INACTIVATION OF MICROORGANISMS IN SEWAGE SLUDGE BY STABILISATION PROCESSES

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Commission of the European Communities, Directorate-General Science, Research and Development

ELSEVIER APPLIED SCIENCE PUBLISHERS
LONDON and NEW YORK

ELSEVIER APPLIED SCIENCE PUBLISHERS LTD
Crown House, Linton Road, Barking, Essex IG11 8JU, England

Sole Distributor in the USA and Canada
ELSEVIER SCIENCE PUBLISHING CO., INC.
52 Vanderbilt Avenue, New York, NY 10017, USA

British Library Cataloguing in Publication Data:

Inactivation of microorganisms in sewage sludge by stabilisation processes.
1. Sewage—Purification
I. Strauch, D. II. Havelaar, A. H.
III. L'Hermite, P. IV. Commission of the European Communities, *Directorate-General for Science, Research and Development*
628.3 TD745

ISBN 0-85334-383-7

WITH 70 TABLES AND 56 ILLUSTRATIONS

Publication arrangements by Commission of the European Communities, Directorate-General Information Market and Innovation, Luxembourg

EUR 9646

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Printed in Great Britain by Galliard (Printers) Ltd, Great Yarmouth

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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Chairmen : E. LUND
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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 - 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).
- 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 l (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 l. 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 l of raw sludge and axis 3 25 l 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 l. 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 l 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 l, 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.

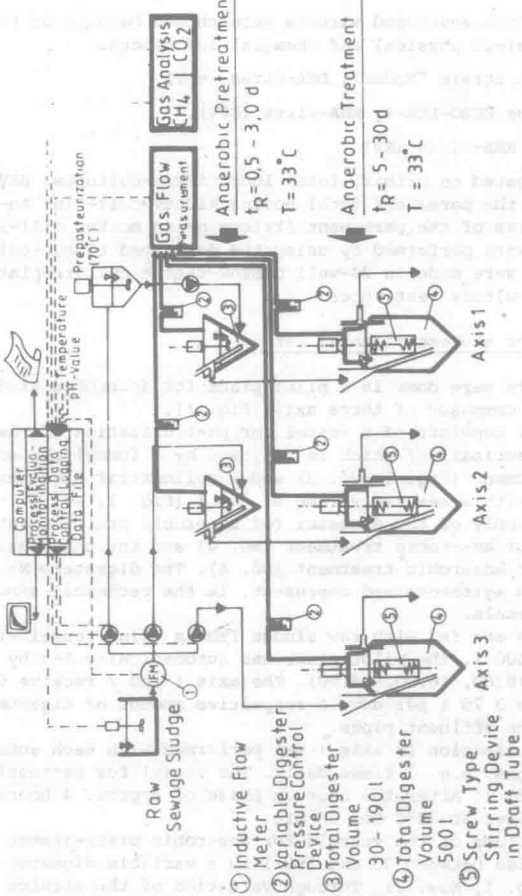

Figure 1. Flow diagram of the pilot-plant

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 10^0 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 pre-pasteurisation 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 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 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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