Hygienic and sanitation requirements in biogas plants treating animal manures or mixtures of manures and other organic wastes

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Introduction

Until relatively recently, anaerobic digestion (AD) plants at farm or industry level treated the organic waste arisings from a single farm or from an individual industrial operation. The installation of centralised AD plants, treating animal manures and slurries from a large number of farms, and the increasing use of co-digestion of manures, food-processing wastes, sewage sludge and the organic fraction of municipal solid waste (OF MSW) have raised the need for effective hygiene and sanitation procedures during operation of these AD plants.

Since the treated effluent is usually disposed of by landspreading on many individual farm holdings in the vicinity of the AD plant, the risk of disseminating pathogens from one farm to another or from sewage sludge, OF MSW, etc. to farmland must be prevented. The increasing emphasis, within the EU, on re-use/recycle of organic wastes, coupled with the need to reduce greenhouse gas emissions, has generated a growing interest in the potential application of centralised and on-farm codigestion in member states.

It is essential, therefore, to develop effective hygiene and sanitation procedures in order to minimise the disease transfer risk posed by centralised or on-farm digestion of organic waste mixtures. However, such regulations, while being effective, should not be so stringent as to cause excessive cost, thereby mitigating against the application of AD technology for organic waste recycle, inorganic nutrient return to land, and renewable energy (methane) generation.

Pathogens present in organic wastes

Wastes of animal or human origin may contain a wide variety of pathogenic bacteria, parasites and viruses. These may be disseminated along the pathways created by the systems of production, the routes of transport of live animals, and by the transformation, distribution and consumption of products of animal origin.[1] The transport of animal manures, their mixing together in AD plants and the return of the treated, mixed effluent for landspreading on farmland creates new potential pathways for disease transfer. The inclusion of abbatoir and fish-processing wastes, sewage sludge and OF MSW in AD plant feedstocks potentially increases the diversity of pathogens that may be landspread and may enter the animal and human foodchains.

The variety of pathogenic species that are regularly present in animal manures and slurries in Denmark are illustrated in Table 1. Böhm and co-workers examined a wide variety of manures, food-processing residues and household wastes for the presence of pathogenic bacteria, fungi and viruses. Table 2 illustrates the range of obligatory and facultative pathogens of man and animals found during this survey.

A diverse range of fungi, pathogenic for a wide variety of vegetable plants (potatoes, onions, tomatoes, peas, beans, carrots, turnips, cucumber, lettuce, etc.), was also isolated from the organic wastes examined in this study.

Clearly, the organic wastes presented for digestion in centralised or on-farm plants may contain a wide variety of human, animal and plant pathogens. Since it is impossible to analyse either the incoming wastes or the treated effluent for all of the pathogenic species that may be present, there is a requirement to identify indicator organism(s) that may be reliably used to evaluate the hygienisation efficiency of the anaerobic treatment process.

Indicator organisms

Indicator organisms are commonly used to detect the possible presence of faecal pathogens in treated potable water supplies, well-water supplies, recreational fresh and salt waters, swimming pools, marine waters used for shell-fish production or harvesting, etc. A variety of indicator bacterial and parasitic species have been evaluated with respect to their reliability as public health indicators, including *E. coli*, Group E *Streptococci, Salmonella, Staphylococcus aureus, Clostridium perfringens*, sulfite-reducing *Clostridia*, and the eggs and larvae of nematodes. The most commonly used indicator for public health monitoring is faecal *E. coli*. Since *E. coli* is present in lower numbers in faecal matter from animals than in man, its use as an indicator of possible faecal pathogen presence from animal sources is generally regarded as unreliable.

Böhm *et al.* (1999) listed the following requirements which indicator organisms should fulfill in the context of AD treatment:-

- Presence, with a high probability, in the raw material or manure/organic waste.
- The indicator organism should not be involved in the biological treatment process (i.e. should not be a member of the complex microbial flora involved in the AD process).
- The indicator organism should not be commonly present in natural soil or water environments.
- The isolation and quantification methods for the indicator organism must be simple, definitive, reliable and preferably low-cost.
- The methods must take into account the very complex microbiology of the wastes undergoing anaerobic digestion (animal manures, food-processing wastes, sewage sludge, OF MSW).

The veterinary research programme instituted by the Danish Energy Agency, the Agency for Environmental Protection in Denmark and the Danish Veterinary Service investigated the survival of bacteria, viruses and parasite eggs in animal manures under varying storage and anaerobic treatment conditions. The bacterial indicator organisms of choice were faecal streptococci (enterococci)(FS). Since the elevated temperatures used during AD treatment constitute the primary killing/inactivation effect on microbial pathogens, FS were chosen as the indicator species because they survive thermal treatment long after pathogenic bacteria, viruses and parasite eggs are killed or lose their viability.

In Germany, the inclusion of sewage sludge and OF MSW (biowaste) in codigestion plants was investigated from a hygiene/sanitation viewpoint. The requirements already put in place concerning

hygienic aspects of aerobic compost production were used as a guideline, and many of the potential indicator organisms used in public health microbiology were rejected because of their existing prevalence in soil and water environments. With respect to co-digestion of OF MSW, Böhm and co-workers concluded that the absence of *Salmonella* provided the best index of effective sanitation in codigestion AD plants. *Salmonella* sp. were shown to be present in >90% of biowaste bins sampled. Consequently, it was proposed to use *Salmonella* as an indicator organism, with the recommendation that *Salmonella* should be absent from tested 50g samples. Unlike the FS method advocated in Denmark, the *Salmonella* test procedure requires pre-enrichment and enrichment cultivation stages in buffered peptone water and selective media prior to positive identification.

The necessity to ensure phytohygiene was also investigated in Germany. Unlike the bacterial system, there are no recognised indicator organisms for potential plant pathogen presence. The only indicator which is widely distributed in household biowastes are tomato seeds. Consequently, the term "phytohygienic safety" has been defined, in Germany, as the absence, in treated wastes and wastewaters, of more than two tomato seeds capable of germination and/or reproducible parts of plants in one litre of treated waste.

The inactivation of animal viruses, faecal enterococci and ascarid ova has been studied in detail in Switzerland by Pesaro, Wellinger and Metzer. Using test methods involving ampoules and novel filter sandwich systems containing the viruses under test, these authors investigated the effect of various thermal regimes and AD treatments on the viability and infectivity of the test pathogens. The essential features of the filter-sandwich technique are the use of virus-loaded nylon filters that are sandwiched between polycarbonate (PC) membranes. The filter-absorbed state of the virus mimics the natural association of virions with solid biomass particles, a phenomenon known to prolong virus survival during aerobic or anaerobic treatment. The filter sandwiches were placed within special filter holders prior to introduction to the biogas plant.

The results obtained in these studies highlighted the effect of temperature on inactivation of viruses, faecal enterococci and parasitic ova. In laboratory trials using ampoules, some test viruses displayed D values (i.e. the time required to achieve 90% reduction of initial titres) lower than those of faecal enterococci and ascarid ova.

However, the parapoxvirus, PaPoV, was shown to be twice as heat resistant at 55°C (D value of 25 min) as *Enterococcus faecalis* (D = 13.2 min) and ascarid ova (D = 12.5 min). The bovine parvovirus (BPV) displayed a striking thermoresistance, with D values of c. 20 h at 55°C. In experiments with sandwich filters within the full-scale thermophilic biogas plant, the D value for BPV was shown to be reduced from 20 h at 55°C to 6.3 h. This was attributed to the effect of soluble inactivants, such as ammonia, present in the digester mixed liquor and capable of permeating through the PC membrane sandwich. With the majority of the viruses under test, heat was found to be the single most important virucidal factor. With the parvovirus, BPV, factors other than heat substantially contributed to overall loss of viability. This is in agreement with the findings of other researchers that factors such as high pH, ammonia, detergents and microbial metabolites may contribute to viral inactivation.

The use of indicator organisms to evaluate potential pathogen kill relies on activation, growth and infectivity of the test organisms. However, many bacterial species may enter a viable, but non-culturable, state (VBNC) when exposed to adverse environmental conditions, thus surviving periods of thermal or nutritional stress. Although VBNC species may not be detected by standard viable enumeration techniques, these organisms have been shown to be capable of causing infections in animals after oral administration. Consequently, reliance on viable growth test procedures for indicator organisms may not adequately reflect the infection potential of AD-treated effluent samples. However, it should be pointed out that public health test procedures for potable water supplies, shellfish production waters, etc. do not currently take the potential presence of VBNC bacteria into account.

Sanitation standards/regulations in EU countries

Only a limited number of countries within the EU have addressed the requirement to set hygiene/sanitation standards in biogas plants treating animal manure mixtures or animal manure combinations with other organic wastes.

The most detailed sanitation studies have been carried out in Denmark on centralised AD plants treating 300-500 tonnes of manure and other feedstocks (generally 20% - 30% of daily feed) per day and involving transport to and from 60-80 animal holdings per plant. The findings of these studies have been published in Danish by Bendixen and consist of eleven individual reports authored by the members of the veterinary research team. The titles of these reports have been listed in English by Bendixen and consist of very detailed studies on thermal inactivation effects on faecal streptococci and viruses during sanitation and AD treatment of manures and manure/organic waste mixtures.

Faecal streptococci (FS) were selected as the indicator organisms of choice in Denmark because of their prevalence in animal manures and their greater thermostability than the majority of bacterial, parasitic and viral pathogens likely to be present in manures, food processing wastes, sewage sludge and OF MSW. Initial studies focussed on the persistence of bacteria, parasites and viruses in manure holding tanks and in digesters operated at various temperatures. In holding tanks, bacteria, parasites and viruses were shown to survive for several weeks to months. Table 3 illustrates the survival times observed for various test bacteria in holding tanks and in mesophilic and thermophilic digesters. With the exception of spore-forming bacteria, such as *Clostridium perfringens* and *Bacillus cereus*, FS species displayed longer survival times in holding tanks and greater thermostability in digesters than the majority of bacteria, parasites and viruses tested.

Standardised test procedures were developed by Bendixen and coworkers to quantify the average FS numbers per gram of manure/slurry/organic waste/digester effluents (wet weight basis). Quantitative analysis of FS numbers in digester feedstocks and treated effluent (in AD plants which included storage and sanitation holding times in addition to digester operation) allowed determination of the **Pathogen Reducing Effect** (PRE) - i.e. the reduction in $\log_{10}$ units of FS numbers/g. For example, a PRE of 3 units is indicated by a reduction in FS numbers/g from 100,000 (10^5) in the raw material to 100 (10^2) in the treated effluent. Table 4 illustrates the PRE obtained in a detailed study of 10 full-scale centralised co-digestion plants in Denmark.

Only some of the ten full-scale plants studied are included in Table 4. The data obtained showed that in mesophilic digesters without sanitation pretreatment, the PRE achieved was generally less than 2 (often below 1), with treated effluent FS levels ranging from 10^3 to 10^5/g. In no case was a PRE greater than 2 $\log_{10}$ units achieved by mesophilic digestion alone.

By contrast, PRE values of 3 to 5 $\log_{10}$ units were obtained in thermophilic digesters without a sanitation treatment (Table 4 and Bendixen, 1999). Inclusion of a sanitation step at 55°C prior to digestion in a mesophilic plant (plant 4, Table 4) was shown to increase the PRE to 3.6 $\log_{10}$ units.

The data obtained in laboratory-scale studies on the thermolability of FS and other bacteria and viruses and the results obtaining by monitoring 10 of the full-scale Danish CAD plants led to recommendations by Bendixen and to a Danish Ministerial Order (1989) for pre-sanitation of high-risk feedstocks prior to anaerobic treatment. Pre-sanitation is preferred to post-sanitation for cost and energy usage considerations since only the high-risk wastes need to be subjected to sanitation procedures.

Controlled sanitation (defined as treatment in a sanitation tank at 70°C for a minimum of one hour) is required under the Danish Ministerial Order for wastes classified as categories C & D (OF MSW, sewage sludge, etc.). With wastes of categories A & B (animal manures, food-processing wastes, animal and fish processing wastes from establishments certified by veterinarians as being free of notifiable diseases), Bendixen sets down a range of alternative operational conditions to pre-sanitation at 70°C for 1 hour (Table 5). These conditions include minimal guaranteed retention times for the feedstock within the digester or in a pre-sanitation tank at temperatures ranging from 52°C to 65°C (Table 5). These requirements entail careful monitoring and record-keeping in full-scale biogas plants. In practice, the regular testing of FS levels in incoming feedstocks, holding tanks, sanitation tanks and in digesters has highlighted operational problems and short-circuiting of raw wastes during plant operation. This has led to improved handling procedures within the plants and to better overall operational performance, as well as to improved sanitation.

In addition to specifying an FS $\log_{10}$ reduction of 4 units for biogas plants treating high risk wastes (categories C & D), the Danish regulation specifies that the final treated product must contain less than 100 FS per gram and that *Salmonella* species must be absent from 25 g samples.

In Germany and Austria, a pre-sanitation treatment at 70°C for one hour for mesophilic AD plants or for 0.5 h for thermophilic plants is recommended (Table 6). In Germany, validation procedures, developed initially for composted organic wastes, have been adopted in the recent "Biowastes Ordinance". These procedures involve the use of *Salmonella senftenberg* W775 (H_2S negative) as test organism in specially-designed test carrier systems. The standards required for AD plants treating high risk feedstocks are no *Salmonella* sp. in 50 g and less than two germinative tomato seeds and reproducible plant parts in one litre of digestate.[2]

Other countries within the EU have not, so far, set hygiene or sanitation requirements for AD-treated feedstocks of agricultural or domestic/municipal origin.

Conclusions

The increasing trend towards co-digestion of food-processing wastes, sewage sludge and OF MSW with animal manures in centralised or on-farm AD plants, coupled with the return of the treated biomass to farmland by landspreading, has raised valid questions re the potential dissemination of human, animal and plant pathogens. Detailed studies in Denmark have resulted in the development of indicator organism test procedures and in the setting of regulations which guarantee the sanitation status of AD-treated feedstocks. These regulations also lead to more efficient operation of centralised AD plants.

Throughout the EU, only Austria and Germany have also adopted regulations concerning the hygiene status of AD-treated biomass. Given the potential pathogen content of organic wastes undergoing digestion in AD co-digestion plants, it is necessary to adopt an EU-wide regulation or directive governing the hygienic quality of AD-treated wastes. Such a regulation or directive should not be so restrictive as to mitigate against the adoption of AD treatment for organic waste re-use/recycle, inorganic nutrient return to land and renewable energy generation.

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Table 1. Pathogenic species occurring regularly in animal manures in Denmark, adapted from Bendixen (1994)

Pathogen	Disease	Main susceptible host
Bacteria		
Salmonella dublin	Enteritis	Cattle
Salmonella typhimurium	Enteritis	Cattle, poultry, man
Mycobacterium paratuberculosis	Paratuberculosis, chronic enteritis	Cattle
Enteropathogenic *E. coli*	Colibrucillosis, enteritis	Cattle, Pigs
Treponema hydrosenteriae	Pig dysentery	Pigs
Clostridium perfringens type C	Necrotic enteritis	Pigs
Erysipelotrix rhusiopathiae	Erysipelas	Pigs
Listeria monocytogenes	Listeriosis, encephalitis, abortion, general infection	Sheep, cattle
Parasites		
Ascaris suum	Ascariasis, "liver spots"	Pigs
Trichostrangylidae	Gastrointestinal disorder, emaciation	Cattle
Dictyocaulus viviparus	Bronchitis, pneumonia, emaciation	Cattle
Coccidiae	Diarrhoea	Cattle, pigs, poultry
Fasciola hepatica	Hepatitis	Cattle, sheep
Viruses		
Rotavirus	Calf diarrhoea	Cattle
Coronavirus	Calf diarrhoea	Cattle
Parvovirus	Abortion, fetal death	Pigs
Parvovirus	Plasmocytosis	Mink
Parvovirus	Panleukopenia	Mink

Table 2. Obligatory and facultative pathogens for man and animals isolated from biological and household wastes in Germany, from Böhm et al. (1999).

Bacteria	*Citrobacter, Clostridium, Enterobacter, E. coli, Klebsiella, Proteus, Pseudomonas, Salmonella, Serratia, Staphyloccus, Streptococcus, Yersinia*
Fungi	*Aspergillus* species (e.g. *Aspergillus fumigatus)*
Viruses	Adenovirus, Coxsackievirus, ECHO-virus, Enterovirus, Hepatitis A virus, *Herpesvirus suis*, Paramyxovirus, Parvovirus, Pestivirus, Poliomyelitis virus, Reovirus

Table 3. Decimation time (T_{90}) of some pathogenic and indicator bacteria in biogas plants and during storage in manure holding tanks, adapted from Bendixen (1999).

Bacteria	Holding tanks		Biogas plants	
	6-15°C	18-21°C	35°C	53°C
	T_{90} (Weeks)	T_{90} (Weeks)	T_{90} (Days)	T_{90} (Hours)
Salmonella typhimurium	5.9	2.0	2.4	0.7
Salmonella dublin	-	-	2.1	0.6
Escherichia coli	8.8	2.0	1.8	0.4
Clostridium perfringens C	NR	NR	NR	NR
Bacillus cereus	-	-	NR	NR
Staphylococcus aureus	7.1	0.9	0.9	0.5
Mycobacterium paratuberculosis	-	-	6.0	0.7
Coliform bacteria	9.3	2.1	3.1	-
Group D Streptococci	21.4	2.7	7.1	-
Streptococcus faecalis	-	-	2.0	1.0

Notes: 1. T_{90} values refer to the time taken to reduce the initial microbial number by 90%; 2. NR = no reduction observed.

Table 4. FS measurements in full-scale centralised AD plants in Denmark, adapted from Bendixen (1999)

	Biogas plant 1	Biogas Plant 2	Biogas Plant 3	Biogas Plant 4	
Digester Temperature	38°C	37°C	56°C	39°C	53°C
Sanitation	None	None	None	55°C	None
MGRT[a]	2 h	6 h	3 h	2 h	5 h
HRT[b]	28 d	23 d	20 d	23 d	19 d
Average FS in receiver tank	193,000	290,000	364,000	2,700,000	610,000
Average FS in Digestion tank	41,000	18,000	620	2,600	<10
Post digestion storage tank	104,000	5,400	8,000	3,300	2,100
PRE[c] (log_{10} reduction)	1.2 (0-4.5)	1.3 (0.6-2.0)	3.2 (2.0-4.5)	3.6 (2.3-5.0)	4.6 (3.4-5.2)

a: MGRT is the minimum guaranteed retention time of biomass in the digestion tank.
b: HRT = hydraulic retention time of feedstock
c: PRE = Pathogen reducing effect of overall process in log_{10} units.

Table 5. Sanitation equivalent to one hour at 70°C as required by Danish Notification No. 823 (Ministry for Energy and Environmental Protection), from Bendixen (1999)

Temperature	MGRT[1] in a thermophilic digestion tank[2]	MGRT[1] in a separate sanitation tank	
		Before or after digestion in a thermophilic digestion tank[2]	Before or after digestion in a mesophilic digestion tank[3]
52.0°C	10 h	-	-
53.5°C	8 h	-	-
55.0°C	6 h	5.5 h	7.5 h
60.0°C	-	2.5 h	3.5 h
65.0°C	-	1.0 h	1.5 h

1. MGRT is minimum guaranteed retention time of feedstock in the digestion or sanitation tank.
2. Thermophilic digestion is defined as 52°C or greater. The HRT must be at least 7 days.
3. Mesophilic digestion between 20°C and 52°C. The hydraulic retention time (HRT) must be at least 14 days.

Table 6. Sanitation requirements for risk substances in biogas plants in Austria, from Amon & Boxberger (1999).

Digester type	Heat treatment before anaerobic digestion		Sanitation during anaerobic digestion	
	Temperature (°C)	Time (h)	Temperature (°C)	Time (h)
Mesophilic biogas plants (20 - 40°C)	70	1	-	-
Thermophilic biogas plants (55°C or greater)	70	0.5	55°C	24