Behaviour of prion-proteins in anaerobic and aerobic treatment of animal by-products

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Introduction

Transmissible spongiform encephalopathies (TSE) are neurodegenerative diseases of animals and human beings which are characterised by the accumulation of proteinase K resistant rods (PrP^{SC}) in brain tissue. Scrapie in sheep and bovine spongiform encephalopathy (BSE) in cattle are the most known TSE diseases in animals. The use of animal by-products for the production of biogas is taken into consideration due to the fact that animal tissues such like blood and fat are able to increase the yield of biogas during anaerobic thermophilic digestion (ATD). But the possible use of animal by-products also involves some concerns about a possible resistance of PrP^{SC} to the fermentation during the biogas process by virtue of the resistance of PrP^{SC} to physical, chemical and biological inactivation. Therefore the question whether the proteases in anaerobic thermophilic digesters or in composting process are able to inactivate the PrP^{SC}The aim of this study was to investigate stability PrP^{SC} in such processes and to find out if proteases isolated from an ATD can digest PrP^{SC} . Therefore the proteolytic activity of the fermentation process was determined in laboratory scale reactors and infected hamster brain homogenate (HBH) was incubated with the isolated proteases from the corresponding processes.

Material and Methods

Laboratory-scale thermophilic anaerobic bioreactors made of stainless steel were placed in a water bath to guarantee a constant temperature of 55°C and was operated for five months before the ATD material was used for this study. The ATD was filled with 750 ml of cattle manure, which was stored at 4°C during the whole study period and agitated periodically every 30 minutes for 2,5 minutes. During the last fermentation period of 35 days samples of 15 ml were taken and replaced by 15 ml cattle manure and 2 g of animal byproduct (crude swine stomach). The 15 ml substrate samples were used to determine the pH values and the proteolytic activity of the ATD. The produced gas was measured constantly by using a gas counter (MilliGascounter®, Ritter Type MGC).

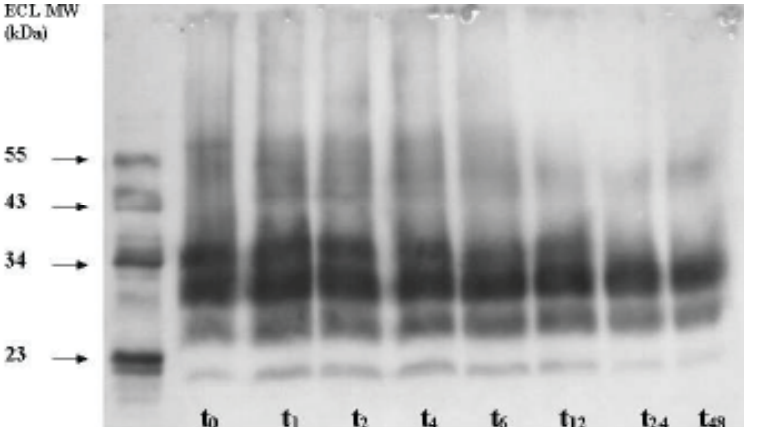
The composting experiments were performed in a pressure ventilated temperature steered (50 °C) semi-technical compost reactor with a volume of 600l. It was fed with source separated biowastes from Stuttgart. Six small gauze sacks filled with brain of infected Hamsters were exposed ad different places in the reactor and exposed for a total time of 12 weeks.

Results

Concerning inactivation of prion protein in the anaerobic process the study showed that the proteases from an ATD was not able to inactivate or to reduce the PrP^{SC} over a period of 35 days. Six out of 35 days of fermentation were chosen to determine the inactivation potential of proteases from ADT for the reduction or degradation of the PrP^{SC}. All tested fermentation days showed similar Western blotting results. The Western blotting result of the final day of fermentation is showed in Figure 1. In general the PrP^{SC} band was observed during the entire fermentation period and the incubation period of up to 48 hours.

A tendency in terms of a reduction of the band strength was observed throughout the 48 h incubation period in all tested samples. During the first 6 hour of the inactivation experiment no change in the intensity of the PrP^{SC} could be detected. After 12 hours of incubation a significant reduction of higher molecular bands has been observed but no reduction of the typical PrP^{SC} band. The same result could be observed after 24 and 48 hour of incubation. The negative control was carried out by using distilled water instead of reactor substrate. Like in the inactivation experiments with reactor substrate no reduction of the PrP^{SC} band was observed during the 48 hour incubation time. The clearly noticeable reduction of higher molecular bands observed in the reactor substrate experiment had not been observed in the negative control. Since during an anaerobic thermophilic fermentation process normal proteins are degraded to peptides and finally to amino acids, an additional control was carried out by using unfermented cattle manure instead of reactor substrate to distinguish between the proteolytic activity of unfermented and fermented cattle manure. Like in the inactivation experiments with the reactor substrate a reduction of higher molecular protein bands could be observed after 12, 24 and 48 hours of incubation, but no reduction or degradation of the characteristic PrP^{SC} band could be detected within 48 hours.(Fig 1) The titer in the eposed prion-protein dropped immediately after filling into the reactor for 2log , butb remained constant at a level of betwewnn 102 to 103 over the 20 day period which resembles the most common mean hydraulic exposure time (Table 1).

Fig. 1: Immunoblot image of the inactivation experiment at the 35th day

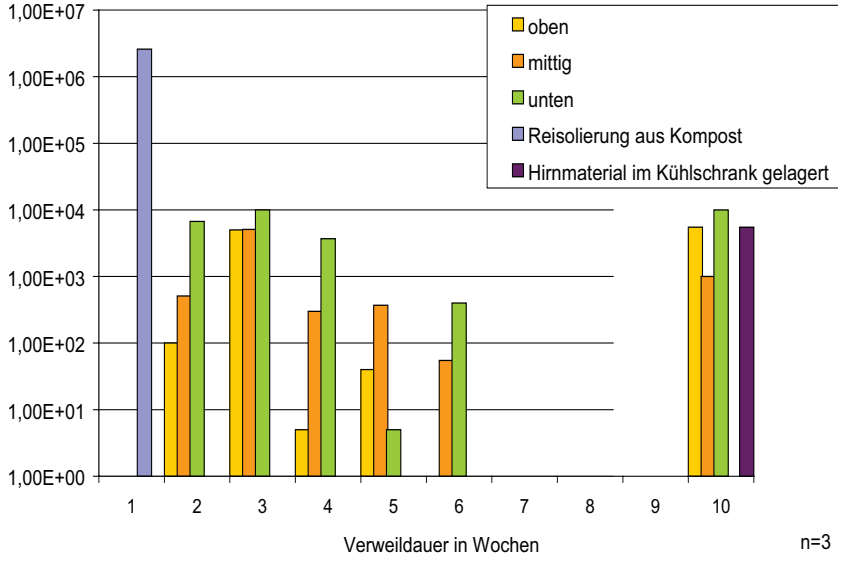


Probennahme	
I. Adaptationsphase	
0-Probe (10 min)	4,0 x 103
1 d	4,6 x 103
5 d	6,4 x 102
10 d	2,4 x 103
15 d	6,2 x 102
20 d	3,8 x 102
Gespikte Proben zur Kontrolle der Methode	3,0 x 105

Discussion

We did not observe a degradation of the PrP^{SC} during our experiments but a reduction of higher molecular bands. Those bands might represent (XX) prion proteins which were not released from the cell membranes after the homogenization of the brain tissue. The infectious PrP^{SC} accumulates at the cell membranes and is able to form polymers (Kazlauskaite et al.

2003; Kourie and Henry 2001; Rymer and Good 2000). For the diagnosis of the PrP^{SC} a treatment with proteinase K is required so that the PrP^{SC} is released into the solution and separated from PrP^{SC} due to its proteinase resistance (Prusiner 1998). The fact that both cattle manure and reactor substrate were able to reduce the detection of higher molecular bands suggests that increased proteolytic activity in the ADT did not contribute to the hydrolysis of HBH protein during the inactivation experiments and indicates that proteases from untreated manure are able to hydrolyze cell membrane as well. This indicates that thermophilic anaerobic treatment does not contribute specific to reduce the remaining risk related to substrates from animal by.-products in any way.



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