Enhancement of biogas production by addition of hemicellulolytic bacteria immobilised on activated zeolite IPUS meth-max®

S. Weiß[1], M. Tauber[2], W. Somitsch[3], R. Meincke[1]†, H. Müller[1], G. Berg[1], G.M. Guebitz[1]

[1]Department of Environmental Biotechnology, Graz University of Technology, Petersgasse 12, 8010 Graz, Austria; [2]IPUS GmbH, Werksgasse 281, 8786 Rottenmann, Austria; [3]Engineering Consultant, Wiedner Hauptstrasse 90/2/19, 1050 Vienna, Austria

Aim

Recalcitrant plant cell structures represent a barrier in the fermentative biodegradation process in single- and two-stage biogas reactors. Since cell wall compounds of cellulose and hemicellulose contribute up to 60 % of the total biomass of energy plants (e.g. maize), approaches concerning a more efficient de-polymerisation of these chemical barriers are required in order to optimise the fermentation efficiency and to increase methane yields. Here we show a new strategy for the enhancement of biogas production from hemicellulose-rich substrates through the application of immobilised microorganisms, specialised on the degradation of xylan, a major constituent of hemicelluloses.

Methods

Common biogas fermenter sludge was used as inoculum to enrich an hydrolytic population in synthetic medium containing xylan powder as sole carbon source, batch-wise fed under anaerobic mesophilic conditions (i.e. 35 ° C). The growth of xylanolytic bacteria was determined by cell counts and the measurement of extracellular xylanase activity (enzyme assay). Furthermore, specific enrichment of hemicellulolytic bacteria during the process was confirmed by using single strand conformation polymorphism (SSCP) analysis based on amplification of the eubacterial 16S rDNA fragments. Enriched hydrolytically active population was then immobilised on trace metal activated zeolite to study effects on the biogas production in batch-culture experiments (VDI conform) by measuring cumulative methane yields and the determination of fatty acids as characteristic intermediates by high performance liquid chromatography (HPLC) analysis.

Results

Xylanase activity increased continuously during subsequent enrichment cycles by up to 162% in synthetic medium grown cultures and was significantly higher ($P < 0.05$) when compared to the origin seeding sludge (fig. 1). Resulting band patterns from SSCP analysis revealed cluster formations for xylan-grown cultures clearly distinguishable from second-stage sludge samples (fig. 2). Sequence analyses of conspicuous bands (fig. 3) resulting from the cultivation on xylan led to the identification of *Bacteroides* sp., *Azospira oryzae* (*Dechlorosoma* sp.) and a wide spectrum of diverse species within the order of Clostridiales (Firmicutes). Since members of the genera *Bacteroides* and *Clostridium* are known to be principally responsible the degradation of major plant structural polysaccharides forming acetate, formiate, lactate etc., a favourable change towards a more specialised and hydrolytically active community structure was achieved. The introduction of immobilised bacteria to batch-culture experiments fed with xylan powder led to an significant increase of methane by about 64.1 l CH_4 kg^{-1} ODM at STP in average (starting from day 21 on) which equals 53% compared to controls with pure zeolite (fig. 4). Overall, acetic acid concentrations increased by 20.4 mM in average which equals 88% in the mean, correlating with simultaneously risen methane amounts observed between day 21 and day 26 of the total fermentation time (fig. 5).

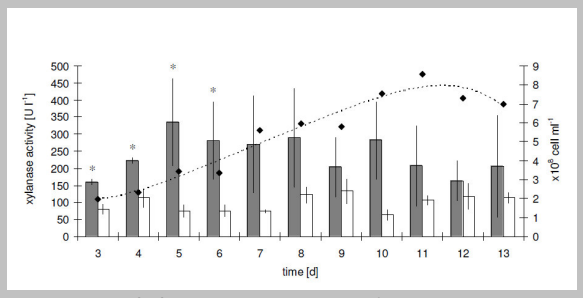

FIG. 1 Xylanase activity in [U l^{-1}] determined during anaerobic cultivation of xylanolytic populations in L47 medium on xylan as mono-substrate (grey columns) and in second-stage sludge without xylan as blank (white columns) over 13 days. Significant differences are defined as $P < 0.05$ and marked with [*] in the diagram. Cell counts ml^{-1} (line) represent the bacterial growth during a single cultivation cycle over 13 days in L47 medium on xylan.

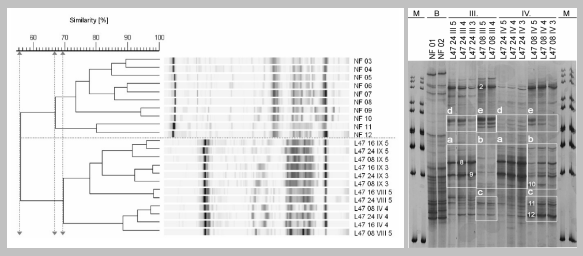

FIG. 2 (left) Dendrogram based on amplified 16S rDNA fragments of bacterial cultures in L47 medium and pure second-stage sludge [NF 03-12] respectively obtained by using eubacterial primers and separated by SSCP. Band patterns were grouped by UPGMA. Double-headed vertical arrows indicate the similarity for the groupings. Sample-Code: L47 = medium, 08/16/24 = replicate number, IV/VIII/IX = cultivation cycle, 3/4/5 = day of cultivation and sample collection. **FIG. 3 (right)** Exemplary non-denaturating polyacrylamide gel from the SSCP-analysis based on 16S rDNA fragments focusing the bacterial community cultured in L47 medium and from second-stage sludge: [M] represents 1 kb DNA-ladder marker lanes. Boxes [2], [8], [9], [10], [11] and [12] represent specific bands selected for DNA extraction and sequencing analysis.

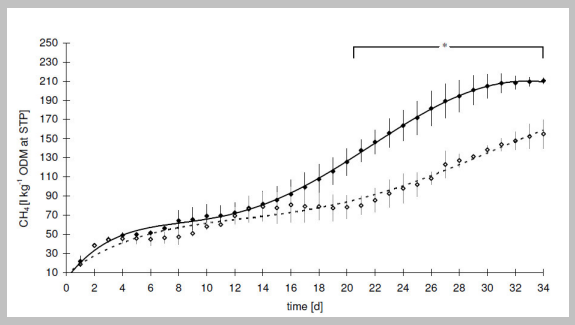

FIG. 4 Cumulative biogas production, i.e. CH_4 in [l kg^{-1} ODM at STP] during discontinuous fermentation in the presence of xylanolytic bacteria immobilised on activated zeolite (black line ●) and control with activated zeolite but without immobilised microorganisms (dotted line ○) at 35 ° C over a total period of 34 days. From day 21 of the fermentation on differences in biogas amounts were significant. Significant differences are defined as $P < 0.05$ and are marked with [*] in the diagram.

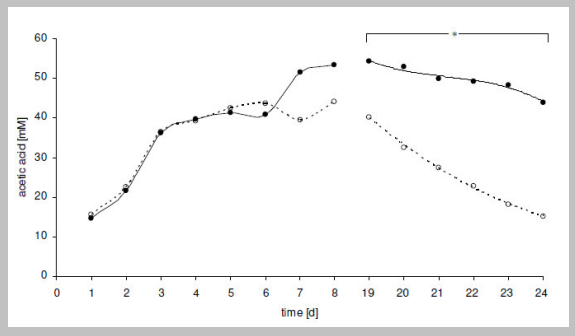

FIG. 5 Acetic acid concentrations obtained from single measurements during discontinuous fermentation at 35 ° C in the presence of xylanolytic bacteria immobilised on activated zeolite (black line ●), control with activated zeolite but without immobilised microorganisms (white line ○). From day 19 on, acetic acid concentrations differ significantly. Significant differences are defined as $P < 0.05$ and marked with [*] in the diagram.