Proceedings of the information symposium under the EEC programme on recycling of urban and industrial waste, Luxembourg, 8–10 May 1984

Secretariat of the symposium and publication arrangements:

P. P. Rotondò
Commission of the European Communities
Directorate-General Information Market and Innovation

ANAEROBIC DIGESTION AND CARBOHYDRATE HYDROLYSIS OF WASTE

Edited by

G. L. FERRERO,

M. P. FERRANTI

Commission of the European Communities

and

H. NAVEAU

Catholic University of Louvain

ELSEVIER APPLIED SCIENCE PUBLISHERS
LONDON and NEW YORK

(4) Calzada, J. F. 1982. Biogas de Subproductos del Beneficio Húmedo de Café. En Simposio Internacional "Avances en Digestión Anaerobia" Organizado por ICAITI, UAM, IMETA. México 25-27 Octubre.

(5) Young, M.A. 1982. Biometización de las Pulpas de Café. Alimentación Alternada o Mezclada con Estiércol de Bovino. En Simposio Internacional "Avances en Digestión Anaerobia". Organizado por ICAITI, UAM, IMETA. México 25-27 Octubre.

(6) Mandujano, M.I., A. Felix and A.M. Martínez. 1981. Biogas, Energía y Fertilizantes a partir de desechos orgánicos. Manual para el Promotor de la Tecnología Olade.

ANAEROBIC CONVERSION OF AGRICULTURAL WASTES TO CHEMICALS OR GASES

P.N. HOBSON, W.G. REID and V.K. SHARMA

Rowett Research Institute, Bucksburn, Aberdeen, Scotland

Summary

Naturally-ocurring, mixed, undefined bacterial cultures can convert agricultural wastes to biogas in anaerobic digesters. From the mixed digester flora, anaerobic bacteria can be obtained which can be used in the production of chemicals from starches or fibres.

An amylolytic bacterium producing acetic, butyric and lactic acids hydrogen and carbon dioxide was selected from about 140 mesophilic an thermophilic amylolytic bacteria isolated from anaerobic digesters. Th paper describes experiments undertaken to obtain maximum production o volatile fatty acids, with maximum butyric acid content and minimum lacti acid. In stirred-tank continuous culture maximum butyric acid productio was obtained at low growth rates. To try to obtain low bacterial growt rates and high hydraulic flow rates, various continuous cultures wit immobilized bacteria were tested. A fixed-film, stirred-tank type o fermentor with pH control gave best results. Good results were als obtained with fed-batch cultures. Both these need to be optimised.

Some 400 cellulolytic bacterial isolates were made over a period fro a mesophilic cattle-waste digester. Analysis showed that at least 1 different species and genera were present with varying abilities to degrade cellulose or straw fibres to mixtures of acids, alcohols and gases.

1. INTRODUCTION

Reserves of oil in the world will decrease and eventually be used up. Coal will probably last longer than oil, but again, is a diminishing asset. Nuclear power has so far not fulfilled its promise and nuclear power stations pose large environmental and other problems. Other sources are, or could be, available to diversify energy production and thus help to conserve fossil fuels in the short term. In the longer term these could become major contributors to the world's energy requirements, particularly in countries with no fossil fuel reserves.

Amongst these potential sources of energy is biomass, in the form of special crops and wastes from the processing of crops grown for human or animal foods, and animal and human excreta and wastes from animal processing for foods. Biomass can be converted to energy, or products that can be used as sources of energy, by chemical and physical means; or, in many circumstances more conveniently and better, by microbiological means.

Microorganisms can convert organic matter to microbial biomass for further processing, and/or to gases or non-gaseous fermentation products which can be used directly as fuels or as feedstocks for a chemical industry producing fuels or other chemicals.

With a naturally-occurring, mixed microbial culture and a suitable environment (an anaerobic digester) organic matter of many kinds can be converted to biogas, a mixture of methane and carbon dioxide. The biogas can be used directly as a fuel for burners or internal-combustion engines, or the methane can be purified for use as a fuel or chemical feedstock. This technology has, for many forms of biomass, been developed beyond the laboratory into full- scale plants (see e.g. 1). However, the laboratory work has shown that the production of biogas involves a number of steps and many bacteria with different properties. The first step in anaerobic digestion of solid wastes such as native vegetable matter and residues of vegetable matter in animal excreta or factory wastes is hydrolysis of polymeric plant structural or reserve polysaccharides to monomers or dimers which are then fermented to lower volatile fatty acids, hydrogen and carbon dioxide, the substrates for methanogenic bacteria. While the presence of the methanogenic bacteria 'guides' the fermentation of the many bacteria in an anaerobic digester towards a limited number of fermentation products, individually the bacteria show activities on many substrates and produce mixtures from a wide variety of fermentation

products.

By suppression, for instance by chemical additives (2), of the methanogenic step of anaerobic digestion a mixture of volatile fatty acids may be obtained. This type of process uses the natural mixed culture of an anaerobic digester, and while it has advantages in being operable under non-sterile conditions there could be problems in control of the reactions and in maintenance of the desired mixed culture. For closer control of the reactions and production of defined acids or other fermentation products, use of pure or defined- mixed cultures is preferable.

Methane is almost insoluble in water and is easily removed from the digester contents. Ethanol is very soluble and its removal from fermentation liquids at present involves processes with high energy inputs. Various methods are theoretically possible for removal of organic acids from fermentation liquids, and these could be less energy-consuming than the distillation of alcohol. These include extraction as acids by organic solvents (e.g. 2), production and extraction of esters (3), precipitation of acids as salts, use of resins and electrodialysis, ultrafiltration and other physical methods. The acids or esters can be used as such in manufacturing processes or converted to alkanes, alkenes, ketones, ethers, etc.

Cellulosic structural polysaccharides of plants, in crops and wastes, represent a vast resource of potential feedstocks for micro-biological processing. On the other hand lignification and other structural complexities of fibres lead to low conversions to sugars and fermentation products unless the fibre is disrupted by chemical or physical means. Starch, however, whilst readily available in grains or tubers of tropical and temperate plants, or as a waste from food processing, can be dissolved in fermentation liquids and is almost completely fermentable.

The present work had two aspects. One investigation was designed to enumerate and characterise the fibre-degrading bacteria of an anaerobic digester as an aid to understanding of the process and to see the potential of these bacteria for fibre fermentations. The other was to investigate the possibilities of butyric acid production from starch, as butyric acid has, itself, manufacturing uses, and can be a precursor of other chemicals and fuels. Since butyric acid is one of mixed-fermentation products the work involved the isolation of a suitable bacterium and the manipulation of the fermentation to maximise butyric

acid production. Genetic manipulation to improve yields might be an extension of the work once the possibilities of the wild-type was explored but could not be attempted in a short-term investigation.

2. PRODUCTION OF BUTYRIC ACID FROM STARCH

Some of this work was described in a paper given at the EEC meeting in Broni (4).

Isolation of bacteria. Previous work (5) had suggested that amylolysis was a common property of digester bacteria although the digester feedstocks contained no starch. Some 75 isolates were made initially by culturing dilutions of fluids from pig- or cattle-waste mesophilic digesters using strictly anaerobic techniques based on those of Hungate (6). Of these isolates the majority produced acetic and lactic acids from starch; 7 produced lactic alone or plus a little acetic, and a minority produced acetic with one or more of the acids propionic, butyric, lactic, succinic. Two isolates were finally selected for ability to produce butyric acid with acetic and some lactic and to grow on ammonia as nitrogen source. These were designated A2012 (the taxonomic position is uncertain) and B211, a Clostridium sp. A2012 was selected after initial batch and continuous culture experiments as B211 produced more lactic acid under all conditions and did not grow as well on ammonia medium. Since then 50 more strains have been isolated but only a minority produced butyric acid and none seemed better than A2012. About 20 strains of thermophilic amylolytic bacteria have been isolated at intervals from a thermophilic pig-waste digester, but all have proved unstable on continued subculture and have died out. A2012 was a Gram-negative rod, 0.5 to 2-6 µm, giving rapid growth (μ_{max} $1.6h^{-1}$) in a medium with maltose or starch plus Casitone and yeast extract under CO_2 and with cysteine as reducing agent. In a medium with only NH_4 as N source μ_{max} was $0.4h^{-1}$ and filamentous growth occurred; yeast extract increased growth in NH_4 medium. Urea was not utilised. It fermented starch, maltose, xylose, glucose, fructose, mannose, lactose, raffinose, sucrose, salicin, but not arabinose, glycerol and sorbitol. The extracellular α-amylase was induced by starch or maltose (e.g. specific activity 16 units in glucose, complex N, medium; 260 in maltose; 243 in starch). Optimum growth was at pH 6.7, there was little growth below 6.4 or above 7.2 and none at pH 5.4 or 7.7. Growth occurred at 25, 35, 39°C but not at 15, 45 or 60°C in 24h incubation. All experimental cultures were at 39°C. There was no growth

under aerobic conditions, and a heavy inoculum was needed to ensure gr in non-reduced media under CO_2: growth could occur under N_2. In experimental cultures hydrogen was produced.

Batch fermentations. The bacterium was not inhibited by h concentrations of substrates or acetic or butyric acids, so high sta concentrations could, theoretically, be fermented. However, sta solutions are very viscous and above 10% concentration become progressi ly stronger gels and this led to problems in handling media. So, continuous culture particularly, starch concentration was usually limi to 2 or 4%, and maltose was used in some experiments.

Preliminary experiments showed that even with 1% maltose or star pH control would be needed to prevent limitation of growth by fall in and the results of some batch cultures with high substrate concentrati and pH controlled at 6.6 are shown in Table I.

Table I. Batch fermentations

Substrate	Fermentation products (mM)				C-recovery (%)† on	
	Acetic	Butyric	Lactic	But:acet	substrate used	substrate added
6% maltose*	65	117	6.6	1.8:1	78.9	76.7
8% maltose*	85	99	28.3	1.2:1	91.5	86.4
10% maltose*	109	215	34.7	2.0:1	102	89.7
6% maltose	50	145	35.4	2.9:1	65.0	64.3
10% maltose	106	260	16.6	2.5:1	80.4	74.6
10% starch*	166	249	29.9	1.5:1	72.1	70.6

* Complex N medium (casitone, yeast extract, NH_4), otherwise simple N (NH_4) medium

† Allowing for dilution of culture by NaOH added for pH control.

In other batch cultures with lower maltose or starch concentration up to 4:1 ratio of butyrate to acetate was found in fermentaton products.

Continuous cultures. Some stirred-tank pH controlled, continuou cultures were run along with the batch cultures. These used an anaerobi culture apparatus with pH control and gas mixing (7). Cultures o maltose in complex and simple N media were run and some typical results o a run of over 1000 hours in the complex medium are shown in Table II.

Table II Continuous culture in complex N medium, 1% maltose, pH 6.6

$D(h^{-1})$	Molar proportions of acids			Maltose* recovered (%)	Maltose† used (%)
	Acetic	Butyric	Lactic		
0.11	20	32.6	0.0	83.2	91.4
0.165	12.2	22.3	0.5	53.2	90.6
0.18	12.1	21.1	3.2	54.5	91.4
0.22	14.7	27.0	4.8	69.1	92.0
0.28	17.0	24.0	6.0	77.9	93.1
0.31	15.4	24.5	6.4	82.3	87.8
0.42	10.6	18.7	12.3	68.5	88.4

* Recovered in fermentation products (including CO_2 and H_2) as % of maltose used.

† As % of maltose in medium.

Formation of bacterial extracellular polysaccharide caused problems of foaming in cultures, and recoveries from other cultures suggested that the maltose used and unaccounted for in fermentation products was converted to extracellular polysaccharide. An analysis of one preparation of this gelatinous material gave 9.8% N (dry wt.) glucose (88%) of carbohydrate), with rhamnose, mannose, fucose, arabinose and ribose.

The results of the continuous culture quoted and others on maltose and starch, including lower dilution rates and up to 4% maltose or starch, together with the results of batch cultures, showed that fermentation products varied with growth rate. Little, if any, lactate was formed below about $\mu 0.15h^{-1}$ and the proportion of butyrate increased with decrease in growth rate.

For controlling growth rate and for optimising acid production, some form of continuous culture would be required. On the other hand a low dilution rate is not compatible with maximum volumetric production rate and a small fermentor. In the next experiments various types of fermentors were tested to try to maximise medium hydraulic flow rates with low bacterial growth rates, and to try to overcome problems of bacterial polysaccharide formation and starch viscosity already becoming apparent. Some typical results are given below.

A U-tube fermentor. A type of sludge-blanket fermentor where the bacteria sedimented in the bottom of a U tube was tested, but starch

conversions and acid productions at high flow rate were not good: e. Hydraulic $D = 1.0h^{-1}$; Acetic, butyric, lactic = 4,6,6.2. Conversion maltose to products 23.7%. pH was uncontrolled.

A fixed-film, column fermentor. This fermentor was a water-jacketed vertical, glass tube packed with unglazed pottery chips fitted wi inlet and outlet valves and a sampling system at the bottom. There was pH control system. The conversion of 2% starch in the simple NH_3 medium varied with hydraulic dilution rate and was higher at the low dilution rates. A maximum ratio of butyrate to acetate of 2.94:1 wa obtained at a hydraulic dilution rate of 0.62 h^{-1}, but fermentatio acids were only 14% of medium starch. Lactic acid was low.

Fixed-film, tank, fermentors. In the previous culture the medium wa pumped in drops on to the top of the pottery-chip column and trickle down the column. The whole system was gassed with CO_2 to preserv anaerobic conditions. The column did not suffer from problems o blockage with bacterial polysaccharide, although a good growth o bacteria was attached to the chips. However, it was thought tha although starch conversion was probably being limited by rate of passag of the medium and by fall in pH, channelling through the chips would hav limited contact of medium and bacteria. So the next cultures used wide-bore tube filled with pottery chips, but with inlet and outlet tube arranged so that although flow of medium was downward or upward over th chips the chips were always completely covered in medium - an 'anaerobi filter' or form of tank fermentor with immobilised bacteria. However although a maximum butyric-acetic ratio of 3.59:1 was obtained at nominal hydraulic dilution rate of 0.10 h^{-1} with the 'upflow' fermentor recoveries were low (17.1% of medium starch in this case) and fermentatio appeared to be limited by pH fall. Build-up of bacterial polysaccharid after some hundreds of hours running stopped the cultures.

A retained-biomass fermentor seemed to show possibilities However, pH control is almost impossible to achieve in a filter type o fermentor with granular support medium so another fermentor wa constructed in which the bacteria were retained on a fine nylon mes supported by a coarser metal mesh convoluted radially. A pH contro electrode and NaOH inlet occupied the centre space of the support mesh an a medium feed tube and tube for inlet of CO_2 for gas stirring were also fitted. In these experiments the complex-N medium was used to try t obtain better growth and 10% maltose was used as substrate. Up to 95% o

the added maltose was used and up to 80% of maltose used was accounted for in fermentation products (with the pH controlled at 6.6). In this case butyrate:acetate was 1.26:1 and lactic acid was 5.5% of acetic + butyric. The maximum ratio of butyrate to acetate was 1.94:1, but here C recovery in fermentation products was only 63% of maltose used.

Build-up of bacterial polysaccharide and associated foaming finally stopped these cultures after some hundred hours and this also made determination of culture liquid volumes and so hydraulic dilution rates difficult.

These cultures showed that high conversions of concentrated maltose (and so starch) could be obtained in a retained-biomass fermentor if pH were controlled, but that bacterial polysaccharide could cause problems. In general, the results suggested that while bacterial growth and substrate conversions were highest at a pH about 6.6, lower pH's possibly tended to increase the proportion of butyric acid in the fermentation products.

Fed-batch fermentors. Although the retained-biomass, tank fermentor showed promise, it seemed that a fed-batch type of fermentor could exploit the features of a batch fermentation while giving continuous production. This type of fermentor could also be better in that viscous starch medium could be pumped in rapidly through a large diameter tubed rather than very slowly as in the continuous cultures and that once the initial batch was fermented the high starch- concentration in the initial medium would be diluted by fermented medium making the whole less viscous.

After some preliminary cultures on a 25 ml scale an anaerobic fermentor was constructed in which a medium reservoir could be filled by a pump and the contents then rapidly ejected by gas pressure into a culture vessel with pH control and gas mixing. Samples of culture could also be taken anaerobically. The vessel was designed to take 200 ml of culture initially and this could be increased to 400 ml before half of the culture was removed.

The preliminary cultures with 1% starch and no pH control suggested that a culture with medium additions at 1-2 day intervals could be used and that the system would then come to a steady-stage. The complex-N medium gave better results than the simple NH_3-N medium, as in other cases.

Further experiments used 8% starch in the complex-N medium; pH was controlled at 6.6 and initial culture volume was 200 ml. Steady states

were obtained in about 5-10 days and 25 or 50 ml medium could be ad
every 48 hours (with 5 ml removed for analysis) to give about
conversion of starch to fermentation products with a butyrate:aceta
ratio of 1.5-1.6:1 and lactate 1.5-2% of the other acids (total about
mM). Daily additions resulted in about 60% fermentation of the starc
Stopping the feeding for an extra 2 days resulted in 100% sta
fermentation. Foaming and bacterial polysaccharide production was not
large problem in these cultures.

The results show that although the hydraulic dilution rate is ve
small compared with even the slowest of the continuous cultures, t
fed-batch cultures do give promising results for acid production.

3. CELLULOLYTIC, FERMENTATIVE BACTERIA FROM AN ANAEROBIC DIGESTER

These bacteria were isolated from a 150 l mesophilic, stirred-ta
digester that had been processing cattle wastes for nearly 5 years (8) a
at the time of the isolations it was treating separated dairy-cattle was
at a retention time of 21 days at 35°C. The majority of isolations we
made from cultures inoculated with dilutions of digester contents taken
11 days over a period of about 3 weeks. The cultures contained
substrates either filter-paper powder, native, ground barley-straw
delignified straw. Anaerobic, cellulolytic bacteria were found up to t
10^6 dilution of digester contents, although numbers varied from day
day. Of the isolates, 367 were tested for ability to hydroly
filter-paper, and representative isolates then tested for str
hydrolysis and in some cases cotton-fibre hydrolysis. The properties
many isolates were investigated in detail and they were provisional
identified with known genera and species of bacteria, or in some cases n
species names were suggested.

Further isolations were made from an anaerobic continuous culture
which medium flowed over cotton thread retained in the culture vesse
This was inoculated from a batch culture containing cotton thread a
inoculated with digester contents. The culture was run for more than 10
hours and actively digested the cotton.

The survey, the most comprehensive so far reported on digest
cellulolytic bacteria, showed that the isolates had a wide range
abilities to degrade prepared cellulose (filter paper) but that degrada
tion of this cellulose was not necessarily correlated with ability
degrade straw. Xylanolytic activity was also found in most strains. Mar

of the bacteria were spore-forming and a large proportion were classified as Clostridium spp. Their temperature and pH optima were suitable for growth in a digester, and the bacteria appeared to exist in a population in a dynamic equilibrium.

Loss in weight of straw in batch cultures ranged from near zero to about 50%. Major fermentation products varied and were various mixtures of formic, acetic, propionic, butyric, lactic, succinic acids; ethanol, butanol, hydrogen and carbon dioxide. The proportions of fermentation products might be altered, and the potential exists for use of these bacteria in direct conversions of cellulose or plant fibres to acids, alcohols and gases of use as chemical feedstocks, fuels or solvents. This work has been described in detail in a thesis (8) and will be published elsewhere.

4. CONCLUSIONS

The results obtained show that by using either retained-biomass tank cultures or fed-batch cultures a continuous fermentation process could be set up to produce volatile fatty acids from starch. High starch concentrations can be fermented and the acid concentrations obtained in these cultures compare well with those produced in other small-scale experiments reported in the literature: e.g. 4.5 and 10 to 15 or 20 g acetic acid l^{-1} in batch fermentations with glucose as substrate (9,10); up to 45 g acetic acid l^{-1} in batch culture and 30-34 g l^{-1} in continuous culture from ethanol (11,12); up to 304 mM mixed acids, 95% acetic, in batch culture with undefined, mixed, bacterial population from CO_2/H_2 (13); about 200-350 mM mixed acids in continuous or batch cultures with undefined, mixed bacteria, from cellulosic substrates (14). The proportion of butyric acid in the fermentation products has varied, but in the cultures producing most acid was rather more than 1.5 x acetic. Higher proportions of butyric than this were obtained and might be produced in the best fermentations by allowing a period for conversion of acetic to butyric acid in the absence of carbohydrate or adjusting the culture pH to slightly more acid values. Problems with bacterial polysaccharide production can occur, but can be overcome. Search for a naturally occurring mutant with no polysaccharide production has not so far been successful.

The recovery of acids at the concentrations produced here should be practically and economically feasible. Recovery of bacterial cells,

amylase, and hydrogen from the H_2/CO_2 fermentation gases formed might a be possible.

The work on digester cellulolytic bacteria showed that it would possible to convert cellulose directly to various acids, alcohols gases and that it should be possible to alter the products by manipulat of the fermentations.

REFERENCES

1. HOBSON, P.N., BOUSFIELD, S. and SUMMERS, R. (1981). Methane Production from Agricultural and Domestic Wastes. Applied Scienc Publishers, London.
2. LEVY, P.F., SANDERSON, J.E. and WIRE, D.L. (1981). Development a process for production of liquid fuels from biomass. Biotechnology and Bioengineering Symposium 11, 239-248.
3. DALTA, R. (1981). Production of organic acid esters from biomass- novel processes and concepts. Biotechnology and Bioengineering Symposium 11, 521-532.
4. HOBSON, P. N. and REID, W.G. (1983). The production of acids from starch by anaerobic bacteria. Fourth Specialized Meeting ANAEROB DIGESTION, Broni, 17-19 May, 1983.
5. HOBSON, P.N. and SHAW, B.G. (1974). The bacterial population of piggery-waste anaerobic digesters. Water Research 8, 507-516.
6. HUNGATE, R.E. (1950). The anaerobic mesophilic cellulolytic bacteria. Bacteriological Reviews 14, 1-49.
7. HOBSON, P.N. and SUMMERS, R. (1967). Continuous culture of anaerobic bacteria. Journal of General Microbiology 47, 53-65.
8. SHARMA, V.K. (1983). Isolation and Characterisation of Cellulolytic Bacteria from a Cattle Waste Digester. Ph.D. Thesis, Aberdeen.
9. SCHWARTZ, R.D. and KELLER, F.A. (1982). Isolation of a strain of Clostridium thermoaceticum capable of growth and acetic acid production at pH 4.5. Applied and Environmental Microbiology 4 117-123.
10. SCHWARTZ, R.D. and KELLER, F.A. (1982). Acetic acid production Clostridium thermoaceticum in pH-controlled batch fermentations acidic pH. Applied and Environmental Microbiology 43, 1385-1392.

11. GHOMMIDH, C., NAVARRO, J.M. and DURAND, G. (1982). A study of acetic acid production by immobilized Acetobacter cells: oxygen transfer. Biotechnology and Bioengineering 24, 605-618.

12. GHOMMIDH, C. and NAVARRO, J.M. (1982). A study of acetic acid production by immobilized Acetobacter cells: product inhibition effects. Biotechnology and Bioengineering 24, 1991-1999.

13. LEVY, P.F., BARNARD, G.W., GARCIA-MARTINEZ, D.V., SANDERSON, J.E. and WISE, D.C. (1981). Biotechnology and Bioengineering 23, 2293-2306

14. PLAYNE, M.J. (1980). Microbial conversion of cereal straw and bran into volatile fatty acids - key intermediates in the production of liquid fuels. Food Technology in Australia 32, 451-456.

DEVELOPMENT OF AN AUTOMATIC EQUIPMENT FOR THE STUDY OF ACID-BASE EQUILIBRIA FOR THE CONTROL OF ANAEROBIC DIGESTION

F. COLIN
Scientific Manager, Institut de Recherches Hydrologiques
NANCY, France

SUMMARY

Search for rapid response methods enabling the early detecti disturbances in the functioning of methane fermentation plants. Sele of methods based on the study of acid-base equilibria in the aq phase of the medium. Modelling of simultaneous equilibria and develo of experimental determination allowing to gain the knowledge o concentrations which characterize the physicalchemical condition o medium as to the danger for the fermentation to be stopped by an in tion of toxicity action:

$[H^+]$, $[OH^-]$, $[CH_3COO^-]$, $[CH_3COOH]$, $[H_2CO_3]$, $[HCO_3^-]$, $p\,CO_2$, $[NH_4^+]$, $[NH_4OH]$

Demonstration of the validity of a method using the acid-base titr curves of the fermenting liquid. Automatisation of the method throug development of a titration apparatus with continuous flow and response.