 of water, left to rest from 12 to 24 h then mixed and roughly sieved. The filtrate is centrifuged at 2800 rpm durirg 15 mn. The centrifugation pellet constitues the material to be enriched.

2.2.2 Enrichment technics

Among the enrichment technics given in the bibliography we have selected two floating technics (1, 5, 19) Their principle is a density differen rence between the parasites and the dilution liquid. Thus, on an aliquot part of the pellet (about 40 g) two enrichment technics were tried.

JANECKSO-URBANYI method using mercury iodid which is a good method to isolate helminth eggs (specific density 1. 44).

DADA and LINDQUIST method using sodium dichromate specially adapted to the research of *Ascaris* eggs in the earth (specific density 1.20).

2.2.3 Microscopic observation

Two plates were studied for each technic In all 208 plates were studied. Parasites have been identified by using number 40 or 100 objectives (8).

3 Results

It should be noted that the microscopic observation was made difficult because of the presence of various types of wastes and numerous structures having the character of artefact.

In addition, many samples incluted detriticols nematods more than 300 eggs in our investigations notably in compost after 3 years. This is the sign of a later vegetal contamination. The results are grouped in 4 tables showing the number of helminths eggs found in the various samples.

x As far as garbage only, the table 1 shows that parasites eggs are found in the final samples at the two sampling levels:

2 *Ascaris* spp eggs, 2 *Toxocara* spp eggs and 1 *Trichris* spp egg. No parasites eggs be found in the compost obtained from garbage only and stocked for 3 years.

x Concerning the garbage plus activated sludge compost (Table 2), it must first be said that no eggs was found in the garbage but that 1 *Ascaris* spp egg and 1 *Trichris* spp egg were found in the activated sludge. The *Trichuris* spp egg was perfectly viable since its growth from 2 to several blastomers could be observed in 3 days. In the compost, globaly 17 parasites eggs were observed: 4 eggs of *Toxocara* spp, 12 eggs of *Trichuris* spp and 1 *Hymenolepis* spp egg . In the samples made at the beginning of the composting process, the parasitological contamination seems higher with 15 eggs isolated than at the end of the composting with only 2 eggs observed.

x In the compost obtained from physichochemical sludge without lime treatment (Table 3), in general, 6 *Ascaris* spp eggs, 2 *Toxocara* spp eggs and 4 *Tricharis* eggs were found. Paradoxically, more eggs were observed at the end of the composting that observation is probably no significant and raises the problem of the consistancy of the samples.

x In the compost with addition of lime (Table 4), 6 Nematodes eggs were found: 2 *Ascaris* spp eggs, 2 *Trichuris* spp eggs, 2 *Toxocara*. spp eggs.

TABLE 1: PARASITOLOGICAL CONTAMINATION OF GARBAGE

GARBAGE ONLY							
SAMPLES	DATES	NUMBER OF OBSERVED PLATES	N E M A T 0 D E S		C E S T 0 D E S		
Ascaris spp	Toxocara spp	Trichuris spp	Hymenolepis spp	Taenia spp			
OM	27.10. 1983	4	–	–	–	–	–
9.11. 1983	4	–	–	–	–	–	
22.11.1983	4	–	–	1	–	–	
OM-100 final 1[x]	7.03.1983	8	1	–	1	–	–
OM-100 final 2[xx]	7.03. 1984	8	1	2	–	–	–
COMPOST 3 YEARS							
OM-100 final 1[x]	20.03. 1984	8	–	–	–	–	–
OM-100 final 2[xx]	20.03. 1984	8	–	–	–	–	–

[x]Final 1 : Sample made by quartering in the upper third of the windrow
[xxx]Final: Sample made by quartering in the lower third of the windrow

TABLE 2: PARASITOLOGICAL CONTAMINATION OF COMPOSTS OM-BB

GARBAGE+ACTIVATED SLUDGE							
SAMPLES	DATES	NUMBER OF OBSERVED PLATES	N E M A T O D E S		C E S T O D E S		
Ascaris spp	Toxoca ra spp	Trichuris spp	Hymenolepis spp	Taenia spp			
OM BB dried	27.10.1983	4	–	–	–	–	–
27.10.1983	8	1	–	1 viable	–	–	
OM-BB	27.10.1983	4	–	2	7	–	–
OM-BB (1)	15.11.1983	8	–	–	2	1	–
OM-BB (1)	20.12.1983	8	–	–	–	–	–
OM-BB							
final 1	22.02.1984	8	–	2	–	–	–
OM-BB	27.10.1983	4	–	2	7	–	–
OM-BB (2)	15.11 .1983	8	–	–	2	–	–
OM-BB (2)	20.12.1983	8	–	–	1	–	–
OM-BB							
final 2	22.02.1984	8	–	–	–	–	–

(1): Sample made by quartering in the upper third of the windrow
(2): Sample made by quartering in the lower third of the windrow

TABLE 3: PARASITOLOGICAL CONTAMINATION OF COMPOSTS OM-BCB

GARBAGE+PHYSICOCHEMICAL SLUDGE WITHOUT LIME							
SAMPLES	DATES	NUMBER OF OBSERVED PLATES	NEMATODES		CESTODES		
Ascaris spp	Toxocara spp	Trichuris spp	Hymenolepis spp	Taenia spp			
OM	22.11.1983	4	–	–	1	–	–
BCBS	22.11.1983	8	–	–	–	–	4
OM-BCB (1)	22.11.1983	4	–	–	–	–	–
OM-BCB (1)	20.12.1983	8	–	–	2	–	–
$OM\text{-}BCB_{final\ 1}$	19.03.1984	8	6	2	–	–	–
OM-BCB (2)	20.12.1983	8	–	–	2	–	–
$OM\text{-}BCB_{final2}$	19.03.1984	8	–	–	–	–	–

(1): Sample made by quarteringin the upper third of the windrow
(2): Sample made by quartering in the lower third of the windrow

TABLE 4: PARASITOLOGICAL CONTAMINATION OF COMPOSTS OM-BCA

GARBAGE+PHYSICOCHEMICAL SLUDGE WITH LIME							
SAMPLES	DATES	NUMBER OF OBSERVED PLATES	NEMATODES		CESTODES		
Ascaris spp	Toxocara spp	Trichuris spp	Hymenolepis spp	Taenia spp			
OM	9.11 .1983	4	–	–	–	–	–
BCAS	9.11.1983	8	2	–	–	–	–
OM-BCA (1)	9.11 .1983	6	–	1	–	–	–
OM-BCA (1)	20.12.1983	8	–	–	1	–	–
OM-BCA $_{final\ 1}$	7.03.1984	8	2	–	–	–	–
OM-BCA (2)	9.11.1983	6	–	–	–	–	–
OM-BCA (2)	20.12.1983	8	–	–	1	–	–
OM-BCA$_{final\ 2}$	7.03.1984	8	–	–	–	–	–

(1): Sample made by quartering in the upper third of the windrow
(2): Sample made by quartering in the lower third of the windrow

4
Conclusion

On the point of view of raw materials, the garbage are slightly contamined since only 1 *Trichuris* spp egg was recovered from 16 observed plates. In the dried activated or physicochemical sludge (18, 20), the contamination is higher with 9 eggs (3 *Ascaris* spp, 4 *Taenia* spp, 2 *Trichuris* spp).

In the mixt compost of sludge and garbage we have revealed the presence of Cestodes and Nematodes eggs among which *Ascaris, Trichuris* and *Toxocara* are the most numerous. No Trematodes eggs was found.

Helminth eggs were present in 60 % of the final samples after 120 days of composting (14, 15). However it's necessary to know if the recovered eggs are viable or not. For this purpose the viability of the *Ascaris* eggs from the final compost were assessed by incubating a suspension in water at 20°C about 30 days. The microscopically examination showed no developped eggs. This observation agrees with the publications on the survival of parasites eggs (7, 17), but we think that further studies about viability of the recovered eggs are necessary to surely determine if the presence of these eggs in the compost does n't present a risk for animal or human health (11, 16, 21) .

5
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BACTERIOLOGICAL AND PARASITOLOGICAL INVESTIGATIONS ON THE INFLUENCE OF FILTER BEDS COVERED WITH REED ON THE SURVIVAL OF SALMONELLAS AND ASCARIS EGGS

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Summary

In the framework of a joint research project about the effect of reed in filter beds on the dewatering of different kinds of sewage sludge hygienic-bacteriological and parasitological investigations were performed for a period of about one year. Salmonella senftenberg and eggs of Ascaris suum were used as test agents. The sludges which were used for charging the filter beds, the dried sludges in the filter beds and the filtrates were analyzed for the test microbes and for indigenous salmonellas. In addition counts of aerobic total germs, coliforms and enterobacteriaceae were made.

Until today no evidence could be found of an eliminating or reducing effect of the rhizome sphere of the reed Phragmites communis on bacteria like salmonellas, coliforms, enterobacteriaceae or on eggs of Ascaris suum, as described elsewhere (11). The filter beds planted with reed had no significant advantages from the hygienic point of view compared with filter beds without reed.

1. INDRODUCTION

In a current research program under the heading “Investigations on the use of plants for dewatering of sewage sludge” we are engaged with hygienic-bacteriological experiments. In the framework of this project the feasibility is investigated to dewater sewage sludges in filter beds which are planted with reed (Phragmites communis). The investigations are performed in a full-scale pilot plant in the sewage works of a municipality in the vicinity of the City of Ulm. For the experiments digested sludge, aerobically stabilised sludge or not stabilised surplus sludge from predominantly municipal sewage were used.

With regard to the Federal Sludge Ordinance which stipulates that beginning with 1st January 1987 only hygienically safe sludge may be utilized on pasture and forage land and arable land for certain crops it was of interest to investigate the capability of this method to eliminate pathogens from the sludge. Nearly 40 % of the sewage sludges produced in the Federal Republic of Germany are utilized in agriculture and therefore it must be ensured that such sludges are hygienically safe (10).

Besides some other plants which were utilized for the purification of sewage and dewatering of sludge Phragmites communis so far was used most frequently as representative of the sweet grasses (Poaceae). This perennial and strongly competitive plant is growing very densely to a height of 4 m and in flooding water occasionally even up to 10 m. It grows best in inun-

There is not much information available from literature about the influence of plants on parasite stages. It is mentioned that in South Africa the grass Eragnostis curvula is appreciated because of its decontaminating effect in soil against nematodes when used for intercropping (7). In the meantime some more plants are known which are said to have similar effects on the helminth fauna of the soil. Noticeable effects of some higher water plants on the eggs of intestinal helminths in humans have also been described.

2. MATERIAL AND METHODS

2.1 Pilot-plant

In the small town Dornstadt near Ulm a first sewage purification plant was installed in 1960/61 for 5.500 population equivalents. For primary purification an Emscher tank was used and the secondary biological treatment was performed in 4 activated sludge tanks with 100 m^3 volume each. The sludge was stored in a silo or in sludge drying beds with a volume of 80 m^3 each. During later constructional measures for an enlargement of the sewage works these sludge drying beds were put out

of operation and are now available for the investigations with reed as a pilot-plant. These beds were filled with gravel and sand and drained by a pipe-line system. The effluent of each filter bed was separately collected and measured in a specially built station. The 4 beds were divided into an implanted control-bed (16 m^2) and a planted bed (46 $m^{2)}$ (Fig. 1).

2.2
Planting of the filter beds with reed

On 15 April 1981 the beds were planted with reed (Phragmites communis), 8 plants/$m^{2,}$ pot-plants and layers in equal amounts. In 1981 the reed grew only sparse and in 1982 the plants were infested with greenflies. By these circumstances a continuous charging program for the reed beds was delayed and it was only in March 1983 that the experiments could be started as originally planned. The plant devoleped only in spring 1983 a luxuriant formation of rhizoma and subsequently a good growth in length and density (Fig. 2).

2.3
Nature of sludges and volumes used for charging of the reed planted filter beds

For the charging of the filter beds the following sludges were used:

a) Excess sludge (ÜS)
b) Aerobically stabilized sludge with extended aeration (AST)
d) Digested sludge=mixture from primary and secondary sludges (FS).

The charging quantities were as follows:

	Bed 1	(ÜS)				Bed 2	(ÜS)		
Control	bed	(1.1)	1.6	m^3	Control	bed	(2.1)	0.8	m^3
Planted	bed	(1.2)	4.6	m^3	Planted	bed	(2.2)	2.3	m3
	Bed 3	(AST)				Bed 4	(FS)		
Control	bed	(3.1)	0.8	m^3	Control	bed	(4.1)	0.8	m^3
Reed	bed	(3.2)	2.3	m^3	Reed	bed	(4.2)	2.3	m^3

dated miry soils which are rich in nutrients (1, 17). Then the part of the blade which is close to the soil is prolongating but it always stays in the same expanse. With the support of its internodical meristem at every node of the blade it is able to produce secondary roots which are able to trans-form, parch up and mineralize the sludge (15).

Besides the possibilities to dewater sludge by the effects of reed we are especially interested in the influence of reed on pathogens. Certain higher plants are able by adjusting the pH to stimulate bacteria and other microorganisms to a better efficiency in self-purification of the surface waters. On the other hand it is said that such plants are also able by microbicidal excretions of their roots and enrichment of oxygen to inactivate pathogenic microorganisms. The decreasing influence of higher plants on Escherichia coli and salmonellas is stressed by that author (12) . In pot experiments with water plants E.coli was reduced to 99 % by several plants within a contact time of 2 hours and also the numbers of Streptococcus faecalis and salmonellas were decreased considerably (11, 12). In testing a root zone purification system first results relating to praxis showed an elimination of total germs, E.coli and salmonellas up to 99 % (9) .

The assumption of Seidel (11) that excretions of the roots with a bactericidal effect could be the cause of the reduction of these bacteria induced another group of researchers to investigate the antibiotic effects of the rush Scirpus lacustris L. (6, 8). They detected microbicidal substances in the rhizome (syring aldehyde,veratrum aldehyde, vanillin, vanillin acid, paracumar acid) but they could not prove their direct transfer into the zone close to the roots.

The root of Iris pseudocoris L. is excreting the not-proteinogenic amino-acid 3-(3-carboxyphenylalanin) into the substrate. But there it is microbiologically degraded so that the antibacterial effect is doubtful (2). The antibacterial effect of certain algae was investigated during their utilization for self-purification of waters. During heavy growth of the green alga Hydrodictyon reticulatus and of the blue-green alga Aphanotece nidulans a reduction of E.coli was observed and as the cause of this effect the authors supposed a volatile or rather quickly decomposable active substance (5). In accordance with others (11, 19) the authors stated that the oxygen saturation of the water could as well be the cause for the reduction of E.coli. If this is in the range of 10 mg/1 as a simple concomitant phenomenon of the photosynthetic activity of the algae mentioned antibacterial conditions can result.

These remarks elucidate that up to now it was not possible to furnish the unambiguous proof of the bactericidal effect of excretions of higher water plants and algae.

In this context it is referred to the observed relations between Vibrio cholerae and the water hyacinth Eichhornia crassipes. After outbreak of cholerae in Bangladesh large numbers of vibrios were found on water plants. In experiments it could be shown that the cholera vibrios are reduced in the water but are attached chiefly to the surface of the water plants. After 5 days of experiment a 300-fold higher concentration of Vibrio cholerae was found in the root system than in the surrounding water. From that it is concluded that free swimming plants or parts of plants are a vehicle for the spread of cholera vibrios or a reservoir in inter-epidemic phases of the disease (16).

2.4 Test germs and germ carriers

For the bacteriological investigations Salmonella senftenberg was used. The germs were absorbed as suspension to small patches of silk-gauze and dried. The some was done with small patches of leather (4×4 cm). These germ-carriers were given into tea-egg-like receptacles. The reisolation of the salmonellas was made with pre-enrichment in peptone-water (18 h/37°C), enrichment in tetrathionate-broth after Müller-Kauffmann (24 h and 48 h, resp., at 37°C and 43°C), isolation and identification on brillantgreenphenolred-lactose-agar and bromthymol-blue-agar with subsequent biochemical and serological identification. Counts of total germs, coliforms and enterobacteriaceae were done in the usual way.

For parasitological investigations eggs of Ascaris suum were used. One million of eggs were given in bagsmade of silk-gauze and deposited in the mentioned receptacles. For reisolation of the parasite eggs they were resuspended with water in Petri-dishes, incubated for 29 days at 29°C and the development of larva II was controlled. 100 eggs were counted at random and their development via the morula stage to larva II recorded proportionally (0–100) and evaluated as positive. Morphological changes of eggs and larva II as vesicular degeneration were evaluated as negative. To control the infectivity of larvae II occasionally the larvae were given to mice per oral. The finding of larva III in their liver after 48 h was evaluated as positive.

The receptacles with the germ carriers were distributed in the filter beds. Within the filter beds an area of ca. 0.5 m^2 was separated and infected with 0.5 liter of a pure culture of S. senftenberg in broth.

In certain intervals samples of the germ carriers were taken out of the filter beds. From the area which was directly inoculated with salmonella suspension samples were taken from the upper layer of sludge and from the ground directly at the contact surface of sludge and sand.

3. RESULTS

It was possible to reisolate the test salmonellas from the germ carriers brought out in May 1983 until September 1983. In the time between June and August a reduction of positive samples was registered (Table 1).

In the reed bed with excess sludge (ÜS-bed 2.2) and the control bed with aerobically stabilized sludge (AST-3.1) in October 1983 indigenous salmonellas were isolated for the first time (Tables 1 and 9). From that time on no test salmonellas could be reisolated. In November 1983 indigenous salmonellas were isolated from all filter beds. This tendency is lasting until today. In the collected filter effluent only in February 1984 in beds US-2.1 and 2.2 indigenous s. paratyphi B and another serovar, which could not exactly be identified, could be found for the first time (Table 9). In March, April and May more indigenous salmonellas were isolated from the filter effluents. Only from the effluent of bed 3.2, covered with reed and charged with aerobically stabilized sludge, during the whole investigation period no indigenous salmonellas were isolated though they were present in the sludge within the bed (Table 9).

From the sludge which was directly infected with salmonella suspension in a separated area of the filter beds only 3 months salmonellas could be reisolated with the exception of beds 2.1 and 3.1 (Table 1) where salmonellas were never reisolated.

The sludge samples of all filter beds were also investigated for aerobic total germs, enterobacteriaceae and coliforms. In general the total germ numbers varied very little, independently of the vegetation of the beds (Tables 2 and 3) and were in the range of 10^6–10^9/ml corresponding to the values of the charged sludges (Fig. 3).

The total germs of the upper layers of the sludge were also in the range of 10^6–10^9/ml and there was not much difference to the samples taken from the contact surface to the sand. The total numbers of germs in the filter effluent varied between 10^3 and 10^7/ml and were reduced for 2–3 logs compared to the charged sludges. Serious differences between the beds could not be observed (Tables 2 and 3).

The enterobacteriaceae in the ÜS-sludges were in the range of 10^4 -10^7/ml (Ø 1.2 x 10^7), AST-sludges and FS-sludges between 105 and 108 (Ø 2.0 x 10^7; Tables 4 and 5). In the upper layers of sludge more than 10^7/ ml enterobacteriaceae were counted in the mean of all samples and they were slightly above the values of the charged sludge. In the samples from the contact surfaces to the sand of the control beds the values of the enterobacteriaceae were somewhat higher than those of the upper layer. In the reed beds an opposite tendency was observed (Tables 4 and 5, Fig. 4).

The filter bed effluent had 105/ml enterobacteriaceae in the average. This is a reduction of 1–3 logs compared with the sludges charged onto the beds (Tables 4 and 5).

Coliforms were 7.0×10^5/ml in the charged US-sludge and 10^6/ml in AST- and FS-sludges (Tables 6 and 7). The mean values in the upper sludge layers of the beds were slightly higher than those of the charged sludges whereas the values of the contact surface to the sand corresponded roughly with those of the charged sludges (Tables 6 and 7). The values of the filter bed effluent were in general ca. 2 logs below those of the charged sludges and of the sludges in the beds, respectively (Tables 6 and 7, Fig. 5).

The ascaris eggs given into the beds in July 1983 were viable for 9 month, excepted the beds 2.1 and 4.3 (Table 8). Since May 1984 their viability was interrupted except in bed 3.1. Serious differences in the results of the beds with an without reed were not observed. Remarkable is the decrease of the viability of the eggs between December 1983 and January 1984 in all of the beds which recovered with the beginning spring.

4. DISCUSSION

The dewatering of sludge by means of plants is an alternative to the now common technical-mechanical methods.

After exposure of the salmonella-infected germ carriers to the charged sludges in the filter beds in May 1983, salmonellas were reisolated from them only until September 1983. After that there were no more positive isolations (Table 1). It can be discussed whether the described influence of microbicidal substances produced in the rhizome system of the reed (11, 12, 14) was involved in this process of inactivation of the salmonellas. But it seems that this could not have been the sole reason because salmonellas were reisolated also only for 4 months from the germ carriers in the filter beds without reed. From those separated areas of the beds which were infected directly with a suspension of salmonellas the test germs could also be reisolated for 3 months. Only from the control beds (ÜS-2.1 and AST-3.1 salmonellas could never be reisolated. During this period all the control beds without reed (2.1; 3.1; 4.1) were heavily overgrown with weeds which had a very dense root system extending into the layer of sand and gravel. The test salmonellas were not drained into the filter effluent. It could therefore also be discussed whether the roots of the weeds could excrete antibacterial substances and influence the test salmonellas by that.

The numerous indigenous salmonellas in the beds were partly also isolated from the charged sludges. Their isolation in the various beds, depths and filtrates do not indicate a definite preference of the beds covered with reed. Only in the filtrate of the reed-covered AST-bed 3.2 (Table 9) no salmonellas could be found until now whereas from the sludge of that bed salmonellas were isolated.

Generally the results of the bacterial counts of all samples of all beds were similar regardless of the vegetation with or without reed and the kind of sludge (Tables 2–7). A possible explanation for the circumstances of the numbers of germs in the US-beds is offered by an observation of Zacher (20) that a large amount of the sludge immediately after charging does not stay on the surface of the older sludge layer but does ooze into the deeper layers of sludge thus displacing them upwards and causing a homogenization of stored and freshly charged sludge.

Aerobically stabilized and digested sludges were apparently not as good mixed due to their higher dry matter content and the more dense packing Thus the liquid phase oozed into the drainage system but the dry matter sedi mented to a large extent on the surface of the already stored sludge. The age of the sludge therefore is increasing from above to below. The humified layer close to the contact surface of the sand in the AST-bed 3.2 and the FS-bed 4.2 confirm these observations. The oozing of a large part of the liquid phase gives an explanation for the slightly higher germ numbers of the upper layers (SO) compared to those of the charged sludges (BS; Tables 2–7). Since the water content of the charged sludges is unequally higher than that of the stored sludge (SO) in the upper layers, the higher amounts of germs in this layer is not the result of a propagation but only the result of a loss of water.

In the majority of the experiments the germ numbers in the lower layers of sludge (SU) were lower than those in the upper layers (SO; Tables 2–7) even those beds without reed (2.1; 3.1; 4.1). Therefore this cannot be attributed to the influence of the reed with its rhizome but rather to the anaerobic conditions in the deeper zones of the beds which adversely affect the aerobic bacterial flora. This possibility corresponds to the statement of Dettwiler (3) that anaerobic conditions may results in certain effects of hygienization.

Contrary to the conditions within the filterbeds there were considerable differences in the germ numbers of the deeper zones (SU) of all beds and the filter bed effluent (Tables 2–7), compare SU and SW). Depending on the type of bacteria and bed the difference was 1–3 log and it must be added that in the majority of cases the filtrates of the beds with reed had lower numbers of germs than those without reed. But this difference is statistically insignificant. Therefore the assumption that the germ numbers have been reduced by the influence of the reed is doubtful. In fact this could correspond to the opinions of Kickuth (8) and Seidel (11) but the results available are not appropriate to differentiate between an eventual germ-reducing influence of the reed and the influence of the gravel-sand layer particularly since the reed is rooted in that filter layer.

The results of the filter effluent of the control beds without reed (2.1; 3.1; 4.1; Tables 2–7) indicate that the gravel-sand layer is involved in the reduction of germs. The germ numbers of these filtrate samples differ only insignificantly from those of the beds with reed. The opinion that bacteria and viruses die-away after passage of the sludge liquor caused by the changing living conditions is supported by Frank and Schmidt (4) based on their experimental findings. During the passage of sludge liquor through the filter organic particulate matter is kept back by filtration and adsorption. Especially bacterial populations with their gel capsule offer good possibilities for adsorption.

Based on our results the charged sludges are often contaminated with indigenous salmonellas which are drained into the filter beds and are persisting there for a longer time. An eliminating efficiency of the reed or the gravel-sand layer for salmonellas cannot be definitely proved, especially because in the filtrates of all beds, with one exception (3.2; Table 9), salmonellas were found. For hygienic reasons the filtrate therefore should not be drained via the effluent of the sewage works into the receiving waters.

Therefore we have to start out that even a humified material derived from sludge can be contaminated with salmonellas and eggs of parasites for a longer time. Also reproductive residues of the rhizome of the reed may disturb the agricultural utilization of that material. For these reasons a further treatment of the humified material seems to be necessary to eliminate the pathogens and destroy the reproductiveness of rhizome residues. Preferably this should be done by composting with bulking material. In the framework of this research project we started respective experiments with an 8 years old humified sludge from another treatment plant which is using reed-planted filter beds for dewatering sludge for nearly 10 years.

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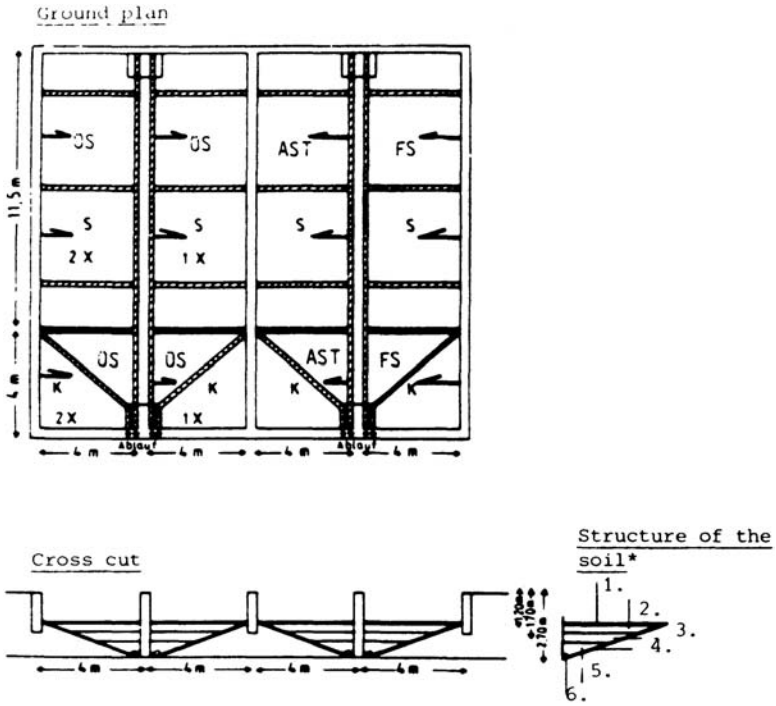

Fig. 1. Ditch reed-planted dehydration-beds at the sewage plant Dornstadt

ÜS/S	2x	Surplus-sludge/	ditch reed (double-charging)
ÜS/K	2x	Surplus-sludge/	control (double-charging)
ÜS/S	1x	Surplus-sludge/	dirch reed (simple-charging)
US/K	1x	Surplus-sludge/	control (simple-charging)
AST/S	Aerobically stabilized sludge/		ditch reed
AST/K		Aerobically stabilized sludge/	control
FS/S		Digested sludge/	ditch reed
FS/K		Digested sludge/	control
□1	=	Sand	
2	=	Finely-grained gravel	
3	=	coarse-grained gravel	
4	=	Broken stone mixture	
5	=	Concrete base	
6	=	Draining pipe system	

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Table 1. The reisolation of test-salmonellas (K) from the germ-carriers, the reisolatlon of the salmonella-suspension (s) and the other salmonella-types found beds (F)

	Bed 2 (DS)						Bed 3 (AST)						Bed 4 (FS)					
Month	**Control-bed**			**Planted-bed**			**Control-bed**			**Planted-bed**			**Control-bed**			**Planted-bed**		
(2.1)			**(2.2)**			**(3.1)**			**(3.2)**			**(4.1)**			**(4.2)**			
K	**S**	**F**	**K**	**S**	**F**	**K**	**S**	**F**	**K**	**S**	**F**	**K**	**S**	**F**	**K**	**S**	**F**	
1983																		
June	+	−	−	+	+	−	+	−	−	+	−	−	+	−	−	+	−	−

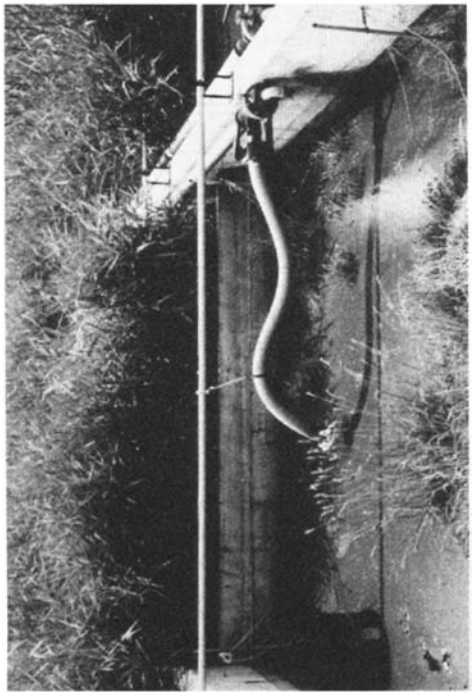

Figure 2. Ditch reed planted dehydration beds in Dornstadt (Dec. 1982)

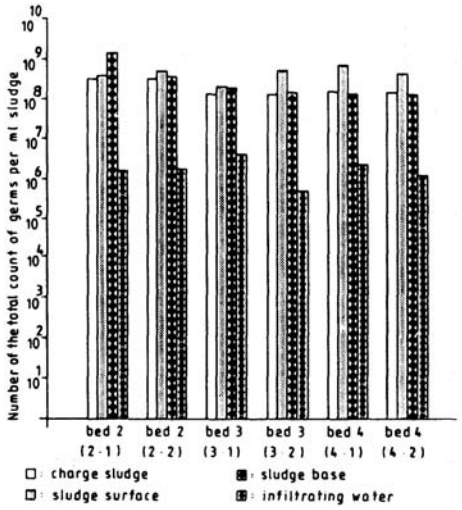

Figure 3. The average of the total count of germs

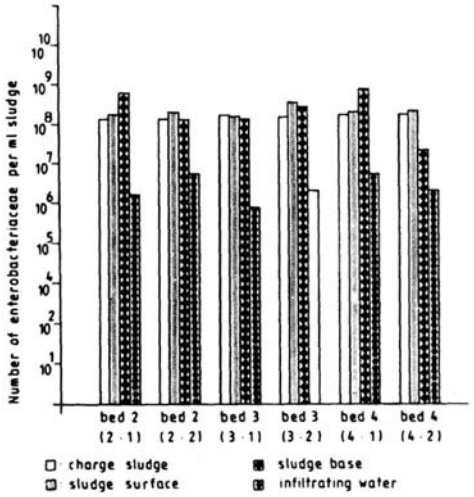

Figure 4. The average of the entero-becteriacea

	Bed 2 (DS)						Bed 3 (AST)						Bed 4 (FS)					
Month	**Control-bed**			**Planted-bed**			**Control-bed**			**Planted-bed**			**Control-bed**			**Planted-bed**		
(2.1)			**(2.2)**			**(3.1)**			**(3.2)**			**(4.1)**			**(4.2)**			
K	**S**	**F**	**K**	**S**	**F**	**K**	**S**	**F**	**K**	**S**	**F**	**K**	**S**	**F**	**K**	**S**	**F**	
July	+	−	−	+	−	−	+	−	−	+	−	−	+	−	−	+	−	−
August	−	−	−	+	+	−	+	−	−	+	+	−	+	+	−	+	+	−
September	+	−	−	+	−	−	+	−	−	+	−	−	+	−	−	+	−	−
October	−	−	−	−	−	+	−	−	+	−	−	−	−	−	−	−	−	−
November	−	−	+	−	−	+	−	−	+	−	−	+	−	−	+	−	−	+
December	−	−	+	−	−	−	−	−	+	−	−	+	−	−	+	−	−	+
1984																		
January	−	−	+	−	−	+	−	−	+	−	−	+	−	−	+	−	−	+
February	−	−	+	−	−	+	−	−	+	−	−	+	−	−	+	−	−	+
March	−	−	+	−	−	+	−	−	+	−	−	+	−	−	+	−	−	+
April	−	−	+	−	−	+	−	−	+	−	−	+	−	−	+	−	−	+
May	−	−	+	−	−	+	−	−	−	−	−	−	−	−	+	−	−	+
June	−	−	+	−	−	+	−	−	−	−	−	−	−	−	+	−	−	+

Bed 2 (OS)=surplus sludge / prtaery sludge

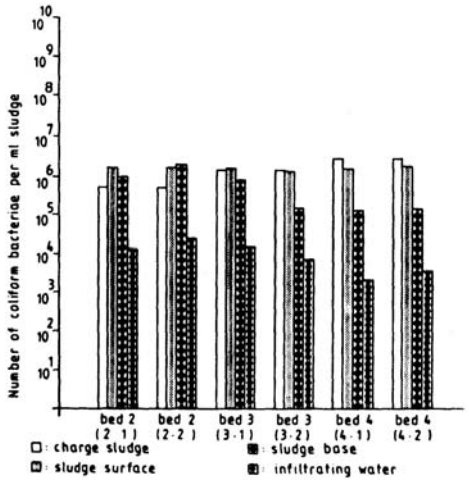

Figure 5. The average of the coliform bacteriae

	Bed 2 (DS)						Bed 3 (AST)						Bed 4 (FS)					
Month	**Control-bed**			**Planted-bed**			**Control-bed**			**Planted-bed**			**Control-bed**			**Planted-bed**		
(2.1)			**(2.2)**			**(3.1)**			**(3.2)**			**(4.1)**			**(4.2)**			
K	**S**	**F**	**K**	**S**	**F**	**K**	**S**	**F**	**K**	**S**	**F**	**K**	**S**	**F**	**K**	**S**	**F**	

2.1=control-bed
2.2=planted-bed
Bed 3 (AST)=aerobial stabilized sludge/secondary sludge
3.1=control-bed
3.2=planted-bed
Bed 4 (FS)=digested sludge
4.1=control-bed
4.2=planted-bed
K=reisolated test-salmonellas from the gern carrier
S=reisolated test-salmonellas from the sludge
F=reisolated other salmonellas from the sludge
+=isolated salwnellas
−=non isolated salmonellas

Table 2. The number of total count of germs per ml sludge of the charge-sludge (BS), the sludge-surface-deposition (SO) and the sludge-base-deposition (SU) and the infiltration waters of every bed

	Bed 2 (OS)				Bed 3 (AST)				Bed 4 (FS)			
Mouth	Control-bed (2.1)				Control -bed (3.1)				Control -bed (4.1)			
BS	SO	SU	SW	BS	SO	SU	SU	BS	SO	SU	SW	
1983												
June	4.6×10^{8}	1.8×10^{8}	4.8×10^{8}	3.0×10^{5}	8.0×10^{6}	3.2×10^{7}	1.6×10^{7}	4.6×10^{5}	2.5×10^{7}	1.2×10^{8}	7.6×10^{7}	4.0×10^{3}
July	6.3×10^{8}	1.4×10^{9}	9.8×10^{9}	8.6×10^{7}	6.2×10^{8}	3.2×10^{9}	7.6×10^{8}	7.2×10^{4}	2.0×10^{8}	2.7×10^{9}	1.2×10^{10}	2.4×10^{3}
August	5.0×10^{8}	1.0×10^{8}	1.7×10^{8}	1.5×10^{5}	7.2×10^{8}	3.0×10^{8}	4.1×10^{8}	1.7×10^{5}	6.4×10^{7}	5.0×10^{8}	2.1×10^{9}	6.5×10^{6}
September	3.0×10^{7}	1.3×10^{7}	3.6×10^{7}	1.2×10^{3}	6.5×10^{6}	9.5×10^{5}	8.2×10^{7}	1.4×10^{4}	5.3×10^{8}	5.8×10^{7}	1.9×10^{8}	1.6×10^{5}
October	1.0×10^{7}	7.1×10^{7}	3.5×10^{7}	3.8×10^{4}	1.6×10^{7}	8.0×10^{7}	3.2×10^{7}	1.2×10^{4}	1.0×10^{8}	3.0×10^{8}	3.4×10^{8}	2.1×10^{5}
November	3.0×10^{8}	3.7×10^{9}	3.8×10^{8}	3.0×10^{3}	1.2×10^{7}	2.3×10^{8}	2.5×10^{8}	9.0×10^{3}	2.2×10^{8}	3.9×10^{7}	1.3×10^{7}	3.4×10^{5}
December	4.4×10^{6}	9.5×10^{8}	2.7×10^{9}	6.0×10^{6}	1.5×10^{7}	5.3×10^{8}	2.6×10^{9}	1.5×10^{6}	6.2×10^{6}	5.8×10^{8}	3.3×10^{7}	2.4×10^{6}
1984												
January	4.5×10^{7}	7.1×10^{7}	1.3×10^{7}	6.0×10^{5}	2.0×10^{6}	3.0×10^{7}	3.7×10^{7}	2.0×10^{4}	6.0×10^{8}	1.6×10^{8}	2.1×10^{7}	3.6×10^{5}
February	1.0×10^{7}	1.8×10^{7}	1.0×10^{8}	8.0×10^{4}	4.7×10^{7}	7.0×10^{7}	3.0×10^{7}	1.0×10^{3}	4.4×10^{7}	3.3×10^{7}	1.2×10^{7}	1.1×10^{5}
March	4.0×10^{6}	4.0×10^{7}	5.4×10^{7}	2.3×10^{5}	8.0×10^{6}	4.1×10^{8}	1.2×10^{8}	3.3×10^{4}	2.1×10^{7}	5.0×10^{9}	5.0×10^{8}	5.5×10^{4}
April	1.0×10^{7}	1.2×10^{8}	1.1×10^{8}	2.2×10^{5}	1.1×10^{7}	3.3×10^{7}	3.2×10^{7}	1.1×10^{5}	2.6×10^{8}	2.1×10^{7}	1.4×10^{7}	1.8×10^{5}

	Bed 2 (OS)				Bed 3 (AST)				Bed 4 (FS)			
Mouth	Control-bed (2.1)				Control -bed (3.1)				Control -bed (4.1)			
BS	SO	SU	SW	BS	SO	SU	SU	BS	SO	SU	SW	
May	2.0×10^{7}	6.7×10^{7}	4.1×10^{7}	3.9×10^{7}	1.2×10^{7}	9.0×10^{8}	3.3×10^{8}	5.6×10^{7}	5.2×10^{7}	9.2×10^{8}	5.5×10^{7}	3.6×10^{7}
June	8.6×10^{7}	2.5×10^{9}	1.1×10^{8}	4.8×10^{6}	7.5×10^{6}	2.5×10^{8}	1.1×10^{8}	2.3×10^{6}	5.4×10^{8}	4.4×10^{8}	2.1×10^{8}	3.7×10^{6}
x̄	5.0×10^{8}	5.3×10^{8}	1.1×10^{9}	1.8×10^{6}	1.1×10^{8}	3.8×10^{8}	3.6×10^{8}	6.3×10^{6}	2.0×10^{8}	8.6×10^{8}	1.3×10^{8}	3.8×10^{6}

Bed 2 (OS)=surplus sludge/primary sludge
2.1=control-bed
Bed 3 (AST)=aerobial stabilized sludge/secondary sludge
3.1=control-bed
Bed 4 (FS)=digested sludge
4.1=control-bed
BS=charge sludge
SO=sludge surface
SU=sludge bas**e**
SH=**infiltration water**

Table 3. The number of the total count of germs per ml sludge of the charge-sludge (BS). the sludge-surface-deposition (SO) and the sludge-base-deposition (SU) and the infiltration waters of every bed

	Bed 2 (OS)				Bed 3 (AST)				Bed 4 (FS)			
Month	Planted-bed (2.2)				Planted-bed (3.2)				Planted-bed (4.2)			
BS	SO	SU	SW	BS	SO	SU	SW	BS	SO	SU	SU	
1983												
June	4.6×10^{8}	1.1×10^{7}	5.5×10^{6}	3.2×10^{6}	8.0×10^{6}	2.0×10^{7}	1.0×10^{7}	3.0×10^{5}	2.5×10^{7}	2.6×10^{7}	3.8×10^{7}	6.2×10^{3}
July	6.3×10^{8}	9.3×10^{7}	3.4×10^{9}	6.6×10^{5}	6.2×10^{8}	6.2×10^{7}	5.3×10^{7}	2.4×10^{6}	2.0×10^{8}	3.6×10^{9}	8.0×10^{8}	2.0×10^{3}
August	5.0×10^{8}	1.6×10^{8}	8.0×10^{7}	3.4×10^{6}	7.0×10^{8}	1.6×10^{8}	8.0×10^{7}	1.7×10^{5}	6.4×10^{7}	6.8×10^{7}	3.2×10^{8}	9.0×10^{4}
September	3.0×10^{7}	2.2×10^{7}	1.7×10^{7}	6.7×10^{3}	6.5×10^{6}	6.4×106	8.6×10^{8}	3.1×10^{4}	5.3×10^{8}	1.6×10^{7}	4.2×10^{7}	2.5×10^{4}
October	1.7×10^{7}	4.1×10^{7}	7.0×10^{7}	2.0×10^{5}	1.6×10^{7}	2.6×10^{8}	3.3×10^{7}	8.0×10^{3}	1.0×108	9.7×10^{7}	1.4×10^{7}	3.2×10^{5}
November	3.0×10^{8}	3.5×10^{8}	3.4×10^{6}	2.0×10^{3}	1.1×10^{7}	2.9×10^{9}	3.8×10^{8}	1.6×10^{4}	2.2×10^{8}	1.7×10^{7}	4.0×10^{6}	4.1×10^{9}
December	4.4×10^{6}	2.0×10^{7}	6.0×10^{6}	1.4×10^{5}	1.5×10^{7}	5.5×10^{9}	7.7×10^{8}	5.4×10^{6}	6.2×10^{6}	4.8×10^{8}	3.0×10^{8}	3.4×10^{6}
1984												
January	4.5×10^{7}	1.9×10^{8}	3.7×10^{8}	1.7×10^{5}	2.0×10^{6}	4.0×10^{6}	5.0×10^{6}	1.0×10^{4}	6.0×10^{8}	6.3×10^{7}	6.0×10^{6}	5.0×10^{4}
February	1.0×10^{7}	1.7×10^{8}	1.8×10^{7}	4.0×10^{5}	4.7×10^{7}	3.0×10^{7}	1.5×10^{7}	1.0×10^{3}	4.4×10^{7}	2.3×10^{7}	2.7×10^{7}	1.0×10^{3}
March	4.0×10^{6}	7.2×10^{9}	1.5×10^{8}	1.9×10^{5}	8.0×10^{6}	2.8×10^{7}	3.0×10^{7}	2.5×10^{4}	2.1×10^{7}	1.7×10^{8}	1.3×10^{7}	1.8×10^{4}
April	1.0×10^{7}	4.5×10^{7}	2.0×10^{7}	3.2×10^{5}	1.1×10^{7}	4.1×10^{7}	8.0×10^{6}	2.4×10^{3}	2.6×10^{8}	9.4×10^{7}	2.8×10^{7}	8.3×10^{5}
May	2.0×10^{7}	3.4×10^{6}	6.0×10^{6}	1.6×10^{7}	1.2×10^{7}	5.5×10^{6}	5.4×10^{6}	2.4×10^{5}	5.2×10^{7}	1.2×10^{8}	4.8×10^{8}	7.8×10^{6}
June	8.6×10^{7}	5.7×10^{8}	2.4×10^{7}	3.5×10^{6}	7.5×10^{6}	6.2×10^{8}	7.7×10^{8}	1.1×10^{6}	5.4×10^{8}	4.0×10^{9}	3.3×10^{8}	8.4×10^{6}
x̄	5.0×10^{8}	6.8×10^{8}	3.2×10^{8}	2.0×10^{6}	1.1×10^{8}	7.4×10^{8}	1.2×10^{8}	7.4×10^{5}	2.0×10^{8}	6.9×10^{8}	1.6×10^{8}	1.7×10^{6}

Bed 2 (OS)=surplus sludge/primary sludge
2.2=planted-bed
Bed 3 (AST)=aerobial stabilized sludge/sec
3.2=planted-bed
Bed 4 (FS)=digested sludge
4.2=planted-bed
BS=charge sludge
SO=sludge surface
SU=sludge base
SW=Infiltrating water

Table 4. The number of enterobacterias per ml sludge of the charge-sludge (BS),the sludge-surface-deposition (SO), and the sludge-base-deposition (SU) and the infiltration waters of the non-planted-beds

	Bed 2 (OS)				Bed 3 (AST)				Bed 4 (FS)			
Month	Control -bed (2.1)				Control -bed (3.1)				Control -bed (4.1)			
BS	SO	SU	SU	BS	SO	SU	SW	BS	SO	SU	SW	
1983												
June	1.6×10^{6}	4.2×10^{5}	4.1×10^{5}	4.2×10^{5}	2.8×10^{6}	3.5×10^{5}	2.0×10^{6}	3.1×10^{4}	4×10^{6}	2.4×10^{6}	2.0×10^{5}	3.1×10^{3}
July	1.6×10^{7}	2.6×10^{7}	3.2×10^{8}	2.2×10^{6}	6.8×10^{7}	2.4×10^{7}	2.6×10^{7}	1.4×10^{3}	1.6×10^{6}	3.2×10^{7}	2.1×10^{8}	1.2×10^{3}
August	2.3×10^{6}	2.1×10^{6}	4.4×10^{6}	5×10^{3}	2.0×10^{6}	1.3×10^{6}	1.0×10^{6}	3.1×10^{3}	3.1×10^{6}	3.0×10^{5}	1.0×10^{6}	2.6×10^{3}
September	5.0×10^{4}	7.0×10^{4}	6.8×10^{7}	ø [3]	2.5×10^{6}	1.3×10^{5}	1.9×10^{6}	5.0×10^{2}	1.0×10^{7}	1.8×10^{7}	2.7×10^{7}	1.0×10^{4}
October	1.2×10^{6}	1.0×10^{7}	3.0×10^{6}	7.6×10^{1}	2.0×10^{6}	6.0×10^{6}	1.2×10^{7}	4.6×10^{3}	1.8×10^{6}	1.8×10^{7}	8.0×10^{6}	3.2×10^{4}
November	6.1×10^{7}	6.0×10^{7}	8.0×10^{8}	6.0×10^{2}	1.8×10^{8}	3.1×10^{7}	1.1×10^{8}	4.1×10^{3}	1.0×10^{7}	2.1×10^{7}	1.1×10^{6}	3.1×10^{5}
December	7.0×10^{5}	1.8×10^{8}	5.2×10^{7}	8.0×10^{3}	1.3×10^{5}	8.6×10^{7}	8.8×10^{6}	3.0×10^{4}	1.0×10^{5}	7.2×10^{7}	8.4×10^{8}	1.2×10^{5}
1984												
January	3.5×10^{7}	1.4×10^{7}	6.1×10^{5}	4.1×10^{3}	1.2×10^{5}	3.1×10^{6}	3.0×10^{6}	1.0×10^{3}	7.0×10^{7}	8.0×10^{6}	1.0×10^{6}	7.0×10^{3}
February	1.6×10^{6}	4.6×10^{6}	7.0×10^{7}	4.0×10^{3}	2.0×10^{6}	4.6×10^{6}	1.0×10^{7}	–	1.4×10^{7}	1.2×1 [6]	1.0×10^{6}	1.0×10^{3}
March	1.0×10^{5}	4.0×10^{6}	1.8×10^{6}	8.0×10^{4}	8.0×10^{6}	6.1×10^{7}	9.0×10^{6}	2.0×10^{4}	1.5×10^{6}	2.0×10^{8}	5.0×10^{7}	4.0×10^{3}
April	4.0×10^{6}	1.2×10^{7}	2.8×10^{6}	3.6×10^{4}	4.2×10^{6}	2.2×10^{6}	1.0×10^{6}	5.0×10^{3}	1.2×10^{8}	2.6×10^{6}	7.6×10^{6}	1.6×10^{4}
May	3.8×10^{5}	8.0×10^{5}	1.8×10^{6}	6.0×10^{4}	6.0×10^{6}	9.6×10^{6}	3.2×10^{5}	7.0×10^{4}	3.0×10^{7}	6.0×10^{5}	8.5×10^{6}	4.2×10^{5}
June	1.0×10^{5}	2.0×10^{6}	6.1×10^{6}	1.2×10^{5}	8.0×10^{5}	1.8×10^{7}	1.8×10^{6}	1.0×10^{6}	3.0×10^{7}	5.0×10^{7}	3.8×10^{7}	8.0×10^{6}
;	1.2×10^{7}	2.4×10^{7}	9.8×10^{7}	2.2×10^{6}	2.1×10^{7}	1.9×10^{7}	1.4×10^{7}	9.0×10^{4}	2.2×10^{7}	3.2×10^{7}	9.1×10^{7}	7.4×10^{5}

Bed 2 (OS)=surplus sludge/primary sludge
2.1=control-bed
Bed 3 (AST)=aerobial stabilized sludge/secondary sludge
3.1=control-bed
Bed 4 (FS)=digested sludge
4.1=control-bed
BS=charge sludge
SO=sludge surface
SU=sludge base
SW=Infiltratinn water

Table 5. The number of enterobacterias per ml, sludge of the charge-sludge (BS), the sludqe-surface-deposition (SO) and the sludge-base-deposition (SU) and the infiltration waters of the planted beds

	Bed 2 (OS)				Bed 3 (AST)				Bed 4 (FS)			
Month	Planted-bed (2.2)				Planted-bed (3.2)				Planted-bed (4.2)			
BS	SO	SU	sw	BS	SO	SU	SW	BS	SO	SU	SW	
1983												
June	1.6×10^{6}	4.0×10^{5}	1.1×10^{6}	4.6×10^{5}	2.8×10^{6}	1.0×10^{7}	2.0×10^{6}	2.6×10^{4}	4.0×10^{6}	2.0×10^{6}	1.8×10^{6}	2.0×10^{2}
July	1.6×10^{7}	2.2×10^{7}	5.2×10^{7}	4.1×10^{5}	6.8×10^{7}	4.0×10^{6}	2.0×10^{6}	6.0×10^{5}	1.6×10^{6}	8.0×10^{7}	2.2×10^{7}	1.8×10^{2}
August	2.3×10^{6}	4.5×10^{6}	8.5×10^{6}	1.0×10^{5}	2.0×10^{6}	3.3×10^{5}	1.5×10^{6}	2.9×10^{3}	3.1×10^{6}	6.0×10^{5}	6.8×10^{5}	1.4×10^{2}
September	5.0×10^{4}	8.5×10^{6}	5.5×10^{6}	–	2.5×10^{6}	1.8×10^{6}	1.6×10^{6}	–	1.0×10^{7}	7.5×10^{6}	6.0×10^{6}	3.6×10^{3}
October	1.2×10^{6}	6.0×10^{6}	4.0×10^{6}	1.7×10^{4}	2.0×10^{6}	1.4×10^{7}	3.6×10^{6}	1.1×10^{3}	4.8×10^{6}	2.0×10^{6}	8.0×10^{5}	1.0×10^{5}
November	6.1×10^{7}	1.7×10^{8}	5.0×10^{7}	3.0×10^{2}	1.8×10^{8}	8.1×10^{8}	5.0×10^{8}	3.1×10^{4}	1.0×10^{7}	6.0×10^{6}	2.4×10^{6}	1.9×10^{4}
December	7.0×10^{5}	6.8×10^{5}	8.0×10^{5}	3.4×10^{4}	1.3×10^{5}	1.5×10^{5}	1.4×10^{7}	2.8×10^{5}	1.0×10^{5}	6.4×10^{7}	2.8×10^{7}	1.8×10^{5}
1984												
January	3.5×10^{7}	5.6×10^{6}	7.0×10^{6}	6.0×10^{3}	1.2×10^{5}	6.0×10^{5}	6.1×10^{5}	–	7.0×10^{7}	9.0×10^{7}	3.0×10^{5}	2.1×10^{3}
February	1.6×10^{6}	8.0×10^{6}	3.0×10^{6}	2.0×10^{4}	2.0×10^{6}	1.6×10^{7}	1.5×10^{6}	–	1.4×10^{7}	2.4×10^{6}	2.4×10^{6}	–
March	1.0×10^{5}	1.0×10^{8}	6.0×10^{5}	4.0×10^{5}	8.0×10^{6}	2.4×10^{6}	2.0×10^{6}	1.5×10^{3}	1.5×10^{6}	1.1×10^{8}	6.0×10^{5}	1.0×10^{3}
April	4.0×10^{6}	9.2×10^{6}	2.8×10^{5}	1.7×10^{4}	4.0×10^{6}	3.0×10^{6}	1.2×10^{6}	–	1.2×10^{8}	1.1×10	9.0×10^{5}	4.6×104
May	3.8×10^{5}	1.2×10^{6}	4.8×10^{5}	1.0×10^{3}	6.0×10^{6}	7.2×10^{4}	6.2×10^{5}	2.4×10^{3}	3.0×10^{7}	5.0×10^{6}	8.0×10^{5}	1.3×10^{5}
June	1.0×10^{5}	1.6×10^{6}	1.7×10^{6}	8.0×10^{6}	8.0×10^{5}	3.2×10^{7}	7.0×10^{6}	3.0×10^{6}	3.0×10^{7}	4.2×10^{7}	1.8×10^{6}	3.0×10^{6}

	Bed 2 (OS)				Bed 3 (AST)				Bed 4 (FS)			
Month	Planted-bed (2.2)				Planted-bed (3.2)				Planted-bed (4.2)			
BS	SO	SU	sw	BS	SO	SU	SW	BS	SO	SU	SW	
x̄	1.2×10^{7}	2.7×10^{7}	1.1×10^{7}	7.2×10^{5}	2.1×10^{7}	8.0×10^{7}	4.1×10^{7}	3.0×10^{5}	2.2×10^{7}	3.2×10^{7}	5.2×10^{6}	2.8×10^{5}

Bed 2 (OS)=surplus sludge/primary sludge
2.2=planted-bed
Bed 3 (AST)=aerobial stabilized sludge / secondary sludge
3.2=planted-bed
Bed 4 (FS)=digested sludge
4.2=planted-bed
BS=charge sludge
SO=sludge surface
SU=sludge base
SW=infiltrating water

Table 6. The number of coliform bacterias per ml sludge, of the charge-sludge (BS).the sludge-surface deposition (SO), and the sludge-base-deposition (SU) and the infiltration waters of every non-planted-beds

		Bed 2 (US)				Bed 3 (AST)				Bed 4 (FS)		
Month		Control-bed (2.1)				Control -bed (3.1)				Control -bed (4.1)		
	BS	SO	SU	SW	SB	SO	SU	SW	BS	SO	SU	SW
1983												
June	3.6×10^{5}	3.2×10^{4}	3.0×10^{4}	3.0×10^{4}	2.5×10^{5}	4.8×10^{4}	7.2×10^{4}	1.8×10^{3}	4.5×10^{5}	2.1×10^{5}	1.2×10^{5}	2.1×10^{2}
July	2.8×10^{5}	1.8×10^{6}	5.2×10^{5}	6.0×10^{4}	2.4×10^{6}	5.2×10^{6}	8.4×10^{6}	2.2×10^{2}	7.2×10^{4}	2.0×10^{6}	1.1×10^{6}	2.0×10^{1}
August	3.0×10^{5}	3.2×10^{5}	2.5×10^{5}	1.5×10^{3}	4.1×10^{5}	2.5×10^{5}	8.5×10^{4}	2.0×10^{2}	2.1×10^{5}	1.6×10^{5}	7.7×10^{5}	1.5×10^{3}
September	5.5×10^{6}	1.0×10^{7}	1.2×10^{5}	–	3.1×10^{6}	1.9×10^{5}	2.2×10^{4}	4.0×10^{2}	3.2×10^{7}	1.5×10^{6}	3.3×10^{6}	–
October	1.0×10^{5}	1.0×10^{6}	8.5×10^{5}	5.0×10^{1}	3.0×10^{5}	8.0×10^{5}	7.0×10^{4}	1.4×10^{3}	2.7×10^{5}	7.0×10^{4}	1.6×10^{5}	2.5×10^{3}
November	9.0×10^{5}	8.0×10^{4}	2.7×10^{6}	1.0×10^{2}	9.0×10^{6}	1.2×10^{5}	1.2×10^{5}	2.0×10^{2}	3.2×10^{6}	4.3×10^{5}	1.3×10^{4}	1.3×10^{4}
December	4.0×10^{4}	2.0×10^{7}	3.0×10^{6}	3.0×10^{3}	2.2×10^{5}	9.0×10^{6}	1.4×10^{6}	1.9×10^{3}	4.8×10^{6}	7.0×10^{6}	3.8×10^{6}	1.0×10^{3}
1984												
January	5.0×10^{5}	9.7×10^{5}	7.0×10^{4}	9.0×10^{2}	5.5×10^{5}	1.0×10^{6}	2.0×10^{5}	–	3.3×10^{7}	2.9×10^{6}	5.0×10^{4}	7.0×10^{2}
February	2.5×10^{5}	1.5×10^{5}	1.5×10^{6}	1.3×10^{3}	1.1×10^{6}	8.0×10^{5}	1.7×10^{5}	–	4.0×10^{6}	1.0×10^{5}	6.0×10^{5}	5.0×10^{2}
March	1.2×10^{5}	7.0×10^{4}	1.9×10^{5}	3.0×10^{3}	3.9×10^{5}	5.0×10^{6}	2.0×10^{5}	6.0×10^{2}	4.0×10^{5}	1.1×10^{7}	3.0×10^{6}	5.0×10^{2}
April	4.6×10^{5}	1.1×10^{6}	7.4×10^{5}	1.3×10^{4}	1.0×10^{3}	4.4×10^{5}	2.1×10^{5}	1.0×10^{3}	9.0×10^{6}	5.6×10^{5}	2.0×10^{5}	5.4×10^{3}
May	3.0×10^{5}	4.2×10^{4}	7.0×10^{4}	1.1×10^{4}	2.4×10^{5}	2.8×10^{5}	1.9×10^{4}	4.2×10^{3}	6.3×10^{5}	4.1×10^{5}	2.3×10^{4}	3.9×10^{3}
June	9.0×10^{4}	4.5×10^{3}	3.0×10^{3}	9.0×10^{3}	8.0×105	6.2×10^{5}	1.1×10^{5}	5.4×10^{3}	3.3×10^{6}	7.2×10^{5}	6.0×10^{4}	9.0×10^{3}
x̄	7.0×10^{5}	2.7×10^{6}	7.7×10^{5}	1.0×10^{4}	1.5×10^{6}	1.8×10^{6}	9.9×10^{5}	7.4×10^{4}	7.0×10^{6}	2.0×10^{6}	1.7×10^{5}	2.9×10^{3}

Bed 2 (OS)=surplus sludge / primary sludge
2.1=control-bed
Bed 4 (FS)=aerobial stabilized sludge/seco
3.1=control-bed
Bed 3 (AST)=digested sludge
4.1=control-bed
BS=charge sludge
SO=sludge surface
SU=sludge base

Table 7. The number of coliform bacterias per ml sludge, of the charge-sludge (BS), the sludge surface deposition (SO), the sludge-base-deposition (SU) and the infiltration waters of every planted bed (5)

		Bed 2 (OS)				Bed 3 (AST)				Bed 4 (FS)		
Month		Planted bed (2.2)				Planted bed (3.2)				Planted bed (4.2)		
	BS	SO	SU	SW	BS	SO	SU	SW	BS	SO	SU	SW
1983												
June	3.6×10^{5}	4.0×10^{5}	1.1×10^{6}	4.6×10^{5}	2.5×10^{5}	1.0×10^{5}	2.0×10^{5}	2.6×10^{4}	4.5×10^{5}	6.8×10^{4}	7.2×10^{4}	2.0×10^{2}

Month	Bed 2 (OS) Planted bed (2.2)				Bed 3 (AST) Planted bed (3.2)				Bed 4 (FS) Planted bed (4.2)			
	BS	SO	SU	SW	BS	SO	SU	SW	BS	SO	SU	SW
July	2.8×10^5	4.9×10^6	3.2×10^7	6.0×10^4	2.4×10^6	3.2×10^6	4.8×10^5	8.0×10^4	7.2×10^4	4.2×10^6	1.4×10^6	1.0×101
August	3.0×10^5	2.8×10^5	5.6×10^5	1.5×10^4	4.1×10^5	1.5×10^5	3.2×10^5	1.5×10^3	2.1×10^5	1.5×10^5	1.6×10^5	5.0×10^1
September	5.5×10^6	3.6×10^5	6.2×10^6	–	3.1×10^6	2.0×10^5	1.7×10^5	–	3.2×10^7	2.2×10^6	1.5×10^7	1.3×10^3
October	1.0×10^5	2.1×10^6	6.0×10^5	4.0×10^2	3.0×10^5	2.2×10^6	2.6×10^5	4.0×10^2	2.7×10^5	1.0×10^5	1.0×10^4	4.7×10^3
November	9.0×10^5	2.4×10^5	9.0×10^4	–	9.2×10^6	1.1×10^6	2.4×10^5	1.3×10^3	3.2×10^6	2.4×10^5	6.3×10^4	2.8×10^3
December	4.0×10^4	6.0×10^5	2.0×10^6	4.0×10^2	2.2×10^5	7.0×10^6	1.9×10^5	1.3×10^3	4.8×10^6	1.8×10^7	7.0×10^5	2.0×10^4
1984												
January	5.0×10^5	1.9×10^6	1.3×10^6	2.0×10^2	5.5×10^5	3.0×10^5	8.0×10^4	–	3.3×10^7	1.0×10^6	6.0×10^4	–
February	2.5×10^5	2.4×10^6	1.7×10^5	3.0×10^3	1.1×10^6	7.0×10^5	5.0×10^4	–	4.0×10^6	3.0×10^5	1.0×10^5	–
March	1.2×10^5	6.0×10^5	3.0×10^5	1.0×10^4	3.9×10^5	2.0×10^6	1.0×10^5	4.0×10^2	4.0×10^5	4.0×10^6	7.0×10^4	1.0×10^2
April	4.6×10^5	1.4×10^6	1.0×10^5	4.3×10^3	1.0×10^6	9.0×10^5	9.4×10^4	–	9.0×10^6	1.3×10^6	9.6×10^4	3.4×10^4
May	3.0×10^5	1.9×10^4	1.2×10^4	1.3×10^3	2.4×10^5	1.8×10^4	3.4×104	1.1×10^3	6.3×10^5	4.5×10^5	1.8×10^5	3.4×10^3
June	9.0×10^4	5.5×10^5	1.3×10^5	1.1×10^3	8.0×10^5	3.7×10^5	1.2×10^5	1.7×10^3	3.3×10^6	4.5×10^5	1.0×10^5	2.0×10^3
;	7.0×10^5	2.5×10^6	3.3×10^6	3.9×10^4	1.5×10^6	1.4×10^6	1.8×10^5	8.9×10^3	7.0×10^6	2.4×10^6	1.3×10^6	5.2×10^3

Bed 2 (OS)=surplus sludge/primary sludge
2.2=planted bed
Bed 3 (AST)=aerobial stabilized sludge/secondary sludge
3.2=planted bed
Bed 4 (FS)=digested sludge
4.2=planted bed
BS=charge sludge
SO=sludge surface
SU=sludge base
SW=infiltrating water

Table 8. The development of the ascaris-eggs shown percentually of every sewage-sludge-filter-beds (starting June 1983)

Month	Bed 2 (OS) Control -bed (2.1)	Planted-bed (2.2)	Bed 3 (AST) Control -bed (3.1)	Planted-bed (3.2)	Bed 4 (FS) Control -bed (4.1)	Planted-bed (4.2)
1983						
August	72	73	69.5	59.5	58	66.5
September	84.5	81	75	49	77	79.5
October	82	81	83	91	97	93
November	80	72	81	76	76	80
December	71	84	36	61	85	38
1984						
January	5	2	5	1	5	13
February	–*	24	24	19	30	1
March	16	3	73	–	58	43
April	–	2	10	3	16	–
May	–	–	16	–	–	–
June	**	**	**	**	**	**

Bed 2 (OS)=surplus sludge/primary sludge
2.1=control-bed
2.2=planted-bed
Bed 3 (AST)=aerobial stabilized sludge/secondary sludge
3.1=control-bed
3.2=planted-bed
Bed 4 (FS)=digested sludge
4.1=control-bed

Month	Bed 2 (OS)		Bed 3 (AST)		Bed 4 (FS)	
	Control -bed (2.1)	Planted-bed (2.2)	Control -bed (3.1)	Planted-bed (3.2)	Control -bed (4.1)	Planted-bed (4.2)

4.2=planted-bed
*=because of frost, no sample charging possible

Table 9. Other salmonellas-types in the sludge-samples and in the infiltration waters of every sewage-sludge-filter-bed

Month	Bed 2 (OS)				Bed 3 (AST)				Bed 4 (FS)			
	Control-bed	SW (2.1)	Planted-bed	SW (2.2)	Control-bed	SW (3.1)	Planted-bed	SW (3.2)	Control-bed	SW (4.1)	Planted-bed	SW (4.2)
1983												
June	–	–	–	–	–	–	–	–	–	–	–	–
July	–	–	–	–	–	–	–	–	–	–	–	–
August	–	–	–	–	–	–	–	–	–	–	–	–
September	–	–	–	–	–	–	–	–	–	–	–	–
October	–	–	S.gold coast	–	S.bovis morbificans	–	–	–	–	–	–	–
November	S.bovis morbificans S.gold coast	–	S.bovis morbificans S.gold coast	–	S.gold coast	–	S.bovis morbificans	–	S.bovis morbificans	–	S.paratyphi B	–
December	S.bovis morbificans	–	–	–	–	–	S.bovis morbificans S.gold coast	–	S.bovis morbificans	–	S.bovis morbificans	–
1984												
January	S.paratyphi B	–	S.gold coast	–	S.paratyphi B	–	S.bovis morbificans	–	S.paratyphi B	–	S.paratyphi B	–
February	S.paratyphi B	S.paratyphi B	S.gold coast	S.paratyphi B	S.paratyphi B S.gold coast	–	S.bovis morbificans S.gold coast	–	S.paratyphi B	–	S.paratyphi B S.gold coast	–
March	S.paratyphi B S.gold coast S.bovis morbificans		S.gold coast S.bovis morbificans	–	S.paratyphi B S.bovis morbificans	S.corvallis S.virchow	S. gold-coast S.bovis morbificans	–	S.bovis morbificans S.virchow	S.typhimurium	S.bovis morbificans S.corvallis	S.typhimurium
April	S.gold coast	S.bovis	S.gold coast	–	S.bovis	S. virchow	S.bovis	–	S.bovis	–	S.bovis	S.bovis

		Bed 2 (OS)				Bed 3 (AST)				Bed 4 (FS)		
Month	Control-bed	SW (2.1)	Planted-bed	SW (2.2)	Control-bed	SW (3.1)	Planted-bed	SW (3.2)	Control-bed	SW (4.1)	Planted-bed	SW (4.2)
		morbificans			morbificans		morbificans		morbificans		morbificans	morbificans
	S.bovis morbificans S.paratyphi B		S.bovis morbificans S.paratyphi B		S.gold coast		S. gold-coast					
May	S.bovis morbificans S.gold coast	–	S.bovis morbificans	–	–	–	–		S.bovis morbificans	S.bovis morbificans	S.bovis morbificans	S.bovis morbificans

Bed 2 05) surplus sludge/primary sludge Bed 4 (FS) digested sludge
SW (2.1) infiltrating water from control-bed SW (4.1) infiltrating water from control-bed
SW (2.2) infiltrating water from planted-bed SW (2.2) infiltrating water from planted-bed
Bed 3 (AST) aerobial stabilized sludge / secondary sludge
SW (3.1) infiltrating water from control-bed
SW (3.2) infiltrating water from planted-bed

CONCLUSIONS

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The objectives of the seminar were to assess the microbial inactivation of sludge stabilisation processes and thus to evaluate their use as sludge hygienization procedures as alternatives to established methods like pasteurization and irradiation. A great variety of sludge treatment processes have been discussed including mesophilic digestion, thermophilic digestion, composting, lime treatment, chemical disinfection, lagooning, drying beds and microwave treatment. The different authors have taken different approaches depending on the objectives of their work, their previous experiences, regional interests etc.. There is as yet little agreement on the choice of test organisms (pathogens or indicator organisms, and which organisms from the particular groups), on the question whether indigenous micro organisms or seeded pure cultures should be used, on recovery methods, on evaluation of data etc.. It is therefore difficult to give a balanced overview of the proceedings of the seminar. Nevertheless, important information has been presented and it is obvious that work on sludge hygiene is given much attention in EEC and affiliated countries. The following is an attempt to summarize the major conclusions:

Mesophilic digestion. Conventional mesophilic digestion (circa 1 month at circa 30 °C) was shown to completely inactivate seeded ECBO and Reo animal virus but not bovine parvovirus. The infectivity of Taenia saginata eggs was greatly reduced. If the process was preceded by a thermophilic anaerobic digestion step (55ºC) the effect on viruses was similar, i.e. bovine parvovirus was still detectable in the final sludge. Prepasteurization at 50 °C did not kill Ascaris suum eggs in all cases but increased the sensitivity of the eggs to the subsequent digestion. Two-stage mesophilic digestion with reduced detention times (2 and 8 days) was reported to eliminate Reo-virus but not ECBO and bovine parvovirus, and even after prepasteurization (30 min 70 °C) the bovine parvovirus still persisted. Post pasteurization led to a final sludge in which this organism was only occasionally found.

Aerobic thermophilic stabilization. In two different papers, varieties of this process were described: three stage, two stage and single stage. In the three stage process significant inactivation of indigenous coliforms, streptococci, salmonellae, MS2 phage but not of clostridial spores was reported if a temperature of 60 C was exceeded for a sufficient period of time. In the two stage and single stage experiments using artificially contaminated germ carriers, temperature limits for inactivation of coliforms, salmonellae and A.suum eggs were in the range of 50–55 °C. A temperature of 55 °C for 3 h was also reported to induce a significant reduction of infectivity of T.saginata eggs. The contrast between these studies and the following discussions have again revealed the influence of hydraulic short circuiting digesters on the microbicidal effect. Until now, a digester is studied as a black box and numbers of organisms in and out are compared. To increase our understanding it is essential that microbiologists and engineers work together to produce models of what goes on inside a reactor.

To increase our understanding it is essential that microbiologists and engineers work together to produce models of what goes on inside a reactor. Data on hydraulic residence time distributions, death rates and influent concentrations should be used to model the number of micro-organisms in stabilized sludge.

Composting. Microbial inactivation in composting processes may vary from hardly any to almost complete disinfection and this again was demonstrated. Windrow composting had little effect on indicator bacteria or helminth eggs while composting in static piles was shown to result in significant inactivation of coliforms and faecal streptococci. Best results were obtained when aeration was by suction. The results of bioreactors were variable main ly depending on bed heigth. Favourable results were reported for vertical reactors.

Lime treatment and chemical disinfection. Various authors presented data on inactivation of micro-organisms by lime and varying ‘threshold’ pH values were reported: salmonellae pH > 12.8 for 3 hours; A.suum eggs pH > 12.5 for 2 months, T.saginata eggs pH > 12.0 for 24 hours while spores of Bacillus anthracis were resistant. An important conclusion to be drawn is the fact that parasite eggs are not fully resistant to liming as has long been believed. Inactivation of Taenia saginata eggs by chemicals can be the result of two mechanisms: hatching inhibition (lime, alkaline formalin, quaternary ammonium compounds) or direct ovicidal (bis-biguanides, copper-ions) or a combination of both (acid formalin, peracetic acid).

Lagooning and drying beds. These processes of long standing can have important effects on micro-organisms as was again shown in this seminar: T.saginata infectivity was decreased by lagooning sludge for a period of 1 month. Planting drying beds with reed did not have an additional microbicidal effect.

Microwaves. Sludge disinfection within seconds can be obtained by using microwave ovens. There is not yet information of the economical aspects but the process seems to have some promise as a stand-by technology.

TECHNICAL VISITS

Visit of the two-stage aerobic-thermophilic treatment plant at Gemmingen
Introduction and guidance: K.BREITEMBÜCHER
Visit of a bio-cellreactor-system Dambach-Schnorr in the sewage works of Gaggenau
Introduction and guidance: W.BIDLINGMAIER

ENGINEERING AND PRACTICAL EXPERIENCES OF AUTOHEATED AEROBIC-THERMOPHILIC DIGESTION

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Mayen

Summary

In West Germany, the investigations of the aerobic-thermophilic digestion processes began in 1968. The development of a special self-aspirating aerator by FUCHS first lead to an application of the process to agricultural wastes. The first aerobic-thermophilic stabilization full-scale plants (ATS), processing municipal sludges, were built in 1977. Nowadays more than 20 ATS-plants are operating in municipal wastewater treatment facilities and even more are in the design stage. Today the ATS represents an important technology of sludge treatment and pasteurization in the Federal Republic of Germany. Comprehensive hygienic and economic investigations, which were made during the last years, showed the advantages of the ATS in sludge quality and treatment costs, compared with conventional treatment processes like anaerobic mesophilic digestion, extended aeration, raw sludge dewatering etc. These advantages are significant in the range of 5.000–50.000 population equivalents, especially if land application of sludge is wanted without public health risk. The following report includes some essential results, practical experiences and process design criteria. This is proved by dates of ATS-plants, which have been operating successfully for more than 7 years. In particular, some results of the ATS-plant Gemmingen are submitted. A federal research project was realized there from 1980 to 1982.

1. INTRODUCTION

A definitive removal of wastewater sludges is achieved by recycling into the natural circulation. This solution has to be aimed at, especially for small and middle-sized purification plants in rural areas. However, expensive sludge treatment technologies raise great problems in such communities. The cheapest sludge removal can be seen in the application on nearby agricultural areas. The utilization of liquid sludge proves to be favourable. It is a precondition to produce a fertilizer which may be used without risks in all respects. The sludges must be toxically and hygienically unobjectionable. A nearly odourless disposal should be guaranteed as well as economically reasonable transport quantities in relation to the fertilizer value.

Up to now, mainly anaerobic processes have been used for the stabilization of sewage sludge. In smaller-sized purification plants, an extended aeration is often made. The degree of stabilization and the dewaterability of such sludges are moderate; a pasteurization effect doesn't take place.

Anaerobic mesophilic digesters need big reaction volumes due to long retention times (20–30 d). Expensive installations are necessary for heating and gas utilization.

The high investment costs for heated anaerobic mesophilic digesters don't admit a cost effective use. It is at least questionable compared with possible other treatment technologies for plant-sizes up to 100.000 population equivalents.

Various investigations concerning the profitability of digester gas utilization in sewage sludge treatment works have shown that the very low energy costs have a relatively minimal influence on the total annual costs also in this range. The relatively high capital-costs cannot be balanced. The results of the investigations showed a total economical advantage of the aerobic-thermophilic sludge stabilization in smaller and middle-sized sewage works (1), (2).

Besides the high technological requirements of heated anaerobic digesters which are susceptible to disturbances (particularly for toxic matters), there are additional restrictions for an agricultural utilization of sludge: Anaerobic stabilized sludge still contains a lot of pathogens, which are not or not essentially reduced in the usual mesophilic processes and thus represent a significant health risk (3), (4).

2.
BACKGROUND AND THEORY OF THE AEROBIC-THERMOPHILIC STABILIZATION (ATS)

In West Germany, the aerobic-thermophilic processes have been applied for more than 10 years to the digestion of agricultural liquid wastes.In the meantime, numerous plants have proved reliable in the treatment of municipal sewage sludge.

The exothermic metabolism of aerobic microorganism leads to an autoheating of the sludge up to the thermophilic range (over 45° C) by appropriate treatment technologies with heat insulated reactors and special aerators. However, the biological theory and the technological background of the process have already been reported in detail in previous studies (6), (7).

3.
PATHOGEN DESTRUCTION EFFICIENCY

If certain operational conditions are observed, a pasteurized sludge can be produced by the ATS process. This is proved by many investigations (e.g. 3), 4), 8)) and will not be the subject of this report. The hygienic point of view is a very important one for the Federal Republic because legislation doesn't allow to apply sludges being epidemically critical on grassland and forage plant land.

4.
PROCESS DESIGN CRITERIA

4.1.
Introduction

The potential for autoheating can of course be predicted by using energy and mass balances. This procedure is too uncertain and cannot be applied for practical plannings. There are meanwhile sure parameters for the design of the ATS-plants based on long-term experiences.

Before establishing an ATS-plant, experiments with mobile pilotscale reactors are only made, if sludges with a high portion of industrial wastewater have to be treated.

Normally the ATS can be successfully set in cases where typical wastewater sludges (mixture of primary and secondary waste activated sludge) occure. The process is proved for high-rate trickling filter sludges or surplus sludges. Certain sludge loadings in the biological stage of wastewater purification have to be observed (6).

The following conditions are necessary for the ATS:

4.2.
Sludge characteristics

Source	: primary and secondary waste activated sludge
Total solids concentration (TS)	: ≥ 3,5 %
Organic solids concentration (total volatile solids; TVS)	: ≥ 2,5 % (≥ 60 % of TS).

A mechanical pre-dehydration is only justified for surplus sludges which are very difficult to thicken and which can be found in purification works with a high portion of industrial wastewaters (e.g. dairy, brewery).

4.3.
Aeration system

The applied aeration system is absolutely decisive for the success of the process and it is the main point of the ATS-planning. It should have the following characteristics:

- self-aspirating aerators
- complete mixed system
- superimposing of radial and tangential flows in the reactor (technical and electrical partitioning of the aerator power) depending on the reaction volume

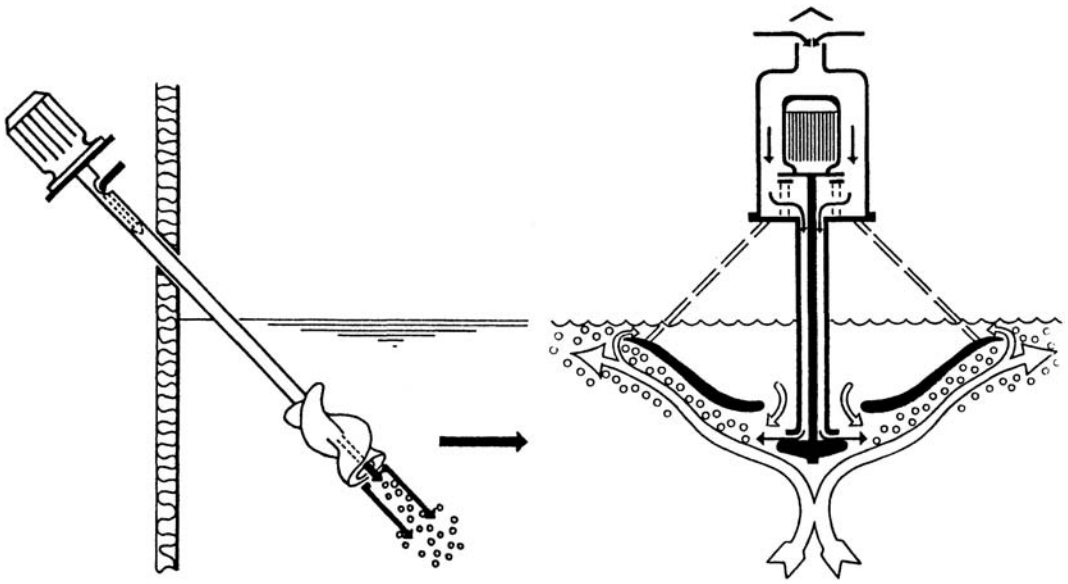

Figure 1 – Schematic view of the aeration system for autothermal aerobic-thermophilic stabilization of sewage sludge

– no barings or packings (the aeration system must be very stable and unsusceptible to disturbances due to the extremely high mixing intensity in the ATS-reactor) in the sludge; no sludge transport through pipes, pumps, stators, etc. inside the reactor.

Experiments with compressed air and separated mixing, injector nozzles or surface aerators showed significantly poorer results in process technological and economical regard than the use of self-aspirating aerators. Fig. 1 shows the schematic construction of the aerators which are mainly used in the ATS.

4.4. Reactor design and construction

The reactors can be designed according to the desired level of pasteurization or—if pasteurization is of less importance—according to the desired degradation level of organics (stabilization).

No special requirements have to be made concerning the form of the tanks because of the flexible aerator system; usually simple cylindrical vessels with a flat bottom are sufficient. Steel has proved the most suitable material. Tanks of reinforced concrete are not sufficiently tested and they include certain risks because of the high, thermical strain. They are very difficult to remodel.

Design of the ATS-plant:

a) for complete stabilization and pasteurization

– 2-stage-operation (or more stages; dependend on size of wastewater treatment plant)
– minimum hydraulic retention time: 6 days or more
– minimum retention time of pathogens: 20 hours per stage or more
– reactor temperatures: R_I=35–50° C
  R_{II}=50–60° C
– PH_{RI} ≥ 7,2
– PH_{RII} ≥ 8,0
– batch or semi-batch operation (feeding 1/day)

b) for minimal treatment for odour and hazard control, e.g. for disposal on cropland without hygienic requirements or for a following dewatering.
  minimum stabilization: 1-stage-operation continous flow retention time ≥ 4 d

The stated parameters and design data are only valid for the most efficient and tested aeration system.

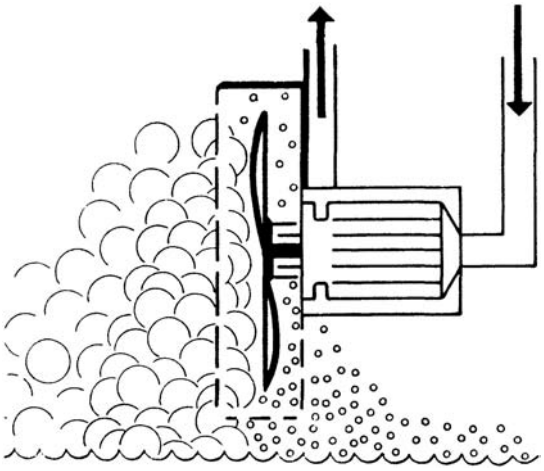

Figure 2 – Foam control system ("Foam cutter"; WerKbild F.G.W.FUCHS)

The foam control system is an important part of the process, too. The ATS-process leads to a desired formation of a strong foam layer on the sludge. Besides the heat-insulation effect, intensive biological degradation processes take place in the foam as well. The oxygen transfer efficiency of the aerators can be obviously increased by a controlled foam treatment. The biological as well as the processtechnical advantages (definite half-life time) of the foam layer (Fig. 2) can be used by the so-called "foam cutters". Equipments where the foam and the air are simultaneously aspirated and limited by the aerators, are not suitable. The specific power requirements may therefore increase up to 40%, the biology of the foam system can be disturbed.

5. OPERATING COSTS AND MAINTENANCE

The ATS aeration system usually has a power input of 85–105 W/m^3 of reactor. This depends on sludge composition, the solids concentration, the number of the reactor stage or the process aims. The according specific energy requirements is 9–15 kWh/m^3 of sludge throughput. Apart from the sludge characteristics, the energy consumption also depends on the process aims. If only a treatment for odour control is required, the average consumption amounts to 10 kWh/m because the reaction times are shorter. If pasteurization is necessary about 13 kWh/m^3 sludge throughput are needed.

The daily operational and maintenance requirements of the ATS-process are very low. About 1 hour per day is necessary for control and maintenance of a 28.000 population equivalents plant for ex. (9). In bigger ATS-plants with pasteurization (two or more reactors), feeding can be realized fully automatically by using micro processors. The daily maintenance demand for a plant of 80.000 population equivalents is lower than 1,5 h/d (10).

One reason for an extremely stable and flexible biological system can be seen in the short generation times of the micro-organism in the thermophilic aerobic milieu (6), (14). Therefore, no essential interferences in the existing plants caused by any toxic substances in the sludge have been known up to now.

Even if there is a long-term breakdown of the aeration system, the process can be reactivated within few hours. The ATS-process has proved a very high reliability compared to other treatment technologies especially, if efficient pathogen kill is necessary. The technological requirements of the ATS-plants are very low compared to other sludge treatment- and pasteurization processes (11). Therefore the service can also be executed by a less qualified staff.

6. HEAT RECOVERY

During the last years, the possibilities of heat recovery are also considered in the planning and construction of ATS-plants. The energy which is contained in the hot sludge and which is biological produced by autoheating is of main interest. These quantities of heat are at disposal and they are not required for the process. The exhaust gases also contain considerable heat quantities. However, the utilization of this energy source is more expensive in a technical view and it is mostly unnecessary for the low requirements of the management buildings (heating and warm water supply).

In post-thickeners and sludge holding tanks simple heat exchanger systems are installed. With coaxial heat exchangers it was possible to gain 25–35 kWh_{therm}/m^3 of sludge throughput in full-scale plants for heating and warm water supply in

management buildings and in buildings for the dewatering equipment. This quantity was sufficient for the total annual heat consumption of a middle-sized sewage work. Theoretically there is of course an even greater potential for energy recovery, but under practical conditions one has to put up with relatively low exchanger efficiency in order to keep small the additional technological equipment (13). The heat recovery data become more interesting when considering that the most efficient ATS aeration system only consumes approximately 13 kWh/m^3 of sludge throughput. It is thus possible to minimize the overall costs of the sludge treatment, because the energy costs of the aeration are a main operation cost factor.

7. OPERATING RESULTS

7.1. System design and Operating

Operating results of some ATS-plants have already been published in detail elsewhere (6), (11), (12).

Fig. 3 shows the flow diagramm of a middle-sized ATS-plant (22.000 population equivalents). The reactors are operated in series. Once a day, they are charged by pumps from a pre-thickener. The treated hot sludge is pumped into a heat-exchanger tank. There the heat is withdrawn for supplying the buildings. The heat recovery system is constructed in a way, that it will be possible in future to heat a municipal swimming-pool. After cooling the sludge is pumped into the sludge holding tank and from there it is disposed to agricultural utilization.

7.2. Reactor temperatures

If the already named technological pre-conditions (aeration system, retention time etc.) are fulfilled, the achieved sludge temperature, produced by autoheating, is the most suitable and simplest parameter for the critical examination of the aerobic-thermophilic process. Fig. 4 presents the typical reaction curve of a two-stage ATS-plant. The influent concentration of organic dry matter (total volatile solids) amounts to about 3%. The first stage of the ATS-plant can reach thermophilic temperature ranges if there are relative highly concentrated sludges. In the second stage the rest stabilization takes place. It is important for the pasteurization effect, that the temperatures of reactorII are relatively constant The necessary retention time for pathogen kill is guaranteed by semi-continuous feed (1/d). The degradation of organic matter is 35–45%.

7.3. Stabilization degree

For judging the stabilization degree it is known that there is no general suitable parameter. In our investigations the measurement of the respiration activity together with a lead acetate-test has proved relatively well especially for the ATS. However, in the ATS-process these datas have to be considered in connection with the reactor temperature (measuring temperature 20° C) the retention time, the aeration system and other treatment technological parameters (11). For official control purposes a TTC-test is meanwhile used to determinate the stabilization degree (9).

In Fig. 5 the typical course of the respiration activity is shown in dependence of the retention time and the reactor temperature. A sta bilized sludge should have a respiration activity below 0, 10 kg 0_2/kg TVS. Such sludges can be stored unobjectionably and disposed without any odour nuisance.

The degree of organic matter degradation is not a sure criterion in the ATS. In the process thermophilic respectively thermotolerant aerobic microorganism are selected. After cooling the treated sludges, there-adaption to the anaerobic milieu takes place only extreme slowly (6), (14); therefore a TVS-degradation of already 25–30% may be sufficient for an odourless storing and disposal of certain sludges because the microbial milieu supplies an additional stabilization effect.

Practical experiences with ATS-sludges have in general shown, that a stabilization degree is reached by this process which is at least as high as by mesophilic digestion processes.

7.4. Sedimentation and dewatering characteristics

If hot ATS-sludge has to be thickened, there is only a modest sedimentation. After cooling the sludges a really good settleability is reached. According to sludge composition and the storing capacity the transport volumes can be reduced by 40–70 % by discharging the sludge liquor. In the operating plants (Fig. 6 and 7) this is usually made by using large storage tanks

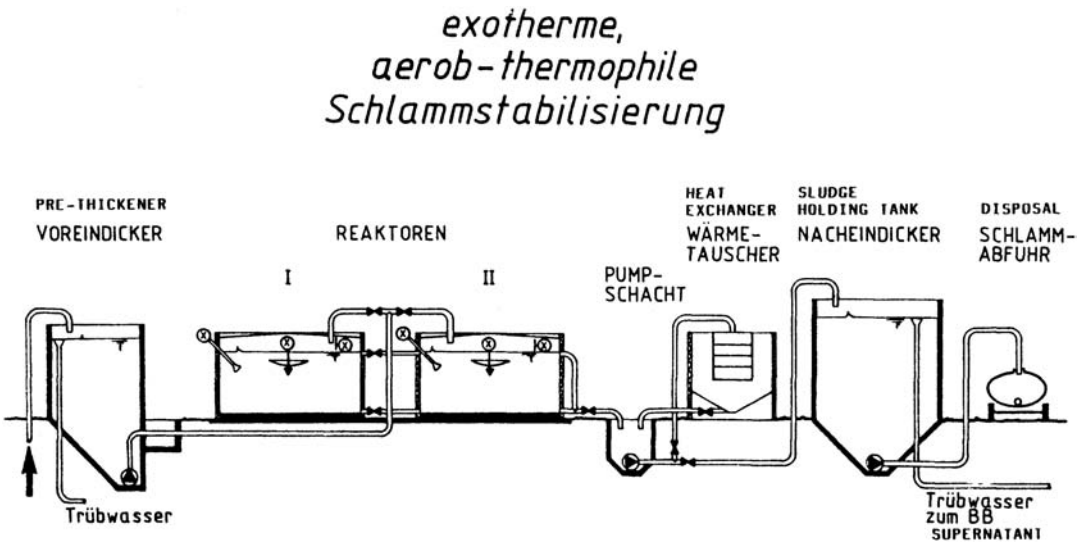

Figure 3 – Flow diagram of a 2-stage ATS facility with heat recovery for the management buildin s (Fassberg; population size: 22 000 P.E.; sludge throughptit: 40 m^3/d; reactor volume 2×120 m^5; in operation since 1983)

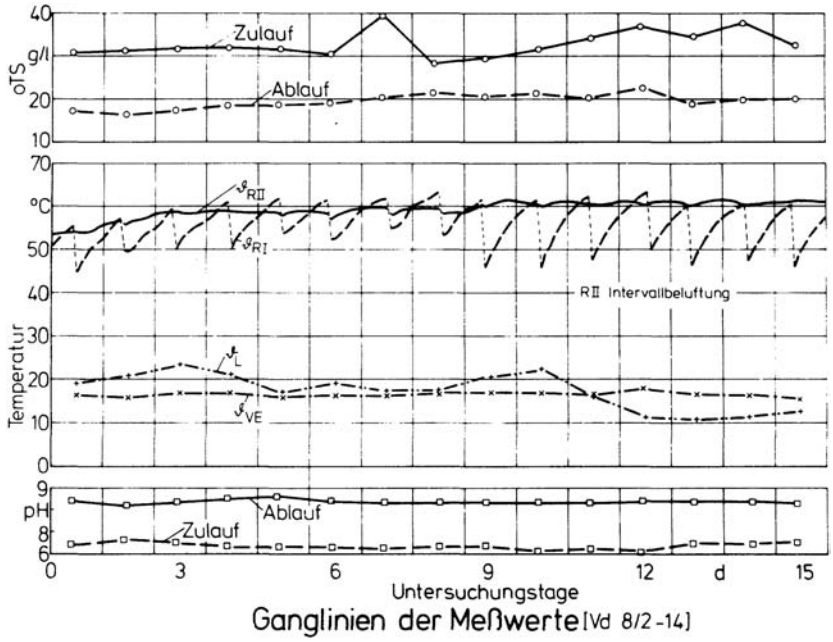

Fig. 4: **Typical reactor (ϑ_{RI}, ϑ_{RII}), ambient (ϑ_L), and feed temperatures (ϑ_{VE}) in a 2-stage ATS facility with corresponding influent (Zulauf) and effluent (Ablauf) data of total volatile solids (oTS) and pH (Gemmingen, population size: 6,000 P.E.; sludge throughput: 7–10 m^3/d reactor volume: 2×24 m^3; in operation since 1980; ref. 6)**

with cooling possibilities (6) or by cooling with heat exchangers (10). In this way a TS-content of 7–12 % is reached for these ATS-sludges. The discharged supernatant is only insignificantly polluted (BOD below 1.000 mg/1).

There have been reached good results in practice concerning dewaterability of the sludge by centrifuges or belt screen presses.

Comparative tests showed that ATS-sludges can be dewatered with almost the same consumption of coagulants like anaerobic digester sludges (15). An operating test with two decanters and one belt screen press has recently been made in the ATS-plant Vilsbiburg. It showed that the hot sludge could be dewatered to more than 30 % TS by each of the three machines. The requirements of coagulants were approximately 150 g/m^3 (16).

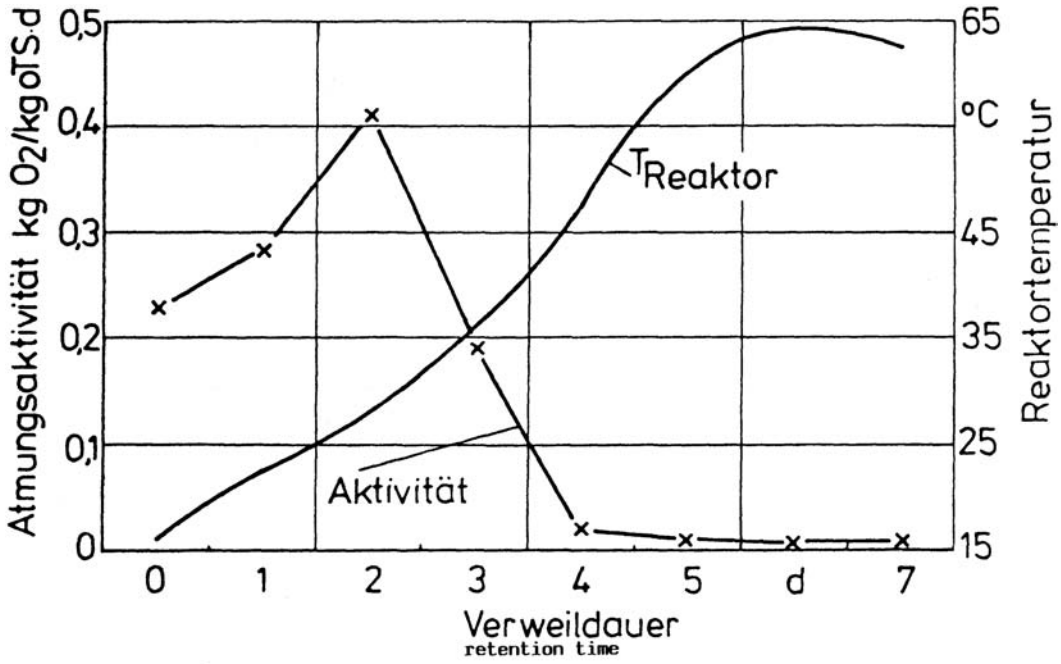

Fig. 5: Respiration activity of aerobic-thermophilic digested sewage sludge as related to reactor temperature.and retention time (Ref. 6)

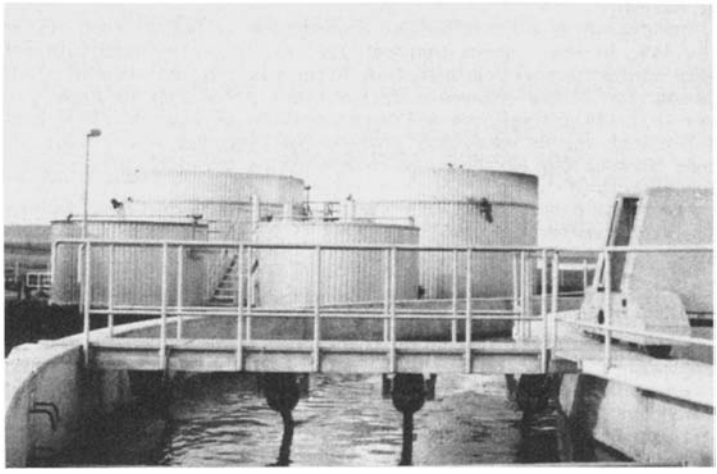

Fig. 6: Aerobic-thermophilic stabilization facility (ATS) for 15,000 P.E. (Vilsbiburg; sludge throughput: 15–20 m^3/d; reactor volume: 2×75 m^3; in operation since 1977)

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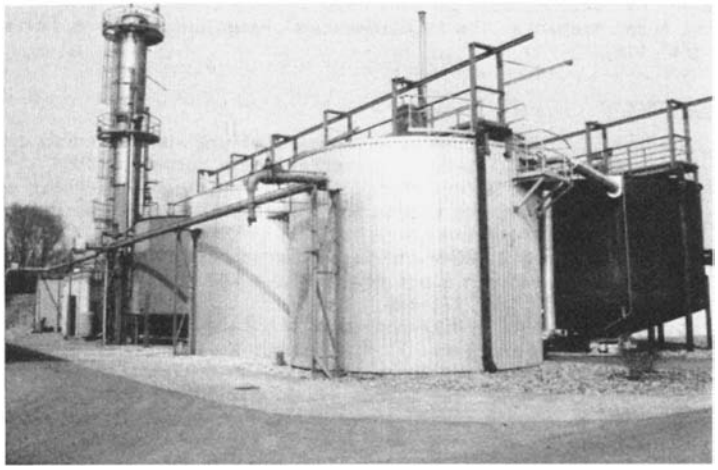

Fig 7: ATS facility for 80,000 P.E. (Niersverband; sludge throughput 75 m^3/d; reactor volume: 2×180 m^3; in operation since 1981) the thermical pasteurization unit in the background left was replaced by the ATS

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THE TREATMENT OF SEWAGE SLUDGE UNDER AEROBIC-THERMOPHILE CONDITIONS

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1. INTRODUCTION

All methods of waste treatment have something in common, namely that the processing of the wastes as a whole leads to the production of new (or secondary) wastes. In the case at hand, for example, it is sewage sludge that is a product of sewage treatment processes.

It is also a fact, that the treatment of these secondary products is usually more problematic than the treatment of the original wastes. A satisfactory solution for the complete problem must thus also comprise treatment of the residual substances, e.g. sewage sludge.

Intensified research activities as well as numerous efforts to develop and patent new process technologies in the area of sewage sludge treatment can be considered to be documentary evidence of the fact, that conventional sludge digestion, as it is often practised, does not represent a final solution to the problems of dewatering the sludge and converting it into a hygienically acceptable state.

The aerobic treatment of sewage slude, i.e. composting, is gaining in importance as opposed to common dewatering procedures.

That composting as a method is nothing new becomes apparent when one studies ancient Indian and Chinese literature. The special problems involved in the composting of sewage sludge will be dealt with briefly in the following text.

2. REQUIREMENTS FOR A GOOD ROTTING PROCESS

Prerequisites for the rotting of organic matter are a sufficient quantity of water and a satisfactory supply of oxygen. These two requirements, however, also give rise to a discrepancy, because the volume of the air pores results in a reduction of the oxygen supply for the microorganisms. The main thing is to find an acceptable compromise for these two parameters when composting sewage sludge.

Microorganisms can assimilate nutrients only in a dissolved form through their semipermeable cell walls, thus a moist medium is a necessity for their metabolic activity. This means that the surface of the rotting matter must always be covered with a water film. This is most usually the case when the waste matter has a minimum water content of 40%. The upper limit for the water content is determined by the requirement of good gas permeability, that as such is made possible by the air spaces between the particles of the material to be composted. If the water content is too high, gas permeability will be limited. As a consequence the aerobic microorganisms will not have a sufficient supply of oxygen. The optimum water content lies between 50 and 60 % for waste matter composting systems known at the time. The so-called most advantageous water content depends, however, on the composition of the material to be composted. The water content can be increased, for example, when roughage, that can fullfill its function as a structuring agent—even at high moisture levels—is present.

Straw, paper and natural fibres are examples of such structuring agents. It is also important that water, as such, is not present in the air spaces between the particles of the material to be composted.

This can be illustrated by the following example. In one case dry straw, in the other Styrofoam particles, were added to sludge with a dry substance content of 20 % in Dewar vessels. The admixture of both substances was so proportioned, that in both cases the arithmetic value of the dry substance content amounted to 40%. During a rotting time of ten days aerobic decomposition took place in the vessel containing straw, but in the vessel containing Styrofoam particles anaerobic conditions prevailed.

In conjunction with this experiment the straw and the Styrofoam particles were then each washed out with a certain amount of water and the water contents of both, as well as of the residues, determined. The results showed that the straw had a water

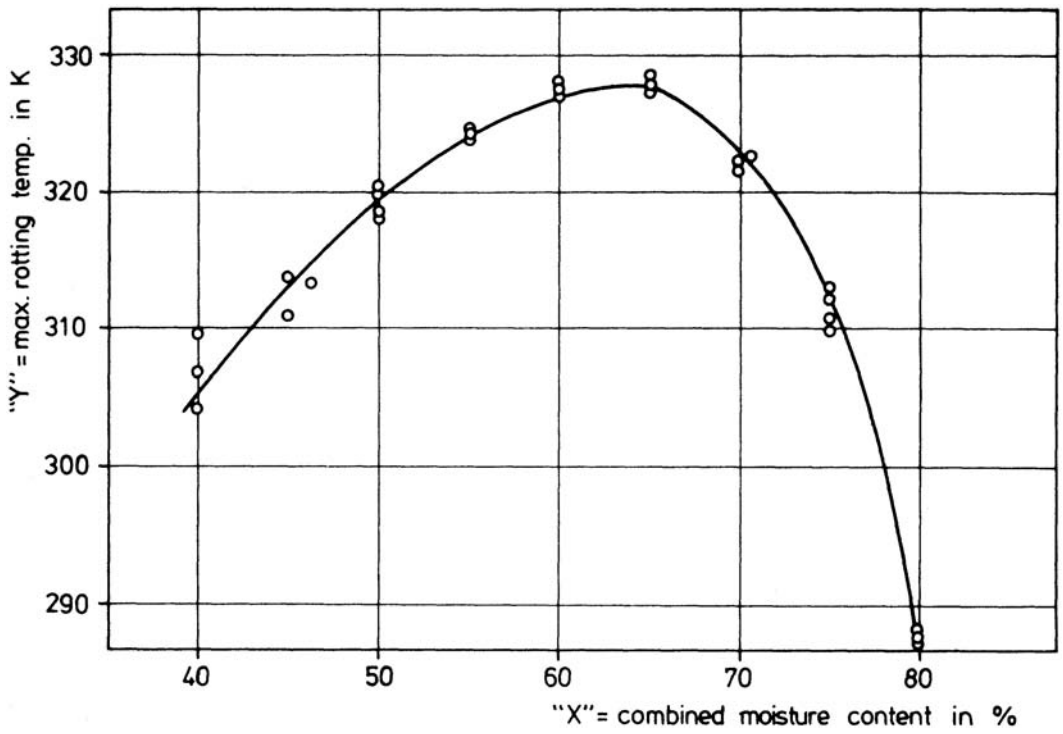

Figure 1

content of 50%, whereas the Styrofoam particles had only 5%. In the latter case the water thus remained in the sludge, i.e. in the spaces between particles, and air had no access to the material.

In order to determine the most suitable water content for the experiments with sewage sludge and paper, different mixtures with varying proportions of dry substance and water were prepared and tested.

The results, as illustrated in Figure I, show clearly that the optimum mixture lies in the range of 55 to 70 % water content.

The maximum temperature generated in each mixture was chosen as the characteristic parameter. It is striking that the slope of the curve in its upward course, i.e. at water contents between 40 et 65%, is less steep than the slope of the curve after its peak. The steeper downward branch thus shows the inhibiting effect that occurs because of a lack of oxygen. In further experiments the water content of the mixtures was therefore adjusted to a value of 65 % from the start.

Furthermore the initial pH—value is also a factor that determines the course of the rotting process.

With increasing initial pH—values better rotting processes take place. Normally the pH— value adjusts itself to a value somewhere around neutrality (pH 7) during the course of a rotting process. It is also noteworthy that after termination of the mesophile phase the pH—value sinks into the acid range. This signalizes the restructuring of the populus of microorganisms.

During the rotting process two temperature phases can usually be observed. During the first phase mesophilic organisms multiply themselves; the temperature increases to values around 313 K. This enormous liberation of energy can be illustrated using the decomposition of glucose as an example:

aerobic cond.

This is twelve times as much energy as is set free during the anaerobic decomposition of the same substance. A second phase in which the decomposition processes continue with thermophile microorganisms follows the first one. In this phase with its high temperatures of more than 338 K, dsinfection of the material takes place.

The C/N—ratio in the mixture is also an essential factor determining the course of the rotting process. In proprietary experiments it was determined that, independent of the mixture used, an initial carbon—nitrogen ratio about 20 is most favourable for the process. Smaller C/N—ratios result in lower rotting temperatures and thus are not conducive to disinfection of the material. Higher C/N—ratios cause extended lag phases, thus increasing the total rotting time.

3.
EXISTING METHODS AND SYSTEMS

In practice one can differentiate between two kinds of methods Firstly, composting methods in which sludge is processed together with domestic wastes. Examples for systems and procedures based on such methods are:

– the Biotank system
– the Blaubeurener System
– the Brikollare System
– the multiple—stage reactor procedure
– the aerated pile procedure
– the rotating drum procedure

These systems and procedures are well-known and therefore need no further comment.

Secondly, there are composting systems that process sludge by itself. Figure 2 represents an attempt to categorize these systems. Two types are conspicious at the firts glance namely those operating with and those operating without supplementary structuring agents.

The former use foreign matter of organic origin, such as sawdust, bark, etc. to form particle structures. The latter used dried compost from the process itself for this purpose.

BAV—System

This system is based on a method for the continuous composting of sewage sludge, in which the sludges moves en bloc from the top to the bottom of a reactor while the air necessary for the rotting process is passed through it. The amount of air is regulated according to the CO_2—content of the exhaust gas and the temperature of the rotting matter. The air is blown through countercurrently in fine bubbles dispersed over the whole cross—section of the reaction mass (See Figure 3).

Before it can be processed in the bio-reactor the raw sludge must be dewatered down to a water content of 75 % at the most The unheated reactor, a double-bottomed, cylindrical container, is charged at the top with 50 % sewage sludge, 40 % recycled end product from the reactor itself and 10 % structuring agent, such as sawdust, shredded straw or felt powder.

Discharge of the finished product is accomplished by means of a chain conveyor at the bottom of the reactor.

Weiss System

The bioreactors manufactured by the Weiss Company operate as closed systems, as opposed to the BAV—plants.

Two bioreactors are situated together with the silo for the structuring agent and the secondary process reactors on the roof of the plant building. The sludge dewatering aggregate and the sludge silo with its discharge unit are located together with the electrical equipment and all other machines and facilities necessary for operation in the plant building.

The raw sludge from the clarifier is dewatered machanically to a water content of 75 % and stored intermediately in the sludge silo. From there the thickened sludge is transported mechanically to the chain conveyor.

The silo for the supplementary structuring agent is filled independently, as far as the operation of the bio-reactor is concerned. The chain conveyor is fed, simultaneously and as necessary, with a certain amount of sawdust, shredded bark, shredded paper or the like from the structuring agent silo (See Figure 4 and 5).

The chain conveyors at the bottom of the bioreactor discharge opening deliver some of the raw compost on one side to the feed chain conveyor, where it is fed back to the reactor system as regulated compost. The feed conveyor unit transports all components of the reaction mass to the blending and spreading equipment on the roof of the plant. Mixing takes place immediately before the material is fed to the bioreactor and thus immediately before the rotting process begins. The two bioreactors can be fed interchangeably, simply by reversing the direction of motion of the feed conveyor. Reactor charging is accomplished by means of a turntable.

The reaction mass moves through the bioreactors from the top of the bottom. Because of the cylindrical construction of the bioreactors, the undercutting ring at the bottom and the layer—wise discharge of the end product (raw compost) from the bottom of the reactor as well as shrinkage of the rotting material the reaction mass passes through the reactor gravitationally by itself.

Air is force-fed at the bottom and exhaust gases are drawn off at the top of the reactor.

Excess water is discharged as vapour together with the exhaust gases. During the aerating and de-aerating procedures the bioreactors are complete closed off, when aerating by means of a slide value, when de-aerating by means of a hydraulically operated damper. The exhaust gases pass through the exhaust duct and are then diverted by means of a blower either to the grit remover or the activated sludge basin of the clarification plant or to the secondary rotting reactor for the de-odourizing.

Dambach—Schnorr System

The method used with this system is also based upon the addition of structuring agents in the form of wood, bark or straw to the sewage sludge.

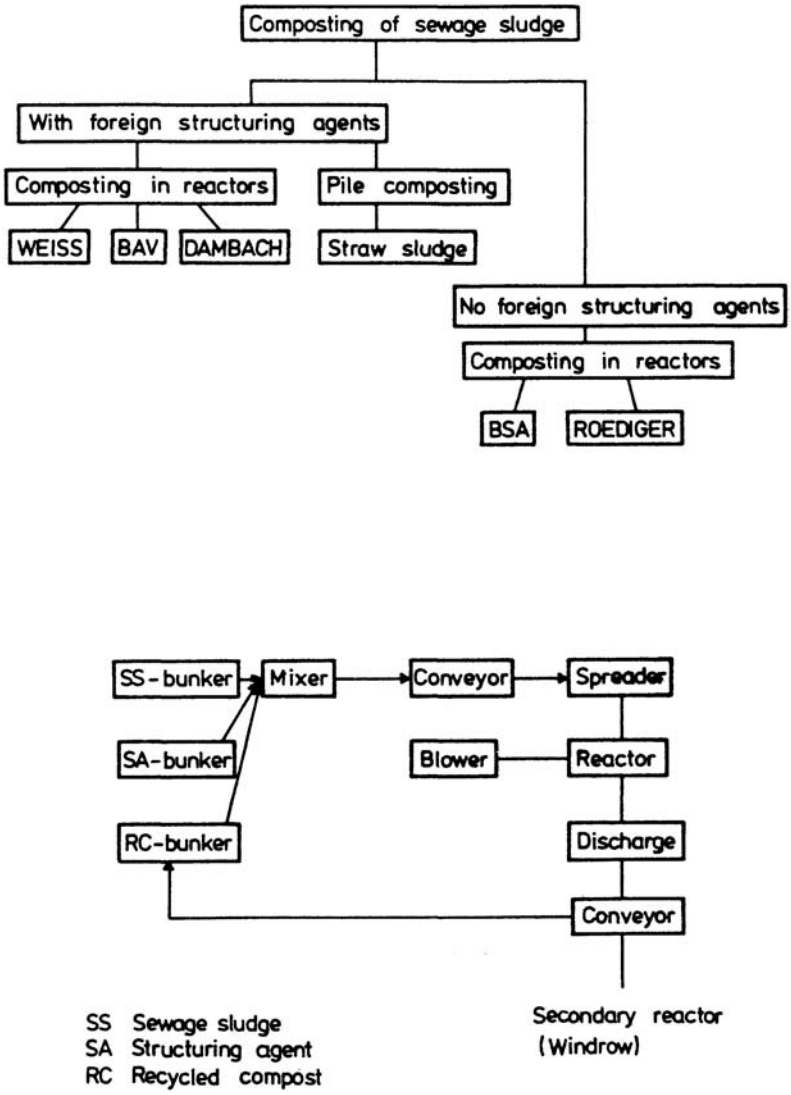

Figure 2

Because of the large height difference to be overcome, the raw substance is fed to the reactor by means of a chain conveyor. The reactor consists of ten platforms, each with a height of about one meter. The individual platforms are partitioned in separate traps. These platform trap units fulfill the following functions:

- Transporting
- Mixing
- Aerating
- Disaggregating

The raw material is distributed over the area of the uppermost platform with chain rakes, remains there on the average for three days and after the trapdoors are opened then falls onto the next platform. Thus the material to be rotted passes through the reactor in (about) 28 days.

The trapdoors are connected to each other through a system of levers and are activated centrally by a hydraulic apparatus. All connections and levers are mounted externally and so that they can be reached more easily when repairs are necessary.

Oxygen is supplied to the rotting material with an aerating system. Each supply line provides air for two platforms. This way, one is supplied from below, the other from above with air and thus with oxygen. The exhaust gases formed in the reactor are drawn off with a ventilator (See Figure 6).

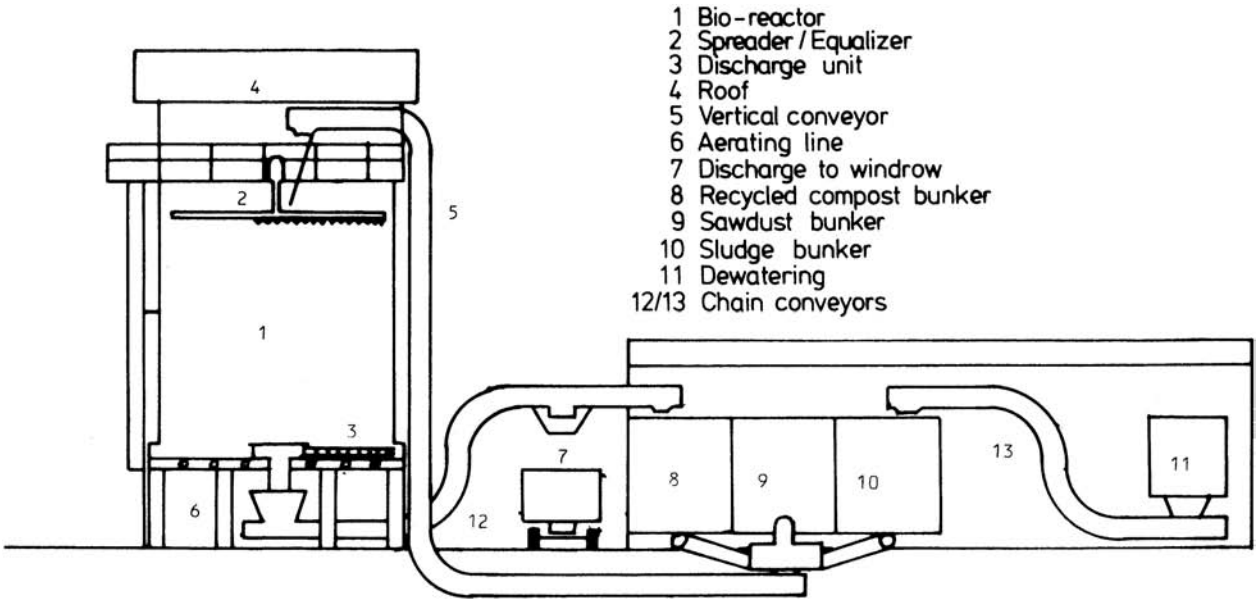

Figure 3

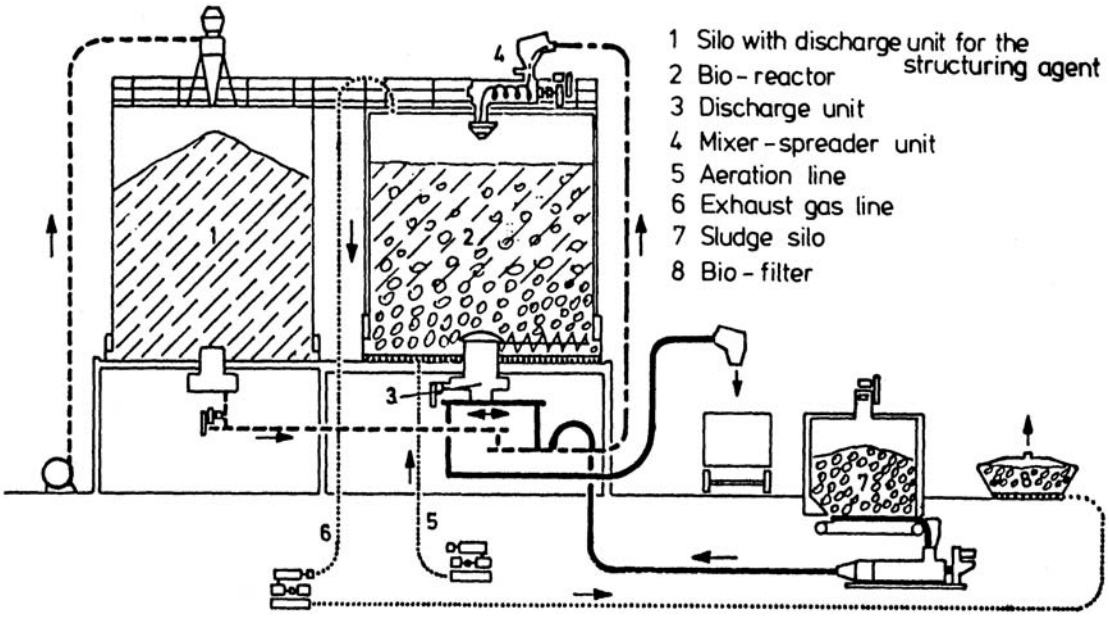

Figure 4

The composting of sludge together with straw, as it is practised for example in Althausen and Pleidelsheim, both FRG, is in principle pile composting. However, the compost heaps are repiled at these installations in regular intervals, as a rule every third day (See Figure 7 and 8). windrows are set up by first placing pressed bales of straw in rows of three and removing the ties from the bales. A certain quantity of sludge is then spread on the straw, and immediately afterwards, the material is thoroughly routed at least two times. A mobile windrow turning machine with its cutting blades rotating opposite to the path of direction is used, so that the routed material is thrown behind. This way the sludge and the straw are mixed with each other and the straw is chopped up. The rotting time until the compost is ready for utilization is about three to six months.

Roediger Fermentation Technik

This system operates without the addition of foreign matter as a structuring agent to the material to be composted. After preliminary dewatering the sludge from the sludge bunker is transferred to a contrarotating dual screw conveyor. Recycled compost material is added to the sludge here until the mixture is then fed to the reactor, which has a square foundation and is bell-shaped. The retention time of the reaction mass is four to six days. Aeration takes place through a grating at the base of the reactor.

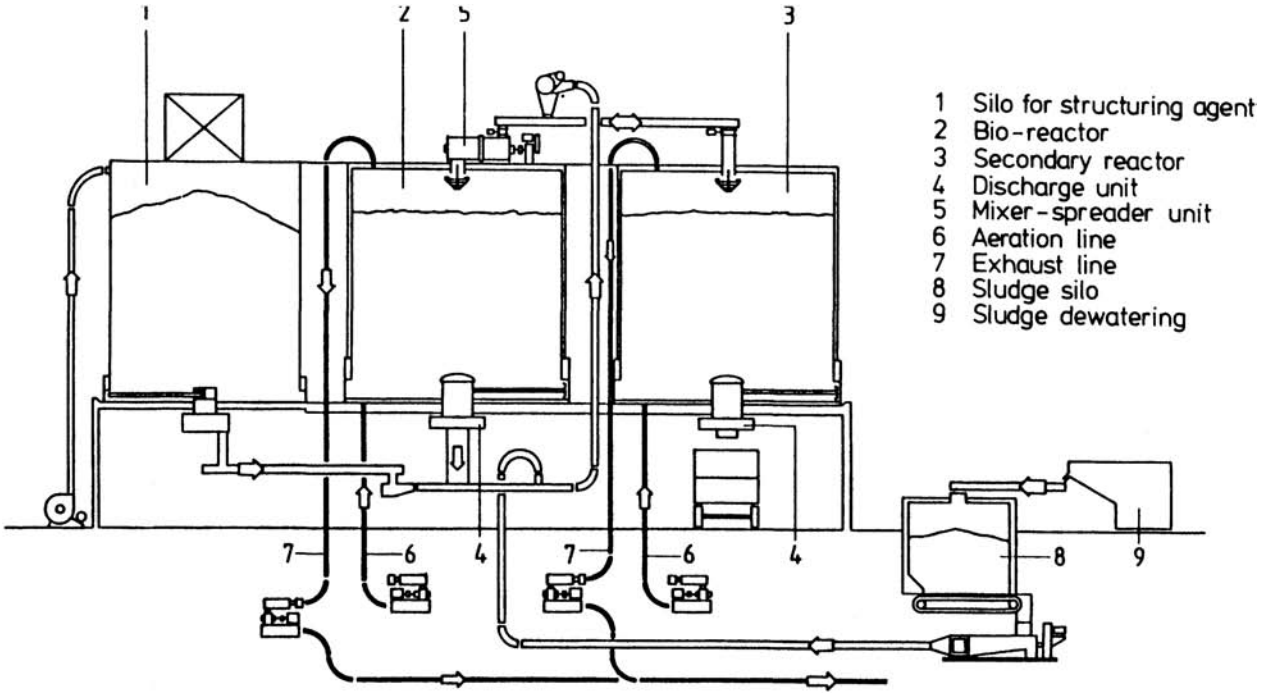

Figure 5

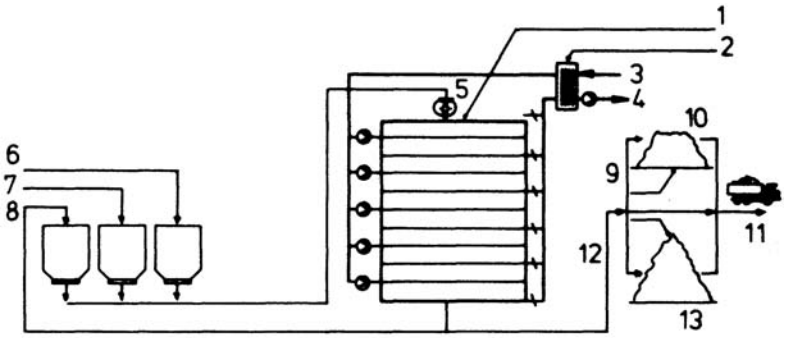

Figure 6

After leaving the reactor, the material is pelletized and transferred to a dryer, where it remains for about four to six days (See Figure 9).

The drying process is considered to be finished when the material has a final moisture content of less than 20%. The dryer is emptied with a suitable discharge unit and the dried pellets transported to a crusher by means of a screw conveyor. Here the material is reduced in size to small particles. Part of this dried and crushed material is diverted to the dry materials silo for use as recycled process compost, the rest goes to the bagging station, where it is packed in sacks.

4. HEAT RECOVERY DURING THE COMPOSTING OF SLUDGE

As a late more and more attempts have been made to utilize the heat generated during aerobic processes, for example in the Erbach clarification plant near Ulm (FRG). This plant in equipped with a Weiss reactor, in which the rotting material is

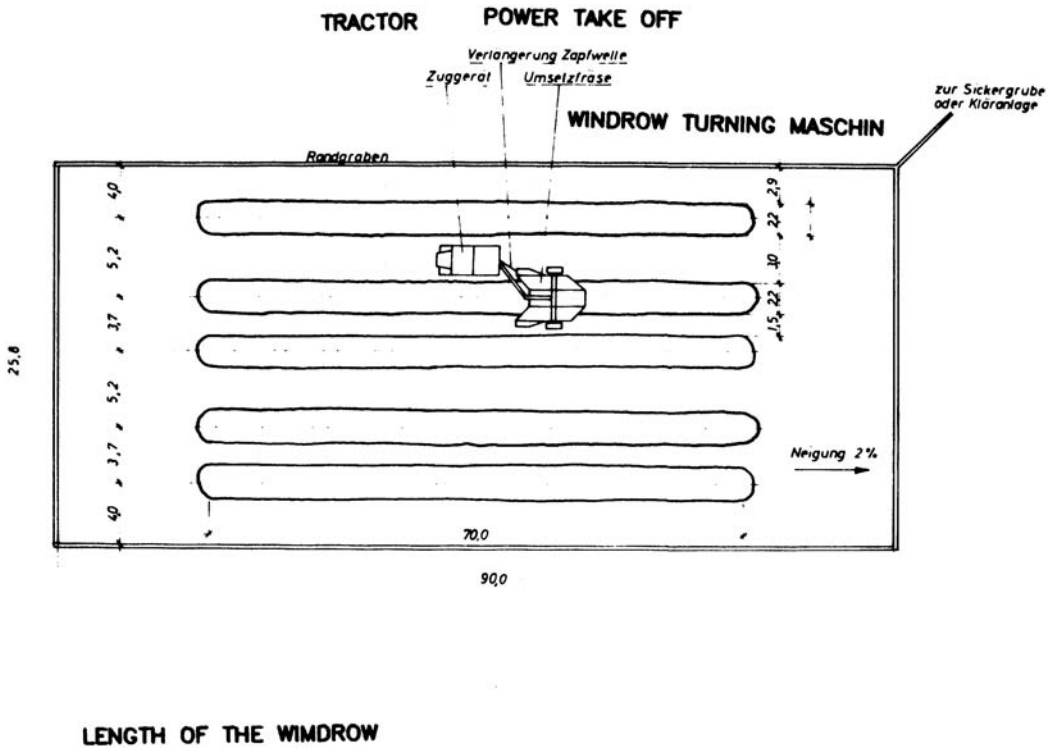

Figure 7

aerated at a rate of 250–300 cubic meters of air per hour. The exhaust gases of the reactor (with a temperature of 60–70° C) are passed through a heat exchanger, where they are cooled down by about 6°C. This heat exchanger (vapourizer Type) is integrated in the process cycle of a heat pump which increases the temperature level of the heat taken from the exhaust gases and then releases it into the central heating plant cycle of the facility. The amounts of heat tranferred have not yet been determined.

Experience gained during the past winter showed, however, that the clarification plant building and a neighboring house could almost be heated completely by the heat pump.

5. COSTS

A summary of the costs involved with the different systems is given in Table 1. The data listed represent the results of a survey carried out by the author. It is obvious that the costs fluctuate from case to case, but in spite of many different imponderabilities certain tendencies can be perceived.

- Independent of the system, the costs for smaller plants are significantly higher than those for larger facilities. The reason for this is that the basic expenditures necessary for personnel, energy and investments do not depend (strong ly) on the size of all plant.
- Investments for Weiss reactors are higher than those for BAV plants because of the more sophisticated technology.
- The lowest operating costs are found for the Dambach system investment costs for this system are, however, the highest.

TABLE I

Plant N°	N° of Pop. Equiv.	Amount of sewage sludge with 4% d.m.		Invest ment cost	Deprec iation 12 % rate	Operating costs		Total yearly costs		Compo st sales returns
m3/d	t/a	DM	DM/a	DM/ td.s,	DM/ Pop. Equiv.	DM/ td.s,	DM/ Pop. Equiv.	DM/m^3		
101	20 000	60,0	876	310 000	37 200	134	5,90	176	7,80	30

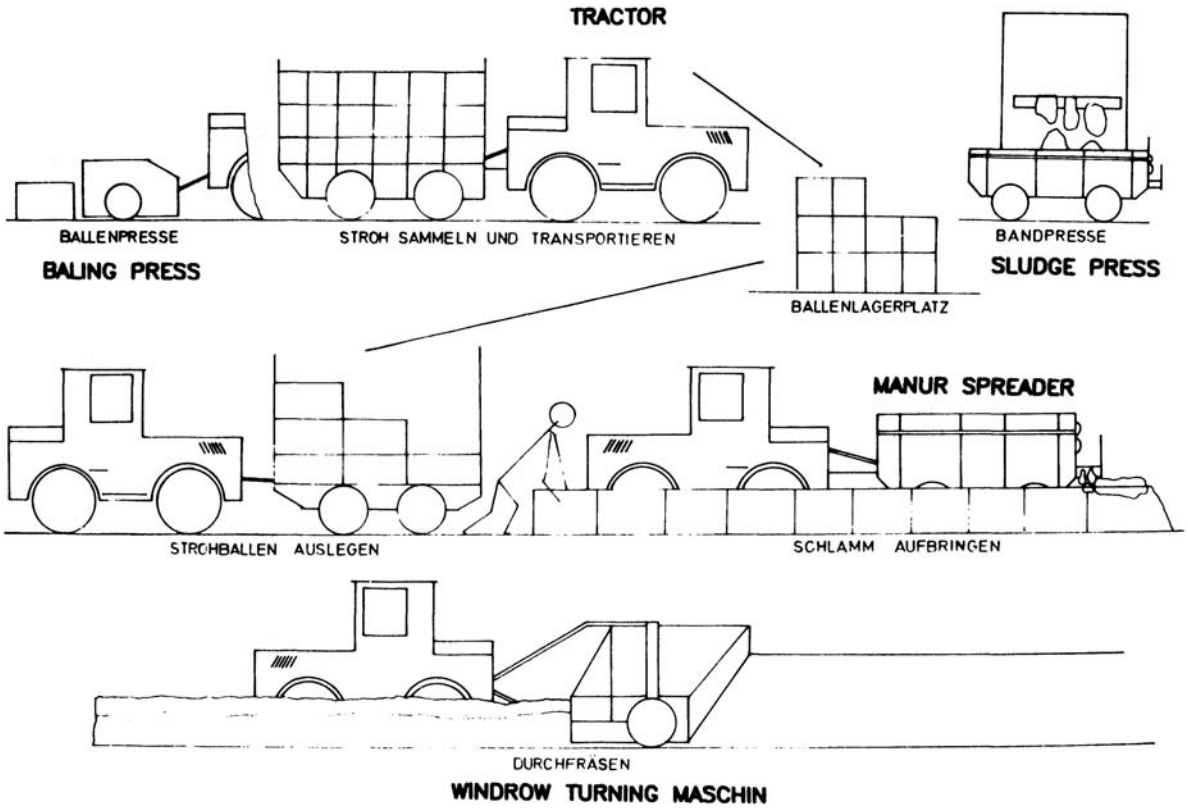

Figure 8

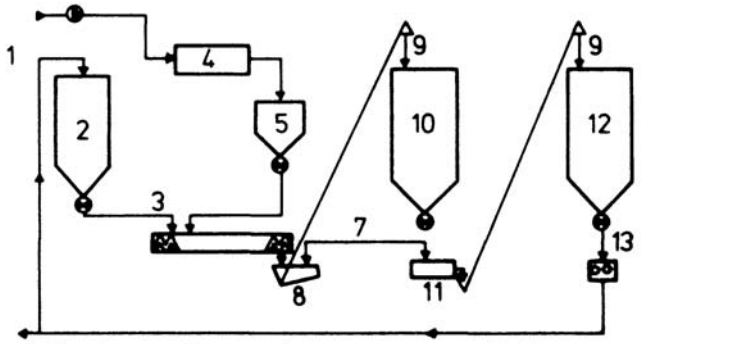

Figure 9

Plant N°	N° of Pop. Equiv.	Amount of sewage sludge with 4% d.m.		Invest ment cost	Deprec iation 12 % rate	Operating costs		Total yearly costs		Compo st sales returns
m3/d	t/a	DM	DM/a	DM/ td.s,	DM/ Pop. Equiv.	DM/ td.s,	DM/ Pop. Equiv.	DM/m^3		
102	15 000	8,5	124	–	–	–	–	–	–	20

Plant N°	N° of Pop. Equiv.	Amount of sewage sludge with 4% d.m.		Investment cost	Depreciation 12 % rate	Operating costs		Total yearly costs		Compost sales returns
m3/d	t/a	DM	DM/a	DM/ td.s,	DM/ Pop. Equiv.	DM/ td.s,	DM/ Pop. Equiv.	DM/m^3		
103	3 000	14,0	204	85 000	10 200	286	19,40	336	22,80	0
104	15 000	45,0	657	500 000	60 000	281	12,30	372	16,80	10
106	6 300	25,0	365	459 000	55 080	176	10,20	327	18,90	30
107	8 000	28,5	416	400 000	48 000	191	9,90	306	15,90	0
108	24 000	25,0	365	400 000	48 000	146	2,20	278	4,20	14
109	10 000	19,0	277	793 000	95 160	–	–	–	–	10
110	9 000	12,0	175	1300 000	156 000	592	11 ,50	1483	28,80	20
111	65 000	187,5	2738	1200 000	144 000	134	5,60	187	7,80	28
112	9 500	6,0	88	437 000	52 440	–	–	–	–	22
113	25 000	70,0	1022	750 000	90 000	137	5,60	225	9,20	25
114	11 000	34,5	504	–	–	–	–	–	–	20
115	6 000	9,5	139	560 000	67 200	–	–	–	–	20
116	10 000	2,2	32	–	–	–	–	–	–	25
117	12 000	22,0	321	400 000	48 000	–	–	–	–	20
118	14 000	18,5	270	472 000	56 640	508	9,80	718	13,90	0
119	13 000	11,3	165	950 000	114 000	–	–	–	–	25
120	8 500	15,0	219	1500 000	180 000	369	9,50	1191	30,70	25
130	8 000	11,0	161	650 000	78 000	–	–	–	–	25
221	10 000	15,0	219	1200 000	144 000	225	4,90	883	19,30	14
222	55 000	67,5	986	3000 000	360 000	81	1,50	446	8,10	0
426	5 800	12,0	175	–	–	–	–	–	–	25
427	17 000	20,0	292	800 000	96 000	239	4,10	568	9,80	35
428	17 000	12,0	175	1100 000	132 000	–	–	–	–	30

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