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DEGRADATION OF PRION PROTEINS IN BIOTECHNOLOGICAL PROCESSES

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Introduction

Transmission of BSE through animal faeces has not been proven so far. For scrapie, however, there are various indications that this way of transmission exists.

So the question was raised whether animal faeces may contain low amounts of prion proteins, undetectable by current methods, thus contaminating the environment. Due to the high resistance of prions to enzymatic digestion and microbial clearance, they might then persist and accumulate. The same scenario would apply to the use of non-disinfected liquid or solid manure from affected farms. Suitable treatments to remove residual infectivity are clearly needed.

There are several aspects of potential inactivation of prions during composting of animal based organic matter, or during their treatment in thermophilic biological gas facilities.

A possible inactivation of the prion protein by proteases had been shown[1,2,4]. One aim of this study is to determine whether the complex bacterial community of an anaerobic and thermophilic digester might have the ability to inactivate prion proteins.

Material and methods

Thermophilic Anaerobic digester

Two digesters, one litre volume each, were run at 55°C under anaerobic conditions in batch mode by adding cattle manure and swine stomach over a period of five weeks. Hamster brain tissue (HBT) of not infected animals was added to one of the digesters to adapt the bacterial population to utilize the brain tissue. The other digester remains as a reference control. Every second day new substrate was added, the pH-value and the total gas volume produced was measured continuously.

Isolation of proteolytic bacteria

A two step procedure was adopted to isolate proteolytic bacteria out of the digester material. In the first step anaerobic and thermophilic bacteria were isolated by using a standard medium (Merck). The second step involved a standard medium with addition of azocasein (1,5%). After 24 to 48 h incubation at 55°C the agar plates were flooded with trichloride acid (10%).

Bacteria which secreted proteases showed a clear zone around the colonies due to the precipitation of not decomposed azocasein.

Determination of the proteolytic activity

For the determination of the proteolytic activity azocasein as a substrate was used. The samples were incubated at 55°C for 24 hours. The proteolytic reaction was stopped by the addition of trichloride acid (10% end concentration) and measured spectrometically at 450 nm.

Zymography

A 10% SDS-polyacrylamide gel which contained 0.1%gelatine was used for the zymography. After electrophoresis the samples were incubated overnight at 55°C and stained with Coomasie blue.

Results

Thermophilic Anaerobic digester

During the digestion process the pH-values of both digesters ranged from 7.5 to 8.0. Total gas volume measured was comparable too (see figure 1).

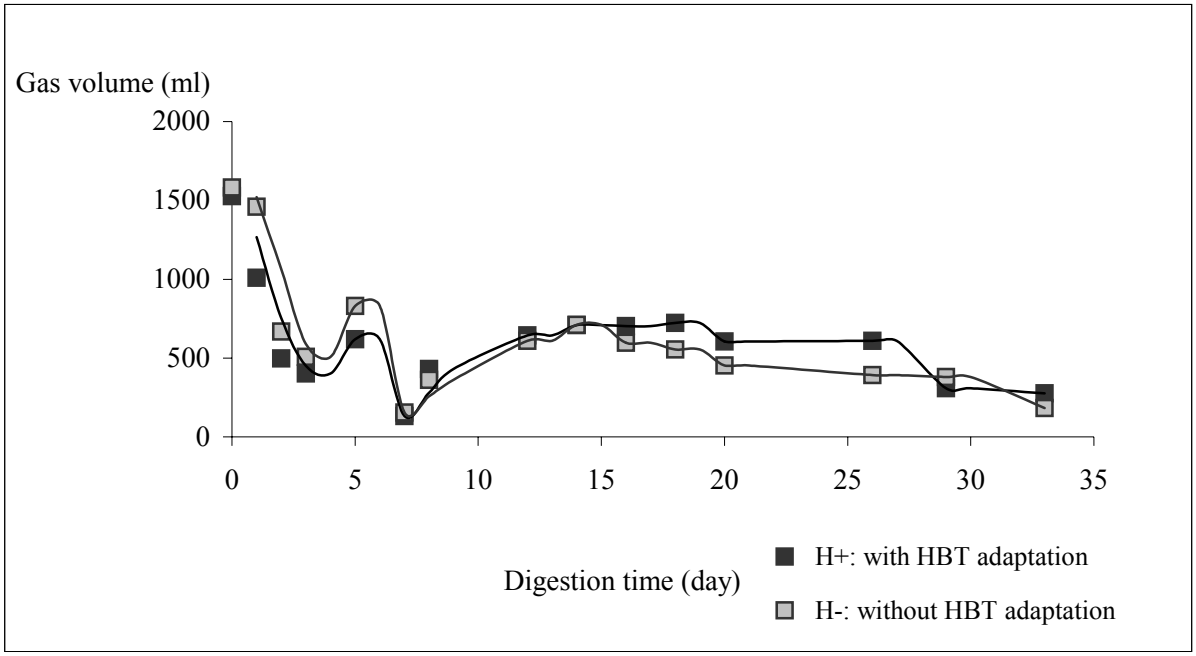

Figure 1: Total gas volume produced from the HBT adapted and the not HBT adapted ***thermophilic anaerobic digesters.***

Isolation of proteolytic bacteria

A total number of 123 bacteria could be isolated out of the digesters (see table 1). 59.3% (73 of 123) of those did not show any proteolytic activity after cultivation on azocasein plates. 40 bacteria (32.6%) showed clear proteolytic activity. Poor activity was determined in 8.1% of the isolated bacteria.

Table1: Total number and percentage of isolated proteolytic bacteria

	Proteolytic activity			
	not proteolytic	proteolytic	Unclear	Total
Total number	73	40	10	123
Percentage (%)	59,3	32,6	8,1	100

Determination of the proteolytic activity

Highest proteolytic activity was measured at the beginning of the digestion (see figure 2). Proteolytic activity tended to increase faster in the HBT adapted digester but also decreased rapidly after approx. 8 days fermentation. After the decrease of the proteolytic activity both digesters showed even similar activity.

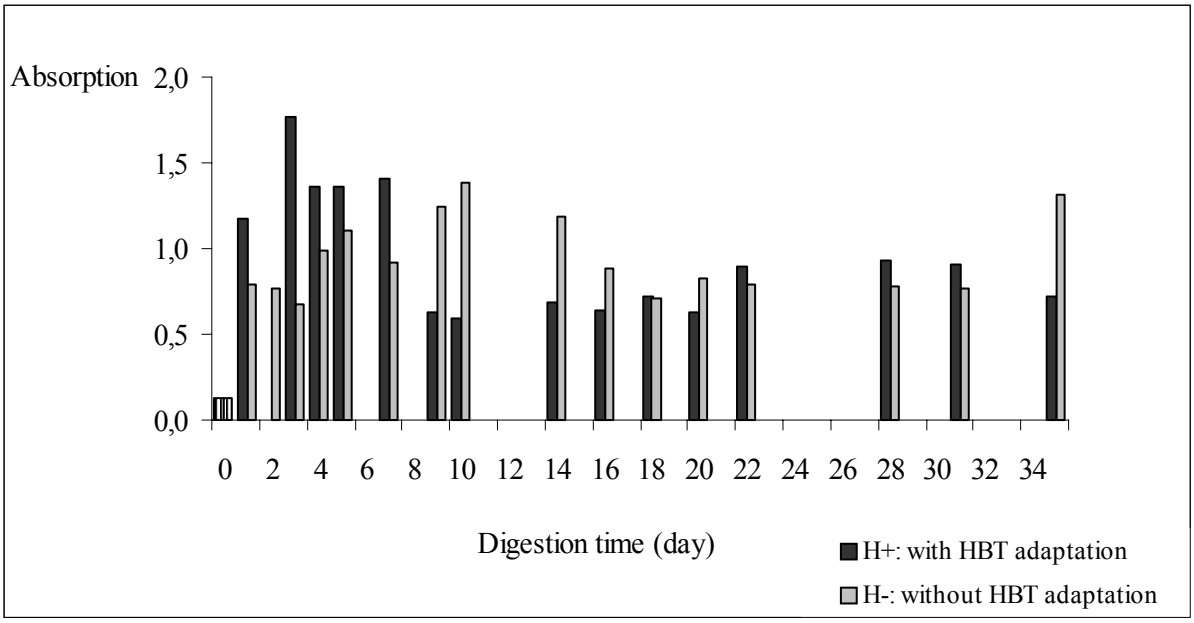

Figure 2: Proteolytic activity determinated during the biogas production

Zymography

In the zymography approximately similar activity was determined in both digesters. On the other hand the distribution of the bands showed that the adapted digester developed a proteolytic activity which varied significant from those found in the reference digester. Characteristic bands seen in the samples of the adapted digester appeared constantly over the whole digestion period.

Discussion

The aim of the study was to investigate the inactivation of prion protein by proteases in a thermophilic biological gas facility during the digestion process. The preliminary results indicate that a specific proteolytic bacterial population was developed in the brain tissue adapted digester. Due to the fact that highest proteolytic activity was found at the beginning of the digestion process degradation of the HBT might be increased in the hydrolytic phase of the digestion process. The proteolytic activity decreased but then remained stable during the process. This finding indicates that the proteolytic bacterial population remained constant too[3].

Ongoing investigations: The *in vitro* inactivation of prion protein by bacterial proteases will be investigated. The isolated proteolytic bacteria will be tested for their capability to inactivate the prion protein. Furthermore it will be examined whether the proteolytic activity of one or more isolated bacteria correlates with the proteolytic activity of the thermophilic anaerobic digester.

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