Production and application of enzyme mixtures for increasing biogas yield in the South of Viet Nam

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1 Introduction

Bioreact GmbH develops new kinds of complex enzyme mixtures (containing cellulases, xylanases, pectinases, esterases, acetylases etc.) by co-cultivation of fungal strains on plant residuals in a solid-state fermentation process. Enzyme mixtures produced in this way are highly efficient in the degradation of plant material in the course of biogas production in Germany, resulting in enhancement of the specific biogas yield, fluidization of the fermenter content and stablization of the anaerobic fermentation process.
The aim of the present project was to adjust this process of enzyme production to the particular conditions (e.g. available substrates, climate), present in the South of Viet Nam. Furthermore, a solid-state bioreactor prototype was developed for the production of small amounts of enzyme mixtures at the University of Can Tho. Using this bioreactor, an enzyme mixture produced in Viet Nam showed systematic effects on the degradation of pig manure and rice straw during batch tests of biogas production from these substrates on the lab scale, which were carried out by the 'AG Stoffflüsse' (University of Bonn). An experiment on the pilot scale in the DESAR-biogas process did not lead to clear results.

2 Material and Methods

2.1 Identification of appropriate fungal strains and substrates

For indentification of appropriate fungi, 11 strains were cultivated in 500-ml-Erlenmeyer flasks on rape meal (50% moisture content) at 30°C under sterile conditions. Fungal growth was observed during cultivation and various hydrolytic enzyme activities were measured after 6 days of cultivation. Eyzyme activities were determined photometrically in aqueous extracts using the method of König et al. (2002). The two best performing fungal strains were cultivated on 5 different substrates as well as on some binary combinations (1:1 WW:WW), afterwards. Screening for the most suited substrate was carried out as before.

2.2 Identification of appropriate bioreactor designs and fermentation protocol

Three lab-scale solid-state bioreactor types were investigated with respect to their applicability for cultivation of the best combination of micro-organisms and substrate achieved so far: screw-type, tubular-type and tray-type. Micro-organisms were grown for several days in these bioreactors and process performance was again assessed by visual characterization of fungal growth and determination of enzymatic activities after the fermentation. The focus was on moisture control, here.
Using the tray-type bioreactor, a fermentation protocol to be applied in Viet Nam was developed by performing fermentations under different material:volume densities and air flow

rates. Now, the focus was on the control of maximum temperature during processing. These studies were accompanied by computer simulations.

2.3 Saccharification and biogas production on the labscale

The potential of the fermentation product produced in the optimal way for saccharification of substrates was investigated by adding small samples of the fermentation product (4 g DM) to 8 g DM of substrate in 100 ml total volume. After 24 h of incubation at 30°C the amount of reducing sugars was determined photometrically using the di-nitro-salicylic-acid method. Control experiments were performed using heat inactivated enzyme preparations. Hydrolysates were filtered and used for investigation of biogas production. For that, 100 mliters of hydrolysate were added to 200 ml of liquid manure and incubated at 37°C for 24 h under gentle stirring. Evolving biogas was collected and measured in upward-down oriented and flooded graduated cylinders.

2.4 Investigation of stability of enzyme mixtures

To measure stability of enzyme mixtures, samples were incubated in the presence of different agents (see Results and Discussion) and the enzymatic activities were determined afterwards as described before. Alternatively, mixtures were dried in a drying chamber.

2.5 Production of enzyme mixture in Viet Nam using a bioreactor prototype

Enzyme mixtures were produced in a prototype of a solid-state bioreactor (developed by Bioreact GmbH) at the University of Can Tho, mainly following the former developed protocol but using a local strain of *A. nig*. The process was cooled by a thermostate (suited for the application in tropical aereas) and a water bath together with using radiator coils mounted at the bottom of the bioreactor. Aeration was performed through small holes in the lower back wall of the bioreactor body and a fan, integrated into the upper back wall, was used for air outflow. 4 trays were arranged in an inclined manner within the bioreactor to facilitate air flow between the trays (see Figure 1). Samples of an enzyme mixture produced in Can Tho were tested on their effect on biogas production from pig liquid manure and rice straw by the 'AG Stoffflüsse' (University of Bonn) as well as in the DESAR-pilot plant.

Figure 1: Bioreactor prototype

3 Results and Discussion

3.1 Identification of appropriate fungal strains and substrates

Table 1 shows the results of screening of the 11 fungal strains for their applicability for enzyme production in Viet Nam.

Table 1: Relative activities of various hydrolases after cultivation of 11 fungal strains on rape meal (FDA = Hydrolysis of fluorescein-di-acetate, Cel = cellulase, Xyl = xylanase, Amy = amylase, Pec = pectinase, Pst = pectinesterase, β-Glu = β-glucosidase, α-Gal = α-galactosidase, β-Xyl = β-xylosidase)

	FDA	Cel	Xyl	Amy	Pec	Pst	β-Glu	α-Gal	β-Xyl	Σ
N. int.	0.59	0.67	0.19	0.07	0.00	0.16	1.00	0.14	0.31	**3,13**
A. nig.	1.00	1.00	0.33	0.06	1.00	0.25	0.55	0.78	1.00	**5.97**
A. tub	0.91	0.47	1.00	0.04	0.77	1.00	0.36	1.00	0.89	**6.44**
A. ory	0.05	0.07	0.03	1.00	0.31	0.33	0.65	0.21	0.02	**2.67**
T. vir	0.10	0.01	0.05	0.02	0.26	0.78	0.27	0.19	0.11	**1.79**
T. har	0.05	0.03	0.11	0.01	0.71	0.34	0.45	0.45	0.32	**2.47**
F. oxy	0.04	0.09	0.03	0.10	0.00	0.00	0.62	0.09	0.14	**1.11**
P. chrA	0.01	0.03	0.01	0.05	0.00	0.05	0.86	0.25	0.02	**1.28**
P. chrB	0.03	0.07	0.06	0.03	0.00	0.12	0.49	0.08	0.03	**0.91**
E. amA	0.09	0.24	0.07	0.00	0.00	0.54	0.18	0.15	0.09	**1.36**
E. amB	0,00	0.04	0.00	0.19	0.14	0.00	0.03	0.21	0.01	**0.62**

From analysis of the enzyme spectra shown in Table 1 it turned out, that *A. nig.* and *A. tub.* produced the highest levels of hydrolytic acitivity during cultivation at the conditions described above. Both strains also grew well at 30°C. Accordingly, these strains were used for further investigation.

Table 2: Relative activities of various hydrolases after cultivation of *A. nig.* and *A. tub.* on 5 substrates and some binary combinations (1:1 WW:WW) (SM = soy meal, SF = soy flakes, SC = soy cake, M = maize, R = rice, G = growth)

		SM	SF	SC	SM/M	SF/M	SC/M	SM/R	SF/R	SC/R	SM/SF	SM/SC	SF/SC
A.t.	**G**	++	++	++	+	+	+	+	+	+	++	+	++
	FDA	1.00	0.37	0.05	0.43	0.30	0.23	0.40	0.12	0.20	0.84	0.75	0.68
	Cel	0.79	0.40	0.07	0.44	0.36	0.26	0.62	0.25	0.74	1.00	0.95	0.50
	Xyl	1.00	0.19	0.52	0.14	0.08	0.08	0.35	0.09	0.18	0.32	0.45	0.21
	Pec	0.24	0.21	0.14	0.02	1.00	0.02	0.20	0.14	0.13	0.28	0.27	0.24
	Est	0.23	0.04	0.26	0.00	0.01	0.01	0.18	0.07	0.06	0.34	1.00	0.51
	Σ	**3.26**	**1.21**	**1.04**	**1.03**	**1.75**	**0.60**	**1.75**	**0.67**	**1.31**	**2.78**	**3.42**	**2.14**
A.n.	**G**	++	++	++								++	
	FDA	1.00	0.69	0.46								0.79	
	Cel	1.00	0.24	0.39								0.65	
	Xyl	0.51	0.15	0.34								1.00	
	Pec	1.00	0.12	0.27								0.59	
	Est	0.45	0.10	0.42								1.00	
	Σ	**3.96**	**1.30**	**1.88**								**4.03**	

Table 2 shows the results of cultivation of *A. nig.* and *A. tub.* on 5 different substrates as well as some binary combinations as described above. Both strains grew well on all substrates and substrate combinations. However, enzyme acitivities developed best on soy meal and a mixture of soy meal and soy cake. Due to the simplicity of using only one substrate, soy meal was selected for the further experiments.

3.2 Identification of appropriate bioreactor designs and fermentation protocol

A mixed culture of *A. nig.* and *A. tub.* was cultivated in different bioreactor types as described in Material and Methods. From these types, the tray type bioreactor turned out to be most suited for cultivation of fungi in Viet Nam due to offering the lowest risk of contamination as a result of moisture accumulation. Moisture could be controlled most suitable within this bioreactor type.

Identification of an efficient fermentation protocol focused on temperature control. For that, temperature development in dependence of substrate:volume density (represented by the number of trays per bioreactor, see Figure 2) and rate of aeration were investigated.

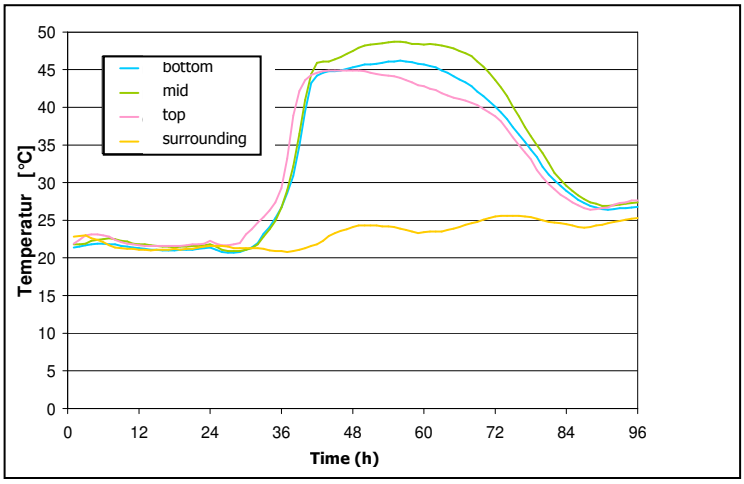

Figure 2: Temperature profile in 3 of 5 trays during fermentation in wooden tray fermenters

In the wooden test fermenters used for the experiments, a number of 3 trays gave the best results with respect to temperature profiles (this corresponds to about 70 kg of substrate per m^3 bioreactor volume). In addition, aeration rates of 3 l/(min·m^3) during the first 2 days and of 50 l/(min·m^3) during the second 2 days gave best results with respect to air supply. Following this way, maximum temperature did not exceed 45 °C during the process.

3.3 Saccharification and biogas production on the lab scale

An enzyme mixture resulting from processing according to the optimal protocol was applied to saccharification of kitchen waste and wheat straw, followed by investigation of biogas production from the hydrolysates (see Material and Methods). The results are presented in Table 3.

Table 3: Effect of application of an enzyme mixture on saccharification of and biogas yield from kitchen waste and wheat straw

	kitchen waste	wheat straw
Additional sugar concentration in mg/mliters	1.9	1.1
Additional biogas yield in mliters	140	18
Additional biogas yield in %	26	11

The results in Table 3 show, that in the case of enzymatic treatment of kitchen waste all of the additionally deliberated suger was transformed into biogas. This gave an increase in specific biogas yield of 26%.

This effect was not that clear in the case of straw. However, even there was an increase in the specific biogas yield of 11%. Hence, these experiments showed clear effects of an enzyme mixture produced according to the protocol to be applied in Viet Nam on the degradation and biogas production from two special substrates classes, which were originally intended to be applied in the biogas plants in Can Tho (DESAR, Hans Huber AG) and Hoa An (B^3 GmbH).

3.4 Stability of enzyme mixtures

To guarantee enough stability of the enzyme mixtures produced in the South of Viet Nam to carry out the pilot experiments under the climate conditions there, different agents were applied and the evolution of enzyme activities over time was observed. The chemicals and substances tested were: glycerine, propionic acid, a starch containing agent, a ligno-cellulose containing agent, rape meal and sugar beat pulp. However, none of them gave satisfactorily results. Hence, drying was investigated, too, and the decrease of moisture in a chamber at 35°C is shown in Figure 3. Under these conditions, there was only little loss in enzyme acitivities during drying.

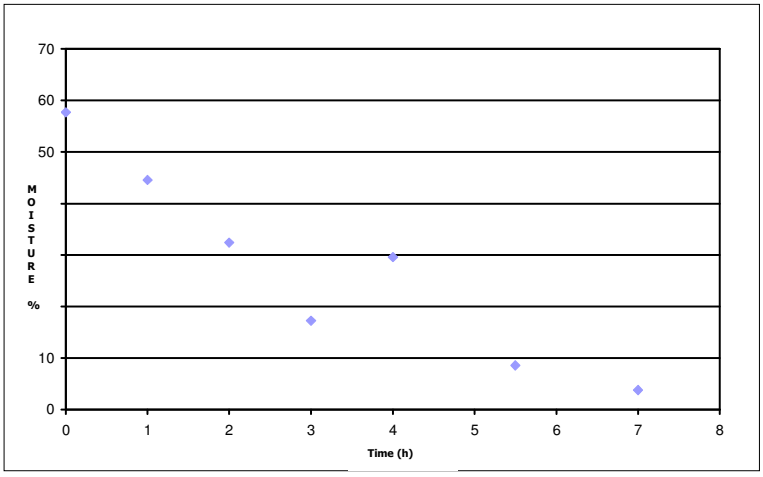

Figure 3: Drying profile of enzyme mixture in a dry chamber at 35°C

3.5 Production of enzyme mixture in Viet Nam using a bioreactor prototype

Based on the above results, a bioreactor prototype (see Figure 1) was developed and taken into operation at the University of Can Tho. Using this prototype and a local strain of *A. nig.*, an enzyme mixture was produced at the University of Can Tho and its effect on biogas production from liquid pig manure and rice straw was investigated by the 'AG Stoffflüsse' (University of Bonn, see there for more detailed information). Experiments were done as batch tests over 21 days on the lab scale. The results showed an increase in the rate of biogas production from both substrates due to enzyme action in the order of 10-20%. However, this increase in biogas production rate was not statistically significant due to scatter between the repetions. Nevertheless, time courses of cumulative biogas yield with and without active enzyme mixtures were as expected from theoretical considerations, that is, there was a transient deviation in the observed yields.

3.6 Test of enzyme mixtures in the DESAR-pilot plant

In addition to that, an enzyme mixture produced by *A. nig.* growing on soy meal together with pulp as an iductor was applied to the DESAR-biogas process running on brownish water. On the lab-scale, slight saccharification of brownish water could be shown using this enzyme mixture. On the pilot scale, there seemed to be an effect visuable on the absolute biogas yield. However, due to the strong scatter in the COD of the daily input and the lack of continuous monitoring, no significant effect on the specific biogas yield could be detected.

4 Conclusions and Outlook

The results optained so far point to the general applicability of enzyme mixtures produced in the South of Vietnam to the optimizationi of biogas production from waste material as well as for other applications. However, further studies have to be done.

5 References

König, J., Grasser, R., Pikor, H. and K. Vogel (2002): Determination of xylanase, β-glucanase and cellulase activity. Anal. Bioanal. Chem. 374, 80-87