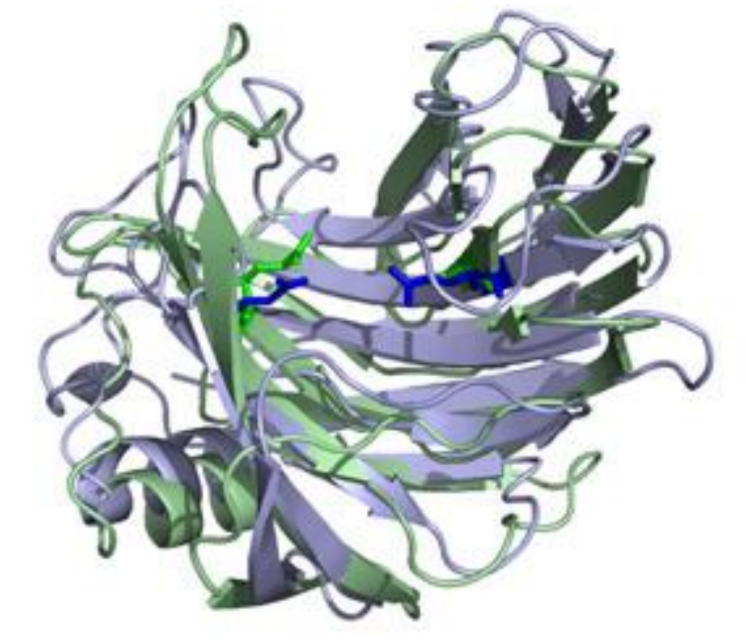


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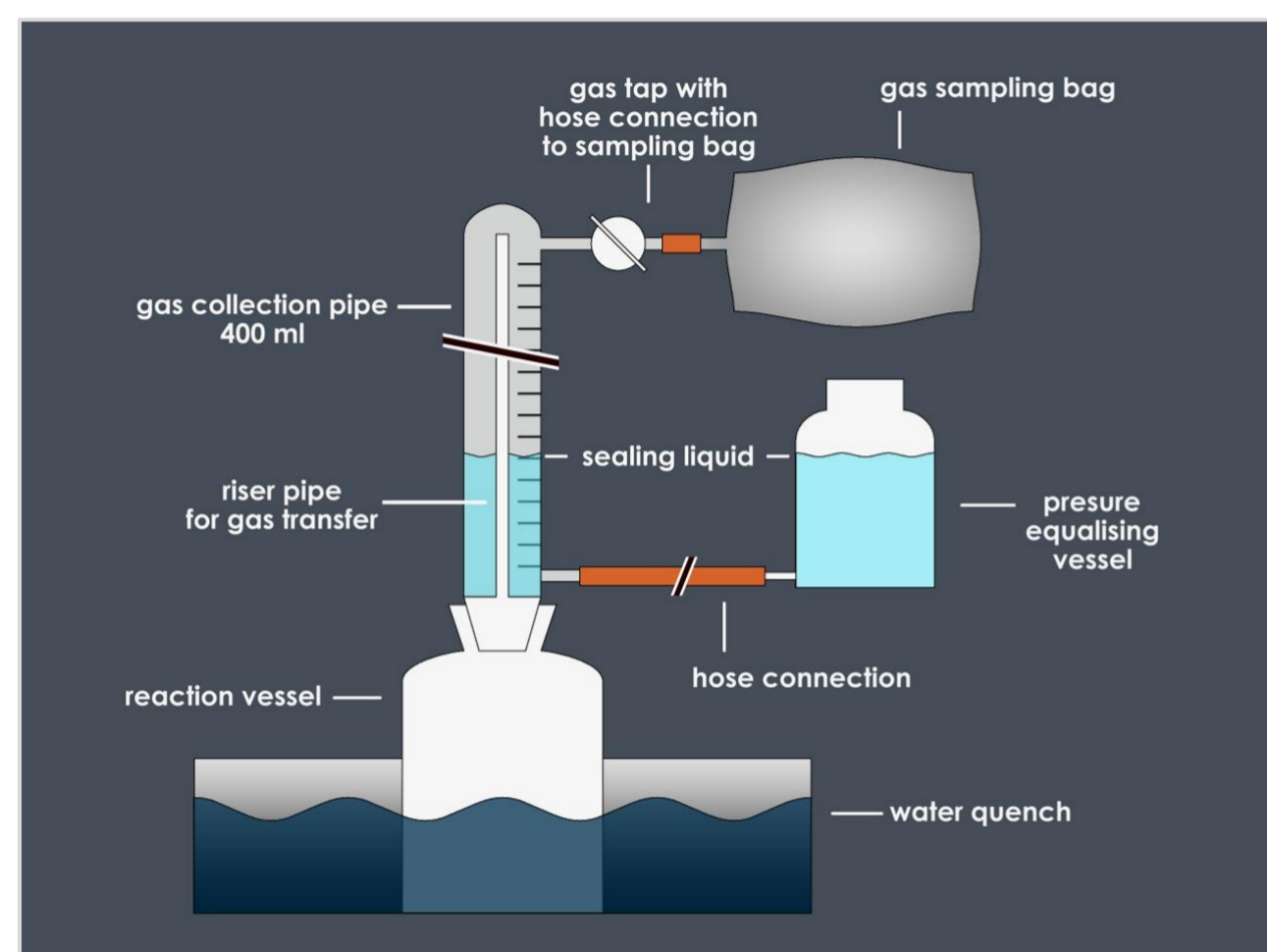
Introduction

To improve substrate utilization and energy production in biogas processes biological pretreatments of biogas substrates can be an option for higher resource and energy efficiency. Therefore commercial or new created enzyme preparations were tested in laboratory and practical trials. New enzyme extracts contain a high variety of enzymes such as pectinase, xylanase and cellulase. Extracts were generated for instance by biorecycling of by-products from edible mushroom production in food industry. Secondly, faeces of herbivores of agricultural industry were examined. Currently snails are under investigation. The main focus is given to organisms which produce their own enzymes for metabolism. By means of catalytic active extracts cost-intensive microorganism cultivation for enzyme production can be saved and industrial waste can be used. The objective is an enhanced conversion of hardly degradable substances of plant cell walls like lignocellulose.



Xylanase + Glucanase, Gilbert 2010

Experimental setup



Laboratory trials

Mesophilic fermentation process
In accordance to VDI Directive 4630
Retention time 30-35/65 d
Inocula: Fermentation residues, biogas plant Pölling or Digested sludge, sewage plant Wansdorf
Organic Dry Matter (ODM)-Ratio 0.4
500 ml fermentation vessels
Determination of samples in triplicate
Gas measured: CH₄, CO₂, O₂
Statistic: Dunnett's test, IBM SPSS Statistics 20



Practical trial

Mesophilic fermentation process
2000 m³ bioreactor
Retention time 63 d
Loading rate 5-5,8 kg ODM/m³*d
Substrates: Maize, sweet sorghum and rye silage, corn
Enzyme concentration 100 ppm
Measured: T, pH, VOA/TAC, content of selected components, degradation rate of lignocellulose, plant performance, gas yields, agitation power, viscosity

Results Commercial enzyme products

A selection of commercial enzyme preparations declared as cellulase, pectinase or laccase from various origins were used in enzyme assays. The selected additives were added separately or as mixture in different stages of the biogas process chain; ensiling, pre-hydrolysis or biogas reactor.

When using a pectinase preparation in combination with a cellulase and laccase preparation in the ensiling process an significant increase in methane yield (l_N/kg ODM or Fresh matter) of 28 % (p<0.001) could be measured. Besides this, enzyme B1 was found to enhance the methane production in biogas reactors when maize or rye silage were used as substrate. The application of enzyme B1 was easily feasible and economical. In addition the named preparation showed an overall positive impact on all kind of lignocellulose rich materials used in laboratory tests (see table

below). The preparation contains a pectinase with highest level of enzyme activity and as side activity hemicellulase and cellulase.

Afterwards the preparation was tested in a bioreactor of a large-scale industrial biogas plant. The following impacts could be measured or were calculated:

- Significant increase in energy production (see table below)
- Significant viscosity reduction (18 %)
- Correlation between viscosity and agitation power
- Improved conversion of ODM, fat and lignocellulose
- Enzyme addition affected fatty acid spectrum in lab-scale tests and concentration in large-scale tests

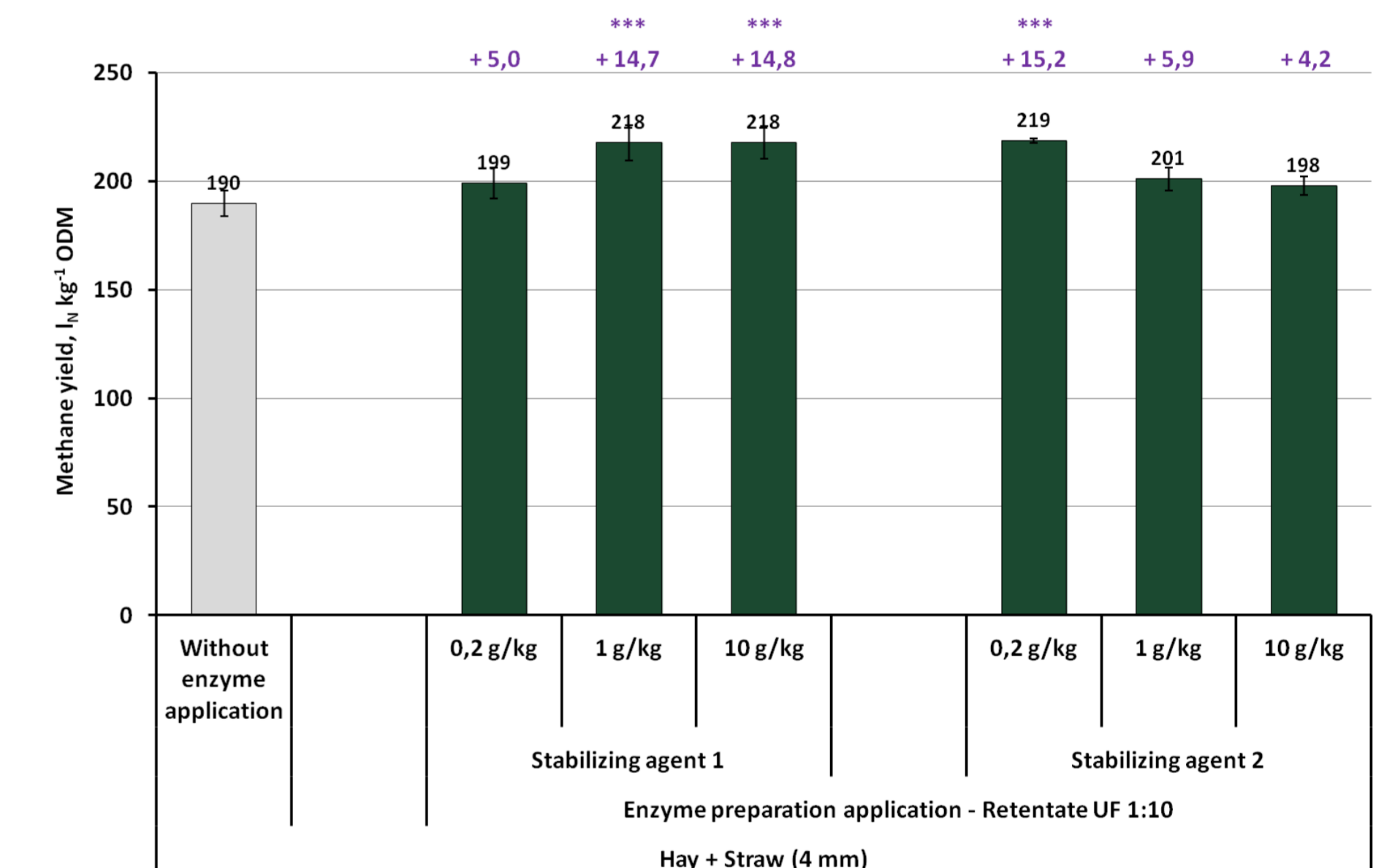
By-products of mushroom cultivation

The by-product of mushroom production, exemplary fungus shiitake, has to be processed as follows: chopping, submerging and pressing the remained cultivation substrate; obtaining a raw enzyme extract.

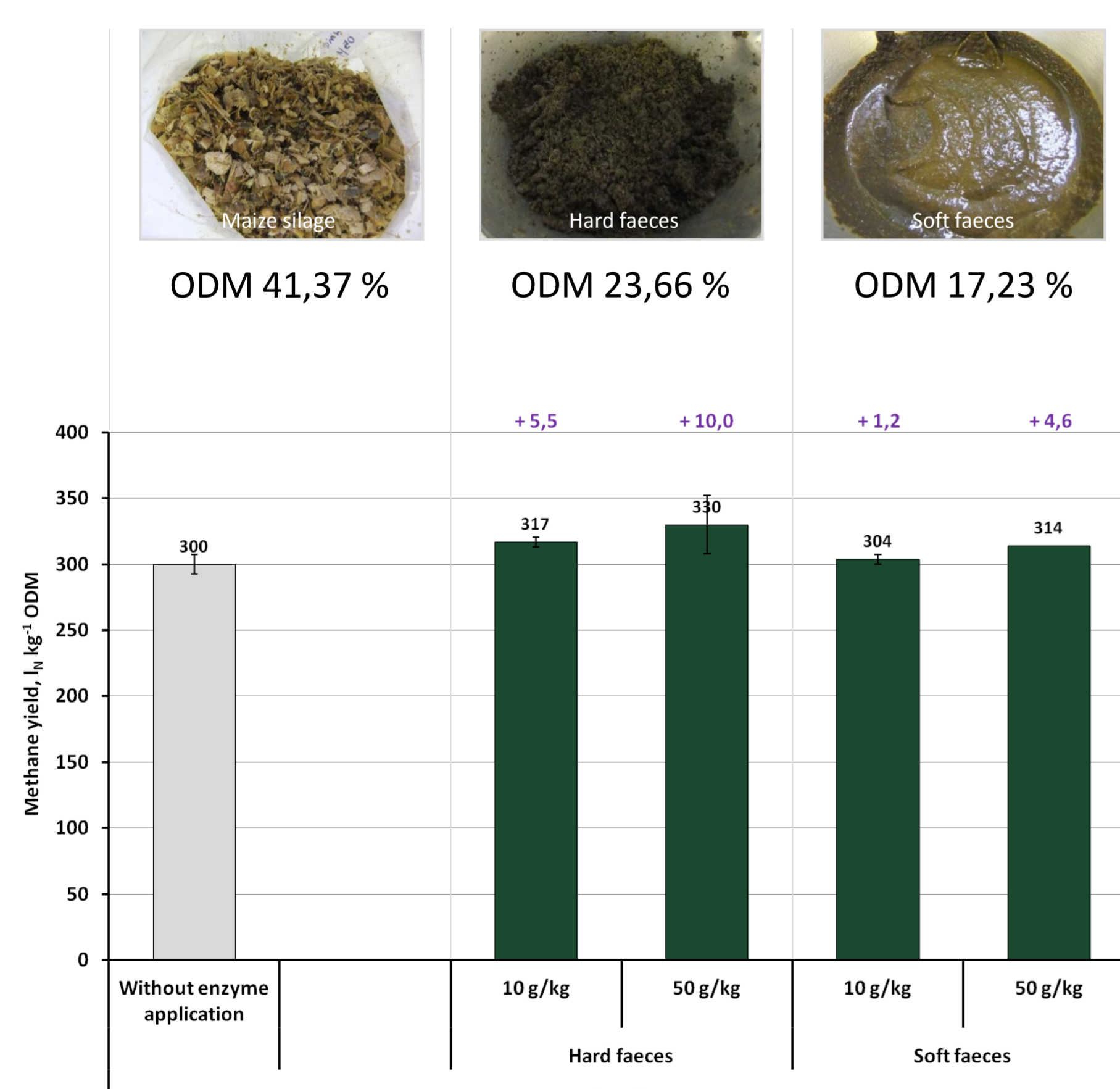
By concentrating the raw enzyme extract via ultra-filtration the protein content of the raw extract could be increased. The obtained enzyme extract was stabilized by two different agents (prototypes) and used like the raw enzyme extract for methane production from hay and straw; grinded to 4 mm. With a concentration of 50 g raw enzyme extract or 0.2 g prototype (agent 2) per kg substrate significant increases in methane yield (p<0.01 or 0.001) were achievable (see figure).



Laboratory trials [35/65 d]						
Enzyme application	Biogas yield l _N /kg ODM		Increase %	Methane yield l _N /kg ODM		Increase %
	Without	With		Without	With	
Maize silage (8 mm)	445/457	484/516	9/13	262/274	286/316	9/15
Rye silage (8 mm)	429/439	444/468	3/7	240/254	255/280	6/10
Waste of mushroom production	104	126	21	51	64	26
Hay + straw	311	340	9	176	189	7
Practical trial [65 d]						
Enzyme application	Specific energy production kWh/t Fresh matter		Increase %			
	Without	With				
Maize, rye and sweet sorghum silage, corn	450	473	5			



Results Faeces of herbivores



Enzyme extracts of molluscs



Several snails are able to use a wide spectrum of substrates for their metabolism, e.g. plants, other snails or dog faeces.



These snails produce an extensive spectrum of enzymes for digestion. In molecular biologic analyses approx. 1000 of proteins and protein domains could be analyzed. Identified were enzymes of all enzyme classes for instance enolase, ATPase, GTPase and transketolase.

In acetone powders of slaughtered, processed molluscs cellulolytic and hemicellulolytic activities were measurable. The named powder, a commercial product as well as further own created enzyme extracts were able to release carbohydrates in hydrolyses of press cakes (solid phase of digestate) of biogas processes. The next step is e.g. an application of snail extracts in methane potential tests.

Conclusion

It could be shown that commercial enzyme preparations can significantly increase the methane yield of substrates rich in lignocellulose in mesophilic lab-scale tests as well as large-scale industrial biogas plants.

By-products of agribusiness and food industry could substitute commercial preparations or serve as beneficial co-substrates; more research is needed.

The enhancement of methane yield is associated with the lignocellulose degradation (data not shown). The studies demonstrated that with increasing enzyme concentrations the degree of decomposition of hemicellulose and/or cellulose and thus the methane production increased. Additionally, it could be shown that the methane yield can decrease by enhancing the enzyme concentration because of product or substrate inhibition.

The use of by-products of mushroom production to stabilize and enhance biogas production was patented.

