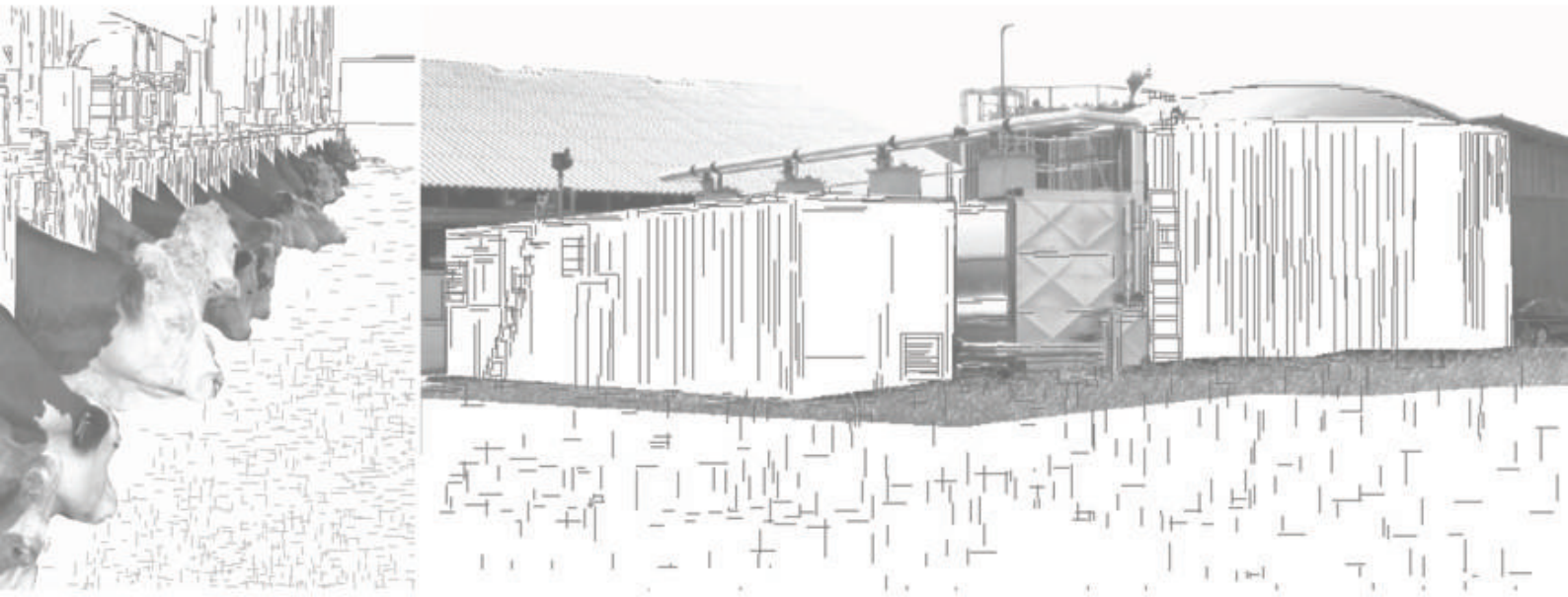
Mathias Effenberger

Influence of temperature and feeding mode on digestion and sanitation efficiency during multiple-stage anaerobic treatment of liquid dairy cattle manure



Cuvillier Verlag Göttingen

Technische Universität München
Lehrstuhl für Agrarsystemtechnik

Influence of temperature and feeding mode on digestion and sanitation efficiency during multiple-stage anaerobic treatment of liquid dairy cattle manure

Mathias Effenberger

Vollständiger Abdruck der
von der Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt der Technischen Universität München
zur Erlangung des akademischen Grades eines

Doktor-Ingenieurs (Dr.-Ing.)

genehmigten Dissertation.

Vorsitzender:
Univ.-Prof. Dr. J. Meyer

Prüfer der Dissertation:
1. Univ.-Prof. Dr. H. Auernhammer, i. R.
2. Univ.-Prof. Dr. K. J. Hülsbergen
3. Univ.-Prof. Dr. Dr. h.c. P. A. Wilderer, i. R.

Die Dissertation wurde am 19.09.2007 bei der Technischen Universität München eingereicht und durch die Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt
am 11.03.2008 angenommen.

Bibliografische Information der Deutschen Nationalbibliothek
Die Deutsche Nationalbibliothek verzeichnet diese Publikation in der Deutschen Nationalbibliografie; detaillierte bibliografische Daten sind im Internet über http://dnb.ddb.de abrufbar.
1. Aufl. - Göttingen : Cuvillier, 2008
Zugl.: (TU) München, Univ., Diss., 2008

978-3-86727-652-8

Nonnenstieg 8, 37075 Göttingen
Telefon: 0551-54724-0
Telefax: 0551-54724-21
www.cuvillier.de

1. Auflage, 2008
Gedruckt auf säurefreiem Papier

978-3-86727-652-8

DANKSAGUNG / ACKNOWLEDGEMENTS

Die vorliegende Arbeit wurde am Institut für Landtechnik und Tierhaltung der Bayerischen Landesanstalt für Landwirtschaft (LfL; vormals Bayerische Landesanstalt für Landtechnik) im Rahmen eines durch das Bayerische Staatsministerium für Landwirtschaft und Forsten geförderten Forschungsprojektes angefertigt.

An den Anfang dieser Danksagung möchte ich das Andenken an meinen ursprünglichen Doktorvater Herrn Prof. Dr. Dr. h.c. Hans Schön stellen, der leider während meiner Promotionszeit plötzlich verstarb. Herrn Prof. Dr. habil. Hermann Auernhammer danke ich, dass er daraufhin ohne Zögern bereit war, die Begutachtung der Arbeit am Lehrstuhl für Agrarsystemtechnik zu übernehmen. Bei den Herren Prof. Dr. Kurt-Jürgen Hülsbergen und Prof. Dr. Dr. h.c. Peter A. Wilderer bedanke ich mich für die bereitwillige und engagierte Übernahme der Koreferate.

Prof. Dr. Milos Tesić danke ich für seine hilfreichen kritischen Anmerkungen.

Dr. Andreas Gronauer gebührt Dank für die fachliche Betreuung der Arbeit und die persönliche Unterstützung. Bei Dr. Michael Lebuhn und Gabriela Garcés bedanke ich mich für die stets angenehme und interessante Zusammenarbeit und die Prise Humor im rechten Moment. Johannes Bachmaier stand mir in so mancher Gülleschlacht bei und verlor irgendwie nie seine niederbayrische Gelassenheit. Ein Danke auch an Robert Kliche, der mich im Labor unterstützt hat. Besonderer Dank geht an die Mitarbeiter des Arbeitsbereiches Mechatronik am Institut für Landtechnik und Tierhaltung, namentlich an Karl-Heinz Bröker. Bei Dieter Nast von der Abteilung Qualitäts- und Untersuchungswesen der LfL bedanke ich mich für die Unterstützung bei der Tracer-Analytik. Allen Kolleginnen und Kollegen im Arbeitsbereich Umwelttechnik der Landnutzung danke ich für ihren Beitrag zu einem lebenswerten Arbeitsalltag.

Herrn Johann Schweiger mit Familie danke ich für die gute freundliche Zusammenarbeit und die kompetente Betreuung der Pilot-Biogasanlage.

Bei Sr. Marie-Helene bedanke ich mich für ihre treue Begleitung und weisen Rat. I thank Emmanuel C. George for encouraging me to keep “climbing up the mountain”.

Meinen Eltern gebührt unschätzbarer Dank, dass sie in vielerlei Hinsicht die Grundlagen dafür gelegt haben, dass ich studieren konnte. Bei Andi bedanke ich mich für manches ermutigende Wort und seinen brüderlichen Beistand.

Unschätzbarer Dank geht auch an meinen unverhofften Schatz.

O.A.M.D.G.

Freising, im September 2007 Mathias Effenberger

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

AD	Anaerobic digestion
ADF	Acid detergent fiber
ADL	Acid detergent lignin
A/VBNC	Active/viable but not cultivable
BHKW	Blockheizkraftwerk
CFU	Colony-forming units
CHPU	Combined heat-and-power-unit
COD	Chemical oxygen demand
CSTR	Continuously-stirred tank reactor
D1	Digester 1 of pilot biogas plant
D2	Digester 2 of pilot biogas plant
D3	Digester 3 of pilot biogas plant
DM	Dry matter
DNA	Desoxyribosenucleic acid
EPDM	Ethylene-propylene-diene-monomer
FC	Fecal coliforms
IE	Intestinal enterococci
FM	Fresh matter
HRT	Hydraulic retention time
L	Liter
MD1	Digester 1 of model biogas plant
MD2	Digester 2 of model biogas plant
MD3	Digester 3 of model biogas plant
Meso	Mesophilic
MPN	Most probable number
MRT	Minimum retention time
NDF	Neutral detergent fiber
NH4-N	Ammoniacal nitrogen
$N_{org.}$	Organic nitrogen
N_{total}	Total nitrogen
PET	Polyethylene-terephthalate
PU	Polyurethane
PVC	Polyvinyl chloride

RRM	Renewable raw materials
RTD	Retention time distribution
RT-qPCR	Retention time-quantitative polymerase chain reaction
Thermo	Thermophilic
TPAD	Temperature-phased anaerobic digestion
USEPA	United States Environmental Protection Agency
VFA	Volatile fatty acids
VS	Volatile solids
WPA	Water protection area

PUBLICATIONS

Paper in refereed publication

Effenberger, M., Bachmaier, J.,Garcés, G., Gronauer, A., Wilderer, P. A., Lebuhn, M. (2006). Mesophilic-thermophilic-mesophilic anaerobic digestion of liquid dairy cattle manure. *Water Sci Tech* 53/8: 253-261

Papers in non-refereed publications

Effenberger, M., Gronauer, A., Lebuhn, M., Wilderer, P. A. (2003): Das Biogasverfahren „MesTherMes“. *Landtechnik* 6/2003: 392-393

Effenberger, M., Lebuhn, M., Gronauer, A. (2005): Leistungsfähigkeit der Teilhygienisierung von Milchviehgülle durch mehrstufige anaerobe Behandlung. *Landtechnik* 6/2005: 348-349

Conference posters and papers

Effenberger, M., Lebuhn, M., Wilderer, P. A., Gronauer, A.: Inactivation of pathogenic and indicator organisms in cattle manure by anaerobic digestion: Assessment by the methods of cultivation and qPCR. 9th International Symposium on Animal, Agricultural and Food Processing Wastes, Raleigh, North Carolina, USA, 11-14 October 2003. American Society of Agricultural Engineers, St. Joseph, Michigan, ISBN 1-892769-32-8: 83-90

Effenberger, M., Bachmaier, J., Garcés, G., Lebuhn, M., Wilderer, P. A., Gronauer, A.: Teilhygienisierung von Rindergülle durch mehrstufige anaerobe Behandlung: Untersuchungen im Pilot-Maßstab. 7. Tagung Bau, Technik und Umwelt in der landwirtschaftlichen Nutztierhaltung, Braunschweig, Germany, 1-3 March 2005. Kuratorium für Technik und Bauwesen in der Landwirtschaft e. V. (KTBL), ISBN 3-7843-2185-2: 571-576

Effenberger, M., Bachmaier, J.,Garcés, G, Gronauer, A., Wilderer, P. A., Lebuhn, M.: Mesophilic-thermophilic-mesophilic anaerobic digestion of liquid dairy cattle manure. 4th International Symposium Anaerobic Digestion of Solid Waste, Copenhagen, Denmark, 31 August-2 September 2005

Effenberger, M., Lebuhn, M., Gronauer, A.: Biogastechnologie zur umweltverträglichen Flüssigmistverwertung und Energiegewinnung in wasserwirtschaftlich sensiblen Gebieten. 14. Symposium Bioenergie, Kloster Banz, Bad Staffelstein, Germany, 24-25 November 2005, ISBN 3-934681-42-5: 366-371

1 INTRODUCTION

Grazing animals and land application of liquid manure are considered responsible for a certain background level of pathogenic microorganisms - as well as nutrients and, possibly, pollutants - in the environment (Lenhart, 2001). Potential causative agents of human waterborne infections that may be present in animal manure include bacteria, protozoa and viruses (Bicudo et al., 2000). Assessing these environmental impacts from livestock farming is a complex endeavour since the above-mentioned agricultural activities form a diffuse source of pollution, in contrast to a point source such as sewage treatment works.

In order to protect water resources from microbial contamination originating from livestock farms, a multiple-barrier approach has been suggested which incorporates the following control points (Stehman, 2000): (1) Pathogen import to the farm concerning all pathways through which pathogenic organisms can be introduced into an animal operation such as feed, water, and treated or untreated manure; (2) pathogen amplification within the animal operation; (3) appropriate collection and treatment of animal waste in order to eliminate pathogenic organisms to the maximum possible extent; and (4) pathogen export from the farm concerning all measures to prevent pathogenic organisms from entering water resources or food chains. This thesis deals with the third control point, particularly the sanitizing treatment of liquid manure by anaerobic digestion.

Water protection areas (WPA) are an important legal means of preventing contamination of drinking water resources. In Germany, they are normally divided into three zones, with the so-called inner protection zone ("Zone II") serving to prevent contamination of drinking water with pathogenic microorganisms (DVGW, 1995). Generally, both application and storage of animal manure are prohibited in this zone. Therefore, the enlargement of existing WPA will clash with the interests of livestock farmers owning agricultural land in the concerned areas. On the other hand, land owners affected by land use restrictions are entitled to compensation by law (Anonymous, 2001).

The enlargement of an existing WPA was the starting point for this research. In the respective area that serves the water supply of three communities in the Bavarian Alpine forelands, drinking water is produced from a gravel aquifer that is prone to contamination from the surface (thickness of overlying strata: 2.8 to 4 m). To mitigate conflicts with agriculture, the public utility company had been looking for options to subject animal waste to a sanitizing treatment, as a possible alternative to the strict prohibition of land spreading. It was decided to examine this within a joint project of water and agricultural authorities. To

ensure the relevance of the outcomes to the practical application, the scientific investigations were to be performed at pilot-scale.

Anaerobic digestion (AD) was identified as the most promising alternative out of various mature technologies for the sanitizing treatment of animal wastes, mainly because of its outstanding advantage of producing the versatile renewable energy source biogas. Additional benefits of AD such as recycling of nutrients, reduction of odor, and improvement of fertilizing effects may be achieved by other treatments also (Wright, 2000). It is known that for thermophilic conditions (typically 55°C or higher) the combination of treatment time and elevated temperature is the chief control for the sanitizing effect of anaerobic digestion. Mesophilic digestion alone (typically operated at 35 to 38°C, *i.e.* at a temperature level similar to that in the intestine of mammals) causes only a relatively slow reduction of less resistant pathogenic organisms due to chemical factors and microbial competition.

From the hygienic point of view, a completely mixed reactor which is by far the dominant form used in agricultural biogas plants in Germany (Weiland et al., 2005; Effenberger et al., 2002) is disadvantageous. As a matter of principle, the minimum retention time in this type of reactor is given by the time interval between withdrawal and feeding. Therefore, effective sanitation in a completely mixed reactor requires long feeding intervals which are on the other hand not desirable with regard to process stability and continuous biogas production. This problem can be tackled by arranging two or more completely mixed reactors in sequence or employing reactors that are not completely mixed.

In a large number of laboratory studies and though less frequently in full-scale plants, the inactivation of various indicator and pathogenic bacteria in animal manure by anaerobic treatment has been demonstrated. Mainly due to methodical difficulties and financial constraints, relatively few studies exist on the inactivation of endoparasites such as *Cryptosporidium* and *Giardia* spp. by AD. The (oo)cysts of these organisms are highly resistant to environmental stresses and chlorine treatment, and can remain viable and infectious in water for up to several months or even longer (Daugschies, 2000; Robertson et al., 1994). Enteric diseases caused by infective (oo)cysts are dangerous for unborn and small children as well as immuno-compromised persons (Janitschke, 1999). A combination of different analytical techniques is required to examine the presence, vitality and infectivity of (oo)cysts in environmental samples. While Doll et al. (1999) could not prove the complete inactivation of *Cryptosporidium parvum* in sentinel chambers during single-stage thermophilic anaerobic digestion, they proposed that passing through mesophilic temperature

conditions prior to thermophilic treatment could improve the inactivation of cryptosporidia by stimulating excystation of the heat-resistant oocysts.

Combining a thermophilic and a subsequent mesophilic digestion step has been demonstrated by a number of researchers to improve anaerobic degradation efficiency of various organic wastes including domestic wastewater sludge, suspended bio-waste and animal wastewater (Sung & Santha, 2003; Christ, 1999; Han et al., 1997). Successful application of this process to treat liquid dairy cattle wastes at full-scale has not been documented to date.

Based on the findings summarized above, it was decided to construct a sequence of three anaerobic digesters that would be operated at mesophilic, thermophilic and mesophilic temperature level. To increase the guaranteed retention time during quasi-continuous operation, the thermophilic digester was designed as a horizontal tubular reactor with baffles. This thesis evaluates the performance of mesophilic-thermophilic-mesophilic anaerobic digestion for the treatment of liquid dairy cattle manure. The above-mentioned joint research project offered the opportunity to investigate this process scheme at bench and full scale in cooperation with researchers and practitioners from the fields of agriculture, microbiology, and water resources management.

2 STATE OF KNOWLEDGE

This thesis focuses on engineering aspects of the investigated anaerobic treatment process. Consequently, the main part of this chapter is dedicated to the discussion of technical aspects of the anaerobic digestion of liquid animal manure. Some general environmental and legislative aspects of the management of organic residues will be outlined first, as this work was prepared within the framework of a joint research project involving water and agricultural authorities. Most of the information refers to the handling of wastewater sludges and bio-wastes which has been regulated in more detail than the handling of animal manures. Methods for controlling the sanitizing effect of different treatment options include microbiological techniques for hygienic monitoring which were in part developed by cooperating microbiologists in the course of this project.

2.1 Environmental Impacts and Health Risks Associated with Livestock Manure

Agriculture is a major contributor to the overload of the nitrogen cycle occurring in developed countries due to emissions of ammonia and N_2O and the input of nitrogen into surface water bodies and groundwater. N_2O damages the ozone layer and is a powerful greenhouse gas (CO_2-equivalent: 310). Deposition of ammonia contributes to the acidification and eutrophication of soil and water bodies. Nitrate has adverse effects on drinking water quality. Additional environmental impacts from agriculture, particularly from livestock farming, are phosphate input into surface waters, the release of methane as a greenhouse gas and emissions of odorous compounds. Raw liquid manure has a rather low nutrient content, and in addition it contains inorganic and organic nitrogen compounds which makes the calculation of nitrogen availability more difficult compared to synthetic fertilizers. Improper application due to the low valuation of untreated liquid manure intensifies negative impacts on the environment (Döhler et al., 1997).

Since many infectious diseases of livestock are connected with the digestive tract, animal wastes also constitute a substantial source for the spread of pathogenic germs (Strauch, 1996). The concentrations and types of pathogens in animal wastes vary with animal species, herd size, geographic location of the farm, and the composition of the manure. The four main areas of health issues related to the handling of livestock wastes are: Public health concerns, hazards to livestock, health risks for farm staff, and food quality issues (Burton & Turner, 2003).

2.1.1 Hygienic Risks of Land Spreading

A risk of infection from animal wastes may occur from contaminated crops, soil, water and air. The hygienic hazard associated with animal wastes is very different depending on whether slurry or manure are going to be used as fertilizer on arable land, as fertilizer on pastures, or as recycled feed (Strauch, 1987). It is extremely difficult to quantify the actual hazards associated with animal wastes applied to land (Strauch, 1996), since not only livestock but also humans and wildlife species can serve as a source of infection from the same pathogens (Bicudo et al., 2000; Shelton, 2000). However, surveys in the United States revealed that in those cases of waterborne disease outbreaks where the microbial agent could be identified, farm animals were the most likely source of those agents (Gerba & Smith, 2005).

In principal, the risk of biological wastes applied to agricultural land can be divided into (i) the epidemiological risk of transmission of animal pathogens to livestock via direct (*e.g.*, through contaminated pastures) or indirect pathways (through contaminated fodder or living vectors) and (ii) environmental risks through dissemination of pathogens or bacteria resistant to antibiotics (Böhm, 2002). In the case of animal feces, generally the epidemiological aspect is of greater importance. The manure of clinically healthy livestock that is only used within a farm does usually not pose a significant epidemiological risk. However, the risk of transmission of infectious agents rises abruptly in the case of an epizootic outbreak. The predominant pathogens found in manure that can cause disease in humans are *Salmonella* sp., *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Mycobacterium paratuberculosis*, *Cryptosporidium parvum*, and *Giardia* sp. (Olson et al., 1999; Pell, 1997). The survival and transport of different pathogens shed into the environment with animal feces depends on a number of environmental factors, such as insolation, temperature, humidity, salinity, physical and biological soil conditions (USEPA, 2001). Soil type and soil water content and flow appear to be the most important factors for the vertical movement of microorganisms to groundwater resources (Mawdsley et al., 1995).

2.1.2 Legislation

A potentially economical and environmentally sustainable way for the recycling of nutrients is the application of residues such as wastewater sludge, animal manure and bio-waste to agricultural land. However, this requires minimizing the chemical and hygienic risks associated with the application of these materials to land. As indicated above, there are few cases where disease outbreaks of man or animals arising from land application of sludge or animal slurry could be evidenced. The emergence of new pathogens over the last decade due

to factors such as increasing global transfer of goods and people, improved tools for the identification of pathogens, and evolution of pathogens has raised concerns about associated public health risks (World Health Organization, 2003). The following paragraphs illustrate the multiple-barrier approach to risk reduction that forms the basis of U.S. and European legislation governing land application of residues from different sources.

In the U.S., control of pathogens and vector attraction in sewage sludge is regulated under 40 CFR Part 503 (USEPA, 1992). Public health and animals are to be protected from sewage sludge pathogens by combining measures of (i) reducing the number of pathogens present in the sludge, (ii) reducing the susceptibility of the sludge for disease vectors, and (iii) restricting site use to limit human and animal contact with the sludge after its application. Treated sludges are categorized as Class A or B biosolids according to specified requirements for pathogen reduction. Class A biosolids are not subject to site restrictions as treatment of these sludges is required to reduce the numbers of pathogens (including enteric viruses, pathogenic bacteria, and viable helminth ova) to below detectable levels. Additional requirements with respect to reducing vector attraction apply to both categories. Comparable regulations concerning pathogens in animal manures do not exist (Moss et al., 2002).

As far as hygienic aspects are concerned, the European Commission's Directive on the protection of the environment when sewage sludge is used in agriculture has taken a dual-barrier approach (Carrington, 2001; European Commission, 1986). Pathogen loads have been considerably reduced mostly by mesophilic anaerobic digestion. In order to further minimize the risks, constraints have been put on the use or harvesting of crops from land receiving sewage sludge. The European Commission has proposed to define technical parameters for "advanced" sludge treatment processes that ensure hygienization to such a degree that use of those sludges need not be restricted (see below). The application of "conventionally" treated sludges with a lower degree of hygienization would then be subject to certain constraints (European Commission, 2003a). These regulations would correspond to U.S. Class A and B requirements.

Directive 1774/2002 of the European Commission regulates in detail how to deal with animal by-products not intended for human consumption (European Commission, 2002). Therein, animal by-products are divided into categories 1 to 3 according to decreasing hygienic risks. Animal manure from clinically healthy livestock is found in Category 2, but together with gut contents, milk and colostrum is exempt from sterilization prior to biological

treatment or land application. A waiting period of 21 days applies if these materials are to be spread on pastureland.

In Germany, hygienic requirements for the treatment of biological wastes except sewage sludge and animal by-products prior to land application are addressed in the Ordinance on Biowastes (Anon., 1998). Provided that limit values for heavy metals are not exceeded, the maximum allowable amount of bio-wastes applied per hectare is generally restricted to 30 tons of dry matter over a period of three years. To prevent the microbial contamination of groundwater used for the production of drinking water, protection areas (WPA) are established around drinking water supply wells. The aim of the so-called inner protection zone ("Zone II") is to avoid contamination of the drinking water, especially by pathogenic microorganisms (DVGW, 1995). Both application and storage of animal manure are generally prohibited in this zone. According to the Federal Water Act, land owners affected by land use restrictions have to be reimbursed for economical disadvantages (Anon., 2005). It has been discussed whether exemptions from this strict prohibition are possible if the manure is subjected to a sanitizing treatment. In practice these exemptions are decided about for the individual case of a specific WPA.

To summarize the current regulations to avoid risks to human health associated with land spreading of animal wastewater in Germany: Animal manure from clinically healthy livestock is not subject to sanitation requirements; a waiting period of 21 days has to be kept after application of animal manure to pastureland; and application and storage of animal manure are usually prohibited in the inner protection zone of water protection areas.

2.2 Treatment of Livestock Manure

Livestock manure may be subjected to physical, chemical or biological treatments (Figure 1) with the objectives of reducing the amount of readily degradable organic compounds and pathogens, referred to as the process of stabilization and sanitation, and the removal or recovery of nutrients. Optimizing a treatment with respect to one of these aims does not necessarily lead to achievement of the others. The most common treatment processes for animal wastewater or liquid manure that are currently practiced to varying extent are prolonged storage, solid-liquid separation, aerobic stabilization, and anaerobic digestion (Burton & Turner, 2003; Rückert, 1991).

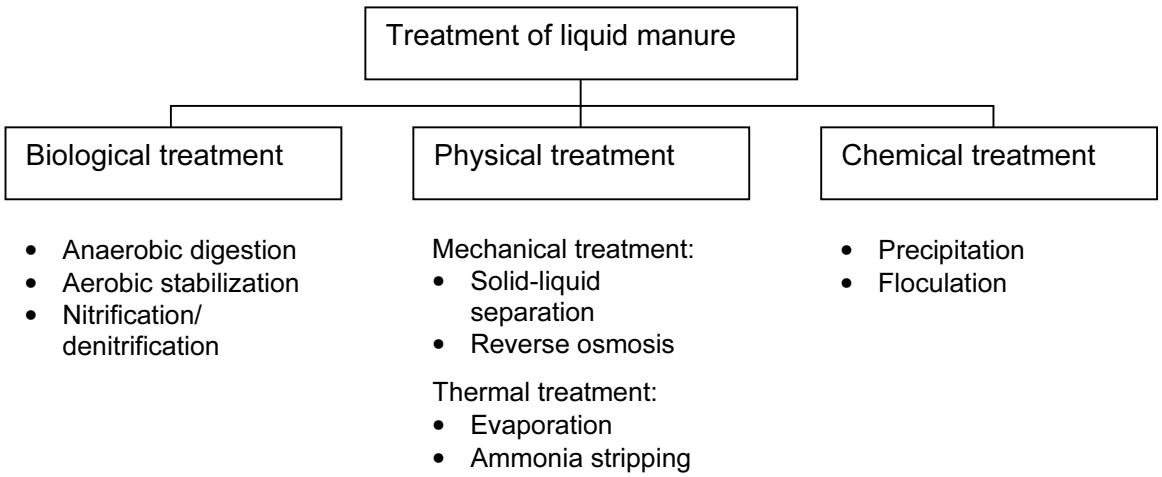


Figure 1. Treatment processes for liquid manure

Anaerobic digestion alone does not reduce the nutrient load of liquid manure. However, due to the effects of the degradation of organic matter, the mineralization of organic nitrogen to ammonia-nitrogen, the improvement of fluidity, and the production of biogas as an energy source, anaerobic digestion is a prerequisite for the application of various further treatments for the recovery of nutrients from liquid manure (Weiland, 1997).

2.2.1 Treatment Options for Sanitation

According to Böhm (2002), since feasible treatment methods can be found for any material and requirements, the mere presence of pathogens in a particular substrate does not justify a general prohibition of its use. “Advanced” treatments for the reduction of pathogens in sewage sludge that have been proposed for amending the respective regulations of the European Communion are (Carrington, 2001): Composting, either in the form of windrows, aerated piles or in vessels; thermal drying; thermophilic digestion (aerobic or anaerobic); heat treatment followed by mesophilic AD; and treatment with lime (CaO). Strauch (1998) suggested that an advanced treatment process should be required to reduce the number of naturally occurring or added Salmonella by 4 logarithmic units and destroy the viability of helminth ova. Pathogenic microorganisms can survive much longer in (semi-) liquid manure than in solid manure which naturally undergoes a process of self-heating, *i.e.* composting (Strauch et al., 1977).

Full-scale studies on the inactivation of *E. coli* as a common indicator organism in the course of the so-called “enhanced” sludge treatments of composting, lime addition, and thermal drying revealed considerable variability in the achieved reduction of microbial concentrations between different facilities operating the same generic treatment process

(Godfree & Farrell, 2005). The reasons for the observed discrepancies could not be elucidated which stresses the importance of process validation and control as outlined in the following chapter.

2.2.2 Control of Pathogen Reduction

A combination of (i) validation of pathogen reduction (process validation), (ii) specification and control of treatment conditions (process control) and (iii) microbiological monitoring of the treated material (product quality assurance), as it is found in the German Ordinance on Biowastes, provides maximum safety that the requirements of pathogen reduction are met in practice (Böhm, 2002). Table 1 summarizes advantages and drawbacks of the different methods for controlling pathogen reduction.

Table 1. Summary and assessment of methods to control pathogen reduction during different treatments (according to Böhm, 2002)

Method	Advantages	Disadvantages
Process validation	• Reproducible and comparable results for a particular technical process • Responsibility for the treatment performance with the planner, manufacturer or vendor of the plant	• Cost- and labor-intensive • Rare event • Does not account for operational faults of the treatment process
Process control	• Easy to accomplish on a continuous basis • Readily available results • No special skills or laboratory investigations required	• Difficulty of finding representative control points • Process validation needed for determining control parameters
Product quality assurance	• Readily accomplished and flexible with respect to sampling procedures	• Difficulty of representative sampling of heterogeneous and complex materials • Difficulty of finding suitable indicator organisms for pathogens that cannot be directly quantified

New processes and treatment plants for sanitation are validated by testing their effectiveness for the inactivation of appropriate surrogates. Upon commissioning of a treatment plant the surrogates are introduced into the reactor in known amounts by using sentinel chambers ("germ carriers"), and the reduction in number is evaluated after the required treatment time. The advantages of process validation are that: it allows to evaluate whether a technical process is at all effective in producing a hygienically safe product; it provides reproducible and comparable results; and, as long as a prototype has been validated, it puts the responsibility for the process performance on the planner, manufacturer, or vendor

of the treatment plant. The disadvantages are that: it is cost- and labor-intensive; it is typically a fairly rare procedure; and it does not account for operational faults of the treatment process (Böhm, 2002).

The performance of a validated treatment process for the inactivation of pathogens may be controlled by monitoring relevant technical parameters. To give an example: For the process of thermophilic anaerobic digestion the German Ordinance on Biowastes specifies a minimum treatment time of 24 hours at 55°C and a minimum hydraulic retention time of 20 days. It is required that the temperature in the reactor is monitored in at least three positions on a daily or possibly continuous basis. Temperature measurements and feeding intervals have to be recorded automatically or manually and stored for at least five consecutive years. Process control measures have the following advantages: They can be easily and continuously accomplished; the results are readily available; and they require no special skills or laboratory investigations (Böhm, 2002). However, measured temperatures are not necessarily representative for the entire process, especially if the material is very heterogeneous. Also, validation of the process at full scale is needed for determining control parameters.

Direct examination of the treated material is readily accomplished and is flexible with respect to sampling procedures. However, with very heterogeneous and complex materials representative sampling is difficult and isolation of actual pathogenic organisms is often impossible. For that reason, indicator organisms are commonly used to assess the performance of sanitizing treatments. These indicators or surrogates should meet the following requirements (Böhm, 2002; Martens et al., 1999): They should be consistently present in the untreated material in large numbers; it should be shown by epidemiological studies that the indicators are transmitted by the treated material; the indicators should neither be a part of the biocoenosis involved in biological treatments nor of the natural biocoenosis of the soil environment; they should be easy to cultivate and reliable to identify from complex matrices such as sludge or animal slurry; and they should not be less resistant to the inactivating factors of the treatment processes than the pathogens for which they are used as a surrogate.

The use of *Salmonella senftenberg* W 775 (H_2S-negative) as indicator organism for demonstrating pathogen reduction during composting and anaerobic digestion has been frequently tested and is regulated in the German Biowastes Ordinance (Anon., 1998). Due to their limited survival time in the environment, fecal coliforms (*Escherichia coli*; FC) have

been used as indicator organisms for relatively fresh contamination of water with human and animal feces. In the U.S. they are used as indicators for pathogen control during sewage sludge treatment.

Intestinal (fecal) enterococci appeared significantly more resistant to increased temperatures than other bacterial and viral indicator organisms used for the evaluation of the sanitation efficiency of anaerobic treatment plants for biowaste (Martens et al., 1999). Due to their higher tenacity compared to bacterial indicators (except for spore-formers) and *Cryptosporidium parvum*, intestinal enterococci may be an ideal indicator organism for the sanitation efficiency of thermophilic anaerobic digestion (Lebuhn & Wilderer, 2006).

The endospores of *Clostridium perfringens* are resistant to temperatures up to 100°C (Schlegel, 1992) and are not reduced during anaerobic digestion. The fact that these organisms are ubiquitous in soil and feces and very resistant to environmental stresses makes them unsuitable as indicators of recent fecal contamination (Lebuhn & Wilderer, 2006).

In order to more fully appreciate the risks of transmission of microbial pathogens from animals to humans, more data on the concentrations of individual pathogens in manures and other residues and their reduction in the course of different treatment processes are needed (Moss et al., 2002). As addressed in the following chapter, this research demand is apparently closely connected with the issue of developing and standardizing reliable and useable detection methods for these organisms (Lebuhn et al., 2003).

It is agreed that effective management of pathogens in biological wastes has to take into account the complete chain of treatment, residues handling and application as well as subsequent processes. A useful tool to achieve this may be quality management systems based on the principles of hazard analysis critical control points (HAACP) which are employed in the UK (Godfree & Farrell, 2005).

2.3 Detection and Quantification of Pathogenic and Indicator Microorganisms in Slurries

Traditionally, the sanitation efficiency of a treatment process is evaluated by cultivation techniques using indicator bacteria such as (fecal) coliforms and intestinal enterococci (fecal streptococci). These culture-based techniques have several drawbacks. Firstly, they require one to several days to produce results. This may be critical if rapid decisions must be made in case of a hygienic hazard. Additionally, cultivation techniques frequently lack sufficient specificity. Active/viable but not cultivable (A/VBNC) and eventually pathogenic cells may

not grow on the (artificial) media but may become virulent in their natural host (Thomas et al., 2002; Lleò et al., 2001; McDougald et al., 1998). This can result in an underestimation of potential pathogens and associated health risks. Finally, protozoan parasites and the Norwalk virus cannot be cultivated at all and thermophilic campylobacters are difficult to cultivate under laboratory conditions. However, these organisms are resistant against most practiced sanitizing measures and are among the leading causes of human enteric diseases worldwide.

One of the most promising molecular biology tools to detect and quantify specific organisms in environmental samples is the real-time quantitative polymerase chain reaction (RT-qPCR) (Heid et al., 1996; Holland et al., 1991). However, a major problem when applying this technique to environmental samples is that these samples can contain PCR-inhibitors such as humic acids which can lead to false negative results (Tebbe and Vahjen, 1994).

Lebuhn et al. (2003) developed a suitable system of DNA extraction and RT-qPCR for the specific and sensitive quantification of pathogenic and indicator bacteria in liquid cattle manure and other environmental samples. In comparison to the culture-based systems for quantification of (indicator) organisms, the qPCR approach has the following advantages: Results are generated much faster; distinct organisms (*e.g.*, pathogens) can be determined specifically; non-cultivable but active and potentially infectious agents can be quantified; and the system provides a high throughput and is cost effective. Using a standard spiking procedure it was possible to compensate for methodological bias, assess the method detection limit, and avoid false negative results (Lebuhn et al., 2004).

Both cultivation and quantitative real-time PCR (qPCR) including optimized DNA extraction and quantification were used to evaluate the hygienization performance of the mesophilic-thermophilic-mesophilic anaerobic digestion system for the treatment of liquid dairy cattle manure that is described in this thesis. The comparison of these two methods with respect to the quantification of pathogenic and indicator bacteria showed that results from cultivation and qPCR targeting DNA were in good agreement for samples of raw manure and digest. However, considerably higher qPCR values were obtained for samples from the digesters. Extrapolating from qPCR results to the number of viable and potentially infectious (micro)organisms was only possible for equilibrated but not for stressed samples, since qPCR also detects the number of genes or genomes of dead organisms whose DNA has not yet been degraded. It was proposed that qPCR should be applied during hygiene monitoring routines for the detection of distinct (pathogenic) organisms, optionally followed by cultivation for

verification. This could reduce analysis time and manpower, warrant hygienic safety by monitoring specific (non-cultivable) pathogens, and allow for prompt action in cases of positive results (Lebuhn et al., 2005).

2.4 Anaerobic Treatment of Liquid Cattle Manure

In most European countries, cattle manure accounts for between 40 and 70 % of the total manure production. Based on their solids content, manures may be characterized as liquid (up to about 10 % DM), semisolid (between 10 and 20 % DM), and solid (above 20 % DM; Ohio State University Extension, 1995). The proportion of livestock manure produced in the form of liquid manure (slurry) varies considerably between countries (Burton & Turner, 2003). While flushing systems are usually not used in Europe, in most countries liquid manure is diluted with waste water from different sources that is collected in the slurry pit. Dry matter (DM) contents of manure samples from Germany were found to vary widely between farms within a range of about 2 to 13 % (Bihler, 1999; Schulz, 1991). In 2,300 samples of liquid dairy cattle manure from Bavaria, the average DM content was 7.4 % (Peretzki & Heigl, 2006).

Cattle wastes can be pumped up to a solids content of around 12 %. Liquid cattle manure shows a pseudo-plastic behavior above a DM content of about 3 % (m/m) and exhibits thixotropy due to a high content of dissolved colloids (Hörnig, 1982; Strauch et al., 1977). This means that its viscosity decreases with increasing shear rate and time of agitation (Sigloch, 1996). These properties are lost during the process of anaerobic digestion.

Beside technological influences such as bedding material and manure collection system, the composition of the manure is also dependent on the animal feed. Because a sufficient supply of roughage (18-22 % (m/m) of feed; Kirchgessner, 1987) is important for a high fat content of the milk, the DM content of manure from dairy cattle typically contains a considerable proportion of raw fiber including lignin which is not anaerobically degradable (see 6.1).

2.4.1 Biochemistry of the Anaerobic Treatment Process

As opposed to the process of aerobic mineralization that yields CO_2, H_2O, large quantities of cell mass, and waste heat, anaerobic degradation of organic compounds yields CO_2 and methane, little cell mass, and little free energy (about 1/7 of the free energy from aerobic mineralization). The process of anaerobic digestion is constrained by the requirement of appropriate internal electron acceptors. The released methane can be used as a source of

energy: One standard cubic meter of methane gas has a heating value of 9.97 MWh which is comparable to the energetic value of one liter of fuel oil (10.08 MWh). Except for minor amounts of nitrogen and sulfur that are released into the biogas, the process of anaerobic digestion does not reduce the nutrient content of the treated material.

Anaerobic degradation is a combination of parallel and sequential processes that involve a variety of microbial consortia. Complete anaerobic digestion to CO_2 and methane proceeds through the four main steps of hydrolysis, acidogenesis (fermentation), acetogenesis, and methanogenesis. For conceptual purposes, the involved microorganisms may be divided into the three groups of hydrolytic bacteria, transitional bacteria, and methanogenic archaea (methanogens). Hydrolytic bacteria solubilize biopolymers by exoenzymes and ferment the produced soluble substrates (monomers) largely to organic acids and alcohols (Figure 2).

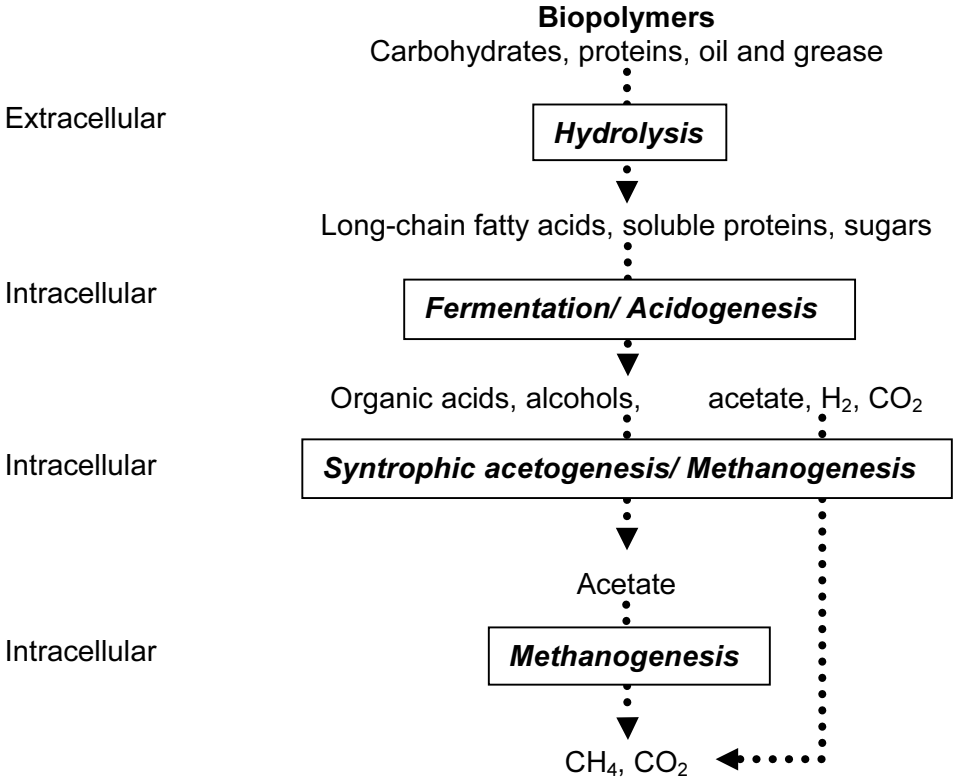


Figure 2. Basic pathways of anaerobic digestion (modified after Batstone, 2000)

The fermentation products are degraded to acetate by transitional bacteria using hydrogen ions or bicarbonate as external electron acceptors. The production of acetate by obligate hydrogen-producing acetogens requires low hydrogen concentrations which is why these organisms grow only in syntrophy with hydrogen-utilizing methanogens (or sulfate

reducers). Fermentative chemoheterotrophic bacteria produce mainly volatile fatty acids, hydrogen, and CO_2, and grow whether or not the produced hydrogen is removed.

Methanogenic archaea are very fastidious, strictly anaerobic microorganisms. Nearly all of them can use H_2 and CO_2 for methanogenesis while only a limited number of methanogens have been isolated that form methane by cleavage of acetate to methane and carbon dioxide (acetoclastic methanogenesis). During the digestion of cattle waste with semi-continuous feeding, between 72 and 86 % of the methane was found to be derived from the methyl group of acetate. The proportion of methane produced from acetate was dependent on time after feeding (Boone, 1981; Mackie & Bryant, 1981).

For most digester feedstocks, the principal source of energy for the bacteria is the degradation of carbohydrate-based particulate matter (Hobson & Wheatley, 1993). Hydrolysis of biopolymers, particularly carbohydrates, is the rate-limiting step during anaerobic digestion of particulate organic waste in digesters with low levels of volatile fatty acids (Boone, 1981). In completely mixed digesters, methanogenic reactions become rate-limiting when the mean cell retention time is lower than the time necessary to maintain stable populations of syntrophic consortia. For syntrophic methane production from lipids and volatile fatty acids, the minimum cell retention time at mesophilic temperatures and within a pH-range of 6.8 to 7.5 is around 2.5 to 4 days (Eastman & Ferguson, 1981).

Inhibitors of the anaerobic digestion process may be divided into two classes: end-products of microbial reactions that are normally part of the digestion process and organic or inorganic compounds in the feedstock. The latter can be high salt loads, heavy metals, antibiotics or other toxic organic substances. During the fermentation of animal waste, hydrogen addition immediately causes instabilities due to increased acetate production and inhibition of acetate dissimilation (Boone, 1981). As mentioned above, simultaneous production and utilization of hydrogen by syntrophic consortia is only possible within a narrow range of low hydrogen levels (Batstone, 2000). High hydrogen concentrations favor the production of reduced fermentation products, particularly propionate and higher volatile fatty acids. Propionate is degraded only at a hydrogen partial pressure below 10 Pa. Propionic acid itself inhibits methanogenesis from $H_2 + CO_2$, acetate, and propionate which results in further build-up of hydrogen (Hobson & Wheatley, 1993).

Other main factors for inhibition of anaerobic digestion are ammonia, sulfide, and pH (Batstone, 2000). Ammonia inhibition is an issue during anaerobic digestion of wastes containing high levels of proteins or ammonia (*e.g.*, chicken manure). Angelidaki &

Ahring (1993) observed poor process performance during anaerobic digestion of cattle manure at temperatures between 40 and 64°C if the calculated concentration of unionized ammonia (NH_3) reached 0.7 g*L^{-1}. Since the distribution of ammonia/ammonium is dependent on temperature and pH, the inhibition could be partly overcome by decreasing the temperature below 55°C. In anaerobic digestion experiments with potatoe juice, granular sludge could adapt to ammonia levels that were 6.2 times higher than the initial toxicity threshold (Koster & Lettinga, 1988). The experiments were performed at 30°C, and the pH in the digester liquid varied between 7.4 and 7.8. Although at greatly reduced specific methanogenic activity, methanogenesis in the adapted sludge was possible up to a level of 11.8 g ammonia-N*L^{-1} while unadapted sludge failed to produce methane at a concentration of 1.9 g ammonia-N*L^{-1}. Ammonia toxicity was found to be more or less reversible. The methanogenic population was first inhibited which caused a build-up of volatile fatty acids.

In anaerobic reactors digesting feedstocks that are rich in protein or sulfate, high sulfide levels are found. Sulfide is produced by sulfate reducers that compete with hydrogen-utilizing bacteria. The main inhibitory agent is undissociated hydrogen sulfide (H_2S) which is in equilibrium with hydrogen sulfide ions (HS^-). Normally, the digester liquid is oversaturated with gases that are produced during the anaerobic digestion process. The degree of oversaturation is dependent on reactor height which may be a limiting factor for the design of reactors for the anaerobic treatment of sulfur-rich wastewater. Up to 500 % oversaturation was measured in upflow reactors as opposed to 40 % in continuously-stirred tank reactors (Witty & Märkl, 1986). During the anaerobic digestion of wastewater from yeast production in lab-scale reactors (reactor volume: 36.5 L, reactor height: 1.7 m) 50 % inhibition of methanogenesis was observed at an H_2S concentration of 95 mg*L^{-1} (Friedmann & Märkl, 1994). The exact determination of inhibitory sulfide levels requires the direct measurement of the concentration of undissolved H_2S. Sulfide control strategies are precipitating sulfide with metal ions or increasing reactor temperature to release more H_2S into the biogas. On the other hand, to avoid problems with corrosion, hydrogen sulfide levels in biogas that is utilized in an engine should be kept at a level below approximately 200 ppm (0.02 %) (see Chapter 4.1.3). In the raw biogas from anaerobic digestion of swine and cattle manures hydrogen sulfide levels typically range between 0 and 0.3 % (Friedmann & Märkl, 1994).

Information about pH inhibition has been summarized by Batstone (2000). Methanogenic bacteria typically require pH values above 6.5 while acidogenic and acetogenic organisms start to become inhibited at a pH below 4.5 to 5.0. A pH above 8.0 to 8.5 appears

to be inhibitory to all relevant microorganisms. Inhibition by sulfide, ammonia, and volatile fatty acids is influenced by pH. The main buffer systems during the anaerobic digestion of animal waste are carbonic acid/hydrogen carbonate with a capacity maximum at pH 6.46 and ammonium/ammonia with a capacity maximum at pH 9.25.

2.4.2 Anaerobic Digestion Systems for Liquid Cattle Manure

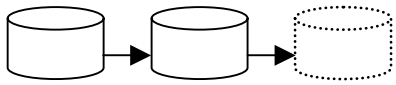
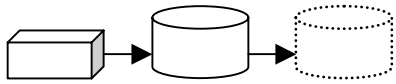
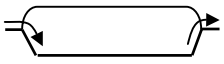
A variety of systems for the anaerobic treatment of liquid manure has been explored, including conventional and biomass-retaining digesters (Zhang, 1998). Conventional completely mixed digesters (batch, fed batch, and (quasi-)continuously fed) are suitable for the treatment of unscreened liquid cattle manure with medium solids content. Since the hydrolysis of the particulate solids is the rate-limiting step during AD of liquid manure, high-rate digestion systems that are designed to increase the degradation rate by retaining the bacteria in the digester are not suitable for treating these materials. The application of biomass-retaining digesters requires dilution and/or pre-treatment of the liquid manure.

According to the characteristics of raw liquid manure and renewable raw materials (RRM) used for biogas production in Germany, the prevalent AD systems operated in agriculture comprise one or several quasi-continuously fed, continuously stirred tank reactors (CSTR) with mechanical agitation (Effenberger et al., 2002). The recent technological development is connected with the trend toward building larger plants, starting from about 350 kW electrical power output (corresponding to a biogas throughput of about 170 to 180 m^3*h^{-1}).

The digestion of RRM with DM contents mostly between 25 and 30 % (m/m) requires suitable devices for solids input, agitation, and pumping. An overview of state-of-the-art agricultural biogas technology can be found in FNR (2005).

In a study of 60 agricultural biogas plants in Germany, vertical completely mixed digesters were operated at almost 90 % of installations (Weiland et al., 2005). Horizontal digesters with slowly rotating paddle agitators were chiefly found as part of multiple-stage processes used for high-solids co-digestion of liquid manure and RRM (Table 2).

Table 2. Overview of the most common concepts of anaerobic treatment plants for liquid manure and agricultural feedstocks

Outline	Typical application	Typical specifications
Completely mixed digesters in series 	Liquid manure and/or renewable raw materials (RRM)	Maximum DM content in primary digester: 10-12 %; mechanical agitation; heating coils (or external heat exchanger); overall HRT: up to 50 days (liquid manure), 60(-120) days (RRM)
Horizontal primary digester and vertical, completely mixed digester(s) in series 	RRM, possibly in combination with (liquid) manure	DM content in primary horizontal digester (cuboid or cylindrical) up to 18 %; mechanical agitation; internal heat exchangers; typically operated with recirculation from secondary to primary digester; overall HRT: 60-90 days
Plug-flow digester 	Semi-liquid cattle manure (U.S.), 13-15 % DM content	rectangular tank, underground; no mechanical agitation; heating coils; HRT: 15-20 days

In the above-mentioned study, the number of plants with two digesters was slightly lower than that of one-stage plants, and 8 out of the 60 installations comprised three digesters. The overall organic loading rate of the investigated plants, *i.e.* the organic loading with respect to the overall active volume of the digesters (Equation 4.6), ranged mostly between 1 and 3 kg VS*(m^3*d)$^{-1}$. Higher system loading rates occurred only at single-stage plants. The first stages of multiple-digester plants were mostly loaded with 3 to 7 kg VS*(m^3*d)$^{-1}$. The largest proportion of plants had hydraulic retention times (HRT) of between 60 and 90 days, with multiple-stage installations accounting for 56 % of plants with more than 90 days retention time. Most biogas plants treating mainly liquid manure had HRTs of less than 50 days. The majority of the investigated plants (95 %) were operated in the mesophilic temperature range between 37 and 43°C. Only a few plants were run at thermophilic temperature level (here: above 50°C) or used a combination of mesophilic and thermophilic stages (Weiland et al., 2005).

Mixing of the digester contents is done to: avoid the formation of scum and sediment layers; distribute the heating energy and maintain a constant temperature throughout the tank;

stir fresh substrate into the digester content; and enhance the release of biogas bubbles from the digester liquid. Most agricultural biogas plants have mechanical agitation devices such as submerged propeller mixers, propeller mixers with engines lying outside, multi-beam paddle mixers, long-axle agitators, or centrally mounted agitators (FNR, 2005; Weiland et al., 2005).

Plug-flow digesters without agitators as they are common in the U.S. appear to be suitable only for the treatment of semi-liquid dairy cattle manure with 13 to 15 % DM (Hills & Mehlschau, 1984; Table 2). Due to significant amounts of fiber from undigested roughage flushed dairy manure with lower solids contents caused scum accumulation in plug-flow digesters (Chen et al., 1984). These systems are typically operated at hydraulic retention times around 20 days and achieve a volatile solids reduction of about 30 % (Wright, 2004).

2.4.3 Sanitation by Anaerobic Digestion

Since many enteric pathogenic and indicator bacteria are mesophilic, *i.e.* there optimum temperature for growth and survival is in the range of 30 to 40°C, temperature will not directly affect their survival during mesophilic anaerobic digestion. Rather, the sanitizing effect of mesophilic AD is mainly due to chemical factors such as pH, redox potential, and elevated concentrations of ammonia, hydrogen sulfide, volatile fatty acids and microbial metabolites.

Ammonia and enzymatic activity appear to be responsible for the inactivation of viruses (Hoferer, 2001). The direct influence of pH on the tenacity of indicator bacteria is supposed to be negligible within the range of pH values typically observed in anaerobic digesters. The inhibitory effects of volatile fatty acids on *Salmonella* spp. in anaerobically digested municipal wastewater sludges were found to depend on pH, temperature, the chain length of the acids, and the acids concentration, and appeared to increase with increasing temperature (Salsali et al., 2006). Fecal coliforms in sewage sludge were reduced to non-detectable levels in an acid-phase digester operated at 21°C and pH values below 6 (Puchadja & Oleszkiewicz, 2004). At mesophilic temperature and pH values above 6.0, fecal coliform destruction was significantly less effective which was attributed to lower levels of VFA.

Generally, the decay of a (homogeneous) population of microorganisms follows the exponential law of disinfection (Chick's Law):

$$X_t / X_o = e^{-kt} \qquad (2.1)$$

with:

X_t = number of organisms at time, t;

X_0 = number of organisms at time = 0;

k = inactivation rate constant; and

t = exposure time to disinfectant.

From this, the decimation time, T_{90}, as the time interval during which the number of microorganisms is reduced by a factor of 10 (or by 90 %), can be calculated as follows:

$$T_{90} = \frac{\ln 10}{k} \qquad (2.2)$$

The sanitizing effect of a treatment process will be dependent on the treatment time, *i.e.* the time the microorganisms are exposed to the specific inactivating factors. In real reactors, there exists no single treatment time but rather a distribution of residence (or retention) times of the micoorganisms in the fluid flowing through the reactor (Levenspiel, 1962). For (quasi-) continuously fed, completely mixed reactors, the minimum guaranteed retention time is the time interval during which neither feeding nor withdrawal occurs.

The rate of inactivation of specific microorganisms in a digester can be determined by monitoring the difference in numbers of native organisms between the feed and the effluent, and/or by measuring the reduction in numbers of organisms that are added to the feed or introduced into the digester in germ carriers. Sanitation as a concomitant of the anaerobic treatment of sewage sludge has been investigated from as early as the 1940s on (von Stromberg, 1985). Various detailed studies on the inactivation of pathogenic and indicator organisms during AD were performed as of the 1980s, again starting from the case of sewage sludge digestion and later on with respect to animal manure and biowaste (see Carrington, 2001 and Hoferer, 2001 for an overview).

Reported T_{90} values of common indicator organisms during AD of sewage sludge and animal slurries in laboratory-scale reactors at mesophilic temperature level are in the range of 1 to 2 days for *Salmonella* spp. and *Escherichia coli*, and 3 to 6 days for fecal enterococci (FE) (Table 3). At thermophilic temperature level, decimation times for these

organisms are less than an hour for *Salmonella* spp. and *Escherichia coli* and a few hours for FE (Table 4).

Table 3. Reported mean decimation times of indicator microorganisms during continuous anaerobic digestion at mesophilic temperature level

Organism	Substrate	T_{90} [d]	References
Salmonella typhimurium	Animal slurry	1.1-2.9	Kearney et al., 1993, Larsen & Munch, 1990, Olsen & Larsen, 1986, Rückert, 1991
Salmonella senftenberg	Cattle slurry + food waste	1.05	Hoferer, 2001
Salmonella duesseldorf	Sewage sludge	1.6	Carrington et al., 1982
Escherichia coli	Animal slurry	0.8-1.8	Kearney et al., 1993, Larsen & Munch, 1990, Munch & Schlundt, 1983, Olsen & Larsen, 1986
Fecal enterococci	Animal slurry	3-6	Munch & Schlundt, 1983
Enterococcus faecium	Cattle slurry + food waste	3.9	Hoferer, 2001

Table 4. Reported mean decimation times of indicator microorganisms during continuous anaerobic digestion at thermophilic temperature level

Organism	Substrate	T_{90} [h]	References
Salmonella typhimurium	Animal slurry	0.6-0.7	Larsen & Munch, 1990, Olsen & Larsen, 1986
Salmonella senftenberg	Cattle slurry + food waste	0.12	Hoferer, 2001
Escherichia coli	Animal slurry	0.4	Larsen & Munch, 1990
Fecal enterococci	Animal slurry	2-4	Munch & Schlundt, 1983
Enterococcus faecium	Cattle slurry + food waste	1.7	Hoferer, 2001

During anaerobic digestion at mesophilic and thermophilic temperature levels, no reduction of the bacterial spore formers *Clostridium perfringens* and *Bacillus cereus* was found (Larsen & Munch, 1990; Olsen & Larsen, 1986).

T_{90} values of *S. typhimurium* and *E. coli* that were introduced into a mesophilic, full-scale digester in semi-permeable nylon bags were comparable to those that were observed when the same bacteria were directly added to the slurry in small-scale digesters. However, in lab-scale tests, T_{90} values for these organisms in nylon-bags were 10-25 % lower than for the suspended bacteria (Olsen & Larsen, 1986).

The reduction in numbers of indicator organisms observed in two thermophilic centralized biogas plants in Denmark varied from 3.8 to 5.4 log units for coliforms and from 4.0 to 5.5 log units for fecal streptococci. Corresponding figures for biogas plants operating at mesophilic temperature level were 2.5 and 1.6, respectively. The minimum guaranteed

retention time in the two biogas plants was 5 and 8 h. Beside animal slurries the incoming material of the centralized biogas plants contained waste from slaughterhouses, fish industries or oil mills. Based on these findings, it was suggested that sufficient reduction of both vegetative bacteria and intestinal parasites during thermophilic anaerobic digestion required a minimum reduction in numbers of fecal streptococci by at least 3 to 4 log units or to a level of 10^2 CFU per mL (Larsen & Munch, 1990).

A study on the inactivation of indicator organisms in 24 full-scale agricultural biogas plants of different design operated at mesophilic temperature level revealed a median reduction of the number of organisms in samples of digest compared to the raw substrate of about 3 log units for coliforms and about 2 log units for intestinal enterococci (Reinhold & Jahn, 2004). The investigated biogas plants represented a range of organic loading rate of 0.67 to 5.49 kg VS*$(m^3*d)^{-1}$, a hydraulic retention time of 22 to 136 days, and a digester temperature of 35 to 44°C. Within this range, significant influences of these parameters on the reduction of indicator organisms were not found. However, in comparison to single-stage plants, a significantly higher reduction of coliform bacteria was observed in plants with two or more digesters in series.

In a simulation study, pathogen removal during mesophilic AD of sewage sludge could be optimized by maximizing stabilization rates and minimizing by-pass flow (Smith et al., 2005). A 2 log reduction in numbers of *E. coli* in a continuously fed anaerobic digester at mesophilic temperature level required that the digester was effectively mixed and by-pass flow was minimized.

The feeding procedure had a significant influence on the microbial reductions achieved during mesophilic (35°C) anaerobic digestion of sewage sludge in continuously-stirred, cylindrical reactors (Farrell et al., 1988). Draw/fill operation (withdrawing digested sludge just before feeding) produced a much larger reduction of microbial indicators than fill/draw operation (feeding, mixing for a short time, then withdrawing). For draw/fill operation the average log reduction at an HRT of 14 days and daily feeding was 2.40 for FC, 2.29 for fecal streptococci (enterococci), and 2.85 log units for total coliforms. For fill/draw operation the corresponding figures were 1.16, 1.21, and 1.57 log units. For physical/chemical parameters that change slowly with time such as VS reduction, the effect of feeding procedure was similar but much smaller.

The studies mentioned and numerous others have shown that at least one thermophilic treatment step is needed for anaerobic digestion processes in order to meet strict sanitary

requirements. Scanlan et al. (2004) provide an overview of so-called "advanced anaerobic digestion" processes that have been designed to meet USEPA Class A pathogen criteria for biosolids. These criteria are: 10^3 MPN*g^{-1} fecal coliforms or less and essentially no enteric virus or helminth ova (USEPA, 1992). The most common processes appear to be so-called temperature-phased processes with a thermophilic first stage and a subsequent mesophilic stage. Other processes consist of a mesophilic or thermophilic first stage and one or more subsequent thermophilic stages. Differences occur between the specified temperature, feeding mode, and hydraulic retention time of the individual stages. In this context, the term "phased" digestion is not always used correctly, as it applies to processes where acid and methanogenic phases occur in different reactors. Typically, this requires a hydraulic retention time of two days or less in the acid stage. Except for the second stage of the "Columbus Biosolids Flow-Through Thermophilic Treatment", that may be a plug-flow reactor, digesters are completely mixed.

Sung and Santha (2003) used a temperature-phased anaerobic digestion system (TPAD) with a thermophilic (55°C; HRT: 4 days) and a mesophilic (35°C; HRT: 10 days) digester in series to treat liquid dairy cattle manure at lab-scale. Fecal coliforms were reduced from a level of between 10^5 and 10^7 MPN*g^{-1} TS in the raw waste to below 1 MPN*g^{-1} TS in the effluent of the thermophilic digester. No further pathogen destruction was observed in the subsequent mesophilic digester.

The addition of a mesophilic acid-phase (HRT: 2 days) upstream of a thermophilic digester (HRT: 13-15 days) improved pathogen destruction in comparison to single-stage thermophilic digestion of sewage sludge at 50°C and 15-20 days HRT (Gray et al., 2006; De León & Jenkins, 2002). This was explained with the combined sanitizing effects of temperature and increased ammonia levels in the thermophilic stage and high levels of VFA at low pH (5.3-5.6) in the mesophilic stage. Reliable pathogen destruction may therefore be achieved at thermophilic temperatures lower than 55°C by using a two-phase meso/thermo process and longer thermophilic HRT. The two-phase process with a thermophilic acid phase had a lower sanitation efficiency than the single-stage thermophilic digestion process. This was due to the fact that the lower temperature in the mesophilic phase resulted in lower levels of toxic ammonia, so that sanitation was only the effect of temperature.

2.4.4 Digestion Performance of "Advanced" Anaerobic Treatment Processes

The question remains how the optimization of stabilization and sanitation performances may be best combined. For a long time it has been recognized that beside efficient sanitation,

thermophilic digestion has the advantages of higher reaction rates and improved dewaterability of digested sludge (Buhr & Andrews, 1977). On the other hand, the authors concluded from computer simulations that process failure can be caused by sudden changes in temperature and that thermophilic digesters require closer control.

Varel et al. (1980) found an increased methane productivity during the anaerobic digestion of beef cattle waste at temperatures above 45°C and short hydraulic retention times (< 6 days) while the kinetic advantage of digesting beef cattle waste at 60°C compared to 50°C was small. Hashimoto (1982) showed that stable anaerobic digestion of beef-cattle manure at 55°C was possible at up to a 3 times higher loading rate than at 35°C. Based on a stress criterion of VFA levels in the digester exceeding 2 $g*L^{-1}$, the maximum loading rate for stable digestion at 55°C was about 20 kg $VS*(m^3*d)^{-1}$, as opposed to 7 kg $VS*(m^3*d)^{-1}$ at 35°C.

Due to the higher methane yield and methane productivity of the thermophilic process, the overall energetic efficiency of the digestion of liquid cattle manure was higher at 60°C than at 40°C if the digesters were operated at high organic loading rates above 9 kg $VS*(m^3*d)^{-1}$ (Mackie & Bryant, 1995).

For co-digestion of liquid manure and industrial organic waste, no differences in process stability could be found between mesophilic (35-38°C) and thermophilic (51-56°C) full-scale biogas plants (Ahring, 1994). By measuring the initial methane production rate of liquid manure digested at 55°C and incubated with different substrates, the highest methanogenic activity was found for an incubation temperature of 60°C. However, at this temperature level, only slight increases in temperature resulted in severe process imbalance. This is due to a dramatic decrease in the growth rates of VFA-degrading microorganisms above 60°C, while the growth rate of hydrogen- and formate-utilizing methanogens continues to increase up to a temperature of 70°C (Ahring, 1995). A temperature range of 52 to 56°C was therefore proposed for the stable operation of full-scale thermophilic biogas plants.

Thermophilic and mesophilic treatment steps in series have been tested to combine the advantages of both processes. Wechs (1985) observed improved digestion performance of sewage sludge for two-stage treatment compared to single-stage mesophilic anaerobic digestion. The highest digestion efficiency was reached for thermophilic-mesophilic treatment. This was attributed to the fact that sewage sludge consists of particulate fractions with different degradability. The first stage served for methane production from easily degradable compounds and partial hydrolysis of resistant fractions which were subsequently

degraded to methane in the second stage. For liquid manure which has sufficient buffer capacity and contains mainly particulate matter that is hard to digest, single-stage anaerobic treatment was considered more suitable (Wechs, 1985).

Compared to a mesophilic-mesophilic system, about 50 % more VS removal from screened dairy manure was achieved in a two-stage anaerobic sequencing batch reactor (ASBR) system with the first reactor run at 55°C and the second one at 35°C (Zhang et al., 2000).

The TPAD system described above achieved a high VS removal from macerated liquid dairy cattle manure of 41.5 % and good effluent quality at an overall loading rate of 5.82 kg VS*$(m^3*d)^{-1}$ (Sung & Santha, 2003). A full-scale system for the treatment of 190 m^3 of liquid dairy manure per day could not be successfully operated as a thermophilic-mesophilic process at steady-state due to technical problems and unclear inhibition of the anaerobic digestion process in the thermophilic reactor (Katers & Schultz, 2003).

Further improvement of the anaerobic digestion efficiency of cattle manure could be reached by applying pretreatment at 68°C before thermophilic digestion at 55°C (Nielsen et al., 2004). The degradation of both the fiber and the liquid fraction of the manure seemed to be improved by the pretreatment. A two stage-process with an HRT of 3 days at 68°C in the first stage and 12 days at 55°C in the second stage achieved a specific methane yield that was 6 to 8 % higher and a VS removal that was 9 % higher in comparison to single-stage thermophilic digestion of liquid cattle manure at 55°C and 15 days HRT. Though the pretreatment reactor accounted for about 7 to 9 % of the total methane production of the system, aceticlastic methanogens and syntrophic consortia degrading VFA were severely affected, reflecting the lower optimal growth temperatures of these organisms compared to hydrogen-consuming methanogens.

Table 5 summarizes information on the sanitation and digestion performance of anaerobic treatment processes. Concerning the effects of staging and phase-separation on the sanitizing effect of anaerobic digestion, there are much more studies for sewage sludge than for liquid manure.

Table 5. Influences on sanitizing effect and performance of anaerobic digestion processes for liquid manure

Process	Pathogen destruction	Digestion performance
Mesophilic, single-stage, completely mixed	Strongly depending on minimum guaranteed RT, hydraulics and chemical parameters, not sufficient for sanitation purposes, typically up to 2 log units reduction for FC, about 1 log unit reduction for intestinal enterococci (IE)	25-50 % VS destruction depending on substrate, VS destruction decreases with increasing loading rate, max. loading rate for liquid cattle manure: about 7 kg VS*(m^3*d)$^{-1}$
Mesophilic, completely mixed digesters in series	Not sufficient for sanitation purposes, improved by about 1 log unit for FC per additional stage	Improved, overall HRT > 50 days is usually not reasonable
Thermophilic, single-stage, completely mixed (48 to 55°C)	Depending on temperature, hydraulics, and minimum guaranteed RT, 24 h hold time at 55°C guarantees sufficient sanitation with respect to bacteria (except spore-formers), viruses, and parasites: 4 to 7 log units reduction for FC; 4 log units for IE	Equal VS destruction achieved at shorter HRT, up to three times the maximum loading rate
Thermophilic, completely mixed digesters in series	Improved, avoids pathogen bypassing	Improved VS removal
Mesophilic acid stage upstream of thermophilic stage	Improved, may allow for lower temperature in the thermophilic stage	May be improved
Thermophilic stage upstream of mesophilic stage	Not significantly improved compared to single-stage thermophilic	Improved

3 THE SCOPE OF THE THESIS

This thesis deals with the evaluation of the performance of a mesophilic-thermophilic-mesophilic anaerobic treatment process with respect to the degradation of organic matter and the inactivation of pathogenic and indicator organisms in liquid dairy cattle manure. The investigations ought to contribute to the assessment of potential treatment options for animal wastes in order to mitigate the input of pathogenic and indicator organisms from livestock farming into the environment.

The anaerobic treatment process was investigated at bench and full scale with the following research objectives:

- Demonstrate continuous mesophilic-thermophilic-mesophilic anaerobic digestion of liquid dairy cattle manure and determine volatile solids degradation and biogas production under steady-state conditions.
- Investigate the sanitizing effect of the treatment on selected pathogenic and indicator organisms.
- Investigate effects of a change in feeding interval from one to four hours with respect to volatile solids degradation and sanitation performance.
- Evaluate the hydraulic efficiency of the horizontal tubular reactor as thermophilic stage.
- Compare the three-stage treatment to two-stage thermophilic-mesophilic treatment with respect to anaerobic digestion and sanitation performance.
- Derive key technical and operational requirements to maximize the sanitizing effect of anaerobic treatment of liquid manure in agricultural biogas plants.

Based on these research goals, the following hypotheses were defined and tested:

1. Continuous anaerobic digestion of liquid dairy cattle manure in a sequence of digesters operated at mesophilic, thermophilic, and mesophilic temperature levels is stable in all stages and provides more efficient conversion of the organic matter of liquid manure into biogas, compared to a single-stage digestion process.

2. With respect to sanitation by thermophilic anaerobic digestion, an upstream mesophilic stage improves the sanitizing effect of the treatment process and guarantees elimination of pathogenic *Cryptosporidium parvum*.
3. Sanitation efficiency of thermophilic anaerobic digestion is seriously affected even by small temperature drops below 55°C.
4. The use of a baffled horizontal tubular reactor as thermophilic treatment stage provides a sufficiently long retention time to significantly reduce levels of pathogenic and indicator organisms in liquid manure during quasi-continuous operation (hourly feeding).
5. The use of such a reactor is thus efficient and economical for the sanitizing treatment of liquid manure.

4 MATERIALS AND METHODS

As the outcomes of the research ought to be directly applicable to the real case (see Chapter 1), a **pilot biogas plant** (pilot plant) was erected on a dairy farm typical in size for the respective region. Based on the requirements specified by the author and co-workers, the plant was designed and built by a professional planner and supplier of biogas plants. It was operated by the farmer according to the specifications given by the author.

A **bench-scale biogas plant** (model plant) was designed and constructed by the author and co-workers at the Institute of Agricultural Engineering, Farm Buildings and Environmental Technology. This model plant was operated simultaneously with the pilot plant. The reasons for the investigations at bench scale were:

- the better control of process conditions than at pilot scale and
- the feasibility of additional experiments with certain pathogens, as only the fate of naturally occurring indicator and pathogenic microorganisms in the liquid manure from the dairy farm could be investigated in the pilot plant.

4.1 Pilot Biogas Plant

4.1.1 Experimental Farm

The pilot biogas plant was erected on a dairy farm in Southern Bavaria. The farmer stables 55 dairy cows and about 30 young cattle in a loose housing system throughout the year. Total area of farmed land is about 41 ha of which 36 ha is grassland. Animals are fed a total mixed ration of grass silage, hey, grain, and mineral mix. The average milk yield of the cows is 6,400 kg*a^{-1} (4.15 % fat, 4.20 % protein).

4.1.2 Collection and Treatment of Liquid Manure

Liquid manure from the stable with partly slatted floor was collected in an underground canal (ca 120 m^3) using automatic scrapers, and pumped into the collection tank of the biogas plant (50 m^3) with an immersed chopping pump in batches of between 20 and 45 m^3 (every five to nine days).

From the collection tank the liquid manure was delivered to a sequence of three anaerobic digesters by progressing cavity pumps. Separate pumps were provided for successive treatment steps in order to avoid microbial recontamination of treated liquid manure (Figure 3).

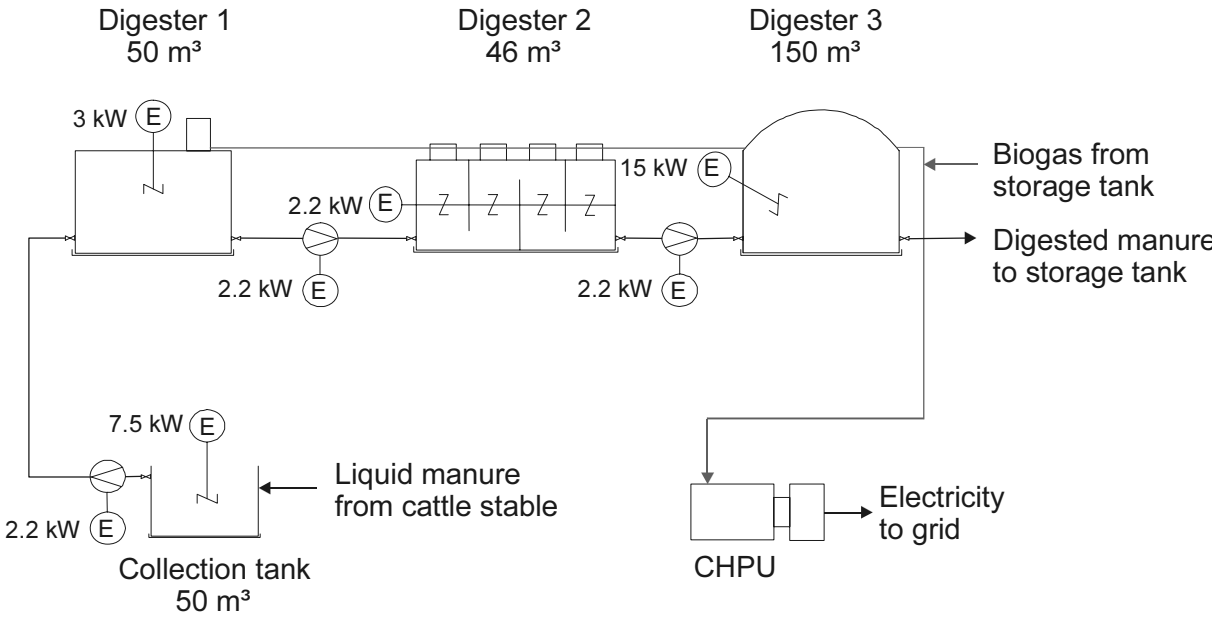


Figure 3. Layout of pilot-scale biogas plant

The treatment process consisted of a stirred-tank digester (Digester 1, D1; usable volume: 50 m^3) supposed to activate (oo)cysts of protozoan parasites and possibly bacterial spores at mesophilic temperature conditions, followed by a horizontal tubular digester (Digester 2, D2; steel; 12 m in length, 2.4 m in diameter, usable volume: 46 m^3) for hygienization at thermophilic conditions, and a stirred-tank digester (Digester 3, D3; usable volume: 150 m^3) for biological stabilization of the substrate. The digested manure from Digester 3 overflowed into a storage tank with a gas-tight cover (800 m^3).

Liquid manure was delivered through PVC pipes (inner ∅: 110 mm) or steel pipes (inner ∅: 100 mm) insulated with polyurethane foam. Digesters 1 and 3 were provided with propeller mixers with engines lying outside, and heated with inside heating pipes on the lower wall section of the tanks. To restrain longitudinal mixing and avoid short-circuiting during quasi-continuous operation, the tubular digester was equipped with a paddle agitator and three baffles, dividing it into four compartments. The baffles also served as bearings for the axle of the agitator (Figure 4).

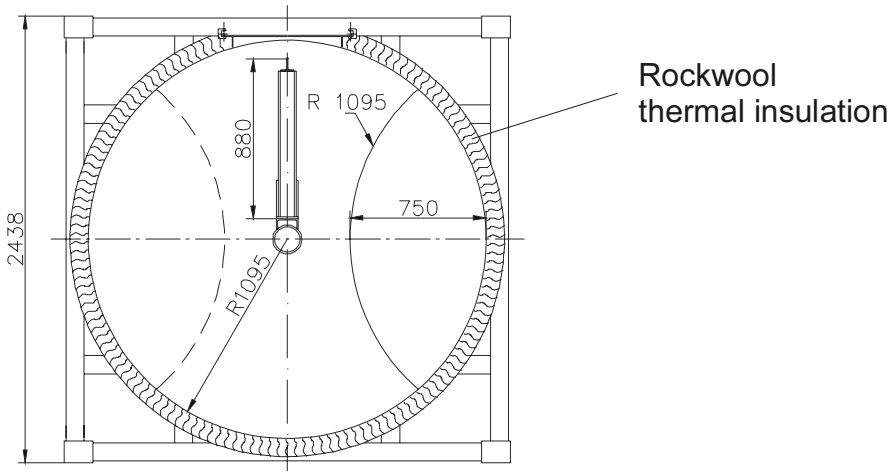


Figure 4. Sectional drawing of Digester 2: design of baffles and agitator paddles

There were three paddles in each of the four compartments, twisted by 150° to each other. Heating of Digester 2 was accomplished with heating pipes inside the axle of the paddle agitator and outside at the bottom of the first and second compartments. Technical specifications of the pilot biogas plant are summarized in Table 6.

4.1.3 Collection and Utilization of Biogas

The produced biogas was collected under a soft hood on Digester 3 and in the head-space of the terminal storage tank. The biogas was conducted to a combined heat and power unit (CHPU) with a pilot injection engine that drove a generator (HJS, Amtzell, Germany; maximum electrical power output: 30 kW). The heat energy from the engine was used for heating the digesters by means of heat exchangers in the exhaust gas stream and the cooling water circuit. Excess heat could be dissipated to the atmosphere by a cooling unit. The generated electrical energy was fed into the grid. To protect the engine from high hydrogen sulfide levels in the fuel gas, small quantities of air (about 7 $L*min^{-1}$) were introduced into the head-space of the first digester with an aquarium air-pump. The atmospheric oxygen was used by sulfide-reducing bacteria to produce sulfur (so-called biological desulfurization).

Table 6. Technical specifications of different components of the pilot biogas plant

Component	Supplier	Specifications	Materials
Collection tank	SCHMACK BIOGAS AG	Partly underground; diameter: 4 m, height: 4.5 m	Concrete, wall and cover
Propeller mixer (collection tank)	SUMA	Powering: 7.5 kW; rotational speed: 1450 min^{-1}	Stainless steel
Supply pump for collection tank	EISELE	Centrifugal chopping pump; powering: 15 kW; feed rate: ca 40 $m^{3*}h^{-1}$	
Digester 1 (D1)	SCHMACK BIOGAS AG	Above ground; diameter: 4 m, height: 4.5 m	Concrete, wall and cover, insulated with Styrodur® (7 cm); sheet-metal lagging on wall
Propeller mixer (Digester 1)	SUMA	Powering: 3 kW; rotational speed: 1450 min^{-1} (specific installed power: 0.06 kW per m^3 net volume)	Stainless steel
Digester 2 (D2)	SCHMACK BIOGAS AG	Above ground; diameter: 2.4 m, length: 12 m; three baffles	Steel, insulated with glass wool (10 cm), sheet-metal lagging
Paddle agitator with longitudinal axle (Digester 2)	SCHMACK BIOGAS AG	Powering: 2.2 kW; rotational speed: ca 2 min^{-1} (specific installed power: 0.048 kW per m^3 net volume)	Steel
Digester 3 (D3)	SCHMACK BIOGAS AG	Above ground; diameter: 7 m, height: 4 m	Concrete, wall insulated with Styrodur® (7 cm), sheet-metal lagging; elastic soft cover (EPDM)
Propeller mixer (Digester 3)	SUMA	Powering: 11 kW, rotational speed: 540 min^{-1} (specific installed power: 0.075 kW per m^3 net volume)	Stainless steel
Supply pumps for digesters	NETZSCH	Progressing cavity pumps; powering: 2.2 kW; rotational speed: 133 min^{-1}; feed rate: ca 14 $m^{3*}h^{-1}$	(Stainless) steel; stator and sleeve: Perbunan
Terminal storage tank	-	Above ground; diameter: 16 m, height: 4 m; propeller mixer with tractor power-take-off drive	Concrete, sheet-metal lagging; soft cover

4.1.4 Process Control and Data Logging

Agitating, feeding, and heating operations of the pilot biogas plant were controlled by a programmable logic controller (Bernecker + Rainer / SCHMACK BIOGAS AG, Schwandorf, Germany) which was programmed by the plant constructor. Quantities of liquid manure fed into Digesters 1 to 3 were measured with electromagnetic flow meters (measuring error as specified: ± 0.5 %). Temperatures at two different heights in the upright digesters were

measured with thermistors placed in immersion sleeves. Temperatures in the thermophilic digester were monitored with thermistors on the inside of the front wall, the three baffles, and the back wall, respectively. Filling levels in the digesters were measured by means of pressure sensors. Inputs and outputs of the controller were continuously logged on a PC on location via a serial connection. Additional data on temperatures and biogas flows were recorded with a separate data logger (logging rate: 1 h^{-1}). The data were transferred to the office on floppy disk about every other week and processed with Microsoft® Excel and Microsoft® Access.

Various readouts and operator's comments were recorded manually in a log on a daily basis and then fed into the computer. Among other data, this included the quantities of electricity which were generated, fed into and obtained from the grid as well as the quantities of heating energy supplied to the individual digesters. The records also served as a backup in cases of failure of on-line data logging. Appendix 3 gives an overview of measuring instruments and data logging at the pilot biogas plant.

4.2 Procedures at Pilot-Scale

The pilot biogas plant was started up in late August 2002 by filling Digesters 1 and 2 with liquid manure diluted with water and heating up to a temperature of 40°C, respectively 55°C. In order to avoid importation of any extrinsic pathogens, the digesters were not seeded with digest from another biogas plant.

Due to faulty design, it was at first not possible to continually ensure a temperature of 55°C in the thermophilic reactor as the foremost requisite for efficient hygienization. The first one and a half years of operating the pilot plant were thus characterized by frequent retrofitting of the thermophilic reactor to meet this requirement. This caused a considerable delay for the course of the project. However, microbiological monitoring data from this period of time provide an indication of the effects of operational shortcomings on the hygienization performance of the anaerobic treatment.

When the thermophilic digester had been retrofitted, the treatment performance of the pilot biogas plant with respect to both volatile solids degradation and hygienization was evaluated for a feeding interval of one hour and four hours. Thermophilic-mesophilic operation of the pilot plant was accomplished by running Digester 1 at a temperature of 20 to 25°C (lower end of mesophilic temperature range according to Schlegel, 1992) after emptying and re-filling it with raw liquid manure. This pre-heating of the liquid manure was necessary because the radiators in Digester 2 were not able to heat up the raw manure from a temperature around 15°C in the collection tank to the required temperature of 55°C in the

thermophilic digester. An overview of the procedures at pilot scale is given in Figure 7. Appendix 1 gives a detailed report of the plant operating period that was evaluated within this thesis.

With a programmed feeding interval of one hour, the daily amount of feed was supplied to the digesters by the logic controller in 21 batches per day. On average, the daily load for the evaluated time period was 5.5 m^3 of liquid manure with the result of a hydraulic retention time of 9.3 days in Digester 1, 8.4 days in Digester 2, and 27.2 days in Digester 3. A feeding cycle was started by drawing effluent from Digester 2 into Digester 3, followed by feeding Digester 2 with effluent from Digester 1 and subsequently feeding Digester 1 with raw liquid manure from the collection tank. Digesters 1 and 2 were supposed to be operated in draw/fill mode, but the programmable logic controller caused some overlap between feeding and withdrawal. With a programmed feeding interval of 4 hours, the daily amount of feed was supplied to the digesters in 5 to 6 batches per day. In this case, the daily load was 5.7 m^3 of liquid manure on the average, resulting in only slightly different mean hydraulic retention times (Table 7).

Table 7. Mean calculated hydraulic retention times (days) in the digesters of the pilot plant during the evaluated time periods

Operating mode	Meso-thermo-meso 21 batches*d^{-1}	Meso-thermo-meso 5-6 batches*d^{-1}	Thermo-meso 21 batches*d^{-1}
Digester 1	9.3	9.1	(9.2)
Digester 2	8.4	8.4	8.2
Digester 3	27.7	27.6	25.5
Chain	45.4	45.1	33.7

During thermophilic-mesophilic operation, the average daily load was 5.5 m^3 of liquid manure, with an average hydraulic retention time of 8.2 days in Digester 2 and 25.5 days in Digester 3. In comparison to the entire HRT, the hydraulic retention time in the thermophilic stage amounted to about 18 % for the three-digester chain and 24 % for the two-digester chain.

During periods of continuous operation, samples of raw liquid manure and digester contents to evaluate performance and stability of the digestion process were taken on a weekly basis. During transition periods, sampling was less frequent. From 0.5 to 2 liters of raw liquid manure and digester content were taken from cocks in the delivery pipes downstream of the pumps (collection tank and Digesters 1 and 2) or from cocks in the tank wall (Digester 3 and terminal storage tank). Sampling from the delivery pipes and the tanks was done shortly after feeding and agitating.

Additionally, for each feeding interval samples of digest from each of the four compartments of Digester 2 were taken on two days by means of special sampling devices. Such a device consisted of a PVC tube with a steel rod that had a rubber stopper affixed to the one end and a handle at the other end. The rubber stopper was pressed on the inlet end with a spring. By pressing down the handle, it could be released to fill the tube with digester content. For the purpose of sampling and in order to avoid discharge of biogas, the agitator of Digester 2 was temporarily halted and dip pipes were installed through special mountings on the gas domes equipped with large ball valves. The sampling devices were then introduced into the digester through the gas domes, and samples were taken from the center area of the digester compartments. Samples for chemical analyses were transported to the lab either after freezing them on site (during the warm season) or in a cooler (during the cold season) and stored in the lab at -18°C until processing.

Samples for microbiological investigations were taken in conjunction with samples for chemical analyses. Two different sampling strategies were applied to evaluate the fate of pathogenic and indicator organisms in the pilot biogas plant: (i) random sampling and (ii) tracing a specific batch of manure by sampling the individual compartments according to the respective calculated hydraulic retention times (Lebuhn et al., 2004). Samples for microbiological analyses were transported to the lab within 2 to 3 hours without cooling to be processed immediately (see section 4.10).

4.3 Model-Scale Biogas Plant

A biogas plant at bench scale (model plant) was designed as a model of the pilot plant at a geometrical scale of approximately 1:6. The model plant comprised a storage tank for raw manure and three digesters in series. The size of the model plant was chosen so that on one hand it would fit into a standard reefer container and on the other hand the liquid manure could be processed continuously with as little additional treatment as possible.

4.3.1 Assembly of Model Plant

The construction of the tubular digester (Model Digester 2, MD2) was meant to be a miniature of the pilot-scale digester at scale 1:6 with respect to the geometries of the vessel, the baffles, and the paddle agitator. The reactor vessel was assembled from four identical tube sections (Figure 5; DN 400, length: 500 mm, thickness: 2 mm) with welded-on flanges (outer ∅: 560 mm, thickness: 15 mm) and front and back covers (outer ∅: 560 mm, thickness: 20 mm).

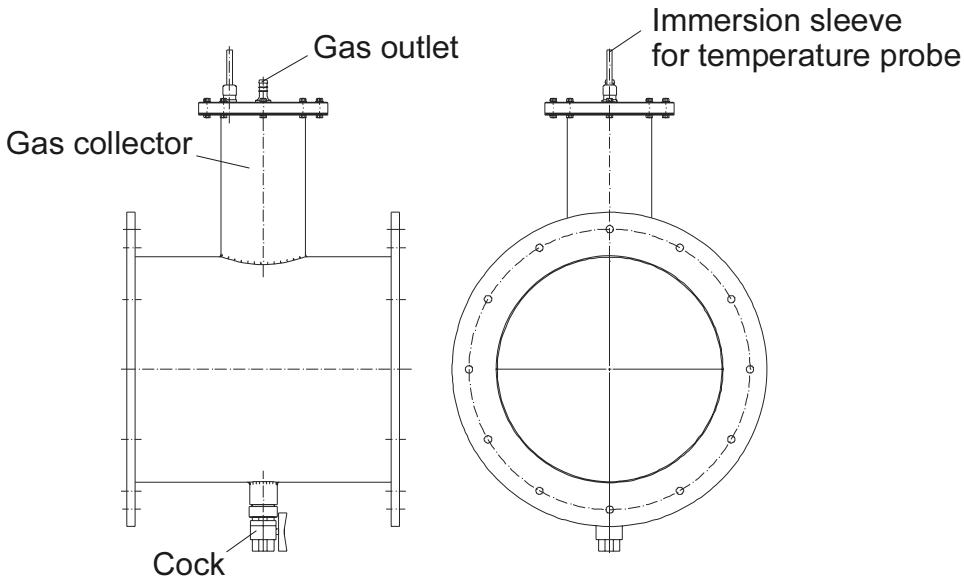


Figure 5. Lateral and front views of one of the four tube sections of Model Digester 2

The three baffles were made of sheet steel (outer ∅: 560 mm, thickness: 1.5 mm) in which apertures were cut by laser. A smaller tube (inner ∅: 150 mm, length: 250 mm, thickness: 1.5 mm) was welded on each of the tube sections to serve as gas collector. The covers and tube sections were connected by means of bolts and nuts, with rubber-seals and baffle sheets clamped in between. All parts that were in contact with manure were made out of V2A stainless steel.

The axle of the paddle agitator (outer ∅: 48.3 mm, thickness: 3.25 mm) had a grooved ball bearing with slide ring seal on the withdrawal end where the drive mechanism was mounted and a plain bearing on the input end of the reactor. The paddles (three in each compartment) were clamped to the axle at an angular offset of 135°. The agitator was powered by an electrical drive motor through a chain drive. The rotational speed of the agitator could be varied by means of a frequency converter.

The tubular digester was equipped with self-adhesive electrical heater mats (thermo Flächenheizungs-GmbH, Rohrbach, Germany), two for each tube section. Temperatures in the four compartments were controlled separately. The top covers of the gas collectors were manufactured from PVC (thickness: 20 mm) and fastened with bolts and nuts. The use of PVC facilitated tapping for the installation of gas pipes, immersion sleeves for temperature probes (stainless steel; thickness 1.5 mm), and level monitors (first compartment only).

Flexible, closed-cell foam mats (Armaflex®: Armacell GmbH, Münster, Germany) were used for thermal insulation of the thermophilic reactor.

The other two digesters were converted from used milk tanks. As a result of this, the usable volumes of these reactors were smaller than it would have been required from the exact scaling factor. The original aluminum lid of Model Digester 1 (MD1) was replaced by a top cover manufactured from PVC (thickness: 40 mm) that was fastened with bolts and nuts and sealed with a rubber-seal. Heating of the digester was achieved by means of an unpressurized water-heating system. A heating tube was installed inside the digester and connected to an external water-quench heating with an electrical heating coil (EGOTHERM®: E.G.O., Oberderdingen, Germany). Heating-circuit water was circulated by means of an electrical pump. The thermal insulation of the original milk tank (18 mm PU-foam) was considered adequate. Connecting pieces for input and withdrawal of substrate were installed at the bottom and at a height of about 2/3 of the normal filling level.

The original stainless steel top cover of Model Digester 3 (MD3) was retrofitted with a PVC lid (diameter: 200 mm, thickness: 20 mm) on which gas pipes, immersion sleeves for temperature probes, and a level monitor were installed. The steel top cover and the smaller PVC lid were fastened with bolts and nuts, and sealed with foam rubber and an O-ring seal, respectively. Apart from the fact that the existing heating coil in the bottom of the tank was incorporated, the heating system was the same as in the case of MD1. Again, the thermal insulation of the original milk tank (53 mm PU-foam) was considered sufficient. Connecting pieces for input and overflow of substrate were installed at the bottom and at a height of about 2/3 of the normal filling level.

Model Digesters 1 and 3 were equipped with centrally mounted propeller agitators (TMR Turbo-Misch- und Rühranlagen, Taufkirchen, Germany) powered by electrical drive motors (Table 8). Slide ring seals were installed to avoid leakage of biogas through shaft bearings.

Manure was delivered through the model plant by means of progressing cavity pumps (ALLWEILER AG, Bottrop, Germany) powered by electrical drive motors. To allow for variable delivery rates, pump 1 was equipped with an adjustable speed belt drive while pumps 2 and 3 were actuated through frequency converters. The conduits connecting the different vessels were made of transparent fabric hose (inner diameter: 40 mm). The storage tank and the three digesters could be shut off with PVC ball valves. Overflowing digest from

Digester 3 was discharged into a sewer through a transparent fabric hose (inner diameter: 50 mm).

The digesters were checked for possible gas leakage by pressurizing with air to about 15 mbar. The biogas from the three digesters was collected and discharged through PVC pipes (inner diameter: 4 mm, wall thickness: 1 mm) with gas-tight fittings (EM-TECHNIK GmbH, Maxdorf, Germany). An additional gas pipe on each digester with an attached gas bag for volumetric compensation was connected to a water trap to avoid overpressure (maximum allowed pressure: about 6 mbar). Biogas flows from MD1, the four collectors of MD2, and MD3 were channeled separately and could interchangeably be switched on the gas analyzer. The biogas was discharged into the atmosphere through a stainless steel pipe, at a height of about 5 m above ground.

The three digesters as well as all the devices for system control, measurements and data logging were installed in a used reefer container, rendering the plant movable. The container was continuously aerated by means of an electrically driven ventilator. The tank for the raw liquid manure (volume: ca 500 L) was placed in front of the container and connected to the feeding pump 1 through a hose coupling. Figure 6 shows the layout of the model biogas plant. Technical data on main electrical components are summarized in Table 8.

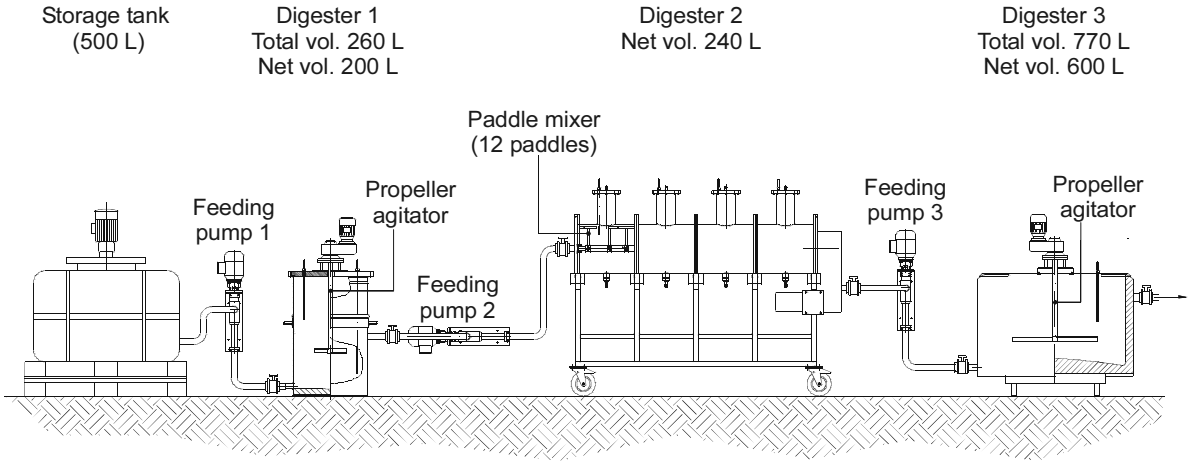


Figure 6. Layout of model-scale biogas plant

Table 8. Technical specifications of the main electrical components of the model biogas plant

Component	Supplier (type)	Specifications	Materials
Supply pumps for digesters	ALLWEILER ANBP 12.2	Delivery rate: 1.67-10 L*min^{-1} Powering: 0.55 kW, 400 V, 50 Hz	Rotor: Stainless steel; stator and sleeve: Perbunan
Propeller mixer (storage tank)	TMR / Self-construction	Powering: 0.55 kW, 400 V, 50 Hz Motor speed: 1360 min^{-1} Output speed: 467 min^{-1}	Parts in contact with manure: Stainless steel (1.4571)
Propeller mixer (Digester 1)	TMR FGMD 0,25/145 Ex	Powering: 0.25 kW, 400 V, 50 Hz Motor speed: 1500 min^{-1} Output speed: 145 min^{-1} Propeller diameter: 400 mm (specific installed power: 1.25 kW per m^3 net volume)	Parts in contact with manure: Stainless steel (1.4571)
Paddle agitator (Digester 2)	Self-construction	Powering: 0.75 kW, 400 V, 50 Hz Rotational speed: 2-30 min^{-1} (specific installed power: 3.13 kW per m^3 net volume)	Stainless steel
Propeller mixer (Digester 3)	TMR FGMD 0,75/83 Ex	Powering: 0.75 kW, 400 V, 50 Hz Motor speed: 1500 min^{-1} Output speed: 83 min^{-1} Propeller diameter: 700 mm (specific installed power: 1.25 kW per m^3 net volume)	Parts in contact with manure: Stainless steel (1.4571)
Heating mats (Digester 2)	thermo	Power: 200 W, 230 V Dimensions: 315 x 490 mm	Metallic ink on PET
Heating coils (Digester 1, 3)	EGOTHERM	Power: 2 x 900 W, 230 V	Stainless steel

4.3.2 Process Control and Data Logging

Agitating, feeding, and heating operations of the model biogas plant except for the heating of Model Digesters 1 and 3 were controlled by a programmable logic controller which was programmed by the supplying company (AWITE Bioenergie GmbH, Langenbach, Germany) according to the specifications given by the author. All recurring operations were controlled on the basis of individual time intervals, *e.g.* to feed the digesters every hour, the respective interval for operating the pumps was set to 1 hour (3600 seconds) and the duration of the process was determined by timing the starting and finishing point (between 0 and 3600 s).

The volume of liquid manure delivered to the digesters was controlled by the combination of feeding rate and operating time of the pumps. For this purpose, the delivery rates of the pumps were measured on a volumetric basis. The disadvantage of this procedure was its limited accuracy (approximately ±5 %).

Model Digesters 1 and 2 were equipped with level monitors to control the filling levels. If the filling level reached the respective minimum or maximum value, the withdrawal or feeding pump was temporarily locked until the level was again within the given limits. The level of MD2 was controlled in such a way that the reactor vessel was always completely filled.

The following measured values were logged by the controller on a CompactFlash™ every ten minutes: Temperatures in the thermophilic digester; total biogas volume produced; filling time of the gas collection bag; methane and hydrogen sulfide contents of the biogas in the collection bag (latest measurements). A separate data logger was employed to record hourly means of the temperature outside, in the gas meter, and in Digesters 1 and 3. All the data were stored on a PC and transferred to the office on floppy disk or CompactFlash™ on a weekly basis. Appendix 4 gives an overview of measuring instruments and data logging at the model biogas plant.

4.4 Procedures at Model-Scale

The model biogas plant was started up by filling the reactors with contents of the respective full-scale digesters of the pilot plant and immediately commencing feeding with raw liquid manure. Liquid manure was delivered from the experimental farm to the feed tank of the model biogas plant every other week. The feed for the model plant was taken from a branch pipe during refilling of the collection tank of the pilot plant. The liquid manure in the feed tank was stored under ambient temperature conditions and stirred intermittently.

The model biogas plant was operated for a time period of about 6 months in 2003 and about 7 ½ months in 2004. Throughout the year of 2003, steady operation of the plant was impaired by frequent clogging of the delivery pump of MD1 and failure of the float switches that had initially been installed as level monitors. These problems could be solved by pumping the raw liquid manure for the model plant through a macerator to cut blades of grass and hairs to a maximum length of about 1 cm and by replacing the float switches with potentiometric devices.

Due to these complications, only the data of 2004 were used to evaluate treatment performance of the model biogas plant. After a complete shut-down during the cold season the model plant was re-filled and re-started at the end of April 2004. Repeated blockage of the overflow of MD3 could be avoided by increasing the diameter of the pipe and installing a branch pipe that served as a trap and could be used for flushing the overflow pipe from time to time.

In the case of the model plant, the daily amount of feed was added to the digesters in 24 and 6 batches, according to the specified feeding interval. During operation with a feeding interval of one hour, the daily load varied between 24.5 and 26.5 L of liquid manure, resulting in average hydraulic retention time of 8.1 days in MD1, 9.6 days in MD2, and 23.4 days in MD3. During the period with the longer feeding interval, the daily load varied between 28.6 and 29.1 L of liquid manure, resulting in average hydraulic retention time of 7.5 days in MD1, 8.4 days in MD2, and 20.8 days in MD3. The proportion of the hydraulic retention time in the thermophilic stage of the model plant amounted to about 23 % of the total HRT in the digester chain.

After re-starting in March 2005, the model plant was operated as a mesophilic-thermophilic process for about three months, while only the biogas production from MD1 was measured. The daily load during this period was 15.4 L of liquid manure, resulting in a hydraulic retention time in MD1 of about 14 days. An overview of the procedures at model scale is given in Figure 7. Appendix 2 gives a detailed report of the procedures with the model plant.

Table 9. Mean calculated hydraulic retention times (days) in the digesters of the model plant during the evaluated time periods

Operating mode	Meso-thermo-meso 24 batches per day	Meso-thermo-meso 6 batches per day	Single-stage meso 24 batches per day
Model Digester 1	8.1	7.5	14
Model Digester 2	9.6	8.4	-
Model Digester 3	23.4	20.8	-
Chain	41.1	36.7	-

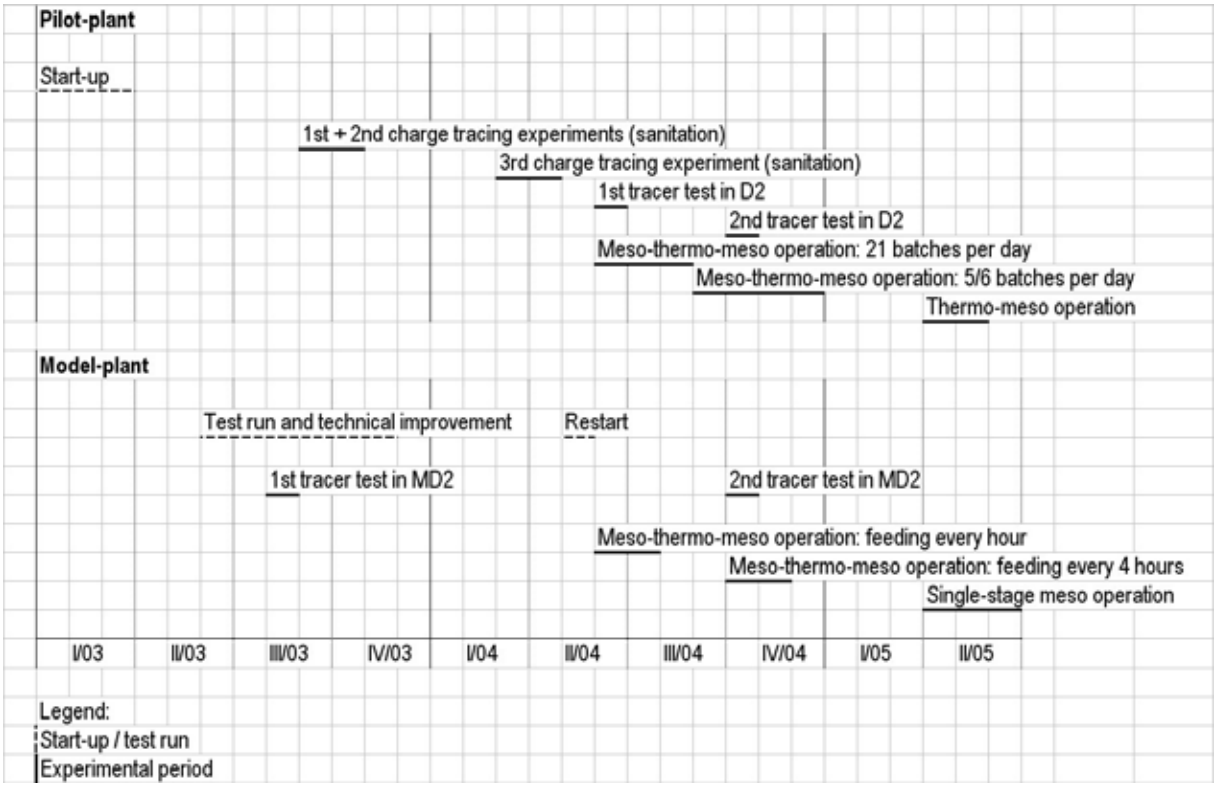


Figure 7. Overview of pilot and model plant operations

Samples of raw liquid manure were taken regularly when liquid manure was delivered to the storage tank of the model plant and infrequently in between refilling the storage tank. In order to evaluate performance and stability of the digestion process at model scale, every 2 to 5 days during periods of continuous operation and less frequently during transition periods samples of digester contents were taken. Approximately 0.5 liters of digester content were drawn from a cock in the withdrawal pipe of Model Digester 2 and from cocks in the tank walls of Model Digesters 1 and 3. Sampling from the withdrawal pipe and the tanks was done shortly after feeding and agitating. As required, samples for microbiological investigations were taken in conjunction with samples for chemical analyses.

4.5 Analytical Procedures

Liquid samples of raw and digested manure were analyzed based on German Standard Methods for the Analysis of Water, Wastewater, and Sludges (Anonymous, 1981) and analytical methods for feed analyses (VDLUFA, 1997).

4.5.1 Dry matter

Dry matter content of liquid samples of raw and digested manure was determined by measuring the reduction of weight after drying a sample of about 50 g of fresh material in a hot-air cabinet (105°C) overnight. The results are specified in per cent, on basis of the mass of the fresh material.

4.5.2 Volatile Solids

The volatile solids (VS) represent the organic fraction of raw manure or digest which is lost during combustion. The remaining ash content of raw manure indicates how much grit is collected together with the manure. VS content was determined by measuring the reduction of weight after incinerating a sample of 0.5 to 1 g of dried and ground material in a muffle furnace (550°C). Depending on the pH of the liquid sample, some VS can already get lost during the process of drying (section 4.5.1). The results are specified in per cent, on basis of the mass of total solids.

4.5.3 Chemical Oxygen Demand

Chemical oxygen demand (COD) was used for the calculation of a mass balance (section 4.7). COD was determined by treating a sample of dried, ground material with a solution of dichromate in sulfuric acid at 148°C, in the presence of a catalyst and over a period of two hours. The results are specified as mass of oxygen (g) per mass of sample (kg).

4.5.4 Parameters Derived from Feed Analyses

Chemical analyses used for evaluating nutritive properties of feedstuffs may be applied to estimate anaerobic digestibility of animal waste. Forage dry matter can be divided into two fractions, corresponding to the cellular contents which are essentially available for anaerobic digestion (lipids, soluble carbohydrates, most proteins and other water-soluble matter) and the plant cell wall constituents (cellulose, hemicellulose and lignin) whose availability is determined by structural features. Having passed the digestive tract, feces of forage-fed ruminants do not contain water-soluble carbohydrates anymore (Van Soest, 1967). The content of water-soluble carbohydrates in liquid manure may thus be used as an indicator of undigested forage arriving in the manure collection system.

Starch was determined by solubilization in hot diluted hydrochloric acid and subsequent filtration. After precipitation and clarification steps, the filtrate was analyzed in a polarimeter.

This analysis was based on the method described by Ewers, DIN 10300, Blatt 1 (Arbeitsgemeinschaft Getreideforschung, 1978).

4.5.4.1 Neutral Detergent Fiber

The content of neutral detergent fiber was determined with the amount of dry residue after boiling a sample in a neutral detergent solution. NDF is a measure of the content of plant cell wall constituents, *i.e.* hemicellulose, cellulose and lignin.

4.5.4.2 Acid Detergent Fiber

The content of neutral detergent fiber was determined as the amount of dry residue after boiling the NDF fraction in a sulfuric acid detergent solution. Hemicellulose is thereby hydrolyzed and its quantity is calculated from the difference of NDF and ADF.

4.5.4.3 Acid Detergent Lignin

The remainder of the ADF fraction after hydrolysis with concentrated sulfuric acid is termed ADL. Essentially, this fraction consists of lignin.

4.5.5 Ammoniac Nitrogen

Ammonium-nitrogen (NH_4-N) content was determined in diluted samples with an ammonia-sensitive electrode (Thermo Orion, Witchford, UK). Organic nitrogen was determined by the Kjeldahl method (Anonymous, 1981).

The concentration of free ammonia was calculated from measured values of NH_4-N according to Gallert & Winter (1997):

$$NH_3 - N = NH_4^+ - N * 10^{pH} / (\frac{K_b}{K_w} + 10^{pH}) \text{, with } K_b / K_w = e^{(6344/(273+T))}$$

or:

$$NH_3 - N = \frac{NH_4 - N}{(\frac{K_b}{K_w * 10^{pH}} + 1)} \qquad (4.1)$$

A temperature of 38°C and 55°C for the mesophilic and thermophilic stages was used for the calculations if the actual digester temperatures did not deviate significantly from these values. At pH = 8 and 55°C the proportion of free ammonia adds up to approximately 28 %, compared to approximately 12 % at 38°C.

4.5.6 Elemental Analysis

Total amounts of carbon (C), nitrogen (N), and sulfur (S) were analyzed with a "Vario MSX CNS" analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). The flue gas components from catalytic combustion of the sample were separated by selective sorption and quantified with a thermal conductivity detector. C, N, and S contents were calculated from the quantities of CO_2, NO_x, and SO_2 detected in the sample.

4.5.7 Volatile Fatty Acids

Volatile fatty acids (VFA) are intermediates of the anaerobic digestion process and have been identified as a suitable indicator of process imbalance, since their concentrations change promptly after a perturbation (Ahring et al., 1995). Total VFA were determined by steam-distillation at pH 3 and subsequent titration using phenolphthaleine as indicator. The results are given in mmol of acetic acid per mL of sample.

The concentrations of the individual VFA in the distillate were determined with a gas chromatograph (Agilent 6890N; column: HP FFAP, 25 m, ∅ = 0.32 mm; temperature program: 80°C/1 min – 120°C at 20 $K*min^{-1}$, 3 min – 220°C at 6.13 $K*min^{-1}$, 20.13 min – Stop).

4.5.8 pH Value

The pH value of the liquid samples was measured directly after sampling with a hand-held meter (EUTECH Instruments, Nijkerk, Netherlands) and again during processing of the samples in the laboratory.

4.5.9 Alkalinity

Alkalinity was determined by titrating a diluted sample with 0.1 N hydrochloric acid until a pH of 4.3 was reached.

4.5.10 Electrical Conductivity

The electrical conductivity of the liquid samples was measured directly after sampling with a hand-held meter (EUTECH Instruments).

4.5.11 Biogas Production

The nominal biogas production of the **pilot biogas plant** was determined from daily readings of the biogas consumption of the combined heat and power unit measured with a bellows-type gas meter (Elster-Instromet GmbH, Mainz, Germany). These values were corrected by

subtracting the amount of air introduced into the gas system for the purpose of desulfurization. Daily normalized biogas production was calculated on the basis of measurements of ambient air pressure (data from local meteorological stations) and gas temperature in the gas meter. To calculate remaining water vapor content in the biogas after condensation in an underground passage, a mean soil temperature of 8°C was assumed. Normalized methane production was calculated from normalized biogas production and daily measurements of the composition of the mixed biogas (see section 1.6.2).

Biogas flows from Digesters 1 and 2 were measured continuously with two V-Cone Flowmeters (McCrometer, Inc., Hemet, CA, U.S.A.) which were installed in the gas pipe downstream of Digester 1 and 2. These meters operate on the principle of measuring the pressure differences created by a centrally located cone inside the gas tube. For the following reasons it was difficult to find suitable instruments to measure the gas flow between individual digesters of the pilot plant: Pressure differences and flow velocities were typically very small but increased abruptly during feeding of the digesters; direction of gas flow was temporarily reversed during feeding of the digesters; and the biogas had corrosive properties. At first the flow meters appeared to operate satisfactorily even though the accuracy of the measurements was limited, as the instruments could not be calibrated on site. Whereas, with temperatures below zero, the flow meters were prone to freeze and had to be bypassed lest they blocked the gas pipe. When the measurement data from the flow meters were evaluated, they appeared implausible in comparison to the readings of the gas meter at the CHPU and could therefore not be used.

The biogas production of all three digesters of the **model plant** was totaled with a drum-type gas meter (RITTER GmbH, Bochum, Germany). The biogas production of the individual digesters was determined in two ways: (i) Mean values of the biogas flows from individual gas collectors were calculated from the inflation times as indicated by a pressure sensor and the known volume of the gas collection bag; (ii) biogas production of Model Digesters 1 to 3 was totaled by connecting only one digester to the gas meter for a period of two to three days; no values of the total biogas production were obtained during this time. Normalized values of biogas production were calculated on the basis of measurements of gas temperature in the gas meter and ambient air pressure (data from a meteorological station in the area adjusted for the difference in elevation).

4.5.12 Biogas Composition

A commercial gas analyzer (SCHMACK SSM 6000; SCHMACK BIOGAS AG, Schwandorf, Germany) was used to measure the composition of the mixed biogas from all three digesters and the terminal storage tank of the **pilot biogas plant** on a daily basis. Biogas for analysis was withdrawn from the biogas supply pipe inside the CHPU room after dehumidification (see above). Methane and carbon dioxide in the cooled biogas (dew point: 5°C) were quantified by means of the infrared two-beam compensation method with pressure compensation (measuring error as specified: ± 2 %). Hydrogen sulfide (after dilution) and oxygen were measured with electrochemical sensors (measuring error: ± 5 % for hydrogen sulfide in dilution and ± 0.2 % for oxygen).

The composition of the biogas in the head spaces of the individual digesters of the **pilot plant** was measured about once a month using two automatic gas analyzers (AWITE Bioenergie GmbH, Langenbach,Germany). Biogas for analysis was withdrawn directly from the gas collectors. Measurement methods were as described above, except that the gas analyzers did not have a gas cooler.

Each of the six biogas collectors of the **model plant** (Model Digester 1, Digester 2/1 to 2/4 and Digester 3) was subsequently connected to a gas collection bag for biogas analysis twice per day, for two hours. Methane and hydrogen sulfide contents of the biogas in the collection bag were automatically measured with an automatic gas analyzer (AWITE Bioenergie GmbH). All gas analyzers were calibrated once per year.

4.6 Characteristics of Liquid Dairy Cattle Manure

Both, the pilot and the model biogas plant were fed solely with liquid manure from the underground storage canal of the dairy cattle stable as described above. The manure contained chopped straw from bedding and slight amounts of forage. Additional inflows occurred in the forms of washing water from the milking parlor (about 700 L per day) and some sewage from a washroom. Despite the inflow of washing water, a gradual thickening of the liquid manure in the storage canal was observed, until the material could hardly be pumped anymore. To keep the mixture pumpable, about once a month a batch of thinner liquid manure was supplied from a neighboring dairy cattle farm to the underground storage. This manure accounted for about 15 % of the total volume of liquid manure processed in the pilot plant.

The mean dry matter content of 26 samples of liquid manure taken from the collection tank of the pilot plant was 7.8 % (m/m). The DM of the liquid manure contained about

22 % (m/m) of ash which was mainly fine grit from the concrete floor of the stable, and a high percentage of lignin. The observed differences in composition of liquid manure samples from the pilot and the model plant were not significant (Table 10 and Table 12). The low starch content of between 0.11 and 0.44 % (m/m) of DM in a few samples indicated that the liquid manure did not contain significant amounts of undigested feed which would have raised the biogas yield.

Table 10. Chemical characteristics of samples of liquid manure taken from the collection tank of the pilot biogas plant during 2004 (mean value ± standard deviation of up to 26 samples)

DM	% (m/m)	7.8 ± 0.8
VS	% (m/m) of DM	77.6 ± 3.0
VS	$g*kg^{-1}$	60.8 ± 6.4
COD	$g*kg^{-1}$	86.7 ± 8.6
pH	-	7.4 ± 0.1
Total VFA	$mg*L^{-1}$	6844 ± 530
NH_4-N	$mg*L^{-1}$	2220 ± 710
Alkalinity	g $CaCO_3*L^{-1}$	12.1 ± 0.5

Table 11. Composition of the dry matter of liquid manure samples taken from the collection tank of the pilot biogas plant during 2004, % (m/m)

VS	77.6 ± 3.0
NH_4-N	2.4 ± 0.5
$N_{org.}$	2.4 ± 0.1
Total P	1.0 ± 0.3
Raw protein	15.0 ± 0.4
Raw fat	4.9 ± 0.9
Raw fiber	16.4 ± 2.8
Cellulose	11.4 ± 4.2
Hemicellulose	12.9 ± 6.7
Lignin	23.9 ± 5.5

Table 12. Chemical characteristics of samples of liquid manure taken from the feed tank of the model biogas plant during 2004 (mean value ± standard deviation of up to 18 samples)

DM	% (m/m)	7.4 ± 0.5
VS	% (m/m) of DM	79.3 ± 0.5
VS	$g*kg^{-1}$	59.0 ± 3.8
COD	$g*kg^{-1}$	86.9 ± 11.7
pH	-	7.5 ± 0.2
Total VFA	$mg*L^{-1}$	5912 ± 986
NH_4-N	$mg*L^{-1}$	2005 ± 483
Alkalinity	g $CaCO_3*L^{-1}$	11.1 ± 1.3

4.7 Evaluation of Treatment Performance

Due to the fact that the produced biogas was not stored in a separate gasholder but in the headspace of Digester 3 and the terminal storage tank, the actual biogas flow from the **pilot plant** could not be metered directly. Depending on the running-time of the engine, readings of the daily biogas consumption of the CHPU which was metered instead varied considerably and were thus not representative of actual fluctuations of total biogas production. In order to identify time periods of relatively steady biogas production, moving means of the readings of biogas consumption were calculated (Despite these limitations the term biogas production will be used in the following sections to simplify matters). The pilot biogas plant was considered to basically run at steady state when the 8-day moving mean of daily biogas consumption did not vary by more than 5 % from day to day. Additional criteria that were taken into account were methane content of the biogas and chemical composition of liquid samples.

The cumulated biogas flow from the **model plant** was recorded automatically every 10 minutes and manually whenever the plant was visited. Due to a fault in the electronics, the automatic system had recorded larger biogas volumes than actually produced. Therefore, the values had to be corrected by comparing them with the manually logged data.

Characteristic values describing the anaerobic digestion of liquid manure in the experimental plants were determined as follows:

- Biogas yield with respect to the feed of liquid dairy cattle manure:

$$Y_{Biogas,FM} = V_{Biogas} / Q_{FM,fed} \qquad (m^3*m^{-3}) \qquad (4.2)$$

with V_{Biogas}: cumulated volume of biogas production (m^3), $Q_{FM,fed}$: amount of liquid manure fed (m^3).

- Biogas yield with respect to the feed of volatile solids:

$$Y_{Biogas,VS} = V_{Biogas} / Q_{VS,fed} \qquad (m^3*kg\ VS^{-1}) \qquad (4.3)$$

with $Q_{VS,fed}$: amount of VS fed (kg).

- Biogas productivity with respect to digester volume:

$$\dot{V}_{Biogas} = V_{Biogas} / (\sum_{i=1,2,3} V_{Fi} * \Delta t) \qquad (m^3*(m^3*d)^{-1}) \qquad (4.4)$$

with V_{Fi}: usable volume of Digester i (m^3), Δt: evaluation period (d)

- Methane yield (with respect to the feed of liquid manure / VS):

$$Y_{CH_4,FM/VS} = \sum V_n(CH_4) / Q_{FM/VS,fed} \quad (m^3*m^{-3}) / (m^3*kg\ oDM^{-1}) \quad (4.5)$$

- Organic loading rate:

$$OLR = Q_{VS} / (V_F * \Delta t) \quad (kg\ oDM*(m^3*d)^{-1}) \quad (4.6)$$

- Degree of VS degradation up to Digester i:

$$VSD_{Fi} = \frac{Q_{VS,fed} - Q_{VS,i}}{Q_{VS,fed}} * 100\ \% \quad (4.7)$$

with $Q_{VS,i}$: amount of VS discharged from Digester i (m^3).

- Biogas / methane yield with respect to the amount of VS degraded:

$$Y' = V_{Biogas/Methane} / (Q_{VS,fed} - Q_{VS,3}) \quad (m^3*kg\ oDM^{-1}) \quad (4.8)$$

- Theoretical (stoichiometric) biogas yield:

$$Q_{Biogas} = \frac{\Delta COD}{2.86 * f_{CH_4}} \quad (m^3) \quad (4.9)$$

with ΔCOD: amount of VS degraded (kg), f_{CH4}: volumetric methane content in the biogas.

4.8 Determination of The Methane Yield of Digested Manure in Batch-Tests

Digested manure from the terminal storage tank was subjected to batch tests in laboratory digesters. Such a digester consisted of a double-wall cylindrical vessel (diameter: 250 mm; height: 750 mm) that was equipped with a centrally mounted agitator and connected to a water heating system. Biogas production was measured with a Milligascounter® (Ritter GmbH, Bochum, Germany). The composition of the produced biogas that was collected in a bag was analyzed with an automatic gas analyzer (AWITE Bioenergie GmbH).

About 20 L of digested manure were taken from the storage tank on 1 June 2004 and tested in triplicate to determine maximum and residual biogas and methane yield. The tests were run without inoculation, at a digester temperature of 38°C and over a period of 70 days.

4.9 Investigations of Residence Time Distributions and Mixing Conditions in Tubular Horizontal Digesters by a Tracer Method

As stated before, in order to achieve a decoupling of minimum retention time and feeding interval the thermophilic digester was designed as a horizontal tubular reactor with baffles. Tracer studies were performed to prove this and to assess the mixing conditions in the tubular reactors.

4.9.1 Selection of Tracer

Lithium in the form of lithium chloride (LiCl) was chosen as tracer due to the following reasons:

- An analytical system for determination of lithium was readily available;
- Lithium has been used for tracer studies on anaerobic digesters of different designs (Schomaker, 2002; Langenhoff & Stuckey, 2000) and its use has been proposed to be included in German legislation on monitoring sanitization performance of biological waste treatment plants (KTBL, 2004);
- Background concentrations of lithium in animal waste were assumed to be low; and
- With the applied concentrations and considering dilution, harmful effects on digester microorganisms and agricultural land were not to be expected.

4.9.2 Tracer Injection and Sampling Procedures

Samples taken out of pilot- and model-scale Digesters 2 prior to injection of the tracer served as blanks to prepare standards as described in section 4.9.3. Both at model and pilot scales a tracer test was performed for each of the two different feeding intervals. The tracer was introduced into Digester 2 of the **pilot plant** through a cock in the feeding pipe. About 80 L of digester content were withdrawn from this cock between two feedings. A known amount of dry LiCl sufficient to achieve a theoretical initial concentration of around 3 mmol Li/L was stirred into the digester content in a plastic barrel. The tracer solution was then pumped back through the cock and thus delivered into the digester during the next feeding operation. To give a pulse-like injection, the volume of the tracer batch was chosen smaller than the volume of the feeding pipe (about 140 L).

In the case of the **model plant** the tracer was introduced into Digester 2 by means of a funnel connected to a three-way ball valve in the delivery pipe from MD1 to MD2. A batch of digest equivalent to the volume fed to the digesters during one feeding operation was taken

out of MD1. A known amount of dry LiCl was stirred into about two thirds of the withdrawn digester content. During the next feeding operation the tracer batch was delivered into MD2 through the funnel after shutting off the withdrawal pipe of Digester 1. The remaining digest was subsequently used to flush the funnel.

To determine the washout of the tracer, samples of digester contents were taken immediately downstream of the withdrawal pump of the pilot-scale Digester 2 and from a cock in the withdrawal pipe of the model-scale Digester 2. Sampling was done every 2 to 4 hours during the first 24 h after injection of the tracer and then once a day for a time period of up to about three times the hydraulic retention time of the tubular digesters. Samples were stored at –18°C until processed collectively. One tracer experiment was done for each feeding interval, as summarized in Table 13.

Table 13. Summary of tracer experiments in horizontal tubular digesters

	Pilot plant	**Model plant**
Approximate digester volume	45,600 L	240 L
Mass of tracer (LiCl)	6119 / 6320 g	44 / 60 g
Theoretical initial concentration, c_0	22 / 23 mg $Li^{+}*L^{-1}$	30 / 40 mg $Li^{+}*L^{-1}$
Sampling period	24 / 22 d	15[§] / 26 d

[§], Experiment was terminated prematurely.

4.9.3 Tracer Determination

Since attempts to digest and subsequently filtrate liquid manure samples in a reproducible way failed, it was decided to analyze the lithium contents of the samples after drying. The dry matter content (DM) of the liquid sample was determined as described in section 4.5.1, and the dry residue was ground in a laboratory mill. An aliquot of 0.5 g of ground dry residue was then digested in a closed-vessel microwave digestion system (CEM MARS 5™: CEM GmbH, Kamp-Lintfort, Germany) after addition of 10 mL of concentrated nitric acid, using a method developed for the digestion of plant material (180°C / 15 min).

The digested liquid was passed through filter paper (Blauband: Whatman GmbH, Dassel, Germany) into a 50 mL graduated flask. The filter paper was rinsed twice with analytical-grade water (Millipore GmbH, Schwalbach, Germany). Wetting agent (0.2 %) and Cs-/Al-buffer solution (3 $mmol*L^{-1}$ CsCl and 12 $mmol*L^{-1}$ $Al(NO_3)_3$) were added, and the sample solution was filled up to 50 mL with analytical-grade water. In analogy, standards were prepared from a liquid sample of digester content taken before injection of the tracer substance by adding the respective amounts of a 5 M lithium standard solution. Lithium contents in the samples were analyzed with an atomic absorption spectrometer (ELEX 6361:

Eppendorf AG, Hamburg, Germany) with a propane flame, using a characteristic curve method. The background concentration of Lithium in samples of digester content as determined from standard spiking was 0.58 mmol*L^{-1}. The minimum retention time, MRT, was determined as the time of sampling after injection of the tracer when the tracer was first detectable in the effluent.

4.9.4 Residence Time Distribution Function Analysis

The RTD functions of the horizontal tubular digesters were characterized by using the method of moments approach. Moments of the zeroth, first, and second order were calculated from experimental *C* versus *t* data using the following formula (Haas et al., 1997):

$$I_j = \sum_{i=2}^{n} \frac{t_i - t_{i-1}}{2} (C_i t_i^j + C_{i-1} t_{i-1}^j) \quad \text{for } j = 0, 1, 2 \tag{4.10}$$

The mean residence time, θ, variance, σ^2, and dimensionless variance, ν, were computed from the experimental moments according to:

$$\theta = \frac{I_1}{I_0}; \tag{4.11}$$

$$\sigma^2 = \frac{I_2}{I_0} - \theta^2; \tag{4.12}$$

$$\nu = \frac{\sigma^2}{\theta^2}; \tag{4.13}$$

Alternatively, a mathematical model was fitted to the experimental data by minimizing the following function (Haas et al., 1997):

$$ESS = \sum_{i=1}^{n} [C(t_i) - A\zeta(t_i; \theta, \nu)]^2 \tag{4.14}$$

where $\zeta(t; \theta, \nu)$ is the assumed RTD function and *A* is a scaling constant. The Gamma model is the extension of the tanks-in-series model for any positive value of *N*:

$$f(t) = \frac{\left(\frac{t}{\nu\theta}\right)^{1/\nu} \exp\left(-\frac{t}{\nu\theta}\right)}{t\Gamma\left(\frac{1}{\nu}\right)} \tag{4.15}$$

where Γ is the Gamma function; $N = 1/\nu$. *ESS* was minimized for the Gamma model by unconstrained nonlinear optimization using MATLAB 6.5.

The accuracy of the above-mentioned method to characterize the residence time distribution of the reactors was limited by the sampling period and the detection limit of the applied analytical method. To determine the mixing time and the proportion of dead volume of the reactors, only the first part of the concentration-time curve was analyzed according to the procedure for a real stirred tank without feeding / withdrawal described by Prechtl (2005). Because feeding continued during the tracer experiments, the concentration-time curve was corrected for the amounts of lithium withdrawn from the reactor, using the following formula:

$$C(t_n) = \frac{c(t_n)}{c_0(t_n)} = \frac{(c(t_n) * (V_R(t_0) + V_{in}(t_n) - V_{out}(t_n))}{m_{Li} - \sum_{t=t_0}^{t_n} [(V_{out}(t_n) - V_{out}(t_{n-1})) * \frac{(c(t_n) + c(t_{n-1})}{2}]} \qquad (4.16)$$

The mixing time, t_m, as the time required for distributing the tracer throughout the whole reactor volume was approximated as the point of time when the tracer concentration measured in the outflow appeared to fluctuate by less than 5 %. Dead volume is indicated by the tracer concentration evening out at a value of $c(t_n)/c_0$ greater than 1.

4.10 Accompanying Hygienic Investigations

The sanitation efficiency of the treatment process was determined by the quantification of various indicator and pathogenic microorganisms in samples of raw liquid manure, digester contents, and digest. The investigated bacteria included coliform and fecal coliform bacteria, *Enterococcus faecium* and *E. faecalis*, *Bacillus cereus*, *Clostridium perfringens*, thermophilic campylobacters, and *Yersinia enterocolitica*. The microorganisms were quantified by conventional cultivation methods and by quantitative real-time polymerase chain reaction (qPCR) as described in Lebuhn et al. (2005; 2004; 2003).

Microbial parameters were monitored in samples from the following five compartments: the collection tank for raw liquid manure from the dairy cattle stable, the three digesters, and the storage tank for digest. To evaluate the sanitation efficiency of the treatment process, two different strategies were applied. “Random sampling” on a monthly basis was done by taking samples for microbiological analysis from all compartments at the same point of time. “Charge tracing” describes a procedure where it was attempted to follow-up a specific batch of raw manure. This was done by taking a sample from the collection tank after it was refilled with raw liquid manure, and subsequently sampling the following compartments according to the calculated mean hydraulic retention times in the digesters (Lebuhn et al., 2005). In the case of the model plant, only random sampling was applied.

The fate of *Cryptosporidium parvum* during the anaerobic treatment process was investigated in experiments in the model plant. Oocyst suspensions in sentinel chambers were subjected to different treatments: 4 h mesophilic; 4 and 12 h thermophilic; and 4 h mesophilic – 12 h thermophilic – 4 h mesophilic (simulating treatment in the reactor chain). To determine degradation and inactivation of *C. parvum* oocysts, qPCR as well as excystation and infectivity tests were applied (Garcés et al., 2006).

5 PRESENTATION OF RESULTS

For the analysis of full-scale manure digesters, Mattock & Moser (2000) suggest to allow for an equilibration period of three times the hydraulic retention time and collect data over a successive fourth retention time, provided that no considerable disturbances occur during that period. For the investigated system this translates into an equilibration time of about four and a half months. As described in section 4.2 and Appendix 1, it was not possible to operate the **pilot plant** over this time period without any perturbations.

The daily load target of the pilot plant was reached in February 2004 (Appendix 1). The results presented here are based on data from the following time intervals:

- a period of three months (1 June to 6 September 2004) corresponding to two system hydraulic retention times of mesophilic-thermophilic-mesophilic (meso-thermo-meso) operation with feeding in 21 batches per day;
- a period of one and a half months (21 October to 7 December 2004) corresponding to one system hydraulic retention time of mesophilic-thermophilic-mesophilic operation with feeding in 5-6 batches per day; and
- a period of three weeks (23 May to 12 June 2005) of thermophilic-mesophilic (thermo-meso) operation with feeding in 21 batches per day.

To reduce to effect on the results of several perturbations occurring during this time period, double the hydraulic retention time was evaluated for operation with feeding in 21 batches (Appendix 1). Due to a power blackout during a thunderstorm, the system evaluation during thermo-meso operation was terminated after less than one system hydraulic retention time.

From June to November 2004, the **model plant** which was shut down during the cold season was operated basically in parallel to the pilot plant. The following results are based on data from a time period of two months (5 June to 5 August) with a feeding interval of one hour and one and a half months (26 September to 8 November) with a feeding interval of four hours.

5.1 Digester Temperatures

Due to the large surface area, considerably higher losses of heat energy occurred in the front and rear compartments of the tubular digester compared to the middle section. In the front portion of the thermophilic reactor of the **pilot plant** the feeding with cooler liquid from the

upstream mesophilic stage caused a distinct drop in temperature. Moreover, the rather short immersion sleeves for the thermistors at the front and rear walls were prone to influences from ambient temperature conditions. Therefore, only the measurements of sensors 2 to 4 installed at the baffles were used to check whether the desired temperature of 55°C was maintained within the thermophilic stage of the pilot plant.

After installing additional heating elements and retrofitting the paddle mixer in December 2003, the temperature level in the front section of the thermophilic reactor was increased, while there was still a temperature gradient from sensor 2 to 4 (Table 14). As a result of switching to less frequent feeding in Sep 2004, the longitudinal temperature gradient nearly vanished or was slightly reverted. At the same time, the highest mean temperature was now recorded at sensor 3 (in the middle of the tank) instead of sensor 4. Based on the temperature data recorded during operation of the pilot plant, it was obvious that the target value of 55°C was not continually maintained throughout the thermophilic reactor.

Table 14. Mean temperatures (°C; ± standard deviation) measured in ambient air and inside the thermophilic digester of the pilot plant

Month / year	Ambient temperature	Sensor 1	Sensor 2	Sensor 3	Sensor 4
Sep 03	12.5 ± 2.8	51.8 ± 1.0	53.7 ± 0.7	54.8 ± 0.4	55.2 ± 0.4
Oct 03§	5.3	51.6 ± 1.1	53.7 ± 0.6	54.8 ± 0.5	55.0 ± 0.6
Mar 04§	2.6	52.2 ± 1.0	54.6 ± 0.6	55.3 ± 0.4	55.3 ± 0.4
Apr 04§	8.9 ± 5.8	52.2 ± 1.0	54.2 ± 0.8	55.0 ± 0.6	55.4 ± 0.6
Jun 04	15.4 ± 3.1	52.3 ± 0.8	54.2 ± 0.6	55.0 ± 0.5	55.5 ± 0.4
Sep 04§	13.5 ± 5.0	53.4 ± 0.8	54.8 ± 0.6	54.6 ± 0.5	54.5 ± 0.4
Oct 04	9.8 ± 3.7	53.5 ± 1.0	55.1 ± 0.5	55.0 ± 0.4	54.9 ± 0.1

§, Temperature data incomplete

In the first mesophilic stage, during mesophilic-thermophilic-mesophilic operation temperatures ranged between 37 and 40°C, given a set-point of 38°C. Only when the plant was restarted in early 2004, digester temperatures dropped to a minimum value of approximately 34°C, temporarily.

In the case of Digester 3, downstream of the thermophilic stage, temperatures varied within a broader range, reaching up to 43°C in summer, virtually without any additional heating. Whereas during periods of very cold weather in winter, digester temperatures sank down to 30°C.

The electrical heating of the thermophilic **model-scale digester** with separate temperature controls for the four compartments kept the temperature in the reactor at mean

values of 54.7 to 54.9 C with small variations (Table 15). The largest variation in temperature occurred in the first compartment due to the colder feed from the upstream mesophilic stage.

The temperature control in the mesophilic digesters of the model plant was satisfactory though less accurate than in the thermophilic digester, particularly in MD1. From 28 August to 2 September an operational fault resulted in an overheating of MD1 to 45°C. In late September due to electrical faults the same digester temporarily cooled down to 25°C. Between 5 and 14 June failure of the heating water pump caused the cooling down of MD3. Temperature data from the aforementioned time periods were not included in Table 15.

Table 15. Mean temperatures ± standard deviation (°C) in the digesters of the model plant

Feeding mode	24 batches*d^{-1}	6 batches*d^{-1}
MD1	38.4 ± 1.21	38.1 ± 1.28
MD2/1	54.8 ± 0.16	54.7 ± 0.36
MD2/2	54.9 ± 0.04	54.9 ± 0.05
MD2/3	54.9 ± 0.05	54.9 ± 0.06
MD2/4	54.8 ± 0.10	54.8 ± 0.09
MD3	37.3 ± 0.32	37.9 ± 0.54

5.2 Loading Rates of the Digesters

Because of the serial arrangement of the digesters, the first stage received an organic load that was fairly high for a mesophilic stirred-tank reactor fed with liquid manure (Table 16 and Table 17). However, the system loading rate, *i.e.* the organic load with respect to the total usable volume of the three digesters, was at a fairly low level of 1.4-1.6 kg VS*(m^3*d)$^{-1}$. The highest system loading rate was achieved in the model plant during feeding every four hours (Table 17). With a value of 3.6 kg VS*(m^3*d)$^{-1}$, the loading rate of MD1 during single-digester operation was about half of that during three-digester operation.

Table 16. Mean organic loading rates (kg VS*(m^3*d)$^{-1}$) of the digesters of the pilot plant during the evaluated time intervals

Operating mode	Meso-thermo-meso 21 batches*d^{-1}	Meso-thermo-meso 5-6 batches*d^{-1}	Thermo-meso 21 batches*d^{-1}
Digester 1	6.95	6.81	(6.92)
Digester 2	5.89	6.21	7.23
Digester 3	1.68	1.74	1.89
Chain	1.37	1.49	1.85[§]

[§], neglecting the volume of Digester 1

Table 17. Mean organic loading rates (kg VS*(m^3*d)$^{-1}$) of the digesters of the model plant during the evaluated time periods

Operating mode	Meso-thermo-meso 24 batches*d^{-1}	Meso-thermo-meso 6 batches*d^{-1}	Meso 6 batches*d^{-1}
Digester 1	7.32	7.81	3.64
Digester 2	5.27	5.95	-
Digester 3	1.93	2.16	-
Chain	1.43	1.60	3.64

5.3 Anaerobic Digestion Process

The stability of the anaerobic digestion process was assessed by chemical analyses of liquid samples taken from the digesters and by monitoring biogas flow and composition. Unless otherwise stated, only the data from the evaluated time periods as specified before are reported.

5.3.1 Values of pH and Alkalinity in Digester Samples

Due to the high buffer capacity of liquid manure, alkalinity and pH in digester samples are not suitable to assess process conditions during anaerobic digestion. The mean pH values measured in samples from the digesters of the pilot and model plants were between 7.8 and 8.2 (Table 18) which is between the bounds of the equilibrium points of the two major buffer systems H_2CO_3 / HCO_3^- (pH = 6.46) and NH_4^+ / NH_3 (pH = 9.25).

Table 18. pH-values measured in digester samples (mean ± standard deviation of 10-35 samples)

Operating mode	Meso-thermo-meso 21 batches*d^{-1}	Meso-thermo-meso 5-6 batches*d^{-1}	Thermo-meso 21 batches*d^{-1}
Digester 1	8.0 ± 0.2	7.8 ± 0.1	7.4 ± 0.1
Digester 2	8.2 ± 0.2	8.1 ± 0.1	8.1 ± 0.1
Digester 3	8.1 ± 0.2	8.0 ± 0.1	7.9 ± 0.2
Model Digester 1	8.0 ± 0.2	7.8 ± 0.1	7.9 ± 0.2[§]
Model Digester 2	8.1 ± 0.1	8.1 ± 0.1	-
Model Digester 3	8.1 ± 0.2	8.0 ± 0.1	-

[§], single-stage, mesophilic operation

In the course of the anaerobic digestion process the already high alkalinity of the liquid manure increased significantly (Table 19). As a consequence of over-heating MD 1 for a short time, alkalinity values in samples from this digester decreased down to about 7 g $CaCO_3$*L^{-1}, temporarily.

Table 19. Values of alkalinity (g $CaCO_3$*L^{-1}) in digester samples (mean ± standard deviation of 3-35 samples)

Operating mode	Meso-thermo-meso 21 batches*d^{-1}	Meso-thermo-meso 5-6 batches*d^{-1}	Thermo-meso 21 batches*d^{-1}
Digester 1	14.8 ± 1.4	13.6 ± 0.8	n. d.
Digester 2	15.5 ± 1.4	14.4 ± 0.3	13.2 ± 0.1
Digester 3	17.0 ± 1.2	15.4 ± 0.1	13.9 ± 0.1
Model Digester 1	14.8 ± 1.4	12.5 ± 0.4	10.3 ± 0.9[§]
Model Digester 2	15.3 ± 1.3	12.9 ± 0.3	-
Model Digester 3	16.8 ± 0.7	13.8 ± 0.2	-

n. d., not determined; [§], single-stage, mesophilic operation

5.3.2 Ammonia Levels

Levels of NH_4-N in digester samples ranged mostly between 2000 and 3000 mg*L^{-1}. Between 19 October and 10 November, NH_4-N concentrations in samples of raw liquid manure and digester contents were up by 100 % and subsequently returned to normal levels (see the high standard deviations for the period with the longer feeding interval in Table 20 and Table 21). The reason for this is not clear. As the sharp rise in NH_4-N levels was observed in samples from both the pilot and the model plant which were analyzed at different times, faulty measurements can be excluded.

Table 20. NH_4-N-levels (mg*L^{-1}) in digester samples (mean ± standard deviation of 3-35 samples)

Operating mode	Meso-thermo-meso 21 batches*d^{-1}	Meso-thermo-meso 5-6 batches*d^{-1}	Thermo-meso 21 batches*d^{-1}
Digester 1	2106 ± 216	3064 ± 972	1339 ± 68
Digester 2	2338 ± 197	3604 ± 1196	1390 ± 334
Digester 3	2712 ± 246	3831 ± 1294	1564 ± 136
Model Digester 1	2194 ± 292	2373 ± 819	1653 ± 53[§]
Model Digester 2	2372 ± 207	2106 ± 621	
Model Digester 3	2632 ± 214	2561 ± 1265	

[§], single-stage, mesophilic operation

Apart from the samples taken during the above-mentioned period of time, maximum NH_3-N-levels of approximately 1.5 and 1.0 mg*L^{-1} were calculated for samples from the thermophilic stage of the pilot and model plant.

Table 21. Calculated levels of NH_3-N ($mg*L^{-1}$) in digester samples (mean ± standard deviation of 11-35 samples)

Operating mode	Meso-thermo-meso 21 batches*d^{-1}	Meso-thermo-meso 5-6 batches*d^{-1}	Thermo-meso 21 batches*d^{-1}
Digester 1	231 ± 92	275 ± 128	14 ± 2
Digester 2	758 ± 234	1164 ± 491	464 ± 35
Digester 3	362 ± 167	438 ± 198	123 ± 34
Model Digester 1	290 ± 94	161 ± 102	125 ± 55[§]
Model Digester 2	822 ± 188	525 ± 252	-
Model Digester 3	402 ± 131	208 ± 142	-

[§], single-stage, mesophilic operation

5.3.3 Total Volatile Fatty Acids

The level of volatile fatty acids (VFA) is a suitable indicator of imbalances of anaerobic digestion processes. Based on laboratory-scale experiments, Varel et al. (1980) state that for mesophilic digestion of liquid cattle manure, inhibition is typically to be expected at total VFA concentrations above 2000 $mg*L^{-1}$ acetic acid equivalents.

The rather high VFA concentrations in samples from Digesters 1 and 2 taken at the end of April 2004 indicate that at this point of time the anaerobic digestion process had not yet reached steady-state (Figure 8).

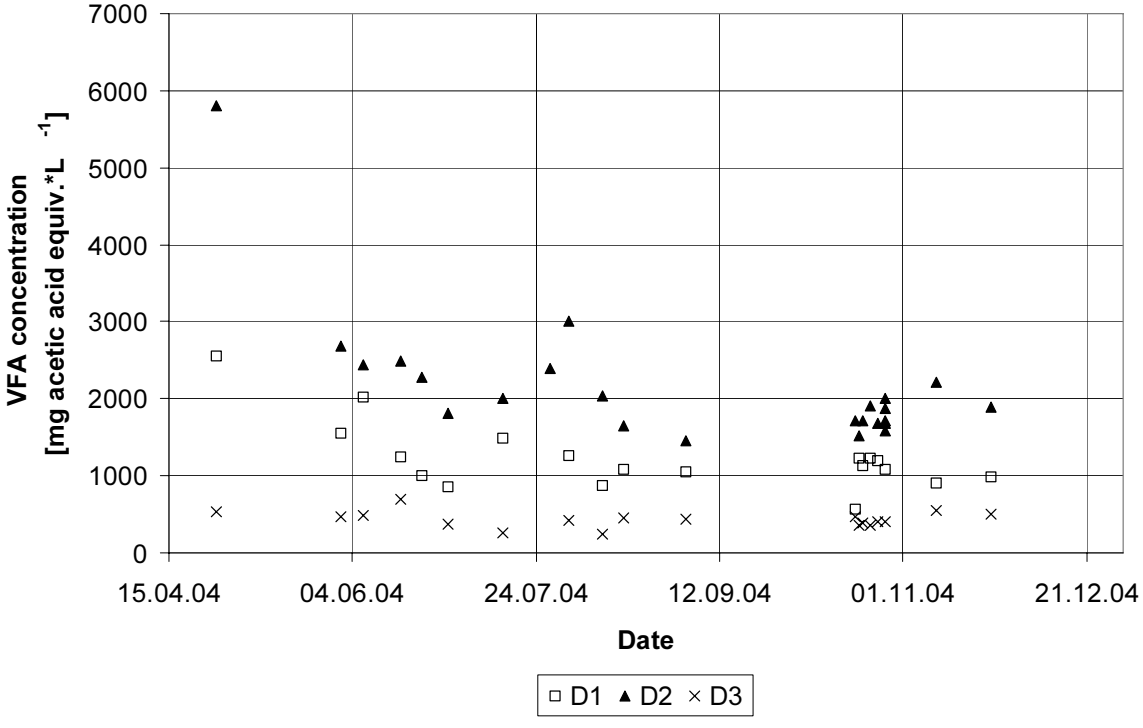


Figure 8. Total concentrations of VFA in samples from Digesters 1 to 3 of the pilot plant

During February 2004, after the design feeding rate had been re-established, VFA levels in samples from D1 were above those in D2, with a maximum value of about 3500 mg*L^{-1}. While the inflow of a large volume of water due to the burst water pipe of 28 March 2004 caused a drop in VFA levels in D1, very high concentrations of up to 6000 mg*L^{-1} of VFA were found in D2 during April. Treatment performance was evaluated from the beginning of June on, when VFA concentrations had decreased to below 2000 mg*L^{-1} in samples from Digester 1 and 3000 mg*L^{-1} in samples from Digester 2 (Figure 8). During the period with less frequent feeding, the overall lower level and variability of the VFA concentration in D1 and D2 indicate that the process was running steadier (Table 22).

Table 22. Total concentrations of VFA (mg*L^{-1}) in digester samples (mean ± standard deviation of 3-34 samples)

Operating mode	Meso-thermo-meso 21 batches*d^{-1}	Meso-thermo-meso 5-6 batches*d^{-1}	Thermo-meso 21 batches*d^{-1}
Digester 1	1241 ± 361	1042 ± 224	7910*
Digester 2	2207 ± 527	1790 ± 209	838 ± 77
Digester 3	427 ± 135	429 ± 73	327 ± 121
Model Digester 1	1481 ± 331	2446 ± 546	363 ± 26[§]
Model Digester 2	1980 ± 484	1342 ± 180	-
Model Digester 3	390 ± 131	381 ± 154	-

*, average of two determinations; [§], single-stage, mesophilic operation

During thermophilic-mesophilic operation, VFA levels in D1 operated at 20-25°C were increased in comparison to the raw manure in the collection tank, indicating some hydrolytic activity. VFA levels in the thermophilic stage were below 1000 mg*L^{-1} and thus significantly lower compared to mesophilic-thermophilic-mesophilic operation (Table 22). During the whole period of observation VFA concentrations measured in samples from Digester 3 were below 800 mg*L^{-1}. During the first months of operation in 2004, VFA levels in samples from Model Digesters 1 and 2 showed a decreasing trend (Figure 9).

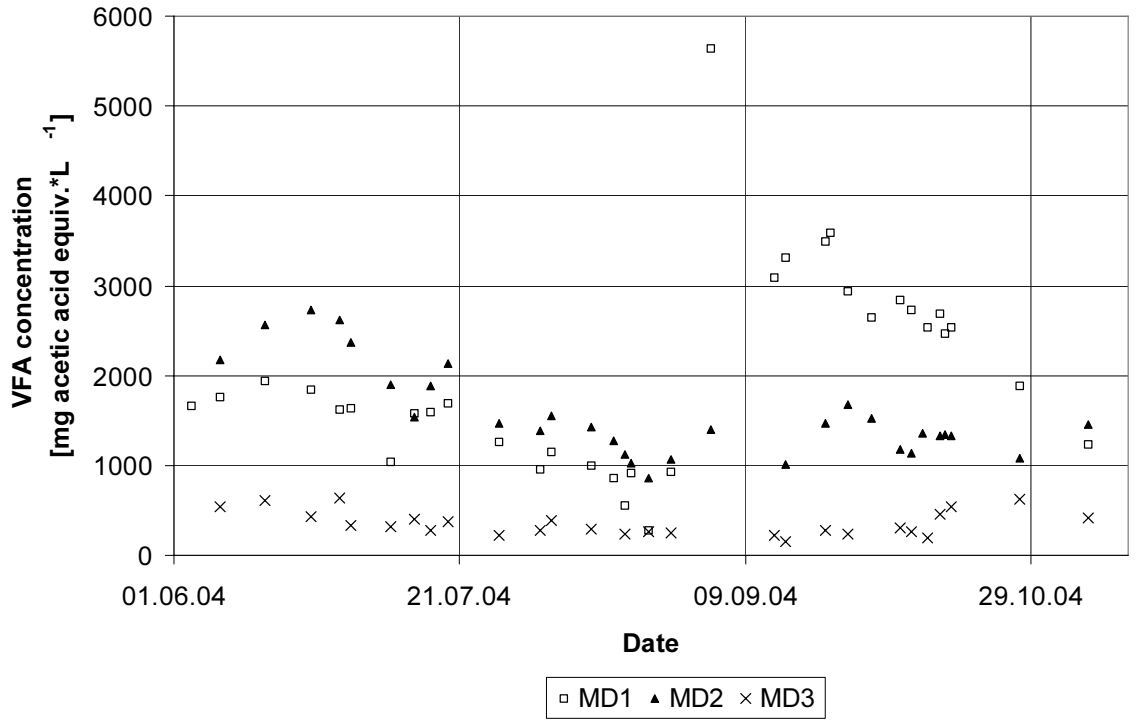


Figure 9. Total concentrations of VFA in samples from Digesters 1 to 3 of the model plant

By the end of the period with hourly feeding, the VFA concentration in MD2 ranged between 1000 and 1500 mg*L^{-1} which was similar to the level of VFA in MD1 and somewhat lower than in samples from the thermophilic digester of the pilot plant. At the end of August, a sharp rise in VFA concentration occurred in MD1 due to overheating. One day after the temperature had been readjusted to 38°C, a value of about 5600 mg*L^{-1} of VFA was measured in MD1. One and a half months later, VFA concentration had decreased to around 2500 mg*L^{-1}, and only at the end of the period of observation did it reach the level that had been observed before the perturbation. A slight rise in VFA levels was also seen in the downstream thermophilic digester. VFA concentrations in samples from MD3 were even below those measured in samples from the corresponding pilot-scale digester. Based on the limited number of samples (between 2 and 8), no significant differences could be found between the total VFA levels in samples from the individual compartments of the horizontal tubular digesters.

5.3.4 Individual Volatile Fatty Acids

A better indicator of process conditions than the total level of VFA is the concentrations of individual acids. Ahring et al. (1995) found that an increase in the concentrations of the

butyric acid isomers was the most accurate indicator of a disturbed anaerobic digestion process.

During the first half of 2004, iso-butyric and butyric acid were detectable in a few samples from Digester 1 at maximum concentrations of 1.3 and 1.0 mM and in samples from Digester 2 at maximum concentrations of 1.5 and 1.2 mM. During thermo-meso operation, butyric acid isomers were undetectable in samples from the thermophilic stage.

During mesophilic-thermophilic-mesophilic operation, iso-valeric acid was measured at a maximum concentration of 1.1 mM in samples from D1 and 1.7 mM in samples from D2. Mean values of acetate and propionate concentrations were 11.7 and 2.5 mM in samples from D1 and 21.4 and 5.1 mM in samples from D2. During thermo-meso operation, the respective values in samples from D2 were 3.0 and 1.2 mM.

As a result of overheating MD1, maximum concentrations of iso-butyric and iso-valeric acid of 2.1 and 3.0 mM were measured. In this case, the increase in the concentration of iso-valeric acid was more pronounced than that of iso-butyric acid. While the concentration of acetic acid sank fairly quick from a maximum measured concentration of 74 mM shortly after MD1 had been overheated, to 25 mM ten days later, the concentration of propionic acid showed a sharper increase and was still about 19 mM, six weeks after the perturbation. In samples from the third, mesophilic stages of both experimental biogas plants VFAs other than acetic acid were virtually not detectable.

5.3.5 Storage of Digest

The volume of biogas metered at the CHPU of the pilot plant included the biogas that was produced from the digest in the terminal storage tank. The biogas production in the storage tank was dependent on the filling level and the temperature of the tank. Occasional measurements of the temperature of liquid samples from the unheated storage tank ranged from 10°C in March 2004 to 28°C in the hot summer of 2003. Withdrawal of digest for land spreading commenced in February and ended in September. After the application of digest had caused severe burns to the grass in summer 2003, the farmer started to dilute the digest in the storage tank with water, before spreading it on grassland. Since the volumes of water added were not quantified, the values of the chemical analyses of liquid samples of digest could not be evaluated from June 2004 on. Older data were therefore included in this report (Table 23). VFA levels in samples of digest were higher than in liquid samples from Digester 3, which indicated an ongoing digestion process in the unheated storage tank.

Table 23. Chemical characteristics of samples of digest taken from the storage tank of the pilot biogas plant (mean ± standard deviation of 9 samples taken between 06/2003 and 04/2004)

DM	% (m/m)	5.5 ± 1.0
VS	$g*kg^{-1}$	38.6 ± 8.0
COD	$g*kg^{-1}$	64.7 ± 9.6
pH	-	7.9 ± 0.2
Total VFA	$mg*L^{-1}$	1094 ± 367
NH_4-N	$mg*L^{-1}$	2334 ± 464
Alkalinity	g $CaCO_3*L^{-1}$	15.1 ± 5.8

Table 24. Composition of the dry matter of digest samples taken from the storage tank of the pilot biogas plant (mean ± standard deviation of 3-8 samples from 2004), % (m/m)

VS	68.9 ± 3.0
NH_4-N	n.d.
$N_{org.}$	2.6 ± 0.1
Total P	1.1 ± 0.2
Raw protein	16.2 ± 1.0
Raw fat	5.3 ± 0.5
Raw fiber	10.1 ± 2.2
Cellulose	8.3 ± 9.0
Hemicellulose	n.d.
Lignin	23.7 ± 2.3

n.d., not determined

5.3.6 Degradation of Organic Matter

For both evaluated time periods during mesophilic-thermophilic-mesophilic operation, based on the analysis of liquid samples of raw manure and digester contents (Appendix 5), a mean VS degradation in the pilot biogas plant of 35 % was calculated (Table 25).

Table 25. Mean values of VS degradation (%) with respect to raw manure in the collection tank and proportion of individual digesters of total VS reduction in the digesters of the pilot plant

Operating mode	**Meso-thermo-meso 21 batches*d^{-1}**	**Meso-thermo-meso 5-6 batches*d^{-1}**	**Thermo-meso 21 batches*d^{-1}**
Digester 1	20	19	8
Digester 2	26	26	22
Digester 3	35	35	31
Proportion of D1	57	55	26
Proportion of D2	17	20	45
Proportion of D3	26	25	29

More than half of the VS reduction occurred in the first, mesophilic stage. Significant differences between the proportions of VS reduction in the individual digesters for the two

evaluated feeding modes were not found. During thermophilic-mesophilic operation, a mean VS degradation of 31 % was calculated, with a share of 45 % in Digester 2. The ratio of VS to COD content in samples of raw and digested liquid manure ranged between 1.4 and 1.6.

Due to the addition of unknown volumes of water to the storage tank (section 5.3.5), VS reduction in the digest could not be determined from chemical analyses. However, the total amount of COD converted into biogas was calculated from cumulated methane production (equation 4.9). Using the mean VS/COD-ratios, the mass of COD degraded in the digesters was then estimated from VS degradation.

The values of COD destruction in the thermo-meso-thermo digester chain and the whole pilot plant were calculated to 35 and 49 % for hourly feeding, and 33 and 46 % for less frequent feeding. The corresponding values during thermo-meso operation were 32 for the digesters and 53 % for the whole plant. For the model plant, the mean calculated VS degradation was 30 % for the period with a feeding interval of 1 h and 35 % for the period with a feeding interval of 4 h (Table 26).

Table 26. Mean values of VS degradation (%) with respect to raw manure and proportion of individual digesters of total VS reduction in the digesters of the model plant

Operating mode	Meso-thermo-meso 24 batches*d^{-1}	Meso-thermo-meso 6 batches*d^{-1}	Meso 6 batches*d^{-1}
Model Digester 1	14	15	22
Model Digester 2	24	23	-
Model Digester 3	30	35	-
Proportion of MD1	47	42	-
Proportion of MD2	30	24	-
Proportion of MD3	23	34	-

The share of the VS reduction in the first mesophilic stage of the model plant was about 20 % lower compared to the pilot plant. With the less frequent feeding, a significantly higher VS reduction was observed in the third, mesophilic stage of the model plant. During single-stage mesophilic treatment, average VS reduction was 22 %.

5.3.7 Conversion of Nitrogen

In the course of the anaerobic digestion process, the proportion of ammonia-nitrogen (NH_4-N) in the dry matter of the liquid dairy cattle manure increased significantly. In comparison to samples of raw liquid manure, the ratio of NH_4-N to total nitrogen in the dry matter of digest samples was increased by 21.6 % (Table 27). This value is within the typical range for the anaerobic digestion of liquid cattle manure (Schulz, 1991). No significant difference was found between the NH_4-N content of samples from MD3 and digest. As organic matter was

degraded and carbon was removed with the biogas, the C/N-ratio in the liquid manure decreased from a mean value of 13.8 in raw manure to 6.4 in digested manure.

Table 27. Mean nitrogen contents (± standard deviation) in samples of raw and digested liquid manure and comparison of means (α = 5 %)

	N_{total}	NH_4-N	NH_4-N/N_{total}	Change in NH_4-N/N_{total}
	% (m/m) of DM	% (m/m) of DM	-	%
Raw manure	2.39 ± 0.08 A	2.73 ± 0.67 A	0.55 ± 0.04 A	-
Digester 3	2.51 ± 0.09 B	4.85 ± 1.38 B	0.65 ± 0.03 B	+17.1
Digest	2.59 ± 0.10 B	5.76 ± 2.93 B	0.67 ± 0.08 B	+21.6

5.3.8 Biogas Composition

The measured values of methane content in the biogas from the **pilot plant** ranged from 52.0 to 60.0% (v/v) during mesophilic-thermophilic-mesophilic operation and from 50.2 to 53.2 % (v/v) during thermophilic-mesophilic operation. The methane contents corrected for the influx of air for biological desulfurization are 54.7 to 60.9 % (v/v) during meso-thermo-meso operation and 53.9 to 57.3 % (v/v) during thermo-meso operation (section 4.5.11). When air was sucked into the biogas collection system due to the withdrawal of digest, methane contents below 53 % were observed (*e.g.*, on 7 September, Figure 10).

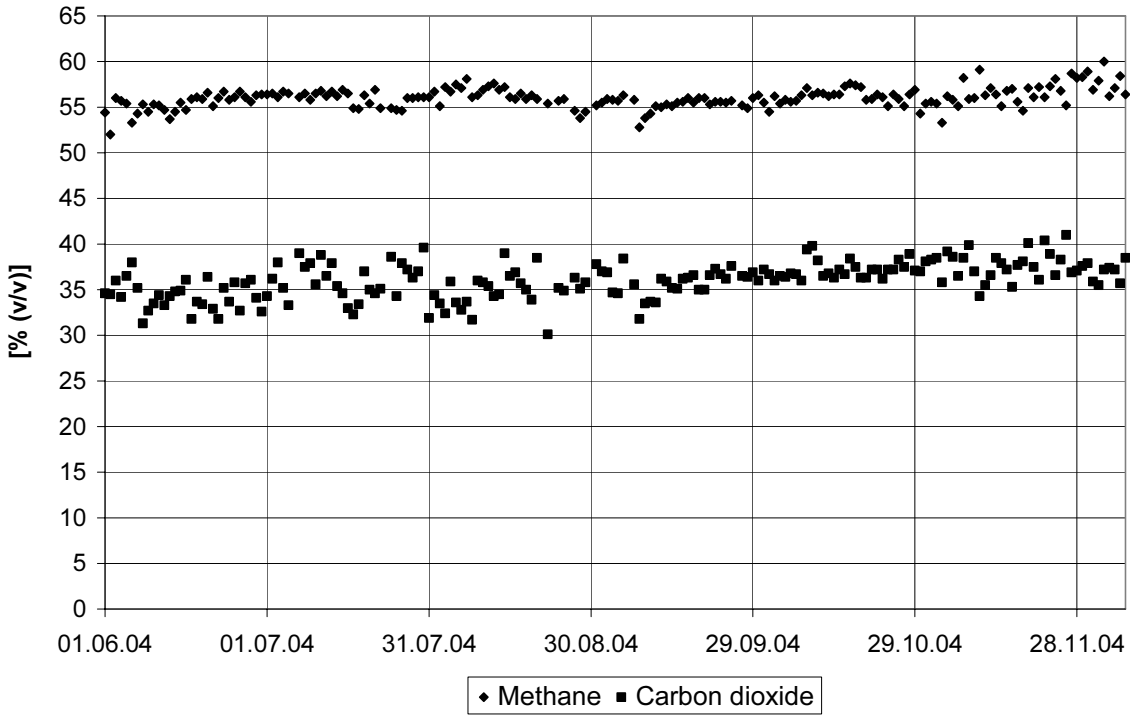


Figure 10. Daily measured values of methane and carbon dioxide concentrations in the biogas supplied to the CHPU at the pilot biogas plant during meso-thermo-meso operation

The concentration of hydrogen sulfide in the biogas that was supplied to the engine fluctuated to a great extent. In particular, elevated levels of hydrogen sulfide were observed during periods of cold weather. However, the guideline value of 200 ppm H_2S specified by the engine supplier was exceeded repeatedly also with mild weather, in conjunction with foaming in Digester 1 but also without any discernible reason in a few cases (Figure 11).

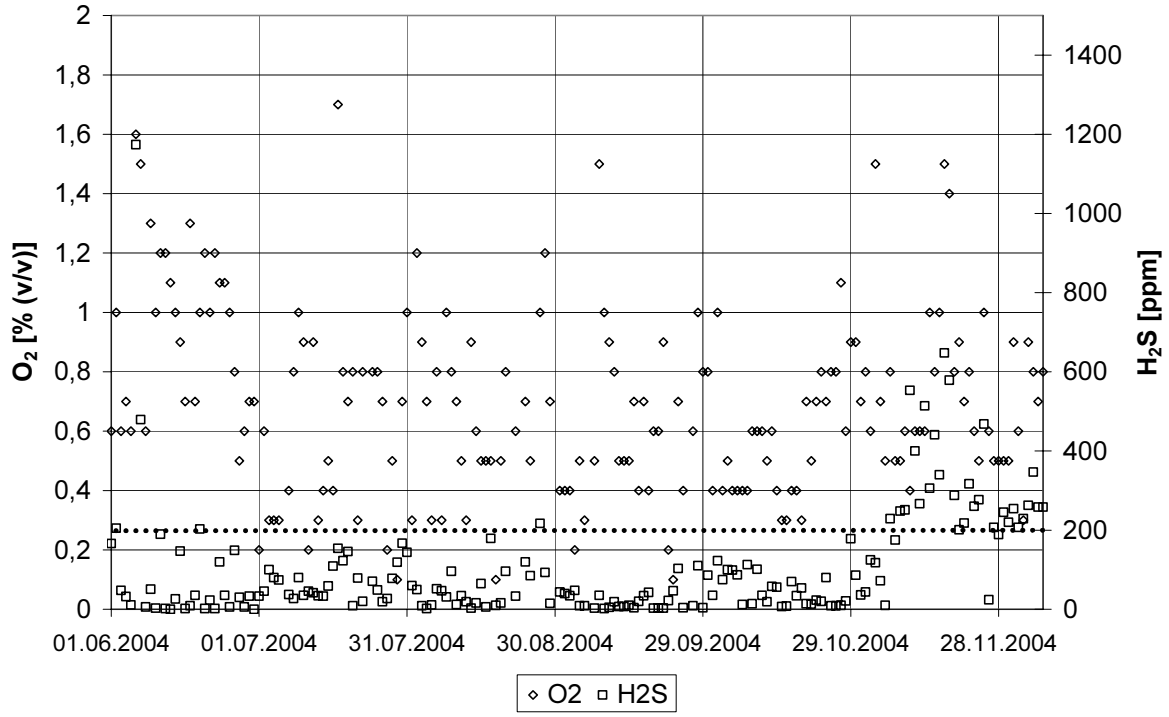


Figure 11. Daily measured values of oxygen and hydrogen sulfide concentration in the biogas supplied to the CHPU at the pilot biogas plant during meso-thermo-meso operation

As an emergency measure for protection of the engine, Fe(II) was used to precipitate sulfide in the digesters. No correlation was found between the concentrations of oxygen and hydrogen sulfide in the biogas. The results of several measurements of the biogas composition in the headspaces of the individual digesters are summarized in Table 28.

Table 28. Measurements of biogas composition in the headspaces of individual digesters and as supplied to the engine at the pilot plant (data from measurements between July and October 2004; mean values ± standard deviation)

	CH_4 % (v/v)	CO_2 % (v/v)	O_2 % (v/v)	H_2S ppm
Digester 1 (n = 11)	53.8 ± 1.1	32.9 ± 3.5	2.4 ± 0.4	n.d.
Digester 2/1 (n = 6)	50.3 ± 2.1	46.4 ± 2.3	0.9 ± 0.3	n.d.
Digester 2/2 (n = 6)	48.8 ± 2.8	49.7 ± 2.0	0.8 ± 0.2	n.d.
Digester 2/3 (n = 6)	50.5 ± 2.7	47.8 ± 2.4	1.0 ± 0.2	n.d.
Digester 2/4 (n = 6)	50.7 ± 1.7	47.2 ± 1.4	0.9 ± 0.2	n.d.
Digester 3* (n = 12)	55.0 ± 1.0	37.0 ± 0.6	1.4 ± 0.4	n.d.
Gas pipe to engine (n = 240)	55.9 ± 1.7	36.2 ± 2.1	0.8 ± 0.4	137 ± 206

n.d., not determined; *, mixed biogas from digesters 1 to 3

The methane content in the biogas from the thermophilic digester was significantly lower than in the biogas from the first mesophilic stage. No significant differences were observed between the biogas composition in the four separate headspaces of the thermophilic digester (α = 5 %). The gas in the headspace of Digester 3 was a mixture of the biogas from the three digesters and the terminal storage tank. The composition of the biogas in the headspace of the storage tank was not analyzed.

Measurements of the composition of the mixed biogas from the **model plant** were not performed. The biogas from the individual digesters was analyzed after collection in a gas bag (section 4.5.11). To avoid false measurements due to mixing of different biogas streams, only the values measured in the second hour after switching from one digester to another were evaluated. Sporadically occurring, implausible values were eliminated manually. From 6 to 14 August and from 22 September to 15 October, data on biogas production and composition of the model plant were lost due to operational faults.

During the time period evaluated for this study, the measured concentration of methane in the biogas from MD1 ranged between 54.9 and 66.5 % (v/v). Overheating of the digester caused a drop in methane content to around 30 %. About ten days after the perturbation, the methane concentration had returned to values above 60 %. The data from this period of time were not included in the analysis shown in Table 29.

Table 29. Mean values, standard deviations, and number of measurements of methane concentration (%) in the biogas from the individual digesters of the model plant

Feeding mode	MD1	MD2/1	MD2/2	MD2/3	MD2/4	MD3
24 batches*d^{-1}						
Mean	60.1	43.5	42.9	44.3	44.7	60.0
Standard deviation	2.0	1.4	1.9	1.9	1.6	1.2
n	96	82	37	67	59	156
6 batches*d^{-1}						
Mean	60.0	46.3	47.5	48.7	49.8	59.4
Standard deviation	1.7	2.0	1.7	2.3	2.2	1.5
n	129	39	19	19	16	106

The hypothesis that the mean concentration of methane in the biogas from MD1 was equal for the two different feeding modes could not be rejected (α = 5 %). The mean methane content in the biogas from MD1 during single-stage operation was 61.2 % (v/v).

A significant increase in the concentration of methane in the biogas from the thermophilic digester was observed following the overheating of the upstream mesophilic stage. This increase was likely due to the elevated levels of volatile fatty acids, particularly acetic acid, in the inflow (section 5.3.3). Again, data from this period of time were excluded from the analysis. In contrast to MD1, the hypothesis of equal mean methane concentrations measured in the biogas from the four compartments of MD2 for the two different feeding modes could be rejected (α = 5 %). The same was true for the mean values of methane concentration in the biogas from MD3.

5.3.9 Biogas Production Rate

After reestablishing the design load in early February 2004, eight-day moving means of daily biogas consumption of the CHPU at the pilot plant increased to a level of about 130 m^3*d^{-1} at the end of March. Due to the inflow of large volumes of water (section 4.4), temporarily the biogas production then dropped by about 15 %. At the beginning of June, the biogas production rate had reached a level of about 150 m^3*d^{-1} (Figure 12).

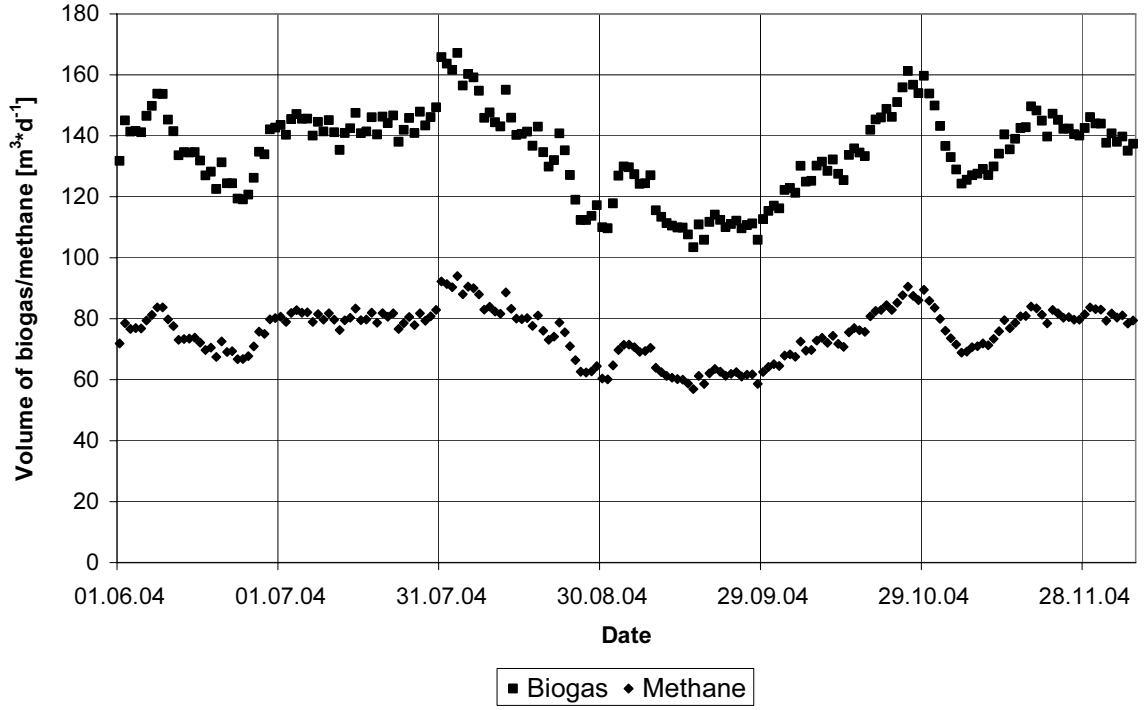


Figure 12. Eight-day moving means of biogas and calculated methane consumption of the CHPU at the pilot plant

Reduced feeding due to a faulty setting of the controller and withdrawal of digest resulted in a decreasing biogas production during June. In the following month, a fairly steady rate of biogas production of 140-150 m^3*d^{-1} was observed, and the maximum value was recorded at the beginning of August. Gas production sank again during the rest of August, as large amounts of digest were withdrawn from the storage tank. In mid-September, when the feeding mode had been changed, the biogas production rate had reached a level of about 110 m^3*d^{-1}. As the storage tank for the digest was filling, biogas production rose steadily to 160 m^3*d^{-1} until a blocked flow meter caused discharge of biogas through the pressure valve. The biogas production appeared to even out after mid-November at about the same level as prior to the change of the feeding interval (Figure 12).

During mesophilic-thermophilic-mesophilic operation, the mean rate of biogas production of the pilot plant for the two different feeding modes was 137 and 146 m^3*d^{-1}; the mean rate of methane production was 81 and 83 m^3*d^{-1}, respectively (Table 30).

Table 30. Means (± standard deviations) of feeding rates (D1), biogas and methane production rates, and methane concentration determined at the pilot plant

Time period	Feeding rate m^3*d^{-1}	Biogas production rate m^3*d^{-1}	Methane production rate m^3*d^{-1}	Average methane conc. % (v/v)
05-12/2004	5.48 ± 0.67	135 ± 28	79 ± 16	58.3
06-09/2004	5.47 ± 0.86	137 ± 31	81 ± 17	59.2
10-12/2004	5.65 ± 0.52	146 ± 21	83 ± 12	56.8
05-06/2005	5.66 ± 0.21	166 ± 22	90 ± 13	54.2

From the cumulated volumes of biogas, an average methane content of 59.2 and 56.8 % (v/v) was calculated for the period with one- and four-hour feeding interval. During the short period of thermophilic-mesophilic operation, the mean rate of biogas and methane production of the pilot plant was 166 and 90 m^3*d^{-1}, resulting in an average methane content of 54.2 % (v/v).

Figure 13 shows the cumulated, nominal volume of biogas from the **model biogas plant** according to manual readings. At the beginning of May, the model plant was restarted with material from the pilot-scale digesters. In mid-May the biogas production rate increased as a result of treating the liquid manure delivered from the pilot plant with a macerator before feeding it to the model plant. The apparently low rates of biogas production in mid-August and mid-October 2004 are due to the fact that during these periods of time, the gas meter was used to measure the biogas flow from individual digesters.

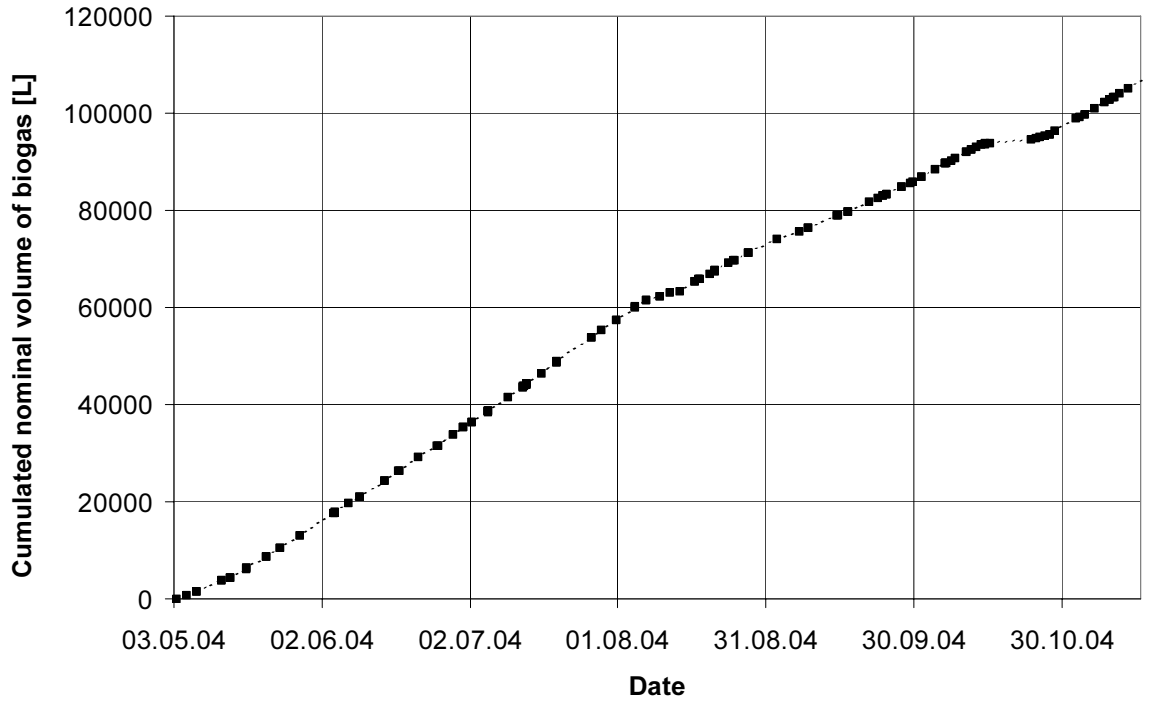


Figure 13. Cumulated, nominal volume of biogas from the model plant (manual readings)

The normalized mean biogas production rate (± standard deviation) of the model-scale digesters during hourly feeding was 583 ± 33 $L*d^{-1}$, as determined from automatically logged readings. Because of a loss of data, the corresponding value over the whole period with the longer feeding interval could not be evaluated.

The biogas collection system with gas bag and pressure sensors was not suitable to measure the average biogas production rates of the individual digesters continuously, since the filling time for the gas bag was influenced by feeding and stirring operations. Individual biogas production rates, as determined by subsequently directing the gas flows from the single digesters to the gas meter for a few days, are available from the period with the 4-hour feeding interval (Table 31). The ratio of the biogas flow from Model Digesters 1 to 3 was 1:0.55:0.38 on average. A normalized total biogas production rate of 562 $L*d^{-1}$ was calculated from the individual biogas flows.

Table 31. Mean biogas production rate (± standard deviation), proportion of total biogas production, and biogas productivity of the individual digesters of the model plant with feeding every four hours

	Biogas production rate	**Proportion of total rate of biogas production**	**Biogas productivity**
	$L*h^{-1}$	%	$L*(L*d)^{-1}$
MD1	12.1 ± 1.5	51.7	1.3
MD2	6.7 ± 0.5	28.6	0.7
MD3	4.6 ± 0.8	19.7	0.2

Due to the higher methane concentration in the biogas from the mesophilic digesters, the proportion of total methane production of MD1 and MD3 was higher than the corresponding values of total biogas flow, with proportions of about 55 and 21 %. About a quarter of the total methane production of the model plant originated from the thermophilic stage (Table 32).

Table 32. Mean methane production rate (± standard deviation) and proportion of total methane production rate of the individual digesters of the model plant with feeding every four hours

	Methane production rate	**Proportion of total rate of methane production**	**Methane yield**
	$L*h^{-1}$	%	$m^3*(kg\ VS_{fed})^{-1}$
MD1	7.3 ± 0.9	55.1	0.11
MD2	3.2 ± 0.4	24.4	0.054
MD3	2.7 ± 0.5	20.5	0.050

5.3.10 Biogas and Methane Yield from Liquid Manure

During mesophilic-thermophilic-mesophilic operation, mean values of biogas and methane yield from liquid dairy cattle manure of 0.41, respectively 0.24 $m^3*(kg\ VS)^{-1}$ were determined for the **pilot biogas plant**. The differences between the two feeding modes were not significant (Table 33).

Table 33. Biogas and methane yield from liquid manure achieved in the pilot biogas plant

Operating mode		**Meso-thermo-meso 21 batches**	**Meso-thermo-meso 5-6 batches**	**Thermo-meso 21 batches**
Biogas yield (VS fed)	$m^3*(kg\ VS_{fed})^{-1}$	0.41	0.41	0.46
Biogas yield (VS degraded)	$m^3*(kg\ VS_{degr})^{-1}$	1.17	1.18	1.51
Biogas yield (COD fed)	$m^3*(kg\ COD_{fed})^{-1}$	0.28	0.29	n. d.
Biogas yield (fresh matter)	m^3*m^{-3}	25.1	25.8	29.4
Biogas productivity	$m^3*(m^3*d)^{-1}$	0.56	0.59	0.85*
Methane yield (VS fed)	$m^3*(kg\ VS_{fed})^{-1}$	0.24	0.24	0.24
Methane yield (VS degraded)	$m^3*(kg\ VS_{degr})^{-1}$	0.69	0.67	0.78
Methane yield (fresh matter)	m^3*m^{-3}	14.9	14.6	15.2
Methane productivity	$m^3*(m^3*d)^{-1}$	0.33	0.34	0.44*

n. d., not determined; *, neglecting the volume of Digester 1

Thermophilic-mesophilic treatment produced a significantly higher biogas yield of 0.46 $m^3*(kg\ VS)^{-1}$, but due to the lower methane content in the biogas, the same figure of 0.24 $m^3*(kg\ VS)^{-1}$ was determined for the methane yield. The same biogas yield with respect to the mass of VS fed was determined for the **model plant** (Table 34).

Table 34. Biogas and methane yield from liquid manure achieved in the model biogas plant

Operating mode		**Meso-thermo-meso 24 batches**	**Meso-thermo-meso 6 batches**	**Meso 6 batches**
Biogas yield (VS fed)	$m^3*(kg\ VS_{fed})^{-1}$	0.41	(0.39§)	0.24
Biogas yield (VS degraded)	$m^3*(kg\ VS_{degr})^{-1}$	1.35	n.d.	1.09
Biogas yield (fresh matter)	m^3*m^{-3}	23.2	n.d.	12.1
Methane yield (VS fed)	$m^3*(kg\ VS_{fed})^{-1}$	n.d.	(0.21§)	0.14
Biogas productivity	$m^3*(m^3*d)^{-1}$	0.56	(0.54§)	0.86
Methane productivity	$m^3*(m^3*d)^{-1}$	n.d.	(0.30§)	0.53

§, estimated from short-term measurements according to Table 31 andTable 32;
n.d., not determined

In the model plant, the biogas yield with respect to the volume of liquid manure fed was slightly lower compared than in the pilot plant. Due to the above-mentioned loss of data, corresponding values of biogas yield for the period with the 4-hour feeding interval could not be determined. The values listed in Table 34 were estimated from short-term measurements

according to Chapter 5.3.9. A methane yield of 0.14 m^3*(kg VS)$^{-1}$ was determined for the single-stage mesophilic process.

As continuous measurements of the composition of the mixed biogas were not performed, values of the methane yield in the model plant are not available except for single-digester operation. The value of methane productivity of the mesophilic-thermophilic-mesophilic process that is stated in Table 34 was calculated from the methane production rates measured for the individual digesters.

5.3.11 Methane Yield from Digest in Batch-Tests

The methane yield of digest from the storage tank as determined in batch-tests amounted to 0.053 m^3*(kg VS)$^{-1}$ or 2.1 m^3 per m^3 of digest. This corresponds to approximately 0.034 m^3*(kg VS)$^{-1}$ with respect to the original VS content in the raw liquid manure, or an additional methane yield of about 14 %.

5.3.12 Own Energy Consumption of the Pilot Plant

An analysis of energy production and own consumption was performed for the pilot plant based on a whole year of operation (15 February 2004 to 15 February 2005). Operating times of the CHPU were dependent on biogas supply and thermal energy demand. The demand for heating energy varied considerably between seasons. Because the CHPU was oversized with respect to the available biogas supply, the engine could not be run continuously at full load. While in summer, the heating energy from biogas utilization was sufficient, during the cold season the engine had to be run on fuel oil for extended time periods to maintain the desired digester temperatures.

Total fuel consumption of the engine (biogas and fuel oil) over the course of one year was 411 MWh. The heating energy supplied to the digesters of the pilot plant amounted to 143 MWh or 35 % of total energy input into the CHPU. About 55 % of this amount was used by the thermophilic reactor with its considerably higher specific surface area compared to the vertical mesophilic digesters (ratio of volume to surface area: Digester 1, 1.22 m^{-1}; Digester 2, 2.05 m^{-1}; Digester 3, 1.19 m^{-1}). Digester 1 and 3 consumed about 40 % and 5 % of the heating energy from the CHPU.

Electrical energy production from running the pilot injection engine on biogas and fuel oil was 94 MWh. In this mode of operation, the average share of fuel oil consumption was 13 % based on heating value. An additional 30 MWh electrical energy were produced from running the engine on fuel oil only. The resulting overall electrical utilization ratio of 30 % is

in accordance with manufacturer's specifications. The pilot plant used 39 MWh of electricity per year, resulting in a share of own electrical energy consumption of 41 %.

5.4 Residence Time Distribution in Tubular, Baffled Digester

From multiple analysis of the same sample from the second tracer experiment, a method detection limit of 0.022 mmol*L^{-1} was estimated. This value corresponds to a lithium concentration in digested liquid manure of 0.14 mmol*L^{-1}. The calculated recovery of Li introduced into the reactor was 98 % for the second tracer experiment, but less than 70 % for the first one.

The minimum retention time was defined as the time after injection of the tracer when the tracer concentration first exceeded the method detection limit. For both tracer experiments in the pilot-scale reactor this occured after 8 hours. From this point on, the normalized tracer concentration in the outflow of the tubular reactors showed an initial quick rise and, after surpassing the maximum measured concentration value, declined in an overall exponential fashion. For the experiment during feeding in 21 batches per day, the maximum concentration was measured in the sample taken 49 hours after injection of the tracer. The concentration of lithium exceeded the method quantification limit of 0.043 mmol*L^{-1} (twice the detection limit) in samples taken from 11 hours to 322 hours after injection of the tracer (Figure 14).

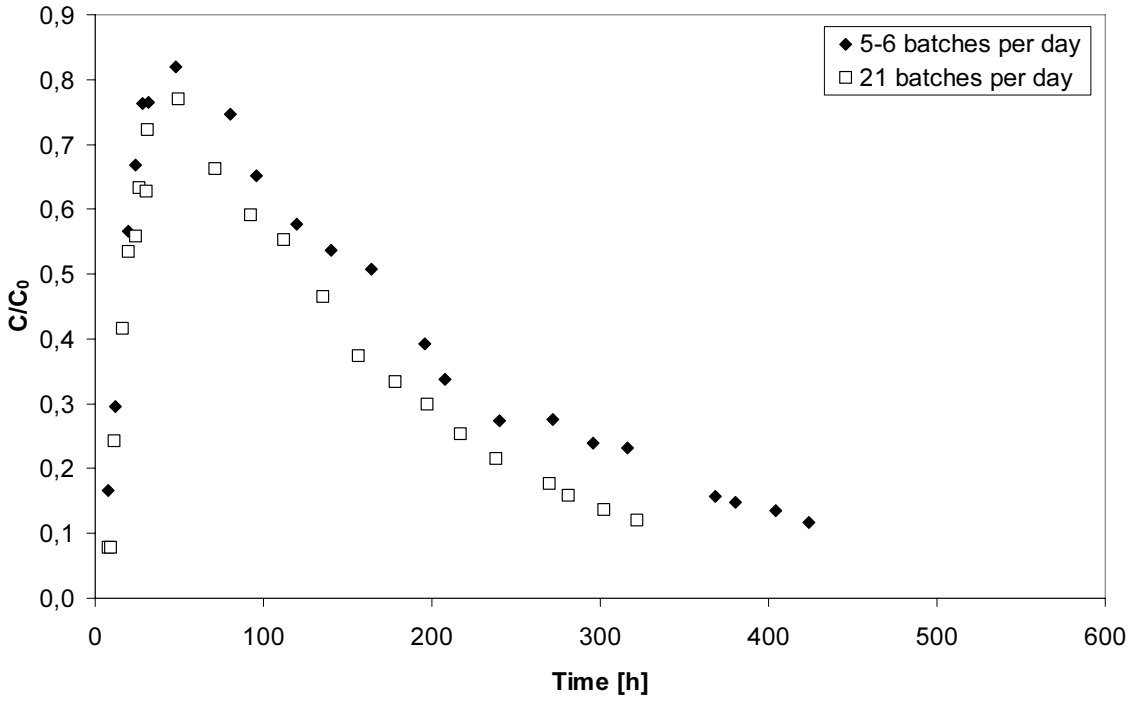


Figure 14. Normalized concentration-time-curves of Lithium tracer measured in samples from the outflow of Digester 2 during feeding in 21 and 5-6 batches per day

For the experiment during feeding in 5-6 batches per day, the maximum concentration value was measured in the sample taken 48 hours after injection of the tracer. The concentration values of lithium exceeded the method quantification limit of 0.043 mmol*L^{-1} in samples taken from 8 hours to 424 hours after injection of the tracer.

For both tracer experiments, a reasonable value for the mixing time could not be derived from the curves of the corrected normalized concentration (Equation 4.16; Figure 15). Both curves and particularly the one for the second tracer test show a pattern of rising and falling concentration values for longer retention times.

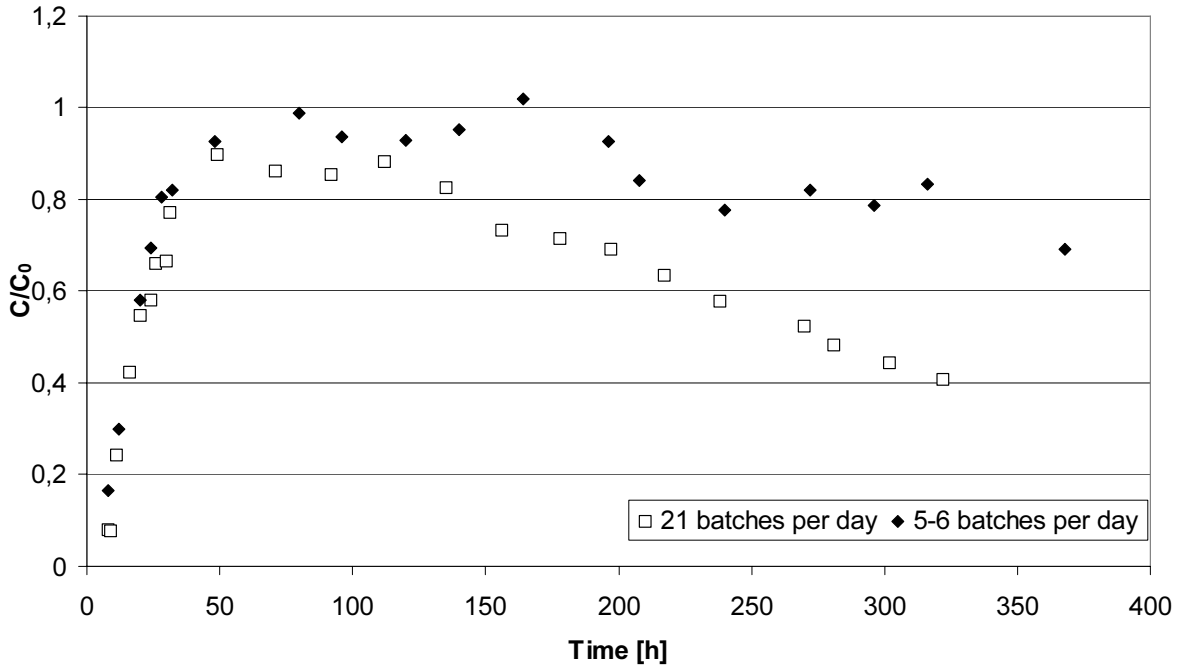


Figure 15. Corrected, normalized concentration-time-curve in the outflow of Digester 2 for the two tracer experiments

The results from the moments and regression analysis of the experimental data are compiled in Table 35 and Table 36. The accuracy of the analysis is limited by the fact that the calculated moments are very sensitive to the concentration values at the tail of the washout curve. The best fit of the Gamma-model to the experimental data is plotted in Figure 16 and Figure 17.

Table 35. Retention time distribution parameters for the horizontal tubular digester of the pilot plant determined from the first tracer experiment (HRT = 8.5 days)

	Moments analysis	**Gamma model regression**
Mean calculated retention time, θ, hours	123.03	132.85
Mean calculated retention time, days	5.13	5.54
Dimensionless variance, ν	0.41	0.56

Table 36. Retention time distribution parameters for the horizontal tubular digester of the pilot plant determined from the second tracer experiment (HRT = 8.7 days)

	Moments analysis	**Gamma model regression**
Mean calculated retention time, θ, hours	155.01	159.14
Mean calculated retention time, days	6.46	6.63
Dimensionless variance, ν	0.47	0.59

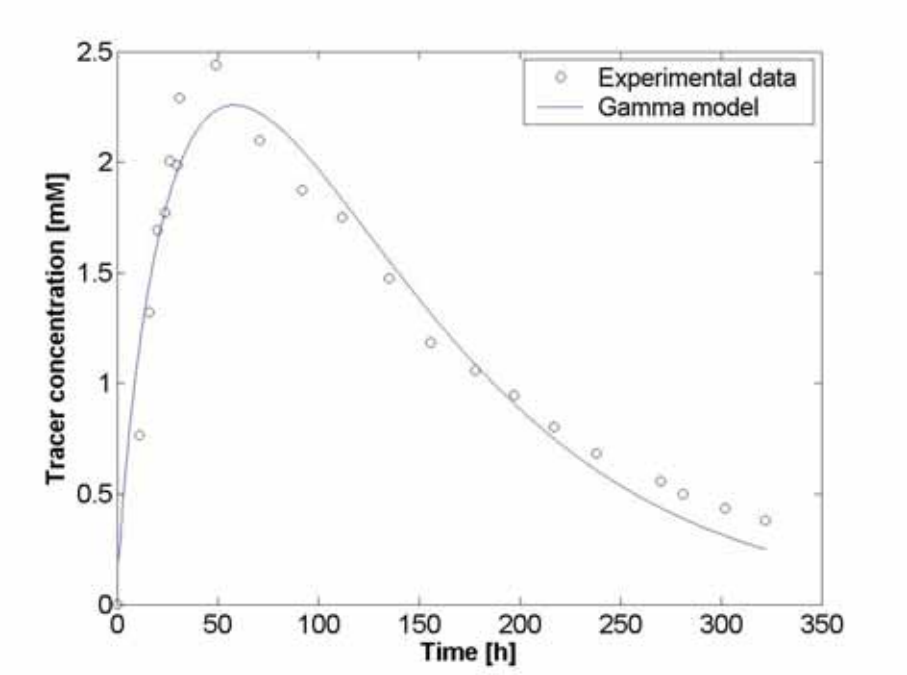


Figure 16. First tracer experiment in pilot-scale reactor; comparison of Gamma-model best fit with experimental data

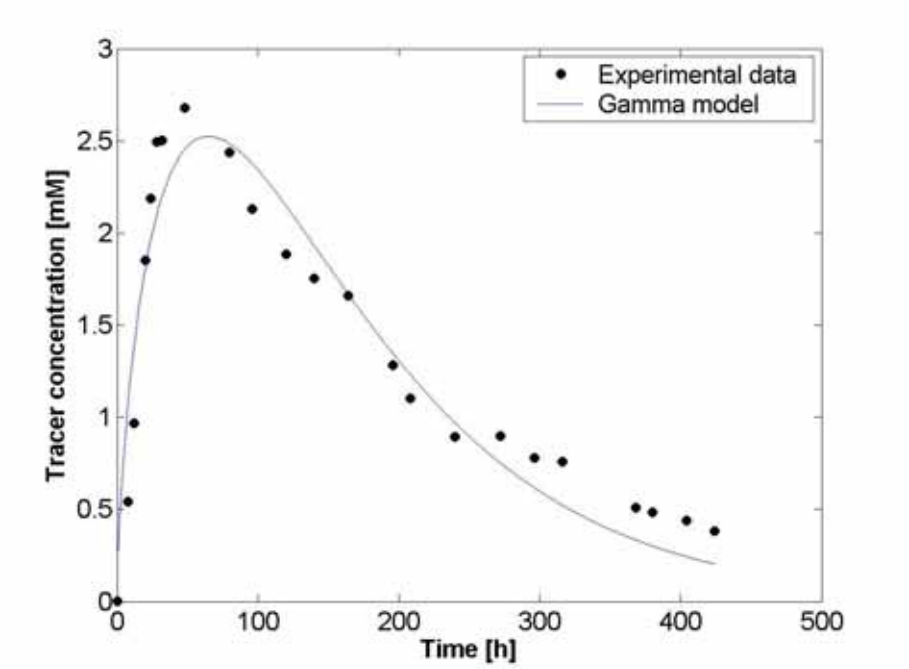


Figure 17. Second tracer experiment in pilot-scale reactor; comparison of Gamma-model best fit with experimental data

The minimum retention time determined from the tracer experiments in the model-scale digester was 4 and 8 hours. Because of an operational failure, the filling level of the horizontal tubular digester of the model plant was not properly controlled during the first tracer test. Therefore, the experiment had to be terminated prematurely and could not be further evaluated (Table 37).

Table 37. Retention time distribution parameters for the horizontal tubular digester of the model plant determined from the second tracer experiment (HRT = 8.3 days)

Feeding mode	Moments analysis	Gamma model regression
Mean calculated retention time, θ, hours	154.69	141.12
Mean calculated retention time, days	6.45	5.88
Dimensionless variance, ν	0.56	0.60

5.5 Sanitation Efficiency

Results presented here concerning the reduction of indicator bacteria are from selective cultivation of fecal coliforms (incubation time: 24 h) and intestinal enterococci according to Lebuhn et al. (2003). Random sampling of the **pilot plant** showed an average reduction of the number of fecal coliforms in raw liquid manure by between 3.7 and 4.7 log units in samples from Digester 2 and between 3.8 and 4.7 log units in samples from Digester 3. Compared to

raw liquid manure, FC densities in samples from the first (mesophilic) stage of the pilot plant were reduced by 0.5 to 1.8 log units (Figure 18).

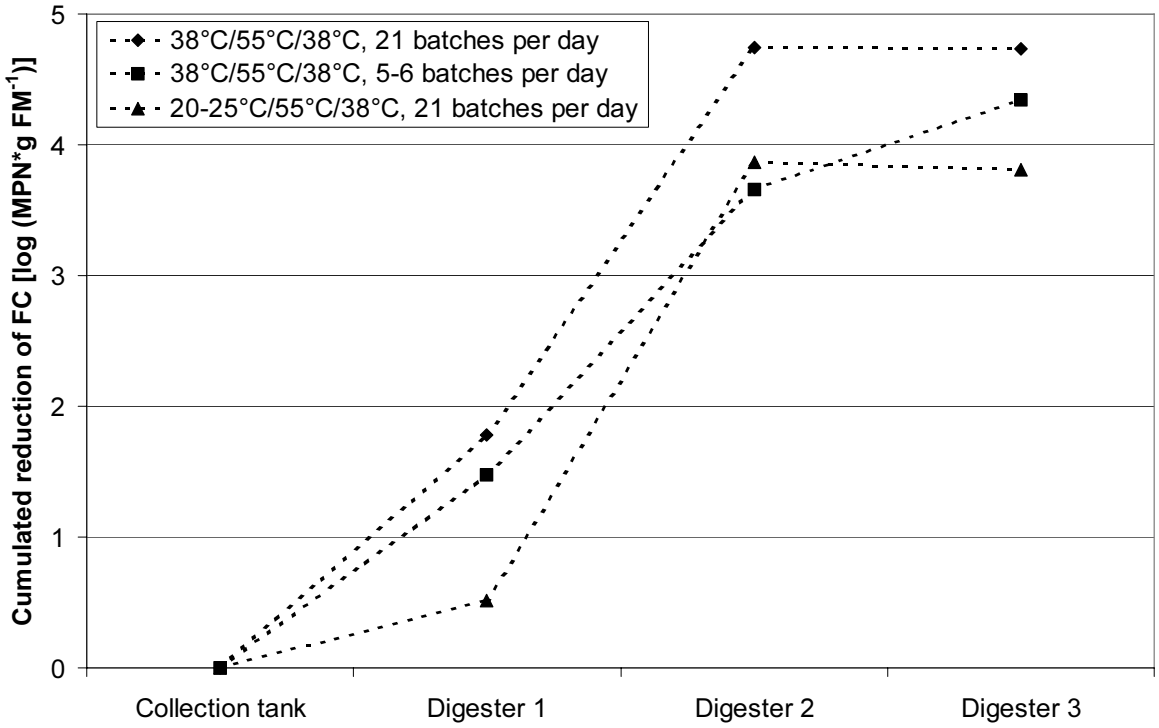


Figure 18. Average cumulated reduction of fecal coliform levels in the pilot plant for different operating modes (random sampling)

FC densities in the raw liquid manure varied considerably between approximately $7*10^3$ and 10^6 MPN*g FM^{-1}. As discussed by Lebuhn & Wilderer (2006), there might have been seasonal influences on FC levels in the raw liquid manure. During the period with less frequent feeding, fecal coliform reduction in the thermophilic stage seemed to be a little lower. For all operating modes mean fecal coliform levels in Digesters 2 and 3 were less than 10 MPN*g FM^{-1} (Figure 19).

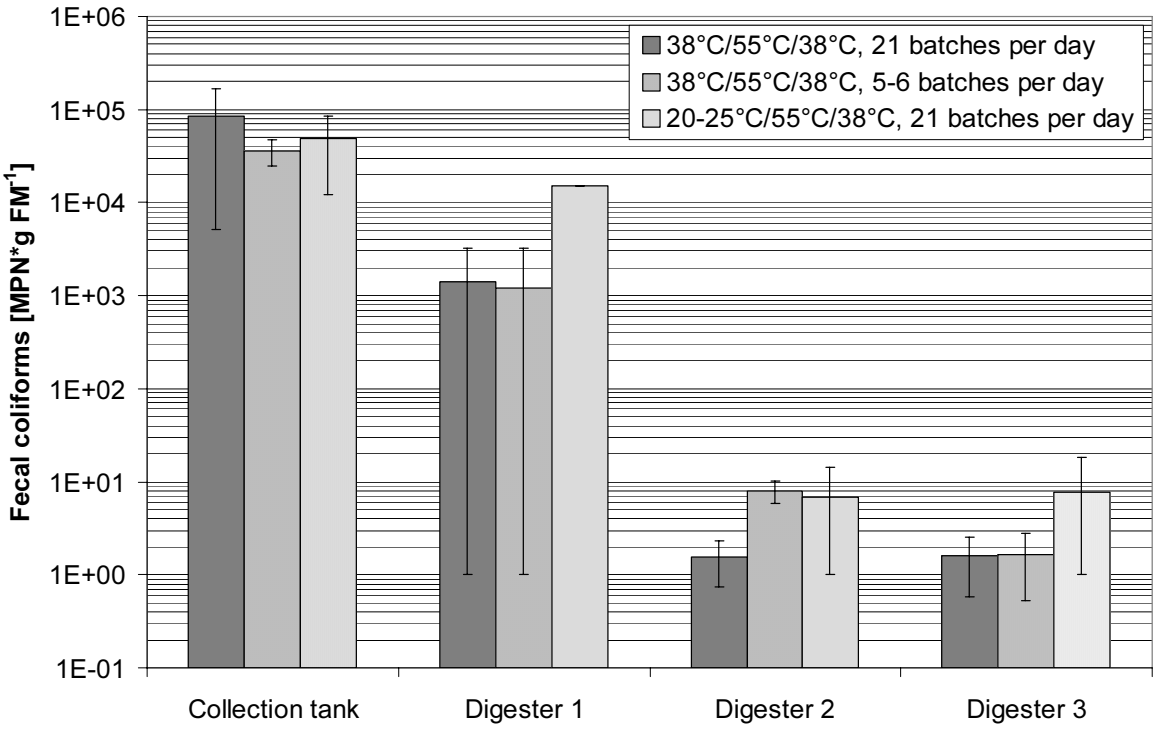


Figure 19. Levels of fecal coliforms (MPN*g FM^{-1}; mean ± standard deviation) in samples from the collection tank and the digesters of the pilot plant for different operating modes (random sampling)

According to the results from random sampling, periods during which the temperature in the thermophilic stage fell below 55°C, temporarily, due to operational problems did not significantly affect fecal coliform levels in this stage, but appeared to result in a rise in the numbers of these organisms in the terminal storage tank (Figure 20).

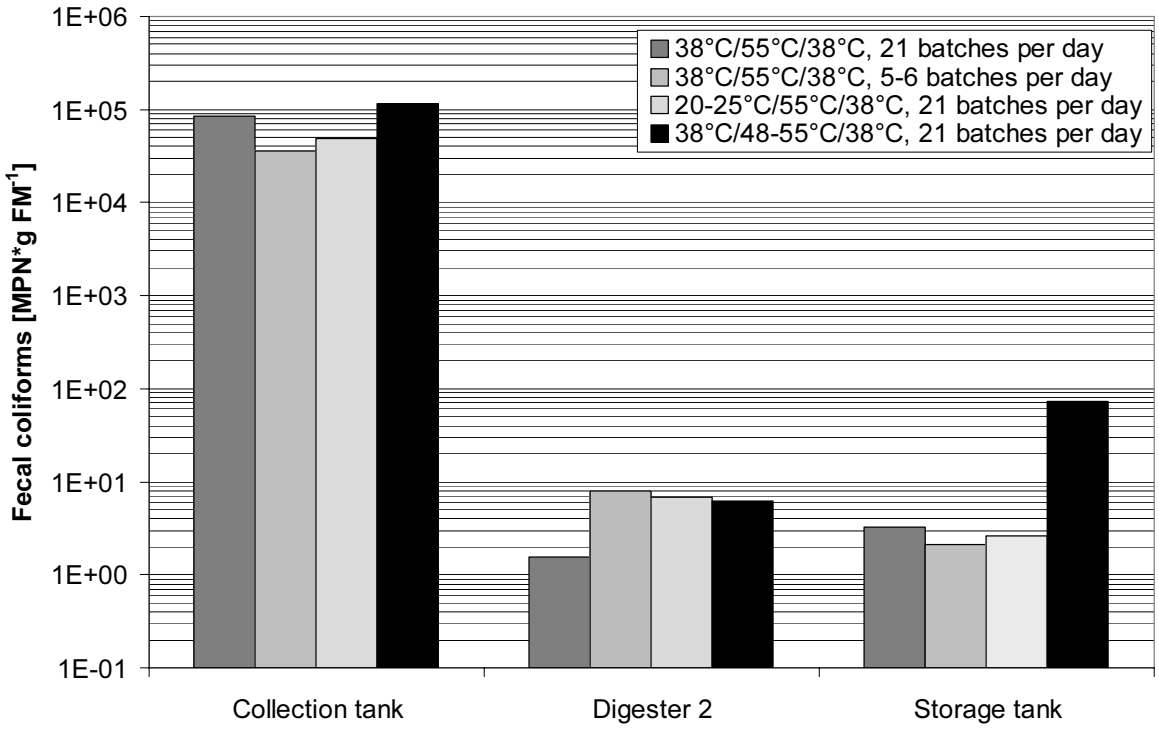


Figure 20. Mean levels of fecal coliforms (MPN*g FM^{-1}) in samples from the collection tank, Digester 2, and the terminal storage tank of the pilot plant for different operating modes (random sampling)

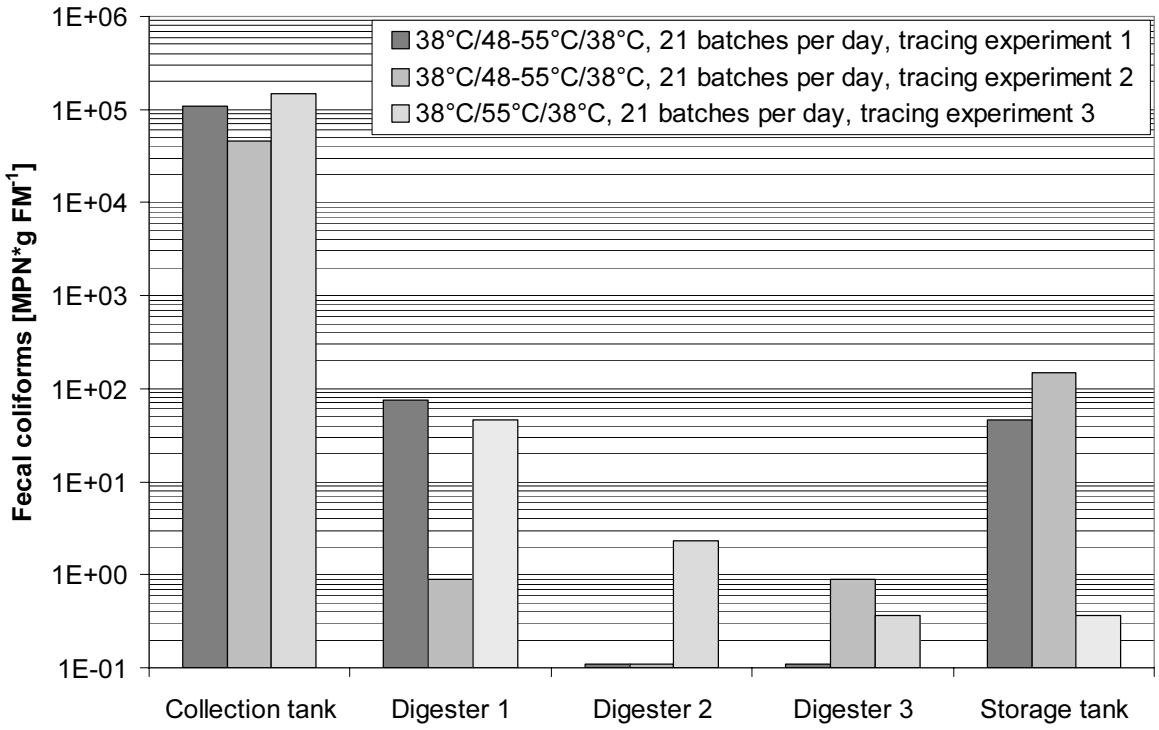


Figure 21. Mean levels of fecal coliforms (MPN*g FM^{-1}) in samples from the collection tank, the digesters, and the storage tank of the pilot plant determined from tracing experiments

The lowest fecal coliform levels in samples from Digester 2 were found in the first two tracing experiments during which failures of the combined heat-and-power unit caused interruptions of feeding and mixing operations as well as a drop in temperature in Digester 2 (Figure 21). A maximum reduction of the numbers of fecal coliforms of 5.6 log units in the storage tank compared to raw liquid manure was observed in the third tracing experiment. Data from tracing experiments during regular meso-thermo-meso operation of the pilot plant with the longer feeding interval and during thermo-meso operation are not available.

With a mean value of 4.3 log units in MD2 and 3.8 log units in MD3, the reduction of fecal coliforms determined from random sampling of the **model plant** was similar to that observed in the pilot plant (Figure 22). Mean fecal coliform levels in MD3 seemed to be higher for the longer feeding interval. However, based on the limited amount of samples (only three and two sampling events, respectively) significant differences in sanitation efficiency between the two feeding modes could not be proven.

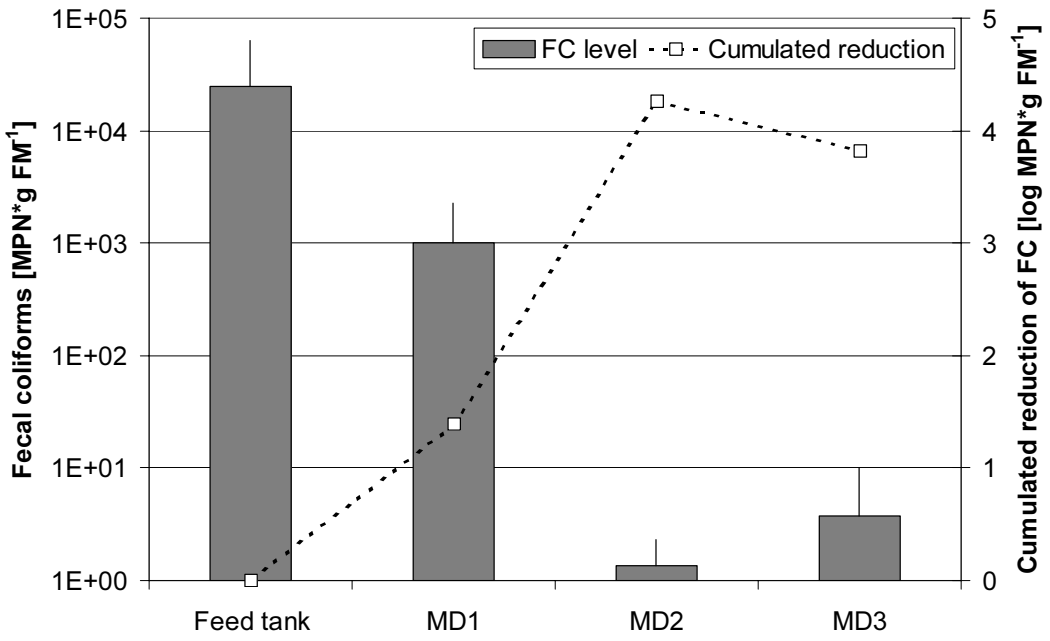


Figure 22. Reduction of mean fecal coliform levels in the model plant (random sampling)

As discussed by Lebuhn et al. (2005), qPCR-measurements targeting DNA were not suitable to determine hygienization performance with respect to indicator bacteria. While the results from quantification of fecal coliforms and *E. coli* by selective cultivation and qPCR were in good accordance for samples of untreated liquid manure, qPCR appeared to

overestimate the number of viable organisms in stressed samples from the digesters and the storage tank.

Both for thermo-meso and for meso-thermo-meso operation, when the thermophilic digester ran at 55°C, levels of intestinal enterococci in samples from this digester were reduced by 2.3 to 2.7 log units on average. During sub-optimal temperature conditions in the thermophilic stage, a reduction of 1.8 log units was observed for the tracing experiment (Figure 23).

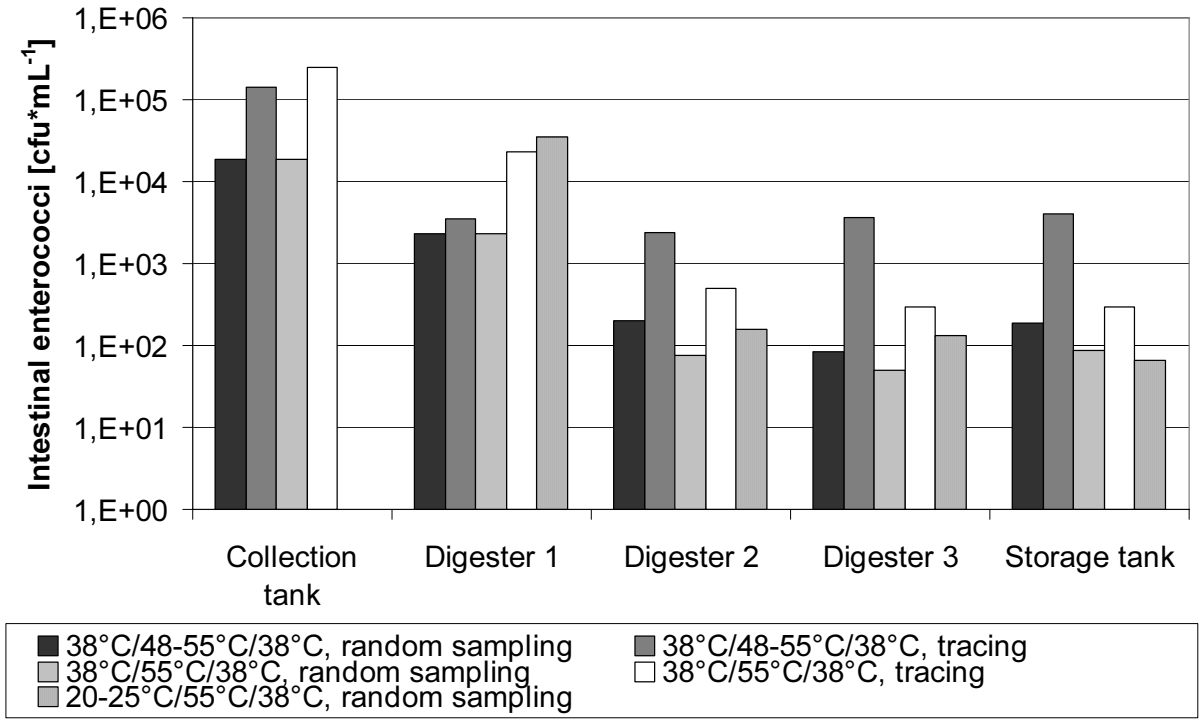


Figure 23. Mean levels of intestinal enterococci (CFU*g FM^{-1}) in samples from the collection tank, the digesters, and the storage tank of the pilot plant for different operating modes (random sampling and tracing)

A slight further reduction of intestinal enterococci levels seemed to occur in Digester 3. For sub-optimal temperature conditions in the thermophilic stage, the levels of these organisms seemed to rise again in the terminal storage tank, although to a much lesser degree than for the case of fecal coliforms. The reduction of intestinal enterococci in the model plant was not investigated.

Levels of *Cryptosporidium parvum* in samples from the different stages of the pilot plant were close to the detection limit of the applied qPCR method (Lebuhn & Wilderer, 2006). It was therefore not possible to evaluate the sanitation efficiency of the anaerobic treatment process at full-scale with respect to these organisms. During thermophilic treatment for 4 hours, the number of infectious oocysts was reduced by more than 5 log units. The same effect was seen for the treatment simulating the mesophilic-thermophilic-mesophilic reactor chain (Garcés et al., 2006).

6 DISCUSSION

The results from the pilot plant are discussed in comparison with conventional and advanced processes for sanitation of liquid cattle manure by anaerobic digestion. A separate chapter is dedicated to the comparison of the findings at pilot and model scale, respectively.

6.1 Performance of the Anaerobic Digestion Process in the Pilot Plant

In comparison to data from literature, the values of VS degradation of between 31 and 35 % that were determined for the mesophilic-thermophilic-mesophilic digester chain of the pilot biogas plant appear rather moderate, considering the relatively long hydraulic retention time of 45 days and the low system loading rate of about 1.5 kg VS*$(m^3*d)^{-1}$.

The mesophilic-thermophilic-mesophilic pilot plant including the storage tank achieved about the same VS removal as the laboratory-scale, thermophilic-mesophilic system operated by Sung & Santha (2003), however, at less than a quarter of the system loading rate. With a VS removal of 31 % during thermophilic-mesophilic operation, the digestion efficiency of the pilot plant with respect to the hydraulic retention time was improved, in comparison to the three-stage process, but still clearly lower than the respective value of about 40 % achieved in the TPAD system at a similar loading rate of about 1.9 kg VS*$(m^3*d)^{-1}$.

The observed VS removal in Digester 1 of about 20 % at a loading rate close to 7 kg VS*$(m^3*d)^{-1}$ is in good accordance to the values reported by Gosch (1984): In his laboratory experiments with mesophilic digestion of liquid cattle manure, the author observed some dependence of the digestion efficiency on the loading rate, with the mean value of VS removal decreasing from 32.2 % over 28.1 % to 22.7 % at a loading rate of 2.9, 4.8, and 7.2 kg VS*$(m^3*d)^{-1}$, respectively. For a single-stage, mesophilic full-scale plant, a VS removal of 29 % from liquid cattle manure at a loading rate of 5.6 kg VS*$(m^3*d)^{-1}$ is reported (Gosch, 1984). A VS reduction of 38.4 % was achieved in a mesophilic, continuously-stirred tank reactor that was operated at an HRT of 25 days and a loading rate of 2.79 kg VS*$(m^3*d)^{-1}$ (Singh et al., 1985).

With a VS reduction of 6 to 7 %, the thermophilic digester of the pilot plant accounted for only 17 to 20 % of the VS reduction observed during mesophilic-thermophilic-mesophilic operation. The thermophilic stage of the TPAD system (Sung & Santha, 2003) accounted for about 69 to 76 % of the total observed VS degradation. Thus, Digesters 1 and 2 of the pilot plant together achieved about 2/3 of the VS reduction in the first stage of the TPAD system (Figure 24).

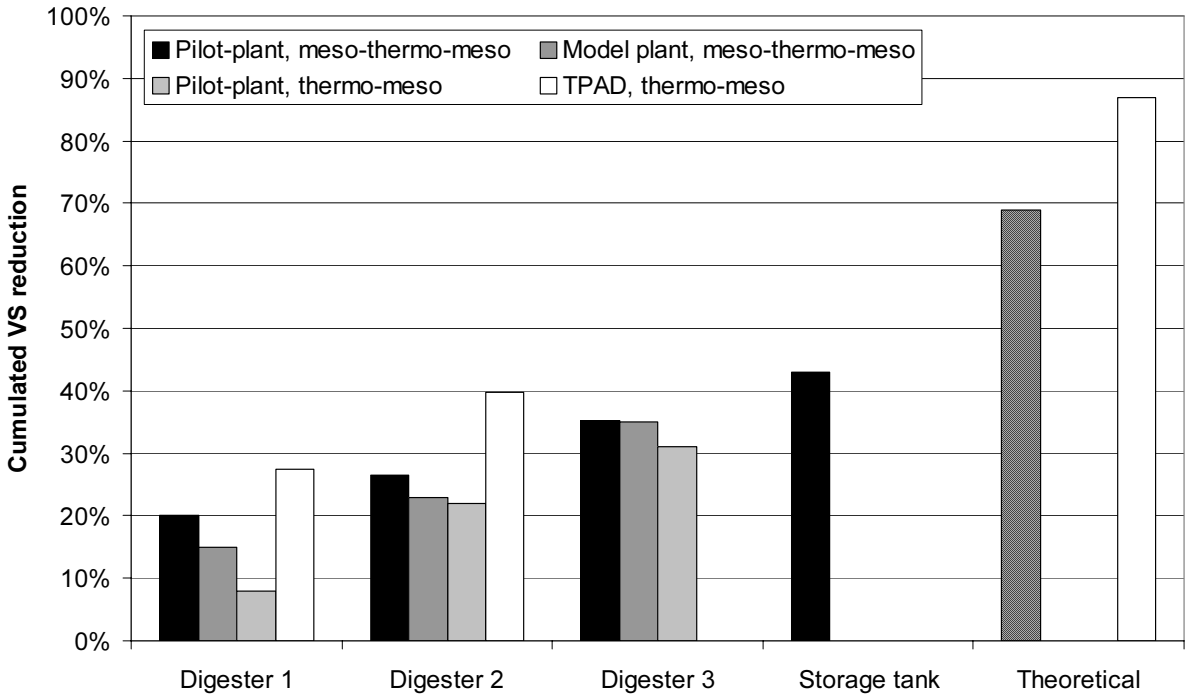


Figure 24. Cumulated VS reduction observed in the pilot and model biogas plants in comparison to the TPAD system of Sung & Santha (2003) and theoretical values

For the TPAD system, the overall value and the share of VS reduction in the thermophilic and mesophilic stage were almost constant for system loading rates of up to about 6 kg VS*$(m^3*d)^{-1}$. The latter value corresponds to a loading rate of the thermophilic digester of 14.5 kg VS*$(m^3*d)^{-1}$. For higher loading rates, a significant decrease in methane recovery was reported.

During thermophilic-mesophilic operation of the pilot plant, the share of VS reduction up to the thermophilic stage was 71 % which is comparable to that in the TPAD system. Despite the low temperatures of 20 to 25°C, there already occurred some digestion activity in the first digester as indicated by VS degradation.

The comparability of the values of VS removal presented here with those reported in literature is compromised by differences in manure composition. In comparison with literature data, the lignin content of the liquid manure from the dairy cattle on the experimental farm was quite high (Table 38).

Table 38. Comparison of data from Weender- and vanSoest-analyses of the organic dry matter of liquid manure from various sources (mean values; % DM (m/m))

Reference	Source/ fodder	Raw protein	Raw fat	Raw fiber	Cellu-lose	Hemi-cellu-lose	Lignin
This work	Dairy cattle/ TMR grass silage	15.0	4.9	16.4	11.4	12.9	23.9
Kaiser, 2005	Dairy cattle/ various	18.4	3.2	27.4	n.a.	n.a.	15.2
Mackie & Bryant, 1995	Dairy cattle/ high-grain, finishing diet	13.5	5.9	n.a.	28.3	18.3	10.3
Wohlt et al., 1990	Dairy cattle/ maize and grass silage	20.0	n.a.	n.a.	20.8	13.1	9.5
Varel et al., 1977	Bulls/ 70 % maize, 20 % oats	n.a.	n.a.	n.a.	17.0	19.0	6.8
Varel et al. 1980	Beef cattle/ 98 % corn, 2 % soybeans	n.a.	n.a.	n.a.	10.6	20.2	3.5
Hashimoto, 1983	Cattle/ 98 %maize	n.a.	n.a.	n.a.	11.9	6.7	2.6

n.a., not available

This was due to the high proportion of grass silage and hay in the fodder, and the fact that ground straw was used as litter in the stable. The lignin fraction (and lignin-protected material which could not be quantified) is not anaerobically degradable. Assuming a mean VS content in the liquid manure of approximately 78 % (m/m) of DM (Table 11), the maximum theoretical proportion of organic dry matter that is anaerobically degradable calculates to about 69 %. This value is within the range of 68 to 76 % stated by Gosch (1984) for the theoretical anaerobic degradability of the organic dry matter of liquid dairy cattle manure. A theoretical digestion efficiency of the VS contained in the liquid manure of 51 % in the digester chain and 71 % up to the storage tank of the pilot plant can be calculated if the maximum observed values of 35 and 49 % VS degradation are related to the above-mentioned theoretical degradability.

Unfortunately, Sung & Santha (2003) do not specify the VS composition of the dairy manure used for their experiments. However, they report on the composition of the high-grain finishing ration fed to the cows which consisted of 30 % (m/m) alfalfa silage, 20 % (m/m)

corn silage, 15 % (m/m) corn glut, and 15 % (m/m) ground corn grain. Assuming a lignin content of 10 % (m/m) of DM and an average VS content of 78 % (m/m), the maximum anaerobically degradable proportion of the dairy manure used in their experiments would be an estimated 87 % (m/m) of VS. With the maximum reported value of VS removal of 41.5 %, the theoretical digestion efficiency of the TPAD system would then be calculated to 48 % which is about the same as the value observed in our digester chain.

The fact that another 13 to 14 % of VS degradation, as estimated from a COD balance, occurred in the storage tank for the digest of the pilot plant indicates that there were considerable amounts of anaerobically digestible VS left in the liquid manure reaching the storage tank (Figure 24). In this respect, the frequent feeding in small quantities may have been disadvantageous. Despite the overlap of feeding and withdrawal in Digester 1, an increase of the feeding interval from one to four hours did not seem to affect overall values and individual proportions of VS reduction. Apparently, both feeding intervals are too short compared to the degradation kinetics of liquid cattle manure which mainly contains slowly degradable particulate matter.

The residuary methane yield of 2.1 m^3 per t of digest from the pilot plant compares favourably to figures of between 5.4 and 14 m^3*t^{-1} that were determined for full-scale plants digesting mainly liquid manure at HRTs below 50 days (Weiland et al., 2005). The actual amount of methane that would have been released to the atmosphere if the storage tank had not been covered and connected with the biogas collection system, cannot be derived from the figure determined in a batch-test.

The reason for the low value of VS reduction that was observed in the thermophilic stage of the pilot (and model) biogas plant during mesophilic-thermophilic-mesophilic operation is not clear. The calculated free ammonia concentrations in samples from Digester 2 of mostly between 500 and 900 $mg*L^{-1}$ are around the value of about 700 $mg*L^{-1}$ that caused beginning impairment of the thermophilic digestion of liquid cattle manure according to Angelidaki & Ahring (1993). Therefore, a slight inhibition of the anaerobic digestion process in the thermophilic stage due to ammonia is neither excluded nor proven.

Average methane concentrations measured in the biogas from the four compartments of the thermophilic digester during meso-thermo-meso operation were between 52.4 and 53.5 % (v/v) after correction for oxygen content. Sung & Santha (2003) report methane contents of 59 to 61 % (v/v) in the biogas from their thermophilic digester.

During mesophilic-thermophilic-mesophilic operation, total VFA concentrations in the thermophilic stage were at a fairly high level for a thermophilic process, in consideration of the given loading rate. At a similar loading rate of 6.7 kg VS*$(m^3*d)^{-1}$, Varel et al. (1980) observed a level of 762 mg acetic acid equivalents*L^{-1} during lab-scale digestion of beef cattle manure at 55°C. During thermophilic-mesophilic operation of the pilot plant, total VFA levels in samples from the thermophilic stage were more than 50 % lower and close to the values observed in the latter study.

In laboratory-studies of thermophilic digestion of liquid cattle manure (high-grain diet) at a loading rate of 6 kg VS*$(m^3*d)^{-1}$, Mackie & Bryant (1995) found an acetate and propionate concentration of 1.6 and 3.0 mM, respectively. Respective values in samples from D2 were similar to these values for thermo-meso operation but much higher for meso-thermo-meso operation.

The relatively high VFA levels, the low methane contents in the biogas, and the small amount of VS reduction observed in Digester 2 of the pilot plant indicate that the thermophilic process was impaired by the upstream mesophilic treatment. During the thermophilic-mesophilic treatment of biowastes, thermophilic digestion at short retention times (< 10 days) provided high concentrations of soluble organic carbon to the downstream mesophilic stage (Christ, 1999). Mesophilic bacteria can degrade soluble compounds more effectively than thermophilic ones, while with a 12 % higher hydrolysis rate, thermophilic digestion was found more efficient for degrading particulate matter. As the second stage in the mesophilic-thermophilic-mesophilic reactor chain, the thermophilic digester received less particulates and was likely inhibited by the degradation products in the effluent of the upstream mesophilic digester.

The third, mesophilic stage of the pilot plant produced an effluent with very low VFA levels. However, in samples of digest from the storage tank, mean VFA levels increased again to above 1000 mg*L^{-1}. Temperatures measured in samples of digest from the storage tank ranged from 10 to 28°C over the year.This illustrates the ongoing digestion process and the fact that the temperature sensitivity of VFA turn-over is higher than that of hydrolysis.

In contrast to the full-scale TPAD system investigated by Katers & Schultz (2003), the thermophilic stage of the pilot plant appeared very stable during thermophilic-mesophilic operation. The loading rate of the full-scale TPAD system is not reported, and the reason for the poor performance of its thermophilic digester could not be clarified. According to the observed VFA concentrations in Digester 2, the pilot plant should have been able to handle

considerably higher organic loading rates. However, due to the limited supply of liquid manure it was not possible to test this.

The methane yield from liquid cattle manure of 0.24 m^3 per kg VS fed that was determined in our experiments is the highest in comparison with values from different references, however, it was achieved at the longest hydraulic retention time and lowest loading rate (Table 39). The same limitations as stated above for VS removal apply to the comparability of the values of methane yield (and loading rate).

Table 39. Compilation of methane yields from liquid cattle manure

System, scale (reference)	**HRT** days	**Loading rate** kg VS*(m^3*d)$^{-1}$	**CH_4 yield** m^3*(kg VS_{fed})$^{-1}$
Guideline value, practice (KTBL, 2005)	n.a.	3.5	0.15
37°C, semi-technical (Lampel, 1984)	20	2.9	0.20
Mesophilic, full scale (Gosch, 1984)	n.a.	4.1	0.17
50°C, laboratory (Elmashad et al., 2001)	20	2.1	0.20
55°C, laboratory (Angelidaki & Ahring, 1993)	15	2.8	0.19
55°C / 38°C, laboratory	14	1.9	0.22
(Sung & Santha, 2003)		5.8	0.22
38°C / 52-55°C / 35-42°C, pilot (this work)	45[§]	1.4-1.5	0.24
20-25°C / 55°C / 38°C, pilot (this work)	34*,[§]	1.9*	0.24
38°C, semi-technical (this work)	14	3.6	0.14

*, neglecting the volume of Digester 1; [§], not including storage of digest; n.a., not available

The low efficiency of the mesophilic-thermophilic-mesophilic system with respect to digester volume is reflected by an average methane productivity of 0.33-0.34 m^3*(m^3*d)$^{-1}$ in comparison to a value of about 1.3 m^3*(m^3*d)$^{-1}$ for the TPAD system described by Sung & Santha (2003). Wechs (1985) states that in a two-stage digestion process the first stage should be designed according to the maximum degradation rate which is dependent on temperature, and the second stage should be dimensioned for maximum stabilization. By adding another, mesophilic stage upstream of the thermophilic stage, the resulting loading rate of the thermophilic digester of the pilot plant was limited by the lower capacity of the mesophilic digester. The loading rate of close to 7 kg VS*(m^3*d)$^{-1}$ in the first stage of the pilot plant corresponds to the maximum loading with liquid cattle manure that could be handled in lab-scale digesters at 35°C (Hashimoto, 1982; Varel et al., 1980). Nevertheless, VFA levels in samples from this stage did not indicate process imbalances during quasi-steady-state operation.

Although identical average values of methane recovery were calculated in the pilot plant for all three operating modes, there were differences between average biogas production rate and methane concentration in the biogas. For mesophilic-thermophilic-mesophilic operation

this could be due to differences in the handling of the digest. While considerable amounts of digest were repeatedly withdrawn from the storage tank for land spreading during the evaluated time period in summer, this was not the case in autumn when the plant was operated with a longer feeding interval. During thermophilic-mesophilic operation, the average methane content in the biogas was significantly lower which reflects the higher share of biogas production from the thermophilic stage.

6.2 Sanitation Performance

Mean FC levels during random sampling of the thermophilic and the subsequent mesophilic stages of both the pilot and model biogas plants were below 10 MPN*g^{-1} in all cases. This is far below the USEPA standard of 1000 MPN*g^{-1} for Class A biosolids. Background levels of FC found in seepage water from agricultural land without fertilization were between 10 and 100 MPN*g^{-1} (Weiß & Popp, 2004). In samples of digestate from the storage tank, FC levels remained at these low levels, provided that a temperature of close to 55°C was reached in the thermophilic stage.

During the experiments at the pilot plant, intestinal enterococci proved to be a very good indicator of sanitation performance with respect to non-spore forming bacteria, viruses, and parasites (Lebuhn & Wilderer, 2006). During random sampling of digestate from the storage tank, the guideline value of 10^2 CFU*mL^{-1} as specified by Larsen et al. (1994), was met only for optimal temperature conditions in the thermophilic stage. This level represents a fraction of intestinal enterococci with higher temperature resistance (Lebuhn & Wilderer, 2006).

According to theory, the sanitation performance of the pilot plant should have been significantly improved due to the longer feeding interval. Assuming first order kinetics for microbial decay, the following relationship between feeding interval and pathogen removal in an ideal stirred tank can be derived (Carrington, 2001):

$$\frac{C}{C_O} = \frac{R}{(10^{DT} - 1 + R)} \tag{6.1}$$

where C and C_0 is the concentration of indicator organisms in the reactor inflow and outflow; R is the fraction of the reactor content replaced during each feeding; D is the decimal decay rate of the respective indicator organism; and T is the time interval between feeds.

The pilot plant may be treated as a sequence of three stirred tanks, using the specifications as described in section 4.1. T_{90} values for fecal coliforms of 1.3 days and

0.4 hours were assumed for mesophilic and thermophilic stages, respectively (see Table 3 and 4). For a feeding interval of 1 hour the model predictions were compared with experimental data from the pilot plant (Figure 25).

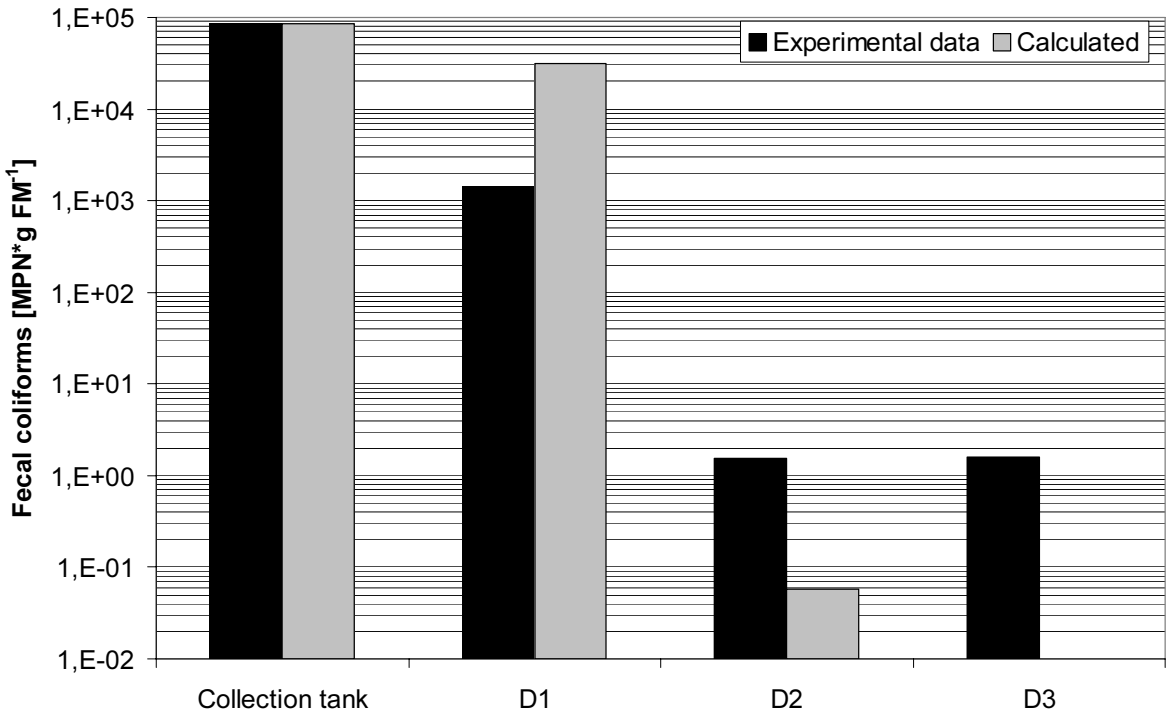


Figure 25. Comparison of mean observed and calculated levels of FC in the pilot plant during meso-thermo-meso operation and a feeding interval of 1 hour

Obviously, this very simplified model does not fit the experimental data in a quantitative manner. It calculates a much higher overall reduction of FC than observed in the pilot plant, however, the resulting absolute FC levels are below detectable values.

Most of the sanitizing effect is predicted to occur in the thermophilic stage, while the calculated FC removal in the first, mesophilic stage is only about a quarter of the experimental effect. The good experimental sanitation performance of D1 was probably due to chemical factors. It also indicates that short-circuiting did not occur in this reactor. Concerning FC removal in the thermophilic stage, the model predicts a reduction of 5.7 log units as opposed to 3.0 log units from experimental data. The model assumes that all the material entering is instantly mixed into the whole reactor volume and heated to the respective temperature. Apparently, this is not a realistic assumption for the horizontal tubular digester of the pilot plant as discussed in Chapter 6.3.

For a feeding interval of 4 hours, the model would predict an even higher FC removal, while with a value of 1.0 and 1.5, respectively, the predicted and observed FC reduction in the

first, mesophilic stage would be in better accordance than for the one-hour feeding interval. In contrast to theory, the experimental data did not show significant differences in sanitation performance between the two feeding modes.

Gray et al. (2006) developed a model for predicting FC densitities in anaerobically treated biosolids. From regression analysis of experimental data they derived the following equation:

$$Log(FC_f) = 4.19 - 0.0516TEMP - 0.0924TMCRT - 0.906STAGED + 0.211Log(FC_i) \quad (6.2)$$

Where FC_f = digested sludge (biosolids) FC density (MPN*g^{-1} TS); *TEMP* = highest average digester temperature (°C) in the digester system; *TMCRT* = digester sludge thermophilic residence time (days); *STAGED* = 0 for a single-stage process, 1 for a two-stage process; and FC_i = FC density in digester feed sludge (MPN*g^{-1} TS). The model is based on data from experiments with single-stage and two-stage digestion of wastewater sludge with the first stage operated at a 2-day HRT and the second stage operated at a 13- to 15-day HRT.

According to this model, with respect to FC removal, a thermophilic-mesophilic process with a 3-day thermophilic (50°C) residence time is equivalent to a single-stage thermophilic process (55°C) with a 12-day HRT. Using the mean FC density of approximately 80,000 MPN*g^{-1} FM corresponding to 1.03*10^6 MPN*g^{-1} DM in the raw liquid manure and the specifications of the pilot plant (*TEMP* = 55°C; *TMCRT* = 8.4 days; *STAGED* = 1), the model calculates a FC density in the effluent of Digester 2 of approximately 9 MPN*g^{-1} DM. Although the model is used beyond its limits, this prediction is in very good accordance with the mean value of 4.3 MPN*g^{-1} FM determined during random sampling of the pilot plant. If the thermophilic-mesophilic operation of the pilot plant is treated as a single-stage thermophilic process, the model predicts a FC density of 66 MPN*g^{-1} DM, as opposed to an experimental value of approximately 7 MPN*g^{-1} DM. In accordance with the experimental data, the deviation may indicate that FC reduction due to chemical factors in the upstream digester operated at 20-25°C was not negligible.

Overall, there seemed to be some small additional sanitizing effect of the third, mesophilic stage on bacterial indicators. Using the simple approach of sampling the individual stages of the pilot plant subsequently, according to their respective hydraulic retention times, it was possible to evaluate the effects of operational irregularities on the sanitation efficiency of the treatment process. Sub-optimal temperature control in the thermophilic stage had a

significant effect on the sanitation efficiency of the treatment process. Interestingly, temperatures below 55°C in the thermophilic stage did not so much affect the level of FC in samples from this and the subsequent mesophilic digesters, but resulted in an increasing level of indicator organisms in the storage tank. The same trend was observed for intestinal enterococci, although the maximum reduction of these temperature-resistant indicators in the thermophilic stage was more sensitive to temperature. This was likely due to the formation of sub-lethally stressed active-but-not-cultivable (A/VBNC) cells (Lebuhn et al., 2005). These cells are not detected by conventional cultivation within an incubation time of 24 hours. The hygienic status of a covered storage tank for digested manure, particularly coliforms (Lebuhn & Wilderer, 2006), may therefore be a good indicator of operational irregularities during anaerobic treatment.

6.3 Hydraulic Efficiency of Horizontal Tubular Digester

According to the results of the tracer experiments, the minimum retention time in the horizontal tubular digester was longer than for a completely mixed tank. However, with a value of 8 hours for the MRT during both feeding modes, the design of the baffled reactor didn't seem to be too effective. For operation with the longer feeding interval, this means that it took only two feeding cycles for the tracer to reach the outlet. Short-circuiting along the bottom of the tank did not occur.

Apparently, there was an analytical problem in the first tracer experiment resulting in a very low level of tracer recovery. The method detection limit for the lithium tracer of 0.022 mmol*L^{-1} corresponded to a normalized concentration of 0.043 or about c_0/23. Beside possible analytical inaccuracies, the relatively high detection limit is due to the complex matrix. This limits the accuracy of the RTD analysis, particularly of the methods-of-moments approach which is very much influenced by the tail of the concentration-time-curve. Also, due to the large difference in tracer recovery, the comparability of the results from the two tracer experiments is compromised.

For both tracer tests, the mean retention times calculated from moments and regression analyses are significantly below the calculated mean hydraulic retention times in the horizontal tubular digesters. This is an indication of considerable dead space in the reactor. The differences between the estimated RTD parameters from moments and regression analyses were larger for the first tracer experiment. For characterizing RTD parameters of continuous flow systems the method of non-linear regression is superior to the method of moments (Haas et al., 1997). Estimates of RTD parameters from non-linear regression are less

sensitive to measurement errors and data truncation. This is illustrated by the changes in RTD parameter estimates if the tail of the concentration-time curve could have been evaluated to the last sample, irrespective of the method quantification limit. While the value of θ estimated from moments analysis decreases by about 20 % because of the truncated tail, the respective estimate from regression analysis changes only by about 1 % (Table 40).

Table 40. Change in estimates of θ for the first tracer experiment in the pilot plant due to data truncation

Moments analysis truncated	Moments analysis full tail	Gamma model truncated	Gamma model full tail
123.03 h	154.07 h	132.85 h	134.44 h
5.13 d	6.42 d	5.54 d	5.60

The share of dead space can thus be best estimated from 1-(θ/HRT) using the value for θ determined from regression analysis. For the second tracer experiment, this comes to a value of 24 % which is remarkably bad. For the first tracer experiment, the proportion of dead space would even be estimated to 35 %. With the reservation that the latter value is inaccurate due to the above-mentioned insufficient tracer recovery, one would expect that mixing of the digester content will be less effective during a shorter interval between feeding and withdrawal. Due to the fact that the paddles of the agitator did only reach the outer section of the cylindrical tank, it is possible that part of the space between the axle and the paddles was insufficiently mixed. During feeding and withdrawal, the flow velocity in the tank will be higher at the baffles, causing preferential flow in the outer section of the tank.

The concentration-time-curves determined from the tracer experiments with an initial quick rise and an overall exponential decline appear typical for non-ideal mixed flow (Figure 14). The fluctuations during the decline may be due to measurement errors, but may also be caused by the baffles: Tracer that initially reached zones of bad mixing, particularly in the first compartment, may have been backmixed at a later point of time.

For qualitative purposes, the Gamma model fits the experimental data from both tracer experiments quite well, overall. During the initial rise of the tracer concentration, experimental and model data are in good accordance. However, the model calculates a lower peak concentration at a later point of time compared to the experimental results. During the concentration decline, the actual data remain below the model curve until about 190 and 250 hours after tracer injection for the first and second tracer experiment, respectively. For longer retention times, the actual concentration-time-curves run above the model curve. This may also be taken as an indicator of backmixing.

Based on the RTD analysis performed within this work, the horizontal tubular reactor can be characterized as a non-ideal mixed flow system that is closer to a series of two stirred tanks than to a single tank, and contains a substantial proportion of insufficiently mixed space. The hydraulic efficiency of the reactor seemed to be improved by increasing the feeding interval. This indicates that the paddles produced incomplete radial mixing and that the hydraulic mixing effect due to increased flow velocities at the baffles during feeding/withdrawal was rather negligible. Because of the low rotational speed and the discontinuous operation of the paddle mixer, a steady-state flow condition is not established in this reactor.

According to the results from monitoring FC levels in the pilot plant, the improved hydraulic efficiency of the thermophilic stage due to the longer feeding interval did not likewise affect sanitation performance. In contrast to theory, mean FC removal even seemed to be a little lower for less frequent feeding/withdrawal. The reasons for this could not be elucidated. Also, the differences in hydraulics between the two feeding modes did not have a significant effect on VS removal in the thermophilic stage.

6.4 Energy Efficiency of the Pilot Biogas Plant

Typically, biogas plants in agriculture consume considerably less than 10 % of their electricity output. At most biogas plants in Germany, all of the electrical energy output is sold, and the electricity required for running the plant is supplied from the grid. This is due to the legally guaranteed prices for electricity from biogas and other renewable energy sources.

The high proportion of own electricity consumption of the pilot biogas plant resulted mainly from the relatively low energy yield from liquid manure and the high effort for mixing the three digesters. The agitator in Digester 1 was operated for about half of the time to avoid overheating of the digester content around the heating coils. Foaming occurred in this digester during failures of the agitator.

Theoretically, all of the heating energy for the digesters could have been supplied from running the CHPU on biogas. In practice, the heating energy from biogas utilization was not sufficient to maintain the desired digester temperatures during the cold season. The reasons for this were insufficient insulation and efficiency of the heat exchangers, particularly of Digester 2, and the fact that the CHPU could only be run on biogas at full load for overall 64 % of the time. Additional heating energy had to be supplied by running the CHPU on fuel oil which is costly and increases greenhouse gas emissions.

Possible measures to improve the energy efficiency of the treatment process are: Improving digester insulation and heat exchanger capacity; recovering the heat from the outflow and using it for pre-heating the inflow of the thermophilic stage; minimizing the effort for agitation of the digesters; employing a high-efficiency burner instead of or in addition to an engine to utilize the biogas.

6.5 Comparison between Pilot and Model Scales

At model-scale, the overall VS degradation and the share of VS degradation in the individual digesters appeared to be somewhat influenced by the feeding mode. The overall lower VS reduction observed in the model plant during more frequent feeding may be attributed to short-circuit flow in MD3 which was equipped with an overflow pipe (Table 26). The higher share of VS reduction in the thermophilic stage of the model plant is in accordance with its higher proportion of the total HRT in the digester chain compared to the pilot plant.

VFA levels in the mesophilic and thermophilic digesters of the model plant were a little bit higher and lower, respectively, compared to the pilot plant which is in agreement with the respective loading rates. The overheating of MD1 during operation of the model plant with feeding in 6 batches per day caused a severe process disturbance in this digester. This was indicated by a fivefold total VFA concentration, increased levels of propionic, butyric, and iso-valeric acids as well as by a reduced biogas production rate and quality. However, after readjusting the temperature, the process recovered without a reduction of the feeding rate.

Unfortunately, a direct comparison between the methane yields from liquid dairy cattle manure in the pilot and model biogas plants was not possible. Concerning VS destruction, the model plant achieved the same performance as the pilot plant, despite the significantly shorter hydraulic retention time of 37 days compared to 45 days. The degradability of the particulate matter might have been increased by macerating the liquid manure for the model plant. Because it was not cooled, some VS degradation already occurred in the feed tank of the model plant. However, this was already accounted for in the mass balance calculations. Chemical analyses of samples taken during the warm season between the points of time when the feed tank was refilled showed that this was less than 1 % of VS per day on the average.

Compared to the thermophilic stage of the pilot plant, the temperature control in Model Digester 2 was much more accurate, and the temperature along the reactor was fairly uniform. At the same time, the mean level of FC in samples from the feed tank was about 1/5 of that in samples from the collection tank of the pilot plant. Nevertheless, FC levels in treated manure from the model plant were similar to those from the pilot plant. Therefore, the time-

temperature-regime in the pilot- and model-scale thermophilic digesters appeared equally efficient with respect to FC removal.

The comparison between the estimated RTD parameters for the model- and pilot-scale tubular digester during less frequent feeding indicates a lower hydraulic efficiency of the thermophilic stage at model scale (Table 41).

Table 41. Comparison of RTD parameters in model- and pilot-scale tubular digesters during less frequent feeding estimated from Gamma-model best fit

	Model-scale	Pilot-scale
Hydraulic retention time, HRT, days	8.3	8.7
Mean calculated retention time, θ, hours	141.12	159.14
Mean calculated retention time, days	5.88	6.63
Dimensionless variance, ν	0.60	0.59

The estimated proportion of dead space is 29 % for the model-scale digester compared to 24 % for the pilot-scale digester. Reasons for this, beside the influence of scale, may be that in the model-scale digester the agitator was only run half of the time, and that feeding and withdrawal occurred simultaneously.

6.6 Scenario for a Real-Case Application

An economical projection for a central biogas plant treating the liquid manure from 550 livestock units of cattle was made. Based on the findings of this work, a thermophilic-mesophilic process consisting of two stirred-tank reactors in series was assumed. A CHPU with 80 kW electrical power output would be required to utilize the yearly biogas production of 267,300 m^3, assuming an average methane content of 56.5 % (v/v). Details of the economical projection for this plant can be found in LfL (2006). The calculated treatment cost amount to 74 EUR per livestock unit or 3.69 EUR per m^3 of liquid manure, including the cost for transport of liquid manure to the biogas plant and land spreading of the digest. If these costs were to be transferred to a drinking water supply of 5.2 million m^3 per year, the water price would have to be raised by 0.78 EUR-cent per m^3 or 0.85 %.

7 CONCLUSIONS AND OUTLOOK

A mesophilic-thermophilic-mesophilic anaerobic treatment process for liquid dairy cattle manure was operated at pilot-scale on an experimental farm for a period of about two years. Parallel experiments were run in a bench-scale plant over about half a year, using the liquid manure from the experimental farm subjected to some additional physical treatment. The evaluation of the anaerobic digestion process was accompanied by hygienic investigations.

The methane yield of 0.24 m^3*(kg VS_{fed})$^{-1}$ that was achieved in the pilot biogas plant from liquid dairy cattle manure with a large proportion of lignin was the highest in comparison with literature data. The system loading rate and methane productivity of the pilot plant during mesophilic-thermophilic-mesophilic operation were limited by the first stage. Also, there were indications that the thermophilic digestion process was impaired by the upstream mesophilic stage. Based on these findings, a two-stage, thermophilic-mesophilic treatment process with a ratio of thermophilic to mesophilic HRT of about 1:3 is considered more efficient.

The mesophilic stage downstream of the thermophilic stage provided an effluent with low VFA concentrations. In a staged process, decreasing the temperature toward the storage tank improves the quality of the digested liquid manure with respect to VFA levels. Particularly in the case of the treatment of liquid manure from dairy cattle fed a high-fiber diet, the terminal storage tank should be covered to capture methane that is produced from residual digestion activity.

The reduction in numbers of bacterial indicators that was observed in the first, mesophilic stage was considerable but not sufficient for sanitation purposes. The sanitation performance of the pilot plant was clearly determined by the thermophilic stage. The pilot plant was able to reduce fecal coliform levels in the liquid dairy cattle manure to mean values below 10 MPN*g FM^{-1} for all operating modes tested. In experiments at model scale, improved inactivation of *Cryptosporidium* oocysts in the thermophilic stage due to the upstream mesophilic treatment could not be proven. Thus, there is no need for the first, mesophilic stage from the point of view of sanitation efficiency.

Temporary fluctuations of temperature in the thermophilic stage between 48, at lowest, and 55°C did not seriously affect the reduction of bacterial indicators. However, maintaining a temperature of 55°C appears critical to achieve efficient sanitation during thermophilic anaerobic treatment and at the same time avoid a rise in levels of indicator organisms during storage of the digested liquid manure. The levels of bacterial indicator organisms in the

terminal storage tank should be monitored, and the storage tank should be covered to avoid extrinsic microbial contamination.

The horizontal, tubular digester increased the minimum guaranteed retention time in comparison to a continuously-stirred tank reactor by a factor of 8 and 2 for a feeding interval of 1 and 4 hours, respectively. Given the expensive design of this reactor, these values reflect a poor hydraulic efficiency. Additionally, the reactor had a high demand for heating energy. Since more frequent feeding of the digesters with liquid dairy cattle manure did not result in better digestion performance, the costly horizontal tubular digester might as well be replaced by a vertical stirred tank, operated in draw-fill-mode. To ensure efficient sanitation, the feeding interval should be 8 hours.

Based on these findings, the following statements can be made with respect to the research hypotheses:

1. Not confirmed: The thermophilic process appeared to be impaired by the upstream mesophilic stage. The first, mesophilic stage limited the system loading rate and methane productivity. A two-stage, thermophilic-mesophilic treatment process was tested and appeared to be more efficient.
2. Not confirmed: Reduction of bacterial indicators and elimination of *Cryptosporidium parvum* was clearly determined by the thermophilic stage.
3. Partially confirmed: Maintaining a treatment temperature of 55°C was critical to avoid a rise in levels of indicator organisms during storage of the digested liquid manure.
4. Confirmed: A minimum guaranteed retention time in the horizontal tubular reactor of 8 hours was determined in a tracer test both for a feeding interval of 1 hour and 4 hours.
5. Not confirmed: The given tubular reactor exhibited a low hydraulic and energetic efficiency. For treating liquid manure, a continuously-stirred tank reactor operated in draw-fill-mode with a feeding interval of 8 hours is a more economical alternative.

For the operation of a biogas plant for the sanitizing treatment of liquid manure, maintaining the required treatment temperature has priority over electrical power generation. To improve energy efficiency of the treatment process, the influent should be pre-heated using heat that is recovered from the effluent of the thermophilic stage. Liquid manure that

has not been sufficiently sanitized due to operational irregularities must not be discharged into the terminal storage tank. Any recontamination of digested liquid manure with material from upstream treatment stages must be excluded.

Despite the efforts for transport of liquid manure and digest, a central treatment plant would enable a local water supplier to manage all the animal waste streams produced within a water protection area. Moreover, anaerobic digestion of animal waste cannot only contribute to prevent drinking water contamination in sensitive areas, but also to reduce pathogen input into the environment in general. Still, if animal waste that has undergone a sanitizing treatment was to be applied to agricultural land in sensitive areas, the hydrogeological site conditions would have to be such that short-circuiting to the aquifer could be ruled out.

Further studies would be required to derive key design parameters for the proposed thermophilic-mesophilic anaerobic treatment process. More than double the loading rate in comparison to the value of about 6 kg VS*(m^3*d)$^{-1}$ tested in this work may be possible in the thermophilic stage. It remains to be investigated whether the hydraulic retention time in the digesters could be significantly reduced without losing too much methane yield. These parameters could be determined at a semi-technical scale that allows the use of the original liquid manure with as little pre-treatment as possible.

A temperature-staged process could also increase the efficiency of biogas production from energy crops. Co-digestion of animal waste and energy crops / renewable raw materials (RRM) could improve the economics of the sanitizing treatment by generating more electrical power. In this case, the different characteristics of liquid manure and RRM have to be taken into account. A revised horizontal digester as thermophilic stage would then be more efficient due to the higher DM contents of RRM. With a CSTR, the longer feeding interval required for efficient sanitation is disadvantageous with respect to the degradation kinetics of whole crop silages of energy crops (such as maize and different grains) with considerable shares of readily degradable starch. Thermophilic anaerobic digestion also has an inactivating effect on specific plant pathogens that may be contained in RRM.

To go one step further, it would be desirable to assess sanitation by anaerobic digestion as an option for the management of liquid manure during a real-case study in the respective water protection area. To minimize the input of pathogenic and indicator microorganisms into the soil, the sanitizing treatment of all the liquid manure that is to be spread in sensitive areas should be combined with other measures, such as constraining the amount of digested manure that can be applied to a specific area and using suitable technology for land application.

8 ABSTRACT

A three-stage, mesophilic-thermophilic-mesophilic anaerobic digestion process for the sanitizing treatment of liquid dairy cattle manure was tested at pilot and semi-technical scale. The aim of the treatment was to provide efficient sanitation of bacterial indicators and protozoan parasites and, at the same time, achieve a high methane yield from liquid manure.

A pilot biogas plant for the treatment of liquid manure from about 100 livestock units of dairy cattle (about 2000 m^3 per year) was erected on a farm in Southern Bavaria. The treatment process consisted of a stirred-tank digester (usable volume: 50 m^3) supposed to activate (oo)cysts of protozoan parasites at mesophilic temperature conditions (around 38°C), followed by a horizontal tubular digester (usable volume: 46 m^3) for hygienization at thermophilic conditions (55°C), and a stirred-tank digester (usable volume: 150 m^3) for biological stabilization of the substrate. The biogas captured from the three digesters and the covered terminal storage tank (800 m^3) was utilized with a pilot-injection engine to generate electricity and produce heating energy for the digesters.

More than half of the VS degradation in the digester chain occurred in the first, mesophilic stage, and the digestion process was stable despite the high loading rate of about 7 kg VS*$(m^3*d)^{-1}$, given mesophilic temperature conditions. As indicated by relatively high VFA levels, low methane contents in the biogas, and a small amount of VS reduction, the second, thermophilic stage appeared to be impaired by the upstream mesophilic process. The third, mesophilic stage of the pilot plant produced an effluent with very low VFA levels, which were increased again in the terminal storage tank due to some ongoing digestion activity.

A methane yield of 0.24 m^3*$(kg\ VS_{fed})^{-1}$ from liquid dairy cattle manure (mean dry matter content: 7.8 %) was achieved during mesophilic-thermophilic-mesophilic operation of the pilot biogas plant, at a hydraulic retention time of 45 days in the digester chain. Changing the feeding interval from one to four hours did not affect the methane yield. Given the high lignin content of the liquid manure from dairy cattle fed a high-fiber diet, the value for methane yield compares very favorably with data from literature. Lowering the temperature in the first stage to between 20 and 25°C resulted in a clearly improved performance of the thermophilic digestion process, while the methane yield remained the same as for mesophilic-thermophilic-mesophilic operation.

During treatment in the pilot plant, the level of fecal coliforms in liquid dairy cattle manure was reduced to mean values below 10 MPN*g FM^{-1} for all operating modes tested,

provided that a temperature of 55°C was maintained in the thermophilic stage. Temperature drops did not so much affect the level of FC in samples from this and the subsequent mesophilic digester, but resulted in an increased level of these indicator organisms in the storage tank. Intestinal enterococci exhibited the same trend, although the maximum reduction of these temperature-resistant indicators in the thermophilic stage was more sensitive to temperature. The inactivation of *Cryptosporidium parvum* oocysts was investigated at semi-technical scale in a biogas plant modeled after the pilot plant at a geometrical scale of approximately 1:6. Treatment at 55°C for 4 hours reduced the number of infectious *Cryptosporidium* oocysts by more than 5 log units. In contrast to previous findings, oocyst inactivation was not improved due to the mesophilic pre-treatment.

A minimum guaranteed retention time in the horizontal tubular digester of 8 hours was determined from tracer tests, for both a feeding interval of 1 and 4 hours. The reactor was insufficiently mixed and exhibited a high demand for heating energy.

A two-stage, thermophilic-mesophilic treatment process using conventional stirred-tank digesters is proposed for the efficient digestion of liquid dairy cattle manure. The ratio of thermophilic to mesophilic HRT should be about 1:3. To insure sufficient sanitation with respect to non spore-forming bacterial indicators and cryptosporidia, the digesters have to be operated in draw-fill-mode with a feeding interval of 8 hours. Insuring the required treatment temperature in a biogas plant for the sanitizing treatment of liquid manure requires efficient heating which has priority over electrical power generation. The influent to the thermophilic stage should be pre-heated using heat that is recovered from the effluent. Liquid manure that has not been sufficiently sanitized due to operational irregularities must not be discharged into the terminal storage tank. A biogas plant for sanitational purposes has to be designed in such a way that recontamination of digested liquid manure with material from upstream treatment stages is excluded. Regardless of treatment temperature, anaerobic digestion of animal waste always reduces the input of pathogenic and indicator organisms into the environment to some extent.

9 ZUSAMMENFASSUNG

Als Verfahren für die weitgehende Hygienisierung von Milchviehgülle wurde ein dreistufiger, mesophil-thermophil-mesophiler anaerober Prozess im voll- und halbtechnischen Maßstab untersucht. Mit dem gewählten Behandlungsverfahren sollte eine wirkungsvolle Verringerung der Keimzahl bakterieller Indikatoren und protozoischer Parasiten sichergestellt und zugleich ein hoher Methanertrag aus Milchviehgülle erzielt werden.

Auf einem Milchviehbetrieb im Oberbayerischen Alpenvorland wurde eine Pilot-Biogasanlage für die Behandlung der Gülle von etwa 100 Großvieheinheiten Rinder (ca. 2000 m^3 pro Jahr) errichtet. Die Behandlungsanlage bestand aus einem Rührkesselreaktor (Nutzvolumen: 50 m^3) mit dem Ziel der Aktivierung der Dauerstadien von Parasiten auf mesophilem Temperaturniveau (um 38°C), in Reihe mit einem liegenden Fermenter (Nutzvolumen: 46 m^3) für die weitgehende Hygienisierung bei thermophiler Temperatur (55°C), in Reihe mit einem weiteren Rührkesselfermenter (Nutzvolumen: 150 m^3) zur Ausgärung des Substrates. Das Biogas aus den drei Fermentern und dem gasdicht abgedeckten Endlager (800 m^3) wurde in einem BHKW mit Zündstrahlmotor zur Erzeugung von elektrischem Strom und zur Bereitstellung von Heizwärme für die Fermenter genutzt.

Mehr als die Hälfte des oTM-Abbaus in der Fermenterkaskade fand in der ersten, mesophilen Stufe statt. Trotz einer für mesophile Bedingungen hohen Raumbelastung von ca. 7 kg oTM*$(m^3*d)^{-1}$ war der Abbauprozess in diesem Fermenter stabil. Vergleichsweise hohe Konzentrationen an flüchtigen Fettsäuren, niedrige Methangehalte im Biogas sowie ein geringer oTM-Abbau zeigten jedoch eine Beeinträchtigung des anaeroben Prozesses in der zweiten, thermophilen Stufe durch die vorgeschaltete mesophile Behandlung an. Der Ablauf der dritten, mesophilen Stufe wies sehr geringe Säurekonzentrationen auf. Im Endlager war das Niveau an flüchtigen Fettsäuren aufgrund fortschreitender Abbauprozesse wieder erhöht.

Während des mesophil-thermophil-mesophilen Betriebes der Pilot-Biogasanlage wurde aus Milchviehgülle (mittlerer TM-Gehalt: 7,8 %) ein Methanertrag von 0,24 m^3*$(kg\ oTM_{zugeführt})^{-1}$ erzielt. Die hydraulische Verweilzeit in der Fermenterkaskade betrug dabei 45 Tage. Eine Verlängerung des Beschickungsintervalls von einer auf vier Stunden hatte keinen Einfluss auf den Methanertrag. In Anbetracht des hohen Ligningehaltes der Gülle, die von Milchvieh mit faserreicher Fütterung stammte, stellt sich der Wert für den erzielten Methanertrag im Vergleich zu Literaturangaben als sehr günstig dar. Nach Verringerung der Temperatur in der ersten Stufe auf 20-25 C war die Leistungsfähigkeit des

thermophilen Prozesses deutlich erhöht, während der Methanertrag im Vergleich zum mesophil-thermophil-mesophilen Betrieb unverändert blieb.

Durch die Behandlung in der Pilotanlage wurde die Keimzahl von Fäkalcoliformen in der Milchviehgülle für alle Betriebsarten auf im Mittel unter 10 MPN*g FM^{-1} verringert, vorausgesetzt dass in der thermophilen Stufe eine Temperatur von 55°C aufrechterhalten wurde. Ein vorübergehender Temperaturabfall hatte auf die Konzentration von Fäkalcoliformen in Proben aus der thermophilen und der nachgeschalteten mesophilen Stufe einen relativ geringen Einfluss, führte jedoch zu einer deutlichen Wiederverkeimung mit diesen Indikatororganismen im Endlager. Für intestinale Enterokokken konnte derselbe Trend beobachtet werden, wobei für diese sehr temperaturresistenten Indikatorkeime der Einfluss verringerter Temperaturen in der thermophilen Stufe stärker ausgeprägt war. Die Inaktivierung von *Cryptosporidium parvum*-Oozysten wurde in einem halbtechnischen Modell der Pilot-Biogasanlage im geometrischen Maßstab von etwa 1:6 untersucht. Eine Behandlung bei 55°C für 4 Stunden verringerte die Anzahl infektiöser Kryptosporidien-Oozysten um mehr als 5 log-Stufen. Im Gegensatz zu vorausgegangenen Studien wurde durch die mesophile Vorbehandlung keine verstärkte Inaktivierung von Oozysten bewirkt.

Aus Markierungsexperimenten wurde im liegenden Fermenter sowohl für stündliche als auch für vierstündliche Beschickung eine gesicherte Verweilzeit von 8 Stunden ermittelt. Der Reaktor war unvollständig durchmischt und hatte einen hohen Bedarf an Heizenergie.

Für die effiziente anaerobe Behandlung von Milchviehgülle in Rührkesselreaktoren wird ein zweistufiges, thermophil-mesophiles Verfahren vorgeschlagen. Das Verhältnis der hydraulischen Verweilzeit in der thermophilen bzw. mesophilen Stufe sollte etwa 1:3 betragen. Um eine ausreichende Keimzahlreduktion von nicht Sporen bildenden Bakterien und Kryptosporidien zu gewährleisten, sind die Fermenter mit einem Beschickungsintervall von 8 Stunden zu betreiben, wobei die Entnahme vor der Beschickung zu erfolgen hat. Um die für eine weitgehende Hygienisierung erforderliche Behandlungstemperatur sicherzustellen, muss beim Betrieb einer solchen Biogasanlage die effiziente Fermenterbeheizung Vorrang vor der Stromerzeugung haben. Die der thermophilen Stufe zugeführte Gülle sollte vorgewärmt werden. Für diesen Zweck sollte Wärme aus dem Ablauf der thermophilen Stufe zurückgewonnen werden. Gülle, die aufgrund von Prozessstörungen bei zu geringen Temperaturen behandelt wurde, darf nicht in das Endlager gelangen. Eine Biogasanlage für Hygienisierungszwecke muss generell so gestaltet sein, dass eine Rekontamination behandelter Gülle durch Material aus einer vorgeschalteten

Behandlungsstufe ausgeschlossen ist. Unabhängig von der Prozesstemperatur leistet die anaerobe Behandlung von Gülle stets einen gewissen Beitrag zur Verringerung des Eintrags von Pathogenen und Indikatorkeimen in die Umwelt.

REFERENCES

[1] Ahring, B. K. (1994). Status on science and application of thermophilic anaerobic digestion. *Water Sci Tech* 30(12): 241-249

[2] Ahring, B. K. (1995). Methanogenesis in thermophilic biogas reactors. *Antonie van Leeuwenhoek* 67: 91-102

[3] Ahring, B. K., Sandberg, M., Angelidaki, I. (1995). Volatile fatty acids as indicators of process imbalance in anaerobic digestion. *Appl Microbiol Biotechnol* 43: 559-565

[4] Angelidaki, I., Ahring, B. K. (1993). Anaerobic thermophilic digestion of manure at different ammonia loads: Effect of temperature. *Water Res* 28(3): 727-731

[5] Anonymous (1981). Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung (German Standard Methods for The Examination of Water, Wastewater and Sludge). Wasserchemische Gesellschaft, Fachgruppe in der GDCh / in Gemeinschaft mit dem Normenausschuss Wasserwesen (NAW) im DIN e.V. (eds.), Weinheim, Wiley-VCH

[6] Anonymous (1998). Verordnung über die Verwertung von Bioabfällen auf landwirtschaftlich, forstwirtschaftlich und gärtnerisch genutzten Böden (Bioabfallverordnung - BioAbfV) (Ordinance on the Utilisation of Bio-Wastes on Land Used for Agricultural, Silvicultural and Horticultural Purposes) vom 21.09.1998 (BGBl. I 1998 S. 2955)

[7] Anonymous (2005). Gesetz zur Ordnung des Wasserhaushalts (Federal Water Act). BGBl I 1957, 1110, 1386; Neugefasst: 19.08.2002 I 3245; zuletzt geändert durch Art. 1 G v. 3. 5.2005 I 1224

[8] Batstone, D. J. (2000). High rate anaerobic treatment of complex wastewater. PhD thesis, University of Queensland, ISBN 1 86499 3863

[9] Bicudo, J. R., Goyal, S. M., Zhu, J. (2000). Animal Production, Manure Management and Pathogens: A Review. Proceedings of the Eighth International Symposium on Animal, Agricultural and Food Processing Wastes, J.A. Moore ed., St. Joseph, Mich., ASAE: 507-521

[10] Bihler, J. (1999). Nährstofftransport und Trennverhalten bei der Feststoffabtrennung aus Flüssigmist. Dissertation, Fakultät IV – Agrarwissenschaften II: Agrarökonomie, Agrartechnik und Tierproduktion, Universität Hohenheim

[11] Böhm, R. (2002). Aspekte der Seuchenhygiene und Anforderungen an die Verwertung von Klärschlamm, Kompost und Gülle. In: Landwirtschaftliche Verwertung von Klärschlamm, Gülle und anderen Düngern unter Berücksichtigung des Umwelt- und Verbraucherschutzes, BMU / BMVEL Wissenschaftliche Anhörung, 25.-26.10.2001, Bonn (KTBL-Schrift 404), Kuratorium für Technik und Bauwesen in der Landwirtschaft e.V. (KTBL), Darmstadt: 61-84

[12] Boone, D. R. (1981). Terminal reactions in the anaerobic digestion of animal waste. *Appl Environ Microbiol* 43(1): 57-64

[13] Buhr, H. O., Andrews, J. F. (1977). The thermophilic anaerobic digestion process. *Water Res* 11: 129-143

[14] Burton, C. H., Turner, C. (2003). Manure Management – Treatment Strategies for Sustainable Agriculture. 2nd Edition, Silsoe Research Institute, 2003. ISBN 0-9531282-6-1

[15] Carrington, E. G., Harman, S. A., Pike, E. B. (1982). Inactivation of *Salmonella* during anaerobic digestion of sewage sludge. *J Appl Bacteriol* 53: 331-334

[16] Carrington, E. G. (2001). Evaluation of sludge treatments for pathogen reduction. Final report, Study Contract No B4-3040/2001/322179/MAR/A2 for the European Commission Directorate General Environment. WRc Medmenham, Ref: CO 5026/1. ISBN 92-894-1734-X

[17] Chen, J. S., Frey, B. C., Brodie, H. L. (1984). Scum accumulation in plug-flow digesters – a problem with flushed dairy manure. *Trans ASAE* 1984: 1894-1896

[18] Christ O. (1999). Leistungscharakteristik der ein- und zweistufigen thermophilen und mesophilen Vergärung von Bioabfällen. Berichte aus Wassergüte- und Abfallwirtschaft, Technische Universität München, Nr. 145

[19] Daugschies, A. (2000). Wasser als Vektor von parasitären Dauerstadien in der Tierhaltung. *Dtsch tierärztl Wschr* 107: 316-319

[20] De León, C., Jenkins, D. (2002). Removal of fecal coliforms by thermophilic anaerobic digestion processes. *Water Sci Tech* 46(10): 147-152

[21] Döhler, H., Kuhn, E., Schwab, M., Metzger, H. J. (1997). Umweltprobleme der Güllewirtschaft und Optionen zur Lösung. In: Umweltverträgliche Gülleaufbereitung und Verwertung, Kuratorium für Technik und Bauwesen in der Landwirtschaft e.V. (KTBL) ed., Arbeitspapier 242, Landwirtschaftsverlag GmbH, Münster-Hiltrup, 1997, ISBN 3-7843-1961-0: 9-16

[22] Doll, L., Meyer, H., Oechsner, H., Philipp, W. (1999). Verfahrenstechnische und mikrobiologische Untersuchungen zur Abtötung von Kryptosporidien in Flüssigmist mittels der Verfahren der aerob-thermophilen Stabilisierung und der thermophilen Anaerobbehandlung. Landesanstalt für Landwirtschaftliches Maschinen- und Bauwesen und Insitut für Umwelt- und Tierhygiene der Universität Hohenheim, Abschlussbericht an das Ministerium für Ländlichen Raum Baden-Württemberg, Projekt Nr. 0056

[23] DVGW (1995). Richtlinien für Trinkwasserschutzgebiete; I. Teil: Schutzgebiete für Grundwasser. Arbeitsblatt W 101. Deutscher Verein des Gas- und Wasserfaches (DVGW) (German Association on Gas and Water)

[24] Eastman, J. A., Ferguson, J. F. (1981). Solubilization of particulate organic carbon during the acid phase of anaerobic digestion. *J WPCF* 53(3): 352-366

[25] Effenberger, M., Schlattmann, M., Kaiser, F., Gronauer, A. (2002). Stand der Biogastechnologie in Bayern. In: Biogasanlagen – Anforderungen zur Luftreinhaltung. Augsburg, Bavarian Environmental Protection Agency

[26] Elmashad, H. M., Zeeman, G., Lettinga, G. (2001). Thermophilic anaerobic digestion of cow manure – effect of temperature on hydrolysis. Proceedings of the 9th World Congress on Anaerobic Digestion, 2 to 6 September, 2001, Technologisch Instituut, Antwerpen, The Netherlands

[27] European Commission (1986). Council Directive 86/278/EEC of 12 June 1986 on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture. Official Journal of the European Communities L 181, 04/07/1986: 0006 – 0012

[28] European Commission (2002). Regulation (EC) No 1774/2002 of the European Parliament and of the Council of 3 October 2002 laying down health rules concerning animal by-products not intended for human consumption. Official Journal of the European Communities L 273/1-95

[29] European Commission (2003a). Draft discussion document for the ad hoc meeting on biowastes and sludges, 15-16 January, Brussels. European Commission Directorate-General Environment, Directorate A - Communications, Governance, Production, Consumption and Waste, ENV.A2 - Production, Consumption & Waste

[30] European Commission (2003b). Draft Commission Regulation of implementing and amending Regulation (EC) No 1774/2002 as regards the organic fertilisers and soil improvers, Brussels, 2003

[31] Farrell, J. B., Erlap, A. E., Rickabaugh, J., Freedman, D., Hayes, S. (1988). Influence of feeding procedure on microbial reductions and performance of anaerobic digestion. *J WPCF* 60(5): 635-644

[32] FNR (2005). Handreichung Biogasgewinnung und –nutzung. Published by: Fachagentur Nachwachsende Rohstoffe e. V., Gülzow, 2005, ISBN 3-00-014333-5

[33] Friedmann, H., Märkl, H. (1994). Der Einfluß der Produktgase auf die mikrobiologische Methanbildung. *Wasser Abwasser gwf* 135(6): 302-311

[34] Gallert, C., Winter, J. (1997). Mesophilic and thermophilic anaerobic digestion of source-sorted organic wastes: Effect of ammonia on glucose degradation and methane production. *Appl Microbiol Biotechnol* 48: 405-410

[35] Garcés, G., Effenberger, M., Najdrowski, M., Wackwitz, C., Gronauer, A., Wilderer, P. A., Lebuhn, M. (2006). Quantification of *Cryptosporidium parvum* in anaerobic digesters treating manure by (reverse-transcription) quantitative real-time PCR, infectivity and excystation tests. *Water Sci Tech* 53(8): 195-202

[36] Gerba, C. P., Smith, J. E. (2005). Sources of pathogenic microorganisms and their fate during land application of wastes. *J Environ Qual* 34: 42-48

[37] Godfree, A., Farrell, J. (2005). Processes for managing pathogens. *J Environ Qual* 34: 105-113

[38] Gosch, A. (1984). Anaerober Abbau von flüssigen Abfällen aus Tierhaltungen. Dissertation, Institut für Landtechnik, Justus-Liebig-Universität Gießen

[39] Gray, D. M. D., Hake, J. M., Ghosh, S. (2006). Influence of staging, mean cell residence time, and thermophilic temperature on the thermophilic anaerobic digestion process. *Water Environ Res* 78(5): 497-509

[40] Haas, C. N., Joffe, J., Heath, M. S., Jacangelo, J. (1997). Continuous flow residence time distribution function characterization. *J Environ Eng, ASCE* 123(2): 107-114

[41] Han, Y., Sung, S., Dague, R. R. (1997). Temperature-phased anaerobic digestion of wastewater sludges. *Water Sci Tech* 36(6-7): 367-374

[42] Hartmann, H., Nielsen, H. B., Ahring, B. K. (2004). Optimization of the Biogas Process Using On-Line VFA Measurement. Proceedings of the 10th World Congress on Anaerobic Digestion, 29 August – 2 September, 2004, Montreal, Canada: 1285-1289

[43] Hashimoto, A. G. (1982). Methane from cattle waste: Effect of temperature, hydraulic retention time, and influent substrate concentration on kinetic parameter (*K*). *Biotechnol Bioeng* 24: 2039-2052

[44] Hashimoto, A. G. (1983). Conversion of straw-manure mixtures to methane at mesophilic and thermophilic temperatures. *Biotechnol Bioeng* 25(1983): 185-200

[45] Heid, C. A., Stevens, J., Livak, K. J., Williams, P. M. (1996). Real time quantitative PCR. *Genome Res* 6(10): 986–994

[46] Hills, D. J., Mehlschau, J. J. (1984). Plug flow digestion of dairy manure at different solids concentrations. *Trans ASAE* 27(3): 889-893

[47] Hobson, P. N., Wheatley, A. D. (1993). Anaerobic Digestion – Modern Theory and Practice. London, Elsevier Applied Science

[48] Hörnig, G. (1982). Berechnung der Druckverluste beim Fördern von Rinder- und Schweinegülle in Gülledruckrohrleitungen. *Agrartechnik* 1982(1): 23-26

[49] Hoferer, M. (2001). Seuchenhygienische Untersuchungen zur Inaktivierung ausgewählter Bakterien und Viren bei der mesophilen und thermophilen anaeroben alkalischen Faulung von Bio- und Küchenabfällen sowie anderen Rest- und Abfallstoffen tierischer Herkunft. Dissertation, Fachbereich Veterinärmedizin, Freie Universität Berlin, ISBN 3-8265-9616-1

[50] Holland, P. M., Abramson, R. D., Watson, R., Gelfand, D. (1991). Detection of specific polymerase chain reaction product by utilizing the 5′⟶3′ exonuclease activity of Thermus aquaticus DNA polymerase. *Proc Natl Acad Sci USA* 88: 7276–7280

[51] Janitschke, K. (1999). Parasitäre Zoonosen. *Dtsch tierärztl Wschr* 106: 358-361

[52] Kaiser, F. (2005). Unpublished data. Bavarian State Research Center for Agriculture, Institute for Agricultural Engineering, Farm Buildings and Environmental Technology, Freising, Germany

[53] Katers, J. F., Schultz, J. (2003). Temperature-phased anaerobic digestion system monitoring project at Tinedale farm. Final report submitted to Wisconsin Department of Administration

[54] Kearney, T. E., Larkin, M. J., Levett, P. N. (1993). The effect of slurry storage and anaerobic digestion on survival of pathogenic bacteria. *J Appl Bacteriol* 74: 86-93

[55] Kirchgessner, M. (1987). Tierernährung. DLG-Verlag, Frankfurt, ISBN 3-7690-0447-7

[56] Koster, I. W., Lettinga, G. (1988). Anaerobic digestion at extreme ammonia concentrations. *Biological Wastes* 25(1988): 51-59

[57] KTBL (2004). Hygiene-Prüfsystem für Vergärungsanlagen und Umsetzung der Ergebnisse, Vorschlag zur Überarbeitung Anhang 2 BioAbfV, Stand: 17.09.2004 (Proposal for the revision of Annex 2 to the Ordinance on the Utilisation of Bio-Wastes on Land Used for Agricultural, Silvicultural and Horticultural Purposes: Safety for use in terms of human, animal and plant health), URL: http://www.ktbl.de/energie/hygiene.pdf (Download: 14.01.2005)

[58] KTBL (2005). Gasausbeuten in landwirtschaftlichen Biogasanlagen. KTBL (Association for Technology and Structures in Agriculture), Darmstadt, Germany

[59] Lampel H. (1984). Energie aus Biomasse: Biogastechnologie – Biogasforschungsanlage Wieselburg. Forschungsberichte der Bundesanstalt für Landtechnik No. 14, Wieselburg, Austria

[60] Langenhoff, A. A. M., Stuckey, D. C. (2000). Treatment of dilute wastewater using an anaerobic baffled reactor: Effect of low temperature. *Wat Res* 43(15): 3867-3875

[61] Larsen, H. E., Munch, B. (1990). Reduction of pathogenic and indicator organisms in biological waste - especially slurry - subjected to various treatments. In: D. Strauch (ed.), Aktuelle Probleme der Desinfektion von Nutztierställen sowie von Fest- und Flüssigmist, Bericht des 3. Hohenheimer Seminars, veranst. vom Inst. für Tiermedizin und Tierhygiene, Universität Hohenheim, Stuttgart, Germany, 18-19 September, 1990, Dt. Veterinärmed. Ges. e. V. (DVG), Giessen: 169-177

[62] Larsen, H. E., Munch, B., Schlundt, J. (1994). Use of indicators for monitoring the reduction of pathogens in animal waste treated in biogas plants. *Zentralbl Hyg Umweltmed* 195: 544-555

[63] Lebuhn, M., Effenberger, M., Gronauer, A., Wilderer, P. A., Wuertz, S. (2003). Using quantitative real-time PCR to determine the hygienic status of cattle manure. *Water Sci Tech* 48(4): 97-103

[64] Lebuhn, M., Effenberger, M., Garcés, G., Gronauer, A.. Wilderer, P. A. (2004). Evaluating real-time PCR for the quantification of distinct pathogens and indicator organisms in environmental samples. *Water Sci Tech* 50(1): 263–270

[65] Lebuhn, M., Effenberger, M., Garcés, G., Gronauer, A., Wilderer, P. A. (2005). Hygienization by anaerobic digestion: comparison between evaluation by cultivation and quantitative real-time PCR. *Water Sci Tech* 52(1-2): 93-99

[66] Lebuhn, M., Wilderer, P. A. (2006). Biogastechnologie zur umweltverträglichen Flüssigmistverwertung und Energiegewinnung in Wasserschutzgebieten: wasserwirtschaftliche und hygienische Begleituntersuchung. In: LfL-Schriftenreihe Nr. 23/2006, Bavarian State Research Center for Agriculture (LfL), Freising, Germany, ISSN 1611-4159: 149-239

[67] Lenhart, B. (2001). Bedeutung und Verminderung diffuser Belastungen im Einzugsbereich "Obere Isar". In: Hygienische Aspekte von Oberflächengewässern aus wasserwirtschaftlicher Sicht, Munich, Germany, Bayerisches Landesamt für Wasserwirtschaft: 167-175

[68] Levenspiel, O. (1962). Chemical reaction engineering. Wiley: New York

[69] Lleò, M.M., Bonato, B., Tafi, M.C., Signoretta, C., Boaretti, M. and Canepari, P. (2001). Resuscitation rate in different enterococcal species in the viable but non-culturable state. *J Appl Microbiol* 91(6), 1095–1102

[70] LfL (2006). Biogastechnologie zur umweltverträglichen Flüssigmistverwertung und Energiegewinnung in Wasserschutzgebieten. LfL-Schriftenreihe Nr. 23/2006, Bavarian State Research Center for Agriculture (LfL), Freising, Germany, ISSN 1611-4159

[71] Mackie, R. I., Bryant, M. P. (1981). Metabolic activity of fatty acid-oxidizing bacteria and the contribution of acetate, propionate, butyrate and CO_2 to methanogenesis in cattle waste at 40 and 60°C. *Appl Environ Microbiol* 41(6): 1363-1373

[72] Mackie, R. I., Bryant, M. P. (1995). Anaerobic digestion of cattle waste at mesophilic and thermophilic temperatures. *Appl Microbiol Biotechnol* 43: 346-350

[73] Martens, W., Fink, A., Philipp, W., Weber, A., Winter, D., Böhm, R. (1999). Seuchenhygienische Bewertung von Anaerobanlagen. In: R. Böhm ed., 7. Hohenheimer Seminar Biologische Abfallbehandlung - Erste Erfahrungen mit der Bioabfallverordnung in Deutschland, Vol. 1. Giessen, Verlag der Deutschen Veterinärmedizinischen Gesellschaft e.V.

[74] Mattocks, R. P., Moser, M. A. (2000). Fate of incoming solids to measure manure digester performance. In: Animal, Agricultural and Food Processing Wastes, Proceedings of the Eighth International Symposium, J.A. Moore ed., St. Joseph, Mich., ASAE: 187-193

[75] Mawdsley, J. L., Bardgett, R. D., Merry, R. J., Pain, B. F., Theodorou, M. K. (1995). Pathogens in livestock waste, their potential for movement through soil and environmental pollution. *Appl Soil Ecol* 2(1995): 1-15

[76] McDougald, D., Rice, S. A., Weichart, D. and Kjelleberg, S. (1998). Nonculturability: Adaptation or debilitation? *FEMS Microbiol Ecol* 25(1): 1-9

[77] Moss, L. H., Epstein, E., Logan, T. L. (2002). Evaluating risks and benefits of soil amendments used in agriculture. Report 99-PUM-1, Water Environment Research Foundation (WERF), Alexandria, VA, ISBN 1-893664-59-7

[78] Munch, B., Schlundt, J. (1983). On the reduction of pathogenic and indicator bacteria in animal slurry and sewage sludge subjected to anaerobic digestion or chemical disinfection. In: D. Strauch (ed.), Hygienic Problems of Animal Manures, Proceedings of a joint workshop of expert groups of the Commission of the European Communities, German Veterinary Medical Society (DVG) and Food and Agricultural Organization, 11-13 October, 1982, Institut für Tiermedizin und Tierhygiene, Universität Hohenheim, Stuttgart

[79] Nielsen, H. B., Mladenovska, Z., Westermann, P., Ahring, B. K. (2004). Comparison of two-stage thermophilic (68°C/55°C) anaerobic digestion with one-stage thermophilic (55°C) digestion of cattle manure. *Biotechnol Bioeng* 86(3): 291-298

[80] O'Connor, G. A., Elliott, H. A., Basta, N. T., Batian, R. K., Pierzynski, G. M., Sims, R. C., Smith Jr., J. E. (2005). Sustainable land application: An overview. *J Environ Qual* 34: 7-17

[81] Oechsner, H., Doll, L. (2000). Inactivation of pathogens by using the aerobic-thermophilic stabilization process. In: Animal, Agricultural and Food Processing Wastes, Proceedings of the Eighth International Symposium, J.A. Moore ed., St. Joseph, Mich.: ASAE, 522-528

[82] Ohio State University Extension, Department of Horticulture and Crop Science (1995). Best management practices: Land application of animal manure. AGF-208-95. In: The Ohio State University Extension, Agronomy Facts. Columbus, Ohio, U.S.A.

[83] Olsen, J. E., Larsen, H. E. (1986). Bacterial decimation times in anaerobic digestion of animal slurries. *Biological Wastes* 21: 153-168

[84] Olson, M. E., Goh, J., Phillips, M., Guselle, N., McAllister, T. A. (1999). *Giardia* Cyst and *Cryptosporidium* oocyst survival in water, soil, and cattle feces. *J Environ Qual* 28: 1991-1996

[85] Pell, A. N. (1997). Manure and microbes: Public and animal health problem? *J Dairy Sci* 80: 2673-2681

[86] Peretzki, F., Heigl, L. (2006). Gülleuntersuchungen stimmen gut mit den Richtwerten überein – Auswertung der Nährstoffgehalte der KULAP-Gülleuntersuchungen. *Bayerisches Landwirtschaftliches Wochenblatt* 7(2006): 38

[87] Prechtl, S. (2005). Optimierte Durchmischung von Biogasreaktoren. Final report to the Bavarian State Ministry for Environment, Health and Consumer Protection, EFRE Project E126

[88] Puchadja, B., Oleszkiewicz, J. (2004). Pathogen inactivation by organic acids in sludge digestion. Proceedings of the 10th World Congress on Anaerobic Digestion, 29 August – 2 September, 2004, Montreal, Canada: 479

[89] Reinhold, G., Jahn, O. (2004). Hygienisierende Wirkungen der Biogaserzeugung auf die Gärsubstrate. 116. VDLUFA-Kongress, 13 – 17 September 2004, Rostock, Germany

[90] Robertson, L. F., Smith, H. V., Ongerth, J. E. (1994). Cryptosporidium and cryptosporidiosis. Part III: Development of water treatment technologies to remove and inactivate oocysts. *Microbiol Europe* 1/2: 18-26

[91] Rückert, V. (1991). Mikrobiologische Untersuchungen zur aeroben und anaeroben Flüssigmistbehandlung. Dissertation, Institut für Tiermedizin und Tierhygiene, Universität Hohenheim

[92] Salsali, H. R., Parker, W. J., Sattar, S. A. (2006). Impact of concentration, temperature, and pH on inactivation of *Salmonella* spp. by volatile fatty acids in anaerobic digestion. *Can J Microbiol* 52: 279-286

[93] Scanlan, P., Santha, H., Fronek, S. (2004). Advanced anaerobic digestion for Class A pathogen removal. Proceedings of the 10th World Congress on Anaerobic Digestion, 29 August – 2 September, 2004, Montreal, Canada: 491-497

[94] Schlegel, H. G. (1992). Allgemeine Mikrobiologie. 7., überarbeitete Auflage, Georg Thieme Verlag, Stuttgart, New York, ISBN 3-13-444607-3

[95] Schomaker, A. H. H. M. (2002). Verblijftijdsspreidingsmeting bij de slibgistingsinstallatie van RWZI-Venray. Ir. A. H. H. M. Schomaker, Division Water, Advisory Group Water Resources & Ecology, Haskoning Nederland B.V., P.O. Box 525, 5201 AM's-Hertogenbosch, Netherlands

[96] Schulz, H. (1991). Stoffliche Veränderungen von Flüssigmist durch Biogasverfahren. In: J. Boxberger, A. Gronauer, L. Popp (eds.), Tagungsband zu Fachgespräch Umweltschonende Verwertung von Fest- und Flüssigmist auf landwirtschaftlichen Nutzflächen, 27./28.06.1990, Freising-Weihenstephan, Germany

[97] Shelton, D. R. (2000). Sources of pathogens in a watershed: Humans, wildlife, farm animals? In: Managing Nutrients and Pathogens from Animal Agriculture, Ithaca, New York: Natural Resource, Agriculture, and Engineering Service (NRAES): 108-115

[98] Sigloch, H. (1996). Technische Fluidmechanik. VDI-Verlag, Düsseldorf, ISBN 3-18-401523-8

[99] Smith, S. R., Lang, N. L., Cheung, K. H. M., Spanoudaki, K. (2005). Factors controlling pathogen destruction during anaerobic digestion of biowastes. *Waste Management* 25(2005): 47-425

[100] Stehman, S. M. (2000). Ag-related waterborne pathogens. In: Managing Nutrients and Pathogens from Animal Agriculture, Ithaca, New York, Natural Resource, Agriculture, and Engineering Service (NRAES): 93-107

[101] Strauch, D. (1987). Hygiene of animal waste management. In: Animal Production and Environmental Health, World Animal Science Vol. B6 (D. Strauch, ed.), Elsevier Science Publishers B. V.: 155-202

[102] Strauch, D. (1996). Hygieneaspekte bei der Cofermentation. In: Internationale Erfahrungen mit der Verwertung biogener Abfälle zur Biogasproduktion (R. Braun, ed.), Tagungsberichte Vol. 14, Bundesministerium für Umwelt, Jugend und Familie: 53-92

[103] Strauch, D. (1998). Pathogenic micro-organisms in sludge – Anaerobic digestion and disinfection methods to make sludge usable as a fertiliser. *European Water Management* 1(2): 12-26

[104] Strauch, D., Baader, W., Tietjen, C. (1977). Abfälle aus der Tierhaltung – Anfall, Umweltbelastung, Behandlung, Verwertung. Eugen Ulmer, Stuttgart, ISBN 3-8001-4328-3

[105] Sung, S., Santa, H. (2003). Performance of temperature-phased anaerobic digestion (TPAD) system treating dairy cattle wastes. *Wat Res* 37(2003): 1628-1636

[106] Tebbe, C. C., Vahjen, W. (1994). Interference of humic acids and DNA extracted directly from soil in detection and transformation of recombinant DNA from bacteria and yeast. *Appl Environ Microbiol* 59: 2657–2665

[107] Thomas, C., Hill, D., Mabey, M. (2002). Culturability, injury and morphological dynamics of thermophilic *Campylobacter* spp. within a laboratory-based aquatic model system. *J Appl Microbiol* 92: 433–442

[108] Trussell, R. R., Chao, J. (1977). Rational design of chlorine contact facilities. *J WPCF* 49(7): 659-667

[109] USEPA (1992). Environmental Regulations and Technology: Control of Pathogens and Vector Attraction in Sewage Sludge (Including Domestic Septage) under 40 CFR Part 503, EPA/625/R-92/013, United States Environmental Protection Agency, Washington, D.C.

[110] USEPA (2001). Protocol for Developing Pathogen TMDLs. EPA/841/R-00/002, United States Environmental Protection Agency, Office of Water

[111] Van Soest, P. J. (1967). Development of a comprehensive system of feed analyses and its application to forages. *J Anim Sci* 26(1): 119-128

[112] Varel, V. H., Isaacson, H. R., Bryant, M. P. (1977). Thermophilic methane production from cattle waste. *Appl Environ Microbiol* 33(2): 298-307

[113] Varel, V. H., Hashimoto, A. G., Chen, Y. R. (1980). Effect of temperature and retention time on methane production from beef cattle waste. *Appl Environ Microbiol* 40(2): 217-222

[114] VDLUFA (1997). Methodenbuch Band III, Die chemische Untersuchung von Futtermitteln (Chemical analyses of feedstuffs). Mit 4. Ergänzungslieferung, VDLUFA-Verlag, Darmstadt

[115] von Stromberg, A. (1985). Mesophiler aerober bzw. fakultativ anaerober Keimbesatz von Frischgülle und Faulschlamm der Biogasgewinnung. Dissertation, Institut für Medizinische Mikrobiologie, Infektions- und Seuchenmedizin der Tierärztlichen Fakultät der Ludwig-Maximilans-Universität München

[116] Wechs, F. (1985). Ein Beitrag zur zweistufigen anaeroben Klärschlammstabilisierung. Berichte aus Wassergüte und Gesundheitsingenieurwesen, Technische Universität München, Nr. 53

[117] Weiland, P. (1997). Stand und Perspektive der Gülleaufbereitung. In: Umweltverträgliche Gülleaufbereitung und Verwertung, Kuratorium für Technik und Bauwesen in der Landwirtschaft e.V. (KTBL) ed., Arbeitspapier 242, Landwirtschaftsverlag GmbH, Münster-Hiltrup, 1997, ISBN 3-7843-1961-0: 136-146

[118] Weiland, P., Rieger, C., Ehrmann, T., Helffrich, D., Kissel, R., Melcher, F. (2005). Ergebnisse des bundesweiten Biogasmessprogramms. In: Biogas – Energieträger der Zukunft, 12./13.04.2005, Osnabrück, VDI-Berichte 1872, ISBN 3-18-091872-1: 117-128

[119] Weiß, K., Popp, W. (2004). Quantifizierung der diffusen Belastung von Gewässern mit Fäkalbakterien aus landwirtschaftlich genutzten Flächen. Schlussbericht, Bayerisches Landesamt für Wasserwirtschaft, Materialien Nr. 111: 1-74

[120] Witty, W., Märkl, H. (1986). Process engineering aspects of methanogenic fermentation on the example of fermentation of *Penicillium Mycelium*. *Ger Chem Eng* 9(1986): 238-245

[121] Wright, P. E. (2000). Manure treatment and handling options. In: Managing Nutrients and Pathogens from Animal Agriculture, Ithaca, New York, Natural Resource, Agriculture, and Engineering Service (NRAES): 156-173

[122] Wright, P. E., Inglis, S., Ma, J., Gooch, C., Aldrich, B., Scott, N. (2004). Preliminary comparison of five anaerobic digestion systems on dairy farms in New York State. Paper presented at the ASAE Annual International Meeting, 1 – 4 August, 2004, Ottawa, Ontario, Paper No. 04-4032. ASAE, 2950 Niles Road, St. Joseph, MI

[123] Wohlt, J. E., Frobish, R. A., Davis, C. L., Bryant, M. P., Mackie, R. I. (1990). Thermophilic methane production from dairy cattle waste. *Biological Wastes* 32(1990): 193-207

[124] World Health Organization (2003). Emerging issues in water and infectious disease. ISBN 92-4-159082-3, ISSN 1728-216

[125] Zhang, R. H. (1988). Biological treatment of animal wastewater. Paper presented at: Research toward optimal swine manure management systems, 19 – 22 July, 1988, Des Moines, Iowa

[126] Zhang, R. H., Tao, J., Dugba, P. N. (2000). Evaluating two-stage anaerobic sequencing batch reactor system for animal wastewater treatment. *Trans ASAE* 43(6): 1795-801

10 APPENDICES

Appendix 1. Description of pilot plant operations (after finishing retrofitting work)

Date	Operating conditions and procedures
28 December 2003	Resumption of feeding at a reduced rate of 2.5 m^3 liquid manure per day after finishing retrofitting the paddle agitator of Digester 2; problems with frozen delivery pipe for raw manure
5 January 2004	Digester 2 reaches normal filling level
January 2004	Increasing daily load to the target value of 5.5 m^3 in steps of about 1 m^3 per week
February to September 2004	Operation with a daily load of about 5.5 m^3 of liquid manure on the average, fed in 21 batches (preset feeding interval: 1 hour); input volumes of Digester 1 vary between 5.3 and 5.7 m^3 mostly; average total hydraulic retention time of digester chain: 45 days
16 to 24 February 2004	Reduced feeding due to repeated plugging of delivery pipe of Digester 1 and failure of feeding pump 1
19 February to 8 March 2004	Blocked up gas collection pipe causes irregular operation of combined heat and power unit
11 to 14 March 2004	Addition of Fe(II) to digesters due to high hydrogen sulfide levels in the biogas of up to 1200 ppm; H_2S levels repeatedly exceed 200 ppm until early June; failure of the agitator causes Digester 1 to foam over
15 March to 5 May 2004	Hygienic monitoring: Tracing experiment
28 March 2004	Burst water pipe in the stable: Approximately 30 m^3 flow into manure canal
29 March to 5 April 2004	Intensified sampling of raw manure and digester contents
25 May 2004	Installation of gas flow meters, discharge of biogas
31 May to 2 June 2004	Digester 1 foaming over again; H_2S in the biogas exceeding 200 ppm
7 June to 5 July	First tracer test in Digester 2
8/9 June 2004	No/reduced feeding due to incorrect setting of level meter of Digester 1
24/25 July 2004	Reduced feeding of Digester 1 due to foreign bodies in delivery pump
12 August 2004	Failure of the agitator causes Digester 1 to foam over
13 to 16 August 2004	Very strong winds cause cooling down of Digester 2 by about 2 K
26 August 2004	Servicing of the engine: Reduced fuel oil consumption and increased thermal energy output exceed capacity of the heating system at full load
7 September 2004	Change of preset feeding interval to 4 hours
7 September to 11 October 2004	Operation with a daily load of about 5.2 m^3 of liquid manure on the average, fed in 5 or 6 batches by turns (preset feeding interval: 4 hours); average total hydraulic retention time of digester chain: 47 days
From 10 September 2004 on	Cooler temperatures require additional heating with fuel oil during nights to maintain thermophilic temperatures

Date	Operating conditions and procedures
20 September to 13 October 2004	Second tracer test in Digester 2
12 October to December 2004	Operation with a daily load of about 5.4 m^3 of liquid manure on the average, fed in 5 or 6 batches by turns (preset feeding interval: 4 hours); average total hydraulic retention time of digester chain: 45 days
19 to 27 October 2004	Intensified sampling of raw manure and digester contents
28 October 2004	Blocked up gas flow meter causes discharge of biogas through pressure valve
26 November 2004	Addition of Fe(II) to digesters due to hydrogen sulfide levels in the biogas exceeding 200 ppm
22 to 25 December 2004	Reduced feeding due to frozen up manure delivery pipe between storage canal and collection tank
23 February 2005	Shut-down of Digester 1; thermophilic-mesophilic operation at reduced feeding rate
5 to 29 April 2005	Emptying and refilling of Digester 1 with raw liquid manure
29 April to 17 June 2005	Thermophilic-mesophilic operation; Digester 1 used for pre-heating the liquid manure to 20-25°C; mean daily load: 5.7 m^3, fed in 21 batches (preset feeding interval: 1 hour), average hydraulic retention time (Digesters 2 and 3): 34 days

Appendix 2. Description of model plant operations

Date	Operating conditions and procedures
16 June 2003	1st filling of the digesters
24 June 2003	Start of feeding
28 August to 12 September 2003	First tracer test in MD2, terminated prematurely due to clogging of delivery pump 1
16 December 2003	Shut-down of model plant for the winter season
29/30 April 2004	Re-start of model plant
7 May to 15 August 2004	Operation with a daily load of 24.5 to 26.5 L of liquid manure on the average, fed in 24 batches; average total hydraulic retention time of digester chain: ca 40 days
16 August to 15 November 2004	Operation with a daily load of 28.6 to 29.1 L of liquid manure on the average, fed in 6 batches (feeding interval: 4 hours); average total hydraulic retention time of digester chain: ca 37 days
27 August to 2 September 2004	MD 1 over-heated to 45°C by mistake
20 October to 15 November 2004	Second tracer test in MD2
15 November to 17 December 2004	Operation of MD2 and 3 only (thermophilic-mesophilic)
17 December 2004	Shut-down of plant for the winter season
15 March 2005	Re-starting of model plant
13 April to 6 July 2005	Operation of MD1 and 2 (mesophilic-thermophilic) with a daily load of 15.4 L of liquid manure, fed in 24 batches, hydraulic retention time in MD1: ca 14 days; gas collection from MD1 only

Appendix 3. Overview of measuring instruments and data logging at the pilot biogas plant

Parameter	**Measurement device (supplier)**	**Automatic data logging**	**Daily manual recording**
Digester temperatures	Pt 1000	Continuous	Yes
Filling levels in digesters	Pressure transducer (ENDRESS + HAUSER)	-	Yes
Volume of manure	Electromagnetic flow meters at all three supply pumps, measuring error: ± 0.5 %	Continuous	Yes
Biogas composition	CH_4, CO_2: IR sensors, dual-beam method, measuring error: ± 2 %; H_2S, O_2: electrochemical sensors, measuring error: ± 5 % and ± 0.2 %, respectively (SCHMACK BIOGAS AG)	Yes, at least daily measurement	Yes
Biogas flow to engine	Bellows-type gas meter (Elster-Instromet GmbH)	-	Yes
Air flow for desulphurization	Rotameter (approximate measuring error: ± 10 %)	-	Yes
Status of CHPU	Px-control (Hans-Jürgen Schnell Anlagenbau, ComAP)	Continuous	-
Fuel oil consumption	Counter	-	Yes
Heating energy	Meters for total heating energy, digesters 1 to 3, and emergency cooler	-	Yes
Electrical energy	Meters for electricity generated, fed into and obtained from the grid	-	Yes
Air temperature on site	Thermometer	-	Yes, daily minimum/maxiumum
Ambient temperature	Sensor at nearest meteorological station	Hourly means	-
Ambient pressure	Sensor at nearest meteorological station	Hourly means	-

Appendix 4. Overview of measuring instruments and data logging at the model biogas plant

Parameter	**Measurement device (supplier)**	**Automatic data logging**
Digester temperatures	Pt 100	Yes
Filling level monitors	Potentiometer (ENDRESS + HAUSER)	Yes
Volume of manure	Operating time of supply pumps	Yes
Biogas composition	CH_4, CO_2: IR sensors, dual-beam method, measuring error: ± 2 %; H_2S, O_2: electrochemical sensors, measuring error: ± 5 % and ± 0.2 %, respectively (AWITE Bioenergie GmbH)	Yes
Total biogas flow	Drum-type gas meter (RITTER), resolution: 0.2 L	Yes + manual recording
Biogas flows from individual digesters	Time for filling a gas bag to a specified pressure	Yes
Biogas temperature	Pt 100 in gas meter	Yes
Ambient temperature	Pt 100 on site	Yes
Ambient pressure	Sensor at nearest meteorological station	Yes

Appendix 5. Values of dry matter and volatile solids contents (mean ± standard error) of samples from the digesters of the pilot and model biogas plants during meso-thermo-meso operation

	DM **% (m/m)**	**VS** **% of DM**	**VS** **% (m/m)**
Digester 1	7.1 ± 0.5	75.6 ± 2.6	5.3 ± 0.18
Digester 2	6.5 ± 0.5	73.0 ± 2.3	4.8 ± 0.16
Digester 3	5.9 ± 0.4	70.5 ± 1.8	4.2 ± 0.09
Model Digester 1	6.5 ± 0.5	75.5 ± 2.0	4.8 ± 0.16
Model Digester 2	6.0 ± 0.2	72.7 ± 5.8	4.3 ± 0.14
Model Digester 3	5.5 ± 0.3	70.5 ± 1.7	3.9 ± 0.20