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).