Biotechnology Letters Vol 6 No 6 379-384 (1984)

ANAEROBIC DIGESTION OF SUGAR BEET PULPS

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SUMMARY

Anaerobic digestion of sugar beet pulps was studied in a 70 l digestor with sequential feeding, after enzymatic hydrolysis by Trichoderma harzianum cellulases. During the 130 days feeding, 3.6 m^3 of biogas were produced with an average content of 58 % CH_4 from 270 l of hydrolysed pulps at 20 g VS/l. Average yield and production rate were respectively 0.67 m^3/kg VS and 0.4 m^3/ kg VS and 0.4 m^3/m^3 of digestor per day.

INTRODUCTION

Bioconversion of agro-industry cellulosic by-products is promising for proteins, enzymes, alcohol or biogas production. Sugar-beet by-products are diversely used: yeasts and various metabolites are obtained from molasses, pulps are used for complementation in animal feeding after ensilage.

Several assays have been made to produce cellulolytic enzymes from pulps by culture of moulds in liquid (CONTRERAS et al., 1982; ROUSSOS et al., 1983) or solid media (ROUSSOS et al., 1983).

We used the culture method of fungal growth in solid state fermentation as described by RAIMBAULT and ALAZARD (1980) to produce cellulases by a strain of Trichoderma harzianum. The enzymes obtained were used to hydrolyse pulps before anaerobic digestion.

MATERIALS AND METHODS

1. Microorganism : the organism used for the production of cellulolytic enzymes was T.harzianum CCM F-470 (Czechoslovack Collection of Microorganisms, Brnö, Czechoslovackia). Stock cultures were maintained on Malt-Agar slants (Difco) at 29°C.

2. Aerobic fermentation medium : 100 g of dry pulps were ground (0.5 mm) and moistened with a solution containing : $(NH_4)_2SO_4$, 9.75 g ; urea , 2.38 g ; KH_2PO_4 , 5 g ; distilled water , 100 ml ; pH 4.2 . The mixture was autoclaved at 110°C for 1 h .

Inoculation was made with a spore suspension in distilled water containing Tween 80 (1% v/v) at the rate of 3.10^7 spores/ g dry material. The moisture content was adjusted to 70%.Then, the final mixture was transferred into incubators according to the method of RAIMBAULT and ALAZARD (1980). These columns incubators were placed in a 29°C regulated water bath and aerated with air saturated with water to maintain aerobic conditions.

According to ROUSSOS et al. (1983), the production of cellulases was 2,000 IU FPA (Filter Paper Activity) and 22,000 IU CMCA (Carboxy Methyl Cellulose Activity) from 100 g of initial dry material. This product, enriched in cellulases, was stored at -25°C before utilization.

3. Pretreatment for pulp hydrolysis : 10 kg of wet pulps (20% dry matter) were mixed with 200 g of cellulases enriched pulps and introduced in a reactor in 100 l of water. Pretreatment

was performed at 50°C for 60 h with continuous mixing. At the end, the whole pulps were liquefied and stored in a deep freezer before utilization.

4. Methanogenic fermentation of solubilized pulps : Anaerobic digestion of hydrolysed pulps was conducted in a 70 l digestor. The 35°C temperature control was obtained by circulation of regulated water in an outside jacket. Intermittent recycling of the digestor contents was made by a pump.

The digestor was inoculated with a mixture containing: swine waste 30 l, fermented manure 30 l, fermented swine waste 5 l and water 5 l. The addition of hydrolysed pulps started only at the end of gas production by this initial mixture.

Introduction of solubilized pulps was made once or twice a day by replacing equal volume of digestor juice content.The V.S. content has been increased in a range of one to four during the 18 weeks of fermentation. From threefold increase, loading was performed twice a day.

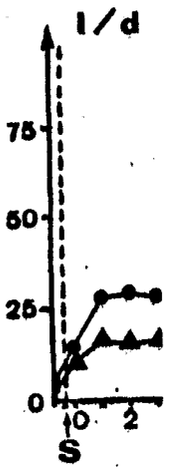
5. Methods of analysis : gas production was measured with a gasmeter and automatically analysed by thermal conductivity with a gas chromatograph (GIRDEL 30) according to the calibration described by GARCIA et al.(1982). Volatiles fatty acids (VFA) were analysed as previously described (GARCIA et al.,1982). The pH value of the digestor contents was followed all along the anaerobic digestion.

RESULTS

During the early 13 weeks, sequential feeding was made with 2 l a day of solubilized pulps (20 g VS/l). Then an increasing dayly load was applied every 2 weeks until it reached four times the initial load. At the end of the experiments, feeding was performed with fermented solubilized pulps in order to observe effects on methanogenesis.

During the 18 weeks of fermentation, the digestor produced 3.617 m^3 of biogas with an average content of 58% CH_4 from 270 l of hydrolysed pulps. Total gas yield was 0.67 m^3/kg VS with an average production rate of 0.4 m^3/m^3 of digestor per day.

As shown in Fig.1, the weekly production of biogas reached a constant value after 1.5 weeks loading time and maintained its rate over the 13 weeks at the same daily load. When double feeding was applied, the gas production increased three times and maintained its rate despite of the next increase of loading. When fermented pulps were added after 18 weeks of fermentation, the gas production decreased dramatically.



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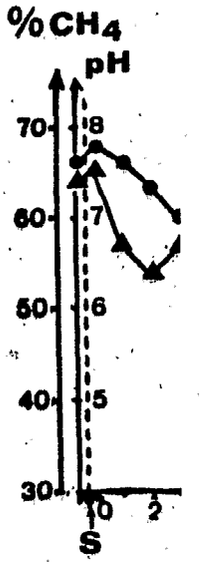
As shown in Fig.2, the gas yield was affected by load increase. The best results appeared for a 4 l of solubilized pulps per day load (80 VS) i.e. 5.7 kg of fresh pulps at 80% moisture per cubic meter per day. The decrease of gas production observed between the 6^{th} and 10^{th} week of fermentation was probably due to the introduction of air during feeding as confirmed by bacterial counts (unpublished results).

Fig.3 shows the relation between pH and CH_4 production. Introduction of fermented pulps brought about an important drop of pH from 7.0 to 4.0 in 2 weeks. Evolution of VFA concentration followed this drop of pH as shown in Fig.4. Propionic acid was the product mainly accumulated. During steady state of anaerobic digestion, VFA concentration was very low. It raised since loading increased threefold.



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DISCUSSION

Current results observed in industrial methanization with solid substrates are : average loading 2 to 6 Kg VS/m^3 of digestor per day, hydraulic retention time 10 to 25 days, gas production 0.5 to 1 m^3/m^3 per day, gas yield 0.2 to 0.3 m^3/kg VS

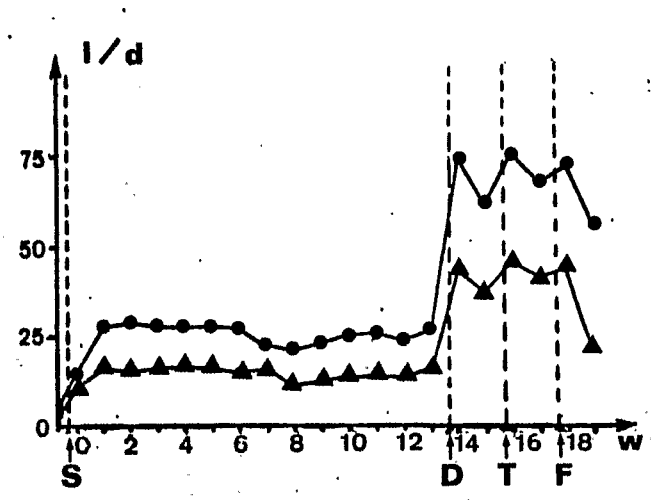

Fig.1- Weekly average production of biogas ● and CH_4 ▲

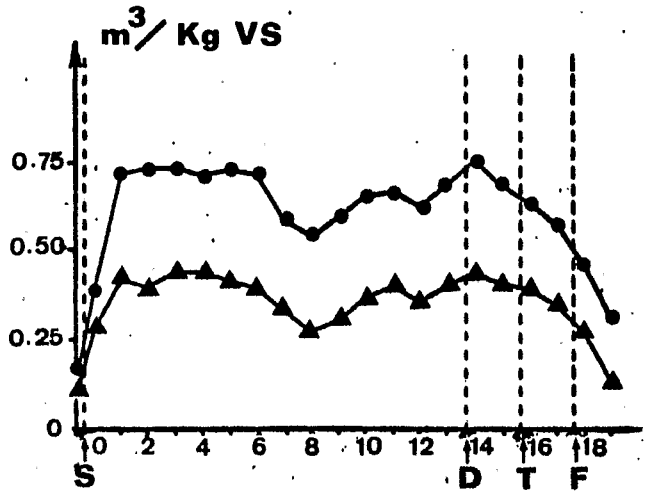

Fig.2- Weekly average yield of biogas ● and CH_4 ▲

S = simple charge loading

D = double charge loading

T = threefold charge

F = fourfold charge

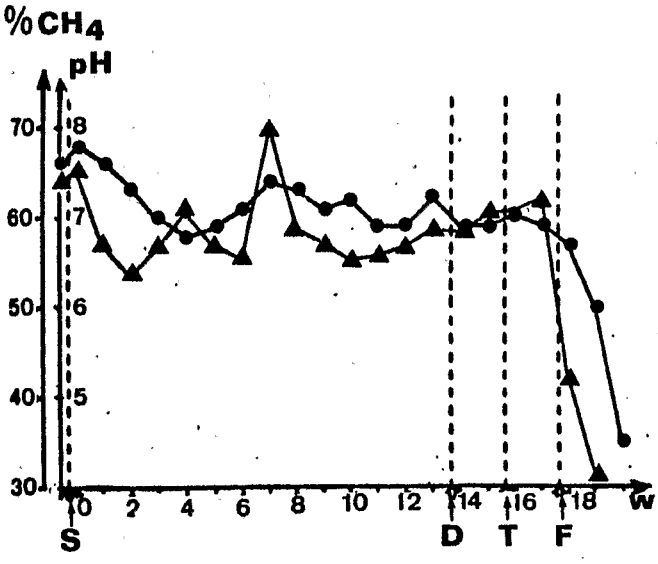

Fig.3- Weekly variations of pH ● and CH_4 evolution ▲ during anaerobic digestion of prehydrolysed sugar beet pulps.

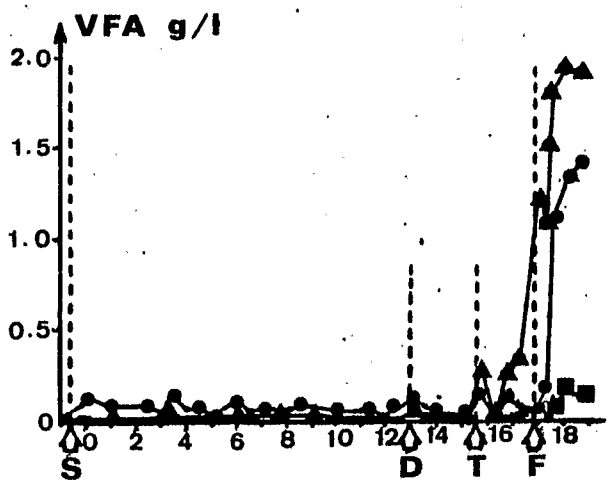

Fig.4- Weekly average production of volatile fatty acids: acetic ● ;propionic ▲ ; butyric ■ .

(NYNS, 1979). Other results obtained with sugar beet pulps by LESCURE (1982), without pretreatment, showed that 88% of the carbon content were digested over 96 days in a 25 m^3 digestor by a two stages digestion.

In our experiments, the best conditions to methanize solubilized pulps seem to be : average loading 1 kg VS/m^3 per day, hydraulic retention time 17 days, gas production 1 m^3/m^3 per day, gas yield 0.74 m^3/kg VS. Enzymatic hydrolysis of pulps releases a lot of metabolizable sugars from cellulose and probably from hemicelluloses and pectines. Given these considerations, the suitable COD loading is not allowed to be as high as for crude substrates. However, biological pretreatment widely increases the susceptibility of carbon, which explains the high yield obtained.

Acknowledgement : this work was supported by a grant n°82.21.091.3261 from the Agence Française pour la Maîtrise de l'Energie (AFME).

REFERENCES

CONTRERAS,I., GONZALEZ,R., RONCO,A., and ASENJO,J.A. (1982). Biotechnol.lett. 4 , 51-56 .

GARCIA,J.L., GUYOT,J.P., OLLIVIER,B., TRAD,M., and PAYCHENG,C. (1982). Cah.ORSTOM,Sér.Biol. 45 , 3-15 .

LESCURE,J.P. (1982). Rapport COMES n°80.75.0168, AFME, PARIS.

NYNS,E.J. (1979). XX^e Cycle Perfect.Génie Chim.Belg., Louvain, 17 pp , Univ.Cathol.Louvain.

RAIMBAULT,M. and ALAZARD,D. (1980). Eur.J.Appl.Microbiol.Biotechnol. 9 , 199-209 .

ROUSSOS,S., GARCIA,J.L.,and RAIMBAULT,M. (1983). Ind.Alim.Agric. 100 , 449-452 .