Hygienic effects and gas production of plastic bio-digesters under tropical conditions

Vo Thi Yen-Phi, Joachim Clemens, Andrea Rechenburg, Björn Vinneras, Christina Lenßen and Thomas Kistemann

ABSTRACT

Plastic plug-flow bio-digesters have been promoted as a good option for improved treatment of manure and wastewater in developing countries although minimal information has been published on their hygienic status. This bench-scale study replicates bio-digester conditions to evaluate the reduction of pathogen and indicator microorganisms at three different hydraulic retention times (HRT) in the anaerobic treatment of pig manures at 30°C for 50 days. Results showed that physicochemical values differed between HRTs. Gas production efficiency was better for longer HRTs. The accumulated sludge at the reactor's base increased with longer HRT. Phages and bacteria examined were reduced, but none was completely eliminated. Log_{10} reduction of bacteria ranged from 0.54 to 2.47. Phages ranged from 1.60 to 3.42. The reduction of organisms at HRT = 30 days was about one log_{10} unit higher than HRT = 15 days and about two log_{10} units higher than HRT = 3 days. The results indicate that the reduction of tested organisms increases with HRT. However the hygienic quality of the liquid effluent does not meet required quality values for surface and irrigation water. Longer HRTs are recommended to increase gas yield and achieve higher pathogen reduction. More barriers should be applied while handling bio-digester outputs to minimise risks to environmental and human health.

Key words | biogas, hygiene, microbial reduction, plastic bio-digester

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INTRODUCTION

In tropical regions plug-flow plastic bio-digesters (PBD) are a cost-effective way to treat animal slurries and produce cooking gas, and have been promoted in many developing countries (An 2002; Yongabi *et al.* 2003; Brown 2006). Bio-digester effluent can be applied to crops (rice, cassava and other perennial crops), vegetables (lettuces, tomatoes, cabbage and water spinach) and in ponds (fish or water plants). Yet their design, construction and operation is unregulated, and linked to environmental and health risks. In particular the pathogen reduction efficacy is not well documented, although a few studies in Vietnam's Mekong Delta region have found high concentrations of the indicator bacteria *Escherichia coli* in bio-digester effluents (Kobayashi *et al.* 2003; Rechenburg *et al.* 2007).

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Unlike biogas plants in Europe the output of tropical PBD is low in dry matter. Solids accumulate at the plant's base, often for years. Hence, methane yields cannot be calculated onsite as gas production strongly correlates to the amount of solids present (Nuber & Tien 2008). Conversely, accumulated solids reduce the hydraulic retention time of PBDs. Of PBDs investigated by Nuber & Tien (2008) 70% showed HRTs of less than six days and the shortest HRT was 1.83 days. Short HRTs may impact negatively on the treatment efficacy of the process as well as the hygienic status of the effluent.

As well as the HRT, factors such as temperature, pH, total solids, volatile fatty acid and batch or continuous digestion, also affect pathogen survival (Henry *et al.* 1983;

Kearney *et al.* 1993*a*; Kunte *et al.* 1998). PBDs in tropical regions are made from cheap and ubiquitous materials such as polyethylene film. To increase PBD lifespan they are not exposed to direct sunlight, they are fenced off from animals, and their internal temperature is kept at 28–30°C. According to An *et al.* (1997), in Vietnam, PBDs are typically fed with fresh pig manure at low loading rates. Via a bench-scale study, *in situ* bio-digester conditions were replicated with the aim of evaluating pathogen reduction at three different HRTs (3, 15 and 30 days) in the anaerobic treatment of pig manures at 30°C.

The objective of this study was to evaluate the gas production and the hygienic quality of the effluent from PBDs in relation to the HRT. The *in situ* bio-digester conditions at three different HRTs were replicated at bench scale. An HRT of 15 days was chosen, because 13–17 days is considered the best anaerobic treatment time for pig slurry (FEC Services 2003). An HRT of 30 days was chosen because a literature review from Thy *et al.* (2005) showed that biogas production peaks at around day 30, and then declines. Finally, an HRT of 3 days was chosen to see the effect of short HRT on the reduction of pathogen and indicator microorganisms, and this HRT represents the present situation in most PBDs in the Mekong Delta, Vietnam.

MATERIALS AND METHODS

Experimental conditions

Polyethylene tubes were used to build reactors of 3 l volume that were filled with 2.5 l of substrate (Figure 1). They are comparable in dimensions to a domestic bio-digester, which has the length:diameter ratio of 5:1.

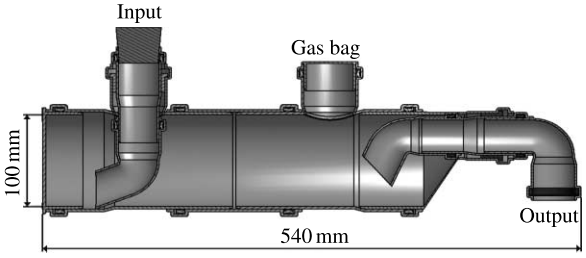

Figure 1 | Constructed reactor.

The triplicate experiment was carried out with three HRTs (3, 15 and 30 days). Reactors were placed in an incubator at 30°C, filled with pig slurry (2 g organic dry matter (ODM) per litre) and seeded with 10% inoculum sourced from a wastewater treatment plant. They were incubated for 8 days without feeding for microorganisms to adapt to the anaerobic conditions. The reactors were then fed once a day with a fixed daily input of 2.5 g ODM of fresh pig manure with different amounts of water for 50 days.

Tested microorganisms included somatic coliphages (ϕX174), MS2 phages and bacteria (*E. coli*, *Salmonella* Senftenberg, *Enterococcus faecalis*). All phages and most bacteria were obtained from the German Collection of Microorganisms and Cell Cultures (DSM) and the American Type Culture Collection (ATCC) (somatic coliphage DSM 4497, MS2 phage ATTC 15597-B1, *Salmonella* Senftenberg DSM 10062, *Enterococcus faecalis* DSM 20478). At the beginning, a batch-wise trial was conducted with similar conditions as in the bio-digesters to compare the reduction of indigenous versus collection strains at low initial concentration (10^2 to 10^3 CFU or PFU ml^{-1}). No significant difference between the reductions of indigenous and collection strains was found, except for *E. coli*. Therefore, *E. coli* was isolated from fresh pig slurry then verified by biochemical tests (Api 20E; Biomerieux) and this indigenous *E. coli* strain was used for further study.

The bacteria suspensions for spiking were made in 0.9% NaCl solution from fresh colonies grown on Columbia blood agar (5% sheep blood, Oxoid) using McFarland (BioMerieux) standards. Bacteria were spiked to the daily feeding material at a final concentration of 10^5–10^6 CFU ml^{-1}. Phages were cultivated according to ISO 10705 and spiked to the feeding material at 10^5–10^6 PFU ml^{-1}.

Sampling

Reactor influents and effluents were sampled daily for pH, dry matter (DM) and organic dry matter (ODM). Chemical parameters (NH_4^+-N, volatile fatty acids (VFA) and total inorganic carbon (TIC)), gas production and tested organisms were analysed weekly. Samples were stored at 4°C and analysed within 24 hours of sampling. The exception were

VFA samples, which were stored in a freezer (−15 to −20°C) and analysed within 3 weeks. Accumulated sludge at the reactor's base was determined at the end of the experiment. The sludge was removed from the reactors and analysed for DM, ODM and the microorganisms concerned.

Physicochemical analysis

COD was analysed by test kit (COD Cuvette test, Merck). The gas quality was analysed by an infrared analyser (VISIT 03). Gas amount in the gasbags was measured with a RITTER gas counter, and then converted to normal conditions (norm litre). Other parameters were determined using *Standards Methods* (1992).

Microbiological analysis

Somatic coliphages and MS2 phages were counted by the single-agar-layer technique as described in ISO 10705-2 and ISO 10705-1, respectively. *E. coli* was counted on Chromocult®Coliform Agar (Merck) after 24 hours of incubation at 36 ± 1°C. *Salmonella* Senftenberg was enumerated on Rambach agar (Merck) after 24 to 48 hours of incubation at 36 ± 1°C. *Enterococcus faecalis* was counted on Enterococcus Selective Agar according to Slanetz and Bartley (Merck) after 48 hours of incubation at 36 ± 1°C.

RESULTS AND DISCUSSION

Characteristics of the feeding materials

In the fresh slurry target phages and bacteria were present in low concentrations before spiking (Table 1). Seeding sourced from the wastewater treatment plant was free of *E. coli* and *Salmonella* spp. but contained 10^2 CFU ml^{-1} of *Enterococcus* spp. Owing to the different amounts of water added to the pig manure, the chemical and microbial characteristics of the feeding materials varied between HRTs, and an overview is given in Table 1.

Performance of reactors

Physicochemical values differed between the different HRTs. Average pH varied from 6.6 to 7.2. Gas yields increased from 12.8 to 40.3 l per reactor; the average amount of gas produced was significantly higher at longer HRTs (Table 2). Yet CH_4 concentration increased with decreasing HRT and pH values.

The influent and effluent ammonium concentrations were below 0.5 g NH_4^+-N l^{-1} over all HRTs. VFA concentrations in the effluents remained low over all HRTs but increased with higher HRT: 108, 235 and 360 mg l^{-1} for HRT of 3, 15 and 30 days, respectively. The COD treatment efficacy (values in the effluents compared with the influents) increased markedly from HRT of 3 days (25%) to 15 days

Table 1 | Average chemical and microbial concentrations of the feeding materials (standard deviations in parentheses)

Parameters	Unit	*n*	HRT = 3 days	HRT = 15 days	HRT = 30 days
ODM	g l^{-1}		3	15	30
pH		22	7.54 (0.18)	7.16 (0.22)	7.17 (0.22)
EC	µS cm^{-1}	21	770 (64)	2,280 (205)	3,160 (543)
TAC	mg HCO_3^- l^{-1}	5	330 (122)	650 (138)	1,190 (388)
NH_4^+-N	g l^{-1}	4	0.07 (0.04)	0.17 (0.08)	0.35 (0.1)
COD	gO_2 l^{-1}	3	2.4 (0.16)	12.2 (0.53)	26.3 (0.4)
VFA	mg l^{-1}	1	115	645	1,290
E. coli	CFU ml^{-1}	2	7×10^3	2.1×10^4	6.3×10^4
Salmonella spp.	MPN/100 ml	2	2×10^0	1.2×10^1	2.0×10^1
Enterococcus spp.	CFU ml^{-1}	2	1.7×10^3	5.1×10^3	1.28×10^4
Somatic coliphage	PFU ml^{-1}	2	3.3×10^2	5.97×10^2	2.97×10^3
MS2 phages	PFU ml^{-1}	2	8×10^1	3.2×10^2	6.32×10^2

Table 2 | Average pH values and sum of biogas produced per reactor for 50 days (standard deviations in parentheses)

Parameters	Unit	*n*	HRT = 3 days	HRT = 15 days	HRT = 30 days
pH		49	6.6 (0.04)	7.0 (0.01)	7.2 (0.03)
Biogas per reactor	l	21	12.8 (1.2)	31.6 (2.1)	40.3 (1.9)
CH_4	%	21	74 (2.6)	69 (0.4)	65 (2.9)
Biogas efficiency	l per kg ODM fed	21	97 (9)	240 (16)	310 (14)

(80%) and 30 days (91%). The accumulated sludge at the base of reactors differed substantially between HRTs: 17%, 35% and 42% of the total ODM fed for HRT of 3, 15 and 30 days, respectively. The TAC–an indicator of process stability–increased gradually for the 15-day HRT to 2,900 $mg\,l^{-1}$ and 30-day HRT to 4,800 $mg\,l^{-1}$ for the duration of the trial while the 3-day HRT had a constantly low value of around 700 $mg\,l^{-1}$. Therefore, a longer HRT is positive for reactor operation, in terms of reactor stability, effluent stability and thereby also gas production.

Effect of operational parameters on PBD performance

The pH of effluents from an HRT of 3 days was lower than that from HRTs of 15 and 30 days (Table 2), which were optimal for the biogas process (FNR 2006). The TAC was positively affected by a longer HRT. At high TAC values, the fermenter may buffer more organic acids produced in the acetogenic and acetic phase of digestion. High TAC values indicate high process stability inside the reactor and thereby also a potential to add other organic material to the digester: for example, if the pig manure source becomes deficient. Besides temperature, pH values and VFA concentration directly affect anaerobic digestion.

COD treatment efficacy was markedly high at HRTs of 15 and 30 days. An HRT of 15 days is acceptable for COD treatment in tropical PBDs. Yet these values do not correlate with real COD treatments owing to the accumulated solids at the digester base. Besides the PBD design, COD treatment efficacy depends on digester operation and maintenance. With the same HRT, effluent COD values may differ as a result of the velocity of influent flow, suggesting that the speed of input flow should be reduced when it enters the digester.

With a fixed daily input of fresh manure, reactors with longer HRTs produced more biogas. A similar trend was described by Thy *et al.* (2005). The higher gas yields at longer HRT may be due to: (1) prolonged digestion time; and (2) a lower velocity leading to increased sedimentation. The fact that sediment can contribute to biogas production is a point supported by Nuber & Tien (2008). In addition, by the end of the trial the accumulated sludge was more homogeneous in the reactors at longer HRTs.

Relations between HRT and biogas production efficiency

Results show that longer HRT positively affects biogas production efficiency in PBDs in tropical regions (Table 2). The higher TAC of the digester's substrate keeps the pH values stable during the anaerobic treatment. This is important for the methanogenesis phase since low pH (<6.5) and high level of VFA have a strongly toxic effect on methanogenic bacteria in the digester (FEC Services 2003). At HRT of 3 days pH and TAC were low while at HRT of 15 and 30 days reactors showed optimal pH for gas production. At low HRTs the methanogenic population is flushed out of the digester because of its long reproduction time, reported to be above 5 days (FNR 2006). As a consequence average methane production per reactor per day increased significantly from HRT of 3 days (0.2 l) to 15 days (0.4 l) and 30 days (0.5 l).

Reduction of microorganisms tested: comparison of influents and effluents

All examined phages and bacteria were reduced during the treatment. Generally, bacteria showed higher resistance to treatments than phages. Log_{10} reduction of bacteria ranged from 0.54 to 2.47. Phages reduction ranged from 1.60 to 3.42. That bacteria were more resistant to mesophilic anaerobic treatment of manure than phages is supported

by Lund *et al.* (1996). In contrast Gessel *et al.* (2004) showed that somatic coliphages were more persistent than *Salmonella anatum* and faecal coliforms in surface soil treated with liquid pig manure. That may be due to the different environmental conditions of the trial. It is documented that several pathogenic and indicator bacteria are very persistent and may even multiply in the biogas digester environment (Gerardi 2003; Sahlström 2003). The longer the HRT the more efficient the reduction of microorganisms. Reduction during 30-day HRT was about one $\log_{10}$ unit higher than that of 15-day HRT, and about two $\log_{10}$ units higher than that of 3-day HRT (Table 3).

E. coli showed less reduction at a HRT of three days compared with *Salmonella* Senftenberg and *Enterococcus faecalis* (Table 3). In a batch experiment with similar conditions *E. coli* showed a lag phase of 1–2 days before their concentration decreased rapidly. It can be inferred that the *E. coli* population found in effluents from reactors with a HRT of 3 days resulted from this lag phase. With HRTs of 15 and 30 days the reduction of the three bacteria investigated was similar. Log_{10} reduction of somatic coliphage was slightly higher than that of MS2 phage. The relation of reduction rates between these two phages was comparable for all HRTs (Table 3).

The reductions in the reactor do not correlate with total reduction efficacy of tested organisms because of the accumulated solids at the digester base. Some pathogens and indicator microorganisms (e.g. helminth eggs) can accumulate in the sludge. However, the concentration of the tested organisms in the sludge at the trial's end was not significantly different from that observed in the liquid output. This concurs with Kearney *et al.* (1993*b*) who reported that the concentration of *E. coli* and *Salmonella typhimurium* in separated solid effluents were slightly higher than in liquid effluents. With longer HRT the sedimentation becomes more efficient. Therefore, not only the effluent, but also the quality of the accumulated sludge, especially the concentration of helminth eggs, should also be taken into account in the different systems. The risk to human health arising from sludge usage has to be further investigated.

Effect of HRT on pathogen reduction

An HRT of 3 days showed a very low reduction of organisms tested, especially for *E. coli*. Kobayashi *et al.* (2003) found no significant difference between the concentrations of *E. coli* at the input and output ends of PBDs. Rechenburg *et al.* (2007) also concluded that indicator bacteria are only slightly reduced in PBDs. The high populations of *E. coli* found in PBD effluents in the Mekong Delta are reflected in the results obtained from the reactors with a HRT of 3 days. When the HRT of the digester is 3 days or less, the $\log_{10}$ reduction of *E. coli* is less than 0.5, while biogas is still produced due to the accumulated sludge at the digester's base. Hence the use of PBDs in such cases does not improve environmental hygiene and poses a health risk if the effluent is not further treated.

Several factors can be related to the higher reduction of pathogen and indicator microorganisms with a longer HRT. One factor is the high level of TAC that, according to Park & Diez-Gonzales (2003) inactivates bacterial pathogens. Another factor is that the longer HRTs result in less easily biodegradable substrates, which affect the survival of facultative anaerobes. Even if the hygienic microbiological quality of PBDs' effluents increases with longer HRTs, an HRT of 30 days was not enough for the effluent to meet WHO (2006) guideline standards for restricted irrigation, which stipulates a reduction in *E. coli* by 4 log units. If effluent is to be used for food production, other safety

Table 3 | Log_{10} reductions of organisms tested comparing inflow and outflow (standard deviations in parentheses; $n = 21$)

Tested organisms	Unit	HRT = 3 days	HRT = 15 days	HRT = 30 days
Somatic coliphage	PFU ml^{-1}	1.60 (0.24)	2.50 (0.36)	3.42 (0.81)
MS2 phage	PFU ml^{-1}	1.17 (0.39)	2.23 (0.45)	3.00 (0.60)
E. coli	CFU ml^{-1}	0.54 (0.43)	1.79 (0.63)	2.43 (0.71)
Salmonella Senftenberg	CFU ml^{-1}	1.23 (0.83)	1.74 (0.84)	2.47 (0.90)
Enterococcus faecalis	CFU ml^{-1}	1.01 (0.36)	1.77 (0.39)	2.30 (0.50)

barriers will be needed. It is not recommended that effluent is discharged directly to surface water, or applied to vegetables that are consumed raw. Therefore, additional health protection measures, such as allowing substantial time to lapse between last irrigation and harvesting, and washing vegetables with clean water prior to consumption, should be applied. Thus the required hygiene levels can be reached, especially for the effluent from reactors with long HRTs.

CONCLUSIONS

From this bench-scale study it can be inferred that the reduction of pathogens common to domestic PBDs in tropical regions increases with HRT. Long HRTs, or factors related to longer HRTs such as high TAC, play a vital role in pathogen reduction, while yielding more gas production as well as improving hygiene for PBD users and the general population more broadly. However effluent quality in terms of microbiological hygiene requirements is not good enough to be discharged directly into surface water or applied to crops that are eaten raw, even with an HRT of 30 days. An HRT of at least 15 days is recommended to increase gas yield and achieve a higher pathogen reduction. In sensitive areas, for example where surface water is used for domestic purposes, an HRT of at least 30 days should be applied.

These findings show that there is a hygienic effect in the anaerobic treatment of pig slurry via plastic plug-flow bio-digesters in tropical regions. More barriers (further treatment, proper practice) should be applied while handling bio-digester effluent to minimise risks to human health and the environment. Domestic PBDs are a low-cost and relatively effective local technology. For their risk-free and sustainable use more knowledge about their pathogen reduction efficacy is required. In addition legislative reform and awareness campaigns are needed to promote and regulate their proper use.

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