Hygiene Aspects of the Biogas Process

By Elisabeth Bagge, National Veterinary Institute, Sweden

To avoid spreading of diseases via biogas plants when digested residues are spread on arable land, a pasteurization stage at 70°C for 60 min before anaerobic digestion gives adequate reduction of most non spore-forming bacteria, such as Salmonella spp., E. coli O157. Some spore-forming bacteria appeared to pass through the biogas process unaffected and thus digested residues should be spread with consideration of the risk of spreading diseases. This risk should be compared with the disadvantages of using artificial fertiliser, i.e. the limited natural resources of phosphorus. Maybe the advantage of using pasteurized digested residues as fertiliser may outweigh the risk of spreading diseases. However, caution should be exercised before digested residues are spread in areas without endemic problems of pathogenic spore-forming bacteria. In Sweden, official recommendation is that digested residues from biogas plants only should be applied on arable land, and not on grasslands for animal pasture.

Biogas, derived from digestion of biowaste, is a renewable source of energy. In addition to the production of biogas, digested residues are rich in plant nutrients and can be used as fertiliser. However, biowaste may also contain pathogenic micro-organisms such as Salmonella spp. Therefore a separate pre-pasteurization step at 70°C for 60 min before anaerobic digestion is required by EC regulation 1774/2002 and EC 208/2006 if the plant process animal by-products. This will reduce non spore-forming bacteria, however, heat treatment at 70°C for 60 min will not reduce spore-forming bacteria such as Bacillus spp. and Clostridium spp. Spore-forming bacteria, including those that cause serious diseases, can be present in substrate intended for biogas production.
In Sweden, full-scale, commercial biogas plants, which process animal by-products, operate a separate pre-pasteurization at 70°C for 60 min. Thereafter, the process is followed by digestion; mesophilic at approximately 35-37°C for 25-30 days or thermophilic at approximately 55°C for 10-12 days.

The purpose of the first study was to establish if, during pasteurization and further processing and handling in full-scale biogas plants, pathogens in biowaste could be sufficiently reduced to allow its use on arable land. Sampling was performed from six locations during biogas production. The samples were analysed quantitatively to detect indicator bacteria and spore-forming bacteria, and qualitatively for bacterial pathogens (Salmonella spp., Listeria monocytogenes, Campylobacter spp. and E. coli O157). In general, the treatment adequately reduced both indicator bacteria and pathogenic bacteria.

Before pasteurization Salmonella spp. was the most frequently isolated pathogen. Spore-forming bacteria were not reduced. However, recontamination and re-growth of bacteria in biowaste was frequently noted after the biogas process. The risk of contamination is particularly high when digested residues are transported in the same vehicles as the raw material. To maintain the standard of the digested residues it must be handled in a strictly hygienic manner to avoid recontamination and re-growth of bacteria.

To assess the effect of pasteurization, selected pathogens and indicator organisms was heat treated under laboratory conditions at 70°C and 55°C for 30 min and 60 min respectively together with unpasteurized biowaste from a full-scale biogas plant, which process animal by-products. Heat treatment at 55°C for 60 min was not sufficient to achieve a hygienically acceptable product. Heat treatment at 70°C for 30 min and 60 min was effective in reducing pathogenic bacteria except spore-forming bacteria, heat-resistant viruses such as porcine parvovirus or Salmonella phage 28B.

Most spore-forming bacteria are harmless inhabitants of the gut or in soil. However, some of them cause dreaded diseases, e.g. blackleg, botulism and anthrax. To investigate the effect of pasteurization and digestion on spore-forming bacteria, manure samples from healthy cattle, samples from slaughterhouses waste intended for biogas production and samples from different stages in the biogas process were collected and analysed. Bacillus spp. and Clostridium spp. were quantified, subcultured and the isolates were identified by biochemical methods and by 16S rRNA sequencing.

The most common Clostridium spp. found was Clostridium perfringens. Clostridium botulinum and Clostridium sordellii were found before and after pasteurization, but not after anaerobic digestion. The most common Bacillus spp. were Bacillus pumilus and Bacillus subtilis. Some of the isolated strains were probably representing new members of the genera Clostridium and Bacillus. The number of species of clostridia decreased after digestion while Bacillus spp. remained constant. Some pathogenic spore-forming bacteria from biowaste can pass unaffected through biogas plants, but the results in this study indicate that the number of species of clostridia seemed to decrease following digestion, likewise the quantity. However, Bacillus spp. seemed to pass unaffected through the biogas process.

In laboratory-scale experiments the effects on clostridia during pasteurization and digestion were investigated. Pathogenic clostridia (Clostridium chauvoei, Clostridium haemolyticum, Clostridium perfringens type C, Clostridium septicum and Clostridium sordellii) were inoculated in substrates from homogenisation tanks and digester tanks from four biogas plants, two with mesophilic digestion and two with thermophilic digestion. Pasteurization and digestion were simulated. To detect and identify clostridia, specific PCR primer pairs for each Clostridium spp. were used after culture on agar plates and DNA preparation. Clostridium chauvoei, Cl. perfringens type C, Cl. septicum and Cl. sordellii were detected both before and after pasteurization. Clostridium septicum and Cl. sordellii were detected following anaerobic digestion whereas neither Cl. perfringens type C nor Cl. chauvoei could be detected throughout the digestion. The results may mean that


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the disease risks associated with spreading of *Cl. chauvoei*, *Cl. haemolyticum* and *Cl. perfringens* type C are negligible.

The impact of anaerobic digestion differs between species of pathogenic spore-forming bacteria, which should be taken into consideration when planning the use of residues as fertiliser. Many pathogenic clostridia are soil bacteria. If digested residues are spread on soil already contaminated with clostridia the risk increase is negligible. But areas with uncontaminated soil, caution should be taken before spreading digested residues.

*Clostridium chauvoei* causes blackleg, an acute disease associated with high mortality in ruminants. The apparent primary port of entry is oral, during grazing on pasture contaminated by spores. Cases of blackleg can occur year after year on contaminated pastures. The use of digested residues as fertiliser may contribute to the spread of *Cl. chauvoei*. A method to determine the prevalence of *Cl. chauvoei* spores on pasture would be useful. Soil, manure and substrate from biogas plants are contaminated with other anaerobic bacteria which outgrow *Cl. chauvoei*. Therefore, detection by PCR would be useful.

This last study applied a PCR-based method to detect of *Cl. chauvoei* in samples from tissue from cattle dead in blackleg, manure, soil and from different stages in the biogas process. The farms where the manure and soil samples were taken had proven cases of blackleg. For detection of *Cl. chauvoei* in the samples, a specific PCR primer pair complementary to the spacer region of the 16S-23S rRNA gene was used.

*Clostridium chauvoei* was detected in 27% (3 out of 11) samples from the biogas plants collected before pasteurization, but samples taken after pasteurization and after digestion all tested negative. *Clostridium chauvoei* was not detected in any soil or silage samples and only one manure samples was tested positive. The standard method for *Cl. chauvoei* detection is culture and biochemical identification, which requires a pure culture. Detection by PCR would be faster and independent of contaminating flora. The PCR-method used could be highly useful for clinical samples, but not applicable for environmental samples.


**Baltic Compass is looking for a biogas feasibility expert**

The Baltic Compass project in cooperation with Agro Business Park is looking for a biogas expert to support Vecauce Research and Training Farm to plan improvements and expansion of their biogas production.

The expert must have a proven track record in biogas feasibility studies, insight into biogas technologies and knowledge to substrates for biogas production. Interested experts must send CV and financial offer (specified in fees, travel costs and per diems) latest Friday 9 September 2011 to Project Manager Henning Lyngsø Foged, hlf@agropark.dk, +45 4034 8625

To find more information about the job, Terms of Reference, Background Report, and a Market Description for the Bioenergy Sector in the Baltics go to: [http://www.balticcompass.org/_blog/Latest_News/post/Baltic_Compass_is_looking_for_a_biogas_expert/](http://www.balticcompass.org/_blog/Latest_News/post/Baltic_Compass_is_looking_for_a_biogas_expert/)
New Interreg project has started: Baltic MANURE

By Knud Tybirk, Agro Business Park, Denmark

In autumn 2010 a new project closely related to Baltic COMPASS was approved by Interreg Baltic Sea Programme and has now published its webpage www.balticmanure.eu.

The overall project goals are to enhance business, improve nutrient cycles, foster renewable energy use in the region, and reduce environmental load from agriculture to the Baltic Sea. If you are interested in news from this project you can sign up for a bi-annual Baltic Manure newsletter at http://www.balticmanure.eu/en/newsletter/newsletter.htm.

Baltic Manure is a Flagship Project in the Action Plan of the EU Strategy for the Baltic Sea Region. It involves 18 project partners from 8 countries with MTT – Agrifood Research as lead partner. The total project budget is € 3.7 million and it is partly financed by the European Union (European Regional Development Fund).

The long-term strategic objective of the Baltic Manure project is to change the general perception of manure from a waste product to a resource, while also identifying its inherent business opportunities with the right manure handling technologies and policy framework. To achieve this objective, research and development within renewable energy and nutrient recycling will be combined with business innovation in the Baltic Sea Region.

Five themes will be treated in the project:

- Innovative technology for animal feeding and housing, processing, storage, and spreading of manure
- Standardisation of manure types with special emphasis on the phosphorus challenge
- Assessing sustainability of manure technology chains
- Energy potentials of manure
- Business innovation

Call for Baltic Manure Handling Award 2011

Have you invented and marketed a novel technology for sustainable manure handling? Are you interested in getting full exposure of your technology and open for market share in the Baltic Sea Region? - Then you should sign up for the Baltic Manure Handling Award!

Criteria for the Manure Handling Award 2011:

The new technology should represent innovation that is market ready and can

- reduce pollution (less leaching of nutrients, less ammonia evaporation, less odor, less climate gas emissions etc.) and/or
- increase plant availability or other sustainable uses/commercialization of manure nutrients (separation, drying, pelleting or other technologies for pretreatment, storage and field application of manure etc.) and/or
- increase the energy output of manure (biogas, fiber incineration, gasification etc.)

Award Call closes at October 1st 2011 and the winner will be published at the joint Baltic Compass, Baltic Deal and Baltic Manure conference “A Greener Agriculture for a Bluer Baltic Sea” in Stockholm on November 2nd. It should be noted, that the Award is only symbolic – the winner will gain from exposure in media and websites.

Please contact Anne-Luise Skov Jensen alsj@agropark.dk for more info and submit your proposal to her. Further info on Baltic Manure project, please contact Communication Manager Knud Tybirk, kt@agropark.dk
Agro Technology ATLAS

Agro Business Park has launched a new website - [http://agro-technology-atlas.eu](http://agro-technology-atlas.eu).

In general, the intention of the website is to provide easy access to validated and impartial information, tools and data about agro-environmental technologies, hereunder handling, treatment and processing of biomass.

The site has been established with financial support from the Baltic Sea Programme 2007-2013 in connection with the implementation of the Baltic Compass project, and the plan is to publish data and information here on an ongoing basis, both originating from Baltic Compass – such as this newsletter – and other projects.

The website will gradually be build up during the coming years. There were recently published a large number of data sets concerning the quality of livestock manure types as well as qualities of end and by-products from various processing of livestock manure.

Sector Study (draft) – concerning prioritized agro-environmental technologies for sustainable food production in the Baltic Sea Region

One of Baltic Compass’ focal points is the acceleration of investments in the best available environmental technologies through innovation networking activities, market analyses, knowledge transfer and investment support.

In order to investigate ways to increase the use of the three prioritized technologies; Biogas production, livestock manure separation, and SCIEN drainage, as well as ensure the implementation of the cross-cutting issues about phosphorus measures, a Sector Study has been developed to identify major barriers and enablers in the ten Baltic Compass countries.

The Sector Study is the result of both desk study and an e-mail survey, and presents the major factors that motivate and deter potential investors within eight descriptive parameters, ranging from the legislative situation in the country to the technological conditions concerning the four technologies.

Companies can with the Sector Study identify the most ready markets for their technologies, while for instance politicians easily can clarify the most relevant instruments to ensure the reaching of policy goals through a higher use of the prioritized technologies.

The present round of National Roundtable Meetings, held under auspices of Baltic Compass, will give stakeholders from each country a possibility to comment on the Sector Study. Anyone is encouraged to comment on the Sector Study, especially the part about their own country, and to comment on the Sector Study in general. Comments can be sent to: CM@agropark.dk.

Phosphorus Indices

New report on “Phosphorus Indices - Status, relevance and requirements for a wider use as efficient phosphorus management measures in the Baltic Sea Region”

The report is mainly based on presentations and discussions from a P-index workshop organized by Baltic Sea 2020 in January 2011. Representatives from science, environment, farmers, farm advisory, and administration in Sweden, Denmark, Norway, Finland, Germany and Poland met and presented the current status for P-indices in their respective countries and discussed the relevance and requirements for a wider use of P-indices as efficient phosphorus management measures in the Baltic Sea Region.

The workshop participants acknowledged P-indices as a relevant tool for avoiding loss of phosphorus from fertilizing to the aquatic environment. They recommended the establishment of a forum for Baltic cooperation in development of efficient P-indices which are widely accepted by farmers and authorities. The objective of such a forum would be to inspire each other to develop functional P-indices in the region (e.g. the suitable geographical area to cover with the P-index).

The report is issued by Baltic Sea 2020 within the frames of their “Intensive Pig Production Programme”, and follows up on earlier reports on the subject of analyzing and discussing best available technologies to reduce loss of plant nutrients from intensive pig production.

### Upcoming events

**Conference: Aquarius – The Farmer as Water Manager**

**Venue:** Fuglsoecentret, near Aarhus, Denmark  
**Date:** 12-13 October, 2011  
**The event:** The conference marks the end of the project: “AQUARIUS – The Farmer as Water Manager” and different stakeholders will get the chance to discuss the results and experiences from AQUARIUS and other related projects at this event.  
**More info:** [http://inbiom.dk/en/20110712_03.htm](http://inbiom.dk/en/20110712_03.htm)

**Conference: A Greener Agriculture for a Bluer Baltic Sea 2011**

**Venue:** Sånga-Säby, near Stockholm, Sweden  
**Date:** 2-3 November, 2011  
**The event:** The event is a must for all those who work with agro-environmental issues in the Baltic Sea Region. The event is the major forum for networking and exchange of knowledge, experiences and ideas.  
**More info:** [http://inbiom.dk/en/20110712_02.htm](http://inbiom.dk/en/20110712_02.htm)

**Seminar: “Wirkung und Folgen der Nutzung von Biomasse zur Biogasgewinnung auf Böden und Gewässer”**

**Venue:** Suderburg, Germany  
**Date:** 12-13 October, 2011  
**More info:** [http://dwa-nord.de/portale/nord1/nord1.nsf/home?readform&objected=E0F1B7FE358CC60AC12578C700361E78](http://dwa-nord.de/portale/nord1/nord1.nsf/home?readform&objected=E0F1B7FE358CC60AC12578C700361E78)