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

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Keywords: biogas, enzymes, saccharification, solid-state fermentation, fungi, bioreactor

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