Pathogen Reduction in Small-Scale Biogas Plants in a Tropical Region - Bench-Scale Experiments

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Introduction

Biogas production is a preferable treatment method to utilize the energy content in and to stabilize the manure, especially in tropical region where most developing countries are located. Tropical temperatures permit the utilization of efficient anaerobic reactors without heating. This is the main factor that makes the use of anaerobic technology applicable and cost-effective (Foresti, 2001). Thus low-cost biodigesters made of polyethylene tubular film and local materials, are promoted in developing countries (An, 2002; Brown, 2006). It is appealing to rural people because of the low investment, fast payback, simple technology and positive effects on the environment (An et al., 1997). Additionally, household hygiene and public health are improved.

Pathogens and indicator bacteria in animal slurries have been reported to reduce under mesophilic anaerobic condition (Juris et al., 1996; Kumar et al., 1999). Yet those studies were conducted at 35-37°C, a higher temperature compared to that of tropical biodigesters (28-30°C). Literature on pathogen reduction efficacy during anaerobic treatment of animal slurries under tropical conditions is scarce. Using Hungate tubes Olsen and Larsen (1987) showed that increasing temperature from 30°C to 35°C significantly shortened average T90 values of *Salmonella typhimurium*, *Streptococcus faecalis* and coliform bacteria. Results observed at 35°C or 37°C may not be applicable to conditions at 28-30°C.

Some studies of small-scale biogas plants in Mekong Delta, Vietnam found *Escherichia coli* with high concentrations in liquid effluents (Kobayashi et al., 2003; Rechenburg, 2007). Unlike biogas plants in Europe the daily output of small-scale tropical biodigesters has low dry matter content. Most of the solids accumulate at the base of reactors for years. The liquid effluent is normally discharged directly to water bodies or used as fertilizer for agricultural crops including those consumed fresh, e.g. water spinach. The digested slurry is managed when needed and in most cases also used as fertiliser, untreated. The situation may pose a human health risk.

This bench-scale study evaluates the pathogen reduction potential in anaerobic treatment of pig and cattle manure by replicating the conditions of small-scale tropical biogas plants.

Materials and Methods

Cattle and swine slurry were sourced from Frankenforst, the Training and Research Center of the Institute of Animal Science, University of Bonn. 500 mL bottles connected to a gas collection system were used as batch digesters. Digesters were fed 300 mL of slurry and seeded with 10% inoculum from a continuous reactor. Substrates were then spiked with low (10^3–10^4 CFU/mL) and high (10^6–10^7 CFU/mL) microbial concentrations. *Ascaris suum* eggs were placed in bags prior to incubation (10,000 eggs per bag). Temperature

conditions replicate an average tropical biogas plant (30°C) with retention time of 45 days, which is longer than the average retention time. The longer retention time was chosen to be able to fully monitor the microbial reduction over time.

Digesters were sampled on days 0, 1, 2, 4, 8, 16, 32 and 45 for analyses of bacteriophages and bacteria. Bags of *Ascaris suum* were removed every two weeks for analysis. Fresh slurry samples were analysed for organic dry matter (ODM), chemical oxygen demand (COD) and NH_4^+-N. The pH values were measured at sampling. Microorganisms included somatic coliphage, *Escherichia coli*, *Salmonella* spp., *Enterococcus* spp., *Clostridium perfringens* and *Ascaris suum*. Phages and bacteria analyses were conducted within 24 hours of sampling.

Results

Characteristic of animal slurries using in the experiment

The solid content in cattle slurries was higher than in swine slurries. Yet NH_4^+-N values were higher in swine slurries (Table 1). The pH and COD values were not significantly different between slurries.

Table 1 Physio-chemical characteristic of the raw substrates

Substrates	pH	Solids (%)		COD	NH_4+-N
		Total	Volatiles	g/L	g/L
swine slurry	7.74 – 7.85	1.8	1.2	30.05	1.55
cattle slurry	7.78 – 7.92	2.6	1.7	30.45	0.85

The presence of concerned phage and bacteria in the fresh substrates were at low concentrations (10^3 – 10^4 PFU or CFU/mL). *Ascaris* spp. was not detected in any raw slurry samples. Seeding used was free of *E. coli* and *Salmonella* spp. but contained 10^3 CFU/100ml and 10^4 CFU/100ml of *Enterococcus* spp. and *Clostridium perfringens* respectively.

The pH status of slurries changed little during the experiment. The NH_4^+-N concentrations differed. Swine slurries ranged from 1.5 to 1.95 g/l and cattle slurries ranged from 0.8 to 1.15 g/l at day 45 of the treatment.

Reduction of tested organisms

Reduction occurred with all phages and bacteria. T90 counts varied from 1.44 to >45 days (Table 2). Initial concentrations and slurry type affected the survival of tested organisms.

E. coli and *Salmonella* spp. showed a lag phase of 1 – 2 days before their concentration decreased rapidly regardless of substrate and treatment, indicating a T90 of 1 – 2 days. In general the time needed for 90% reduction of *E. coli* and *Salmonella* spp. is from 2 – 4 days. No organism was found after 8 days of treatment.

Enterococcus spp. survived longer than *E. coli* and *Salmonella* spp. (T90 varied from 15.22 to 24.79 days) and it was still viable at 45 days. Low numbers of *Enterococcus* spp. were present at day 45 (10^2 to 10^3 CFU/100 mL). *Enterococcus* spp. showed no significant difference between slurries under identical treatments but the survival rate differed ($p < 0.01$) between low and high initial concentrations, this is probably due to the constant presence of *Enterococcus* spp in the biogas slurry as it was already found in the initial seed.

Table 2 T90 (decimal reduction time) values of tested phages and bacteria in different substrates and different initial concentrations at 30°C anaerobic treatment

		Somatic coliphage			E. coli			Salmonella spp.			Enterococcus spp.			Clostridium perfringens		
substrate	treatment	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
swine	low initial conc.	> 45		4	3,13	0,7	4	1,66	0,1	2	23,8	3	4	> 45		2
slurry	high initial conc.	22,5	3,64	4	2,86	0	2	3,5	0,2	2	15,2	4,4	4	> 45		2
cattle	low initial conc.	20,6	3,47	4	2,03	0,1	4	1,85	0	2	23,2	5,7	4	> 45	5,8	2
slurry	high initial conc.	13,9	0,91	4	2,55	0,5	4	1,44	0,2	2	15,3	3,5	4	21,6	2,1	2

Clostridium perfringens showed initial reduction up to 8 days of treatment, and then an adaptation period at concentration of 4 – 6$\log_{10}$ till the end of the experiment (Figure 1), this concentration was in line with the concentration found in the used seed.
Somatic coliphages survived longer in swine than in cattle slurry and high initial inoculation showed higher removal rates than with low initial concentration.

The viability of *Ascaris suum* eggs decreased from 82% to 25% after 45 days in both slurries and corresponded to a T90 of approximately 90 days. After 2 and 4 weeks the viability did not reduce significantly. It indicates a lag phase of 4 weeks before the inactivation occurs.

Discussion and conclusions

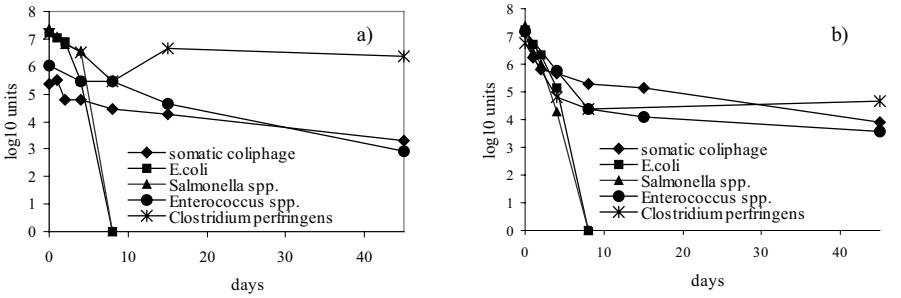
The lag phase in the activation of *Ascaris suum* eggs is corroborated by Nordin (2007) and Pecson (2007). According to Fuchs (2006) the hydraulic retention time (HRT) of the small-scale biogas plants in Vietnam was from 1.2 to 20.4 days, which is shorter than this lag phase. The operation should be adjusted so that *Ascaris* eggs can settle in the accumulated sludge at the digester base. Moreover, the survival time of *Ascaris suum* eggs in the accumulated sludge should be measured.

High concentration of *Clostridium perfringens* found at 45 days reveals a risk to use the digested slurry on the arable land. Some *Clostridium* spp. may cause infection in animals e.g. blackleg (*Clostridium chauveoi*), malignant (*Clostridium septicum* and *Clostridium sordelli* edema), black disease (*Clostridium novyi*), and enterotoxemia (three types of *Clostridium perfringens*). If these diseases are a problem in the region, restrictions in the usage of digested slurry need to be taken into consideration. However, further studies of these specific organisms needs to be done regarding the survival during this treatment.

The results indicate that the viability of tested organisms, with the exception of *E. coli*, related to initial concentration. Except for *Enterococcus* spp. somatic coliphage and bacteria behaved quite differently in swine and cattle slurries. Olsen and Larsen (1987) recognised the significant difference of T90 values in cattle and pig slurry of *Salmonella typhimurium* and *E. coli* serovar. O147, but later researches have not mentioned it (Côté et al., 2006; Kumar et al., 1999).

Survival rates of *E. coli* and *Salmonella spp*. indicate that a minimum retention time (MRT) of 4 days combined with a HRT of >10 days is needed for acceptable (>log 4) removal of these zoonotic organisms, for plug flow reactor a longer MRT may be needed. Other organisms tested require a longer retention time to be noticeably reduced. The high concentrations of *E. coli* found in the effluents in tropical biodigesters may be explained by 1) maintenance failure (e.g. short HRT, too much accumulated sludge in reactors); 2) operation conditions (e.g. the whirl flow of influent go directly to the effluent); and 3) growth within the reactor.

Fig. 1 Survival curves of tested phages and bacteria (high initial concentrations) in swine slurry (a) and cattle slurry (b)



Anaerobic digestion of animal slurries at 30°C has an effect on the reduction of pathogenic and indicator bacteria. Most of organisms tested were reduced in number but not totally eliminated after 45 days of treatment. Thus use of digested slurry on arable land, liquid output in fishponds or directly on crops should be considered in particular conditions to avoid the pathogen transmission route between animals and humans. Besides the known benefits of small-scale tropical biogas plants, the biogas yield and the hygienic quality of sludge and liquid outputs should take into account. Hence the design, construction and operation of these plants should be improved to gain the optimal performance of this simple technology in tropical rural areas. A conclusion from the study is that slurry based on manure from one species should not be used for fertilisation of food/fodder consumed raw by the same species.

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