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Über die Arbeiten gemäss Auftrag: EF-REN (91) 043

Titel des Projektes: Human-, tier- und pflanzenpathogene Keime in der Feststoffvergärung

SUMMARY

Virus Inactivation during Anaerobic Fermentation of Source Separated Waste

Loss of infectivity of six animal viruses was assessed in a newly designed fermenter system (KOMPOGAS) intended for anaerobic-thermophilic treatment of source separated waste. Virus inactivation was followed by being suspended within ampoules and by being exposed as adsorbed and embedded particles. In the later case a unique filter sandwich technique was applied to copy the status of viruses in the environment. During anaerobic thermophilic fermentation a rapid loss of infectivity was observed with all viruses. Temperature was found to be the major factor contributing to virus inactivation. With the most heat stable parvovirus temperature alone led to a D90-value of 20 hr, whereas additional factors in the fermenting biomass exerted a strong synergistic effect leading to an overall D90-value of 5 hr. Together the results indicated that KOMPOGAS will produce a virologically safe compost.

Dauer des Projektes: 1.10.91 - 30.9.92 / 30.6.93

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INTRODUCTION

Treatment of organic matter by thermophilic fermentation is an attractive way to condition source separated municipal waste. A newly developed KOMPOGAS system was designed as a horizontally positioned, continuous-flow fermenter with a net volume of 300 m^3. The unit ist operated under anaerobic conditions at approximately 55°C with a mean retention time ranging from 15 to 21 days. Operation is reliable with waste containing as little as 15% total solids. The daily output of the reactor averages 1300 m^3 biogas and 5.2 m^3 of compost. The compost is intended for use as a fertilizer, provided that it meets with some quality requirements. One of the requirements that should be met with is the lack of microbial pathogens that could represent a health risk for man, animals or plants.

The purpose of this study was to evaluate KOMPOGAS as to the efficiency to inactivate viral pathogens. Results obtained with 6 representative animal viruses and using real scale conditions for virus exposure indicate that compost derived from KOMPOGAS can be regarded as virologically safe.

MATERIAL AND METHODS

Biologics. Viruses and cells used are shown in Tab. 1. Virus propagation and assay followed standard methods. The rotavirus was pretreated with 15 µg/ml trypsin and propagated in cells that were maintained in a medium devoid of fetal calf serum but supplemented with the aforementioned concentration of trypsin. Infectivity titrations were effectuated by the final dilution method in

Table 1. Viruses and corresponding host cells

Virus	Strain (origin)	Host cells
Picornavirus	EMCV[1] (chimpanzee)	MA 104
Rotavirus	UK (bovine)	MA 104
Adenovirus	ZH-91 (bovine)	MDBK
Herpesvirus	BHV2-TVA (bovine)	MDBK
Parvovirus	Haden (bovine)	embryonic bovine lung
Parapoxvirus	ZH-77 (bovine)	embryonic bovine lung

[1]encephalomyocarditis virus.

microtiter plates. Rotavirus infected cells were stained at 15 hr post infection using an indirect immune-peroxidase procedure and a calf hyperimmune serum as primary antibody. This facilitated detection of infected cell cultures. Results were recorded as $TCID_{50}$ per 0.1 ml.

Inactivation of suspended virus particles. Aliquots of virus containing medium were dispensed into glas tubes and incubated in the dark at 50, 55 or 60°C for selected time periods by immersion into a temperature-controlled water bath. For field experiments virus suspensions were filled into screw-capped plastic vials that were additionally sealed with a rubber tape.

Inactivation of adsorbed and embedded virus particles. In natural environments a predominant fraction of viruses is associated with solids, i.e. adsorbed onto or even embedded within solid materials. Therefore the possibility exists that virus survival and dissemination may be facilitated by such protective mechanisms [1-3]. To copy the natural situation a unique filter-sandwich technique was used to follow viral inactivation [4-5] both in the laboratory as well as in the fermenter. Briefly, infectious virus particles were passively bound onto and within positively charged nylon filter disks by simple filtration of virus suspensions. Filters were then positioned between two polycarbonate (PC) membranes and the resulting sandwiches fixed within modified filter holders. The resulting devices were then exposed to elevated temperature in the water bath or they were exposed to the fermenting waste. At selected time intervals the virus was desorbed from the filter membranes using a solution of buffered beef extract. This was followed by titration of residual infectivity. Zero values were determined with samples that were kept at 4°C.
Using PC membranes with pore sizes of 15 nm allowed diffusion of inactivating factors into the filters, but prevented viral escape from the sandwich. To copy embedded virus PC membranes without pores were used. In this case the contribution of temperature together with the effect of permeable gases could be distinguished from total virus inactivation.

Virus exposure in the fermenter. To assure timely recovery of virus containing filter holders and ampoules from the fermenter it was decided to expose the viruses to the fermenting waste by stationary means. For this a perforated holding tube was set up in the middle of the reactor (Fig. 1) that could be capped between experiments to prevent any loss of biogas. Into this holding tube a carrier, equipped with 3 metallic and perforated egg-sized bowls and containing filter holders and ampoules, could be introduced. The carrier was set up so as to assure that the virus samples came into contact with the fermenting waste.

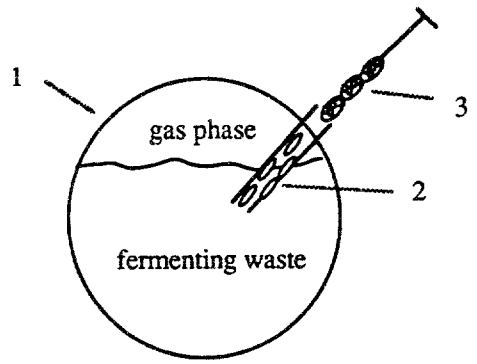

Fig. 1. Cross-sectional view through the middle of the KOMPOGAS-fermenter (1), showing the perforated holding tube (2) and the sample carrier (3) with the three perforated egg-sized bowls that contain filter sandwich devices and ampoules.

Performance date of the KOMPOGAS fermenter [6]. During the experiments temperature and pH within the fermenting waste ranged from 55.1 to 55.7°C and 7.3 to 8.1, respectively. The total solids averaged 27%, and the pressure inside the fementer was 0.5 kPa. Data on the produced biogas, obtained by gas chromatography and mass spectroscopy (Tab. 2), were kindly provided by H. Engeli, c/o Probag Umwelttechnik AG, Grünaustrasse 23, CH-8953 Dietikon.

Table. 2. Composition of biogas produced by KOMPOGAS

	CH_4	CO_2	N_2	H_2S	NH_3
vol. %	51 - 58	39 - 42.5	4.5 - 15.6	0.003-0.012	0.002-0.012

Statistical analyses. The degradation of the viruses under the different experimental conditions was analyzed by linear regression, using the principle of least squares. The rate of viral inactivation was deduced from the regression coefficient determined by regression of the $\log_{10}$ infectivity titer per 0.1 ml versus time (min or hr) and expressed as D_{90}-value, i.e. exposure time necessary to achieve a $\log_{10}$ reduction of initial virus titer ($-\log N_t/N_0 = 1$). The D_{90}-value is the inverse of the regression coefficient. Since most inactivation curves did not strictly follow a first order kinetic the D_{90}-values were additionally deduced from a line that was drawn through the zero time value (N_0) and the latest time value (N_t) of a given experiment. When calculating D_{90} from the slope of this line the resulting value was very similar to the one obtained by the first approach.

RESULTS AND DISCUSSION

Laboratory experiments. It was observed that viral inactivation did not follow a first order kinetic. This is examplified by EMCV being exposed at 50, 55, and 60°C, respectively (Fig. 2). An initial rapid loss of infectivity was followed by an unsteadily virus decay. This may be explained by preferential inactivation of a viral fraction and/or viral aggregation at early times and viral disaggregation phenomena at later times.

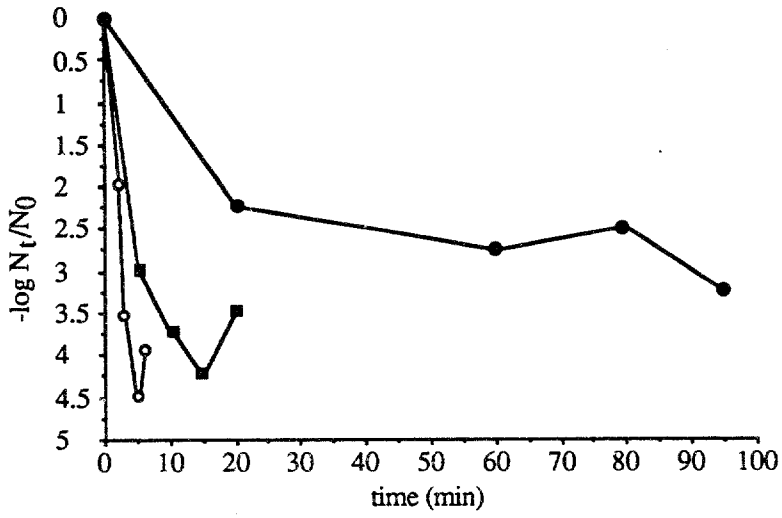

Fig. 2. Thermal inactivation of suspended EMCV at various temperatures. ● = 50°C, ■ = 55°C, o = 60°C

D_{90} -values obtained with the different suspended viruses are shown in Tab. 3. With all but parvovirus D_{90}-values observed at 50, 55, and 60°C were ≤ 31.3, ≤ 25.0, and ≤ 3.3 min, respectively. Parvovirus showed a marked thermo-resistance with D_{90}-values spanning several hours.

Table 3. Thermal inactivation of suspended virus particles[1]

Temperature	EMCV	Adenovirus	Parapoxvirus	Parvovirus
50°C	31.3 min	26.6 min	n.d.	27 hr
55°C	6.7 min	n.d.	25.0 min	20 hr
60°C	1.5 min	1.4 min	3.3 min	11 hr

[1]D_{90}-values, n.d.= not done.

Inactivation of (filter) adsorbed/embedded viruses proceeded more slowly as compared with suspended viruses, as shown for EMCV (Tab 4). This indicated

that adsorbed/embedded virus was more resistant to unfavourable conditions than was suspended virus.

Table 4. Comparison of thermal inactivation (D_{90}) of suspended with adsorbed/embedded EMCV at different temperatures.

	Virus	
	suspended	adsorbed/embedded
50°C	33 min	50 min
55°C	6 min	9 min
60°C	1.4 min	1.8 min

Field experiments in the KOMPOGAS-fermenter. We first conducted two consecutive experiments where the inactivation of suspended viruses was followed (Tab. 5). Virtually identical results were obtained in two experiments.

Table 5. Thermal inactivation of suspended virus particles in the fermenter[1]

	EMCV	Adeno-virus	Rota-virus	Herpes-virus	Parapox-virus	Parvo-virus
Experiment 1	15.6	11.9	18.5	11.1	33.3	16.9 hr
Experiment 2	15.4	10.3	18.2	8.3	33.3	17.5 hr

[1]D_{90} values are given as hr for parvovirus and min for the remainder viruses.

D_{90} for parvovirus approximated 17 hr. Of the remainder viruses parapoxvirus exerted the highest (D_{90} = 33.3 min) and adenovirus, together with the herpesvirus, the lowest resistance (D_{90} = 8.3 to 11.9 min).
When compared with the results shown in Tab. 3 it is evident that inactivation at 55 to 56°C progressed faster with parvovirus and slower with EMCV and parapoxvirus. This could be explained since temperature equilibration in field experiments was likely to be protracted as compared to the laboratory experiments. Such a protracted temperature equilibration would influence apparent D_{90}-values more with thermosensitive than with thermoresistant viruses.

In the next series of experiments the following virus preparations were exposed to the fermenting waste: 1) sandwich devices with perforated PC membranes (showing the effect of all virus inactiving factors), 2) sandwich devices with poreless PC membranes (showing the effect of temperature and possibly permeable gases), and 3) ampoules (thermal effect alone). From the results (Tab. 6) the following can be deduced. D_{90}-values in experiment 1 were significantly shorter than observed in experiment 3. This is likely due to

the combined effect of temperature and (bio-) chemical inactivation. Additionally, D_{90}-values obtained in experiment 3 are virtually the same as in the previous experiment (Tab. 4). With the parvovirus it was observed that D_{90} in experiment 2 was surprisingly low (5.3 hr) when compared with the result from experiment 3 (20 hr). This is likely to be explained by the virus inactivating effect of gases, such as NH_3 or H_2S, that do occur in the fermenter under elevated hydrostatic pressure and that are known to permeate PC membranes [7-8]. The experiment was repeated with parvovirus and the result closely paralleled the previous finding (data not shown).

Table 6. Viral inactivation in the fermenter using different ways to expose the viruses to the fermenting waste[1]

	EMCV	Adenovirus	Rotavirus	Parvovirus
Experiment 1	10.0 min	8.3 min	20.0 min	6.3 hr
Experiment 2	11.1 min	8.3 min	20.0 min	5.3 hr
Experiment 3	15.5 min	12.5 min	21.7 min	20.0 hr

[1]Data shown are D_{90}-values. Exp. 1, sandwich devices with perforated PC membranes; Experiment 2, sandwich devices with poreless PC membranes; Experiment 3, ampoules.

CONCLUSIONS

In this study the inactivation of 6 representative animal viruses was followed in the laboratory and in a biogas plant using suspended and adsorbed/embedded viruses as targets for the inactivating factors. With real scale experiments as effectuated it was found that, depending on the virus, temperature alone exerted the essential virus inactivating effect. With some viruses, as seen with parvovirus, additional factors contributed synergistically to the virus inactivating effect of temperature. The marked stability of parvovirus was confirmed in this study. This virus may thus be of value to serve as indicator for the assessement of waste disinfection processes. It is finally concluded that the KOMPOGAS-system, operating as horizontally positioned anaerobic-thermophilic fermenter, will produce a compost that can be regarded as virologically safe.

LITERATURE

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