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„MesTherMes“ the Biogas Process

Process Engineering and Microbiology for Animal Manure Hygienisation

The performance of a pilot biogas plant in optimised cattle manure hygienisation was investigated in a joint project between research institutions and a water supply company. The plant features three digesters operated in series at mesophilic, thermophilic and again mesophilic temperature conditions („MesTherMes“). During the reported time period the biogas yields were typical for agricultural biogas plants running on cattle manure. Pathogenic and indicator microorganism reduction was investigated using current cultivation methods and a newly developed qPCR-protocol.

Due to hygienic considerations the application of animal manure is generally prohibited within the inner zone of water protection areas. The reduction of different pathogenic microorganisms that might be present in animal manure by anaerobic treatment in biogas plants has been repeatedly shown. While in the mesophilic temperature range (around 35°C), sufficient destruction of relevant pathogens is not achieved within a practicable detention time of 24 h, a thermophilic treatment at 55°C is very effective for the reduction of most pathogenic bacteria, viruses, and parasites in animal manure. The anaerobic treatment process also produces the renewable energy source biogas and valuable organic fertiliser.

Agricultural biogas plants are typically equipped with continuously-stirred tank reactors and operated in the mesophilic temperature range [1]. From practical experience, there are few reliable data on technical and operational requirements of agricultural biogas plants in order to achieve efficient and safe reduction of a broad range of pathogens in animal manure. This problem is therefore investigated at an agricultural pilot biogas plant located in Bad Aibling, Southern Bavaria (*Fig. 1*). The work is a joint project of two research institutions and a water supply company. The major objectives are:

1) to optimise the reduction of pathogenic and indicator microorganisms in cattle manure by anaerobic treatment without the use of an additional hygienisation unit, and
2) to develop and evaluate feasible methods of molecular biology to specifically detect pathogenic microorganisms in semi-liquid manure and digest.

Materials and Methods

Anaerobic treatment process

A sequence of three anaerobic digesters operated at different temperature levels (mesophilic, thermophilic, and again mesophilic - „MesTherMes“) has been constructed in order to inactivate particularly resistant pathogens like spore-forming bacteria and parasites. Both mesophilic fermenters (F1 and F3, respectively) are continuously-stirred tank reactors with mechanical mixers (propeller type). The thermophilic fermenter (F2) is a horizontal tubular reactor that is equipped with a paddle mixer and baffles to reduce

Table 1: Evaluated microbial parameters

Kind of cultivation	qPCR
Coliforme Keime	Enterobacteriaceae
Fäkalcoliforme Keime	Escherichia coli
Intestinale Enterokokken	Enterococcus faecalis + E. faecium

Table 2: Cultivation bases and qPCR-analyses of microorganisms in samples from fresh slurry and from the fermenter/digested slurry

Bacteria / Groups of B.	Slurry	Fermenter 1	Fermenter 2 (48°C)	Fermenter 2 (51°C)	Fermenter 3§	digested$ slurry
Fäkalcoliforme (MPN mL^{-1})	$0.3 \pm 3.5 \cdot 10^5$	$1.1 \cdot 10^3$	$2.4 \cdot 10^1$	2.3	0.1	$3.5 \pm 1.2 \cdot 10^1$
Escherichia coli (Genome mL^{-1})	$1.1 \pm 0.8 \cdot 10^5$	$3.4 \pm 0.2 \cdot 10^3$	$2.6 \pm 0.1 \cdot 10^3$	$2.4 \pm 1.5 \cdot 10^2$	$1.5 \pm 0.3 \cdot 10^2$	$4.3 \pm 0.5 \cdot 10^2$
Enterokokken (KBE mL^{-1})	$1.7 \cdot 10^6$	$3.0 \cdot 10^4$	$3.0 \cdot 10^3$	$1.0 \cdot 10^3$	$6.5 \cdot 10^1$	$1.9 \cdot 10^2$
Fäkale Enterokokken (KBE mL^{-1})	$3.0 \cdot 10^5$	$1.3 \cdot 10^3$	$4.0 \cdot 10^2$	$2.3 \cdot 10^2$	$< 1.0 \cdot 10^1$	$< 1.0 \cdot 10^1$
Enterococcus faecium (Genome mL^{-1})	$4.1 \pm 1.5 \cdot 10^6$	$1.0 \pm 1{,}5 \cdot 10^4$	$1.4 \pm 0.1 \cdot 10^3$	$4.2 \pm 1.7 \cdot 10^2$	$8.2 \pm 3.5 \cdot 10^3$	n.b.

KBE, Kolonie bildende Einheiten. MPN, Most probable number. n.b., nicht bestimmt.
§, Material aus Fermenter 2 bei 48°C; $, Unspezifische Hintergrundflora vorhanden.

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Keywords
Biogas, animal manure, hygiene, pathogens

longitudinal mixing. The pilot plant has been designed to treat the manure of about 100 livestock units of dairy cattle (about 65 animals). It is controlled by a programmable logic controller and operated at quasi-continuous mode (hourly feeding).

Continuous monitoring includes quantity of treated substrate, digester temperatures, quantity and composition of biogas, and quantities of electrical and thermal energy. Samples of fresh manure and digester contents are regularly analysed for total solids (TS), volatile organic solids (V0S), volatile organic acids, ammonia and total nitrogen, chemical oxygen demand, pH, and alkalinity.

Microbiological assays

During the period of operation reported in this paper, the microbiological parameters listed in *Table 1* have been investigated by the methods of conventional cultivation and real-time Polymerase Chain Reaction (qPCR).

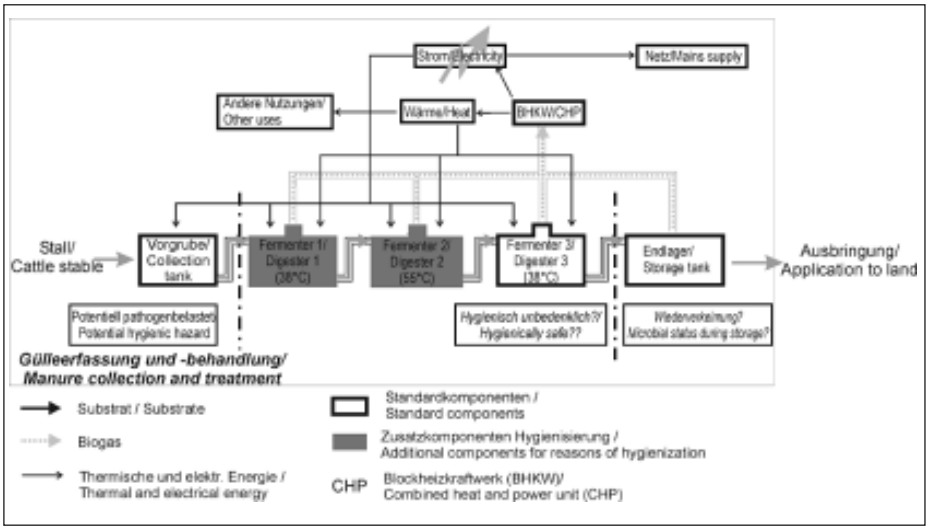

Fig. 1: Overview of the treatment process for animal manure

Results

Anaerobic treatment process

Between January and June 2003, the TScontents on a mass per mass basis in liquid samples taken from the collection tank and the digesters 1 through 3 (*Fig. 1*) ranged between 6.6 and 10.0; 6.1 and 8.2; 5.9 and 7.1; and 7.1 and 6.9 per cent, respectively. The respective contents of (VOS) ranged between 72.5 and 76.9; 69.8 and 73.9; 69.6 and 72.1 and 66.3 and 71.2 %. Based on these values the grade of degradation of VOS can be estimated to between 40 and 50 per cent for the whole digester sequence.

Measured methane contents of the biogas in the collecting pipe to the combined heat-and-power unit reached 54 to 60 % (v/v). After correcting for remaining oxygen contents from biological desulfurisation these values calculate to 63 to 67 % (v/v) of methane which are maximum numbers to be expected for the sole digestion of cattle manure. Between January and June 2003 the average gas yield from all digesters of the pilot plant was 21 net-m^3 per ton of fresh manure (range: 6 to 33 net-m^3 per ton of fresh manure). The average methane yield within this time period was 0.18 net-m^3 per kg of fed VOS, at an average loading rate of 1.6 kg VOS per m3 total used digester volume and day. In practice, typical values of gas or methane yield of cattle manure, respectively, are about 25 net-m^3 per ton of fresh manure or 0.2 net-m^3 per kg of VOS (loading rate: 1.7 kg VOS/m^3•d). As a peculiarity of this plant design, the loading rate of the digester sequence with respect to its total usable volume is comparably low, while the single reactors 1 and 2 exhibit a fairly high loading rate. Specific biogas yields usually decrease with increasing loading rates [2]. Data on the biogas production of the individual digesters were not yet available for this paper. An improved degradation performance for the anaerobic treatment of municipal organic waste has been reported for a sequence of two reactors operated at thermophilic and mesophilic temperature levels, respectively [3]. Data from the pilot biogas plant are currently not sufficient to assess whether this is also achieved with cattle manure. One advantage of having three digesters is that possible process instabilities may be better absorbed.

Microbiology

Data from microbiological analyses are shown in *Table 2*. During the reported time period, the temperatures in the thermophilic digester ranged only between 48 and 51°C. This is considerably lower than the 55°C required by the Ordinance on the Utilisation of Bio-Wastes on Land used for Agricultural, Silvicultural and Horticultural Purposes (BioAbfV) (Animal manure from clinically healthy livestock is, however, not subject to these regulations). Because of the small extraction volume of only 40 µl , the theoretical detection limit of the qPCR protocol was 250 organisms per ml of substrate.

For the investigated microorganisms/ microorganism groups a reduction in numbers by 3 to 5 orders of magnitude was found under the above-mentioned sub-optimal operational conditions. The hygienic performance at a temperature of 51°C in digester 2 appeared slightly better than at a respective temperature of 48°C (*Table 2*). With increasing treatment time, results from qPCR deviated from those based on cultivation toward higher levels. This effect was most pronounced for enterococci, and may be attributed to the detection of dormant or already dead organisms by the qPCR-method. The required time for a complete analyses was 6 to 8 h for qPCR and 24 to 72 h for cultivation, respectively.

Conclusions and Outlook

The detection of specific microorganisms by the method of qPCR offers an excellent alternative to the quantification of (indicator-) microorganisms by conventional cultivation. Within the further course of this project, the developed qPCR-protocol will be applied to investigate the reduction of pathogens including particularly resistant microorganisms in the pilot biogas plant. Economical and ecological assessments will show whether the entire treatment process may be an environmentally friendly and economical contribution to the protection of drinking water resources.

Literature

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