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DATE: 10/19/87

MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18

FROM: AID/SCI, Victoria Ose VO

SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. 2, E-18

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2 E-18

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PROGRESS REPORT

THE CONVERSION OF LIGNOCELLULOSICS TO ETHANOL AND/OR MICROBIAL BIOMASS PRODUCT: THE INTERMEDIATE ACID APPROACH

AID PROJECT NUMBER: 932-5542

Presentado por el ICAITI a

ROCAP
Office of the Science Advisor
AID/Washington

Energy Officer
Bureau of Science and Technology
LAC/DR, AID/W

Guatemala, November 1983

INTRODUCTION

This report describes the activities which have been undertaken during the second six-month period in the project entitled "The conversion of lignocellulosics to ethanol and/or microbial biomass products: The intermediate acid approach" sponsored by the Office of the Science Advisor of AID-Washington. The Limited Scope Grant Agreement covering this project was signed in Guatemala by the Directors of ROCAP and ICAITI on August 27, 1982. The first progress report for the project was presented in April of 1983, and in that document were included the modifications made to the original proposal according to instructions by AID and changes in staff at ICAITI.

The activities which have been carried out during the second six-month period are: establishment of protocols for pre-treatment of substrates, initial digestibility studies of the substrates, acquisition and handling of pure cultures, mantenance of locally obtained mixed cultures described in the previous report, trips to the USA for consulting, technical information and courses, ordering and purchase of equipment and supplies. Details follow.

1. Substrate pre-treatment

1.1 Substrates

The following lignocellulosic substrates are under study in the laboratory:

a) citronella bagasse (*Cymbopogon nardus*)

b) lemon grass bagasse (*Cymbopogon citratus* or *C. flexuosus*)

c) sugarcane bagasse (*Saccharum*) from the EX-FERM process

1.2 Pre-treatments

The following pre-treatment procedures have been developed:

1.2.1 Organosolv

Pretreating raw material with a mixture of ethanol: water (60 : 40, w : w) in the presence of small amounts of sodium hydroxide (2% of the dry material on a weight base, expressed as Na_2O). Solid to liquid ratio is 1:6. Cooking time is four hours, this time including that necessary to reach 175°C, about 140 minutes, and about 100 minutes at 175°C. The digester is then cooled rapidly by submerging it in water. There are 6 two-liter reactors, heated in an oil bath, and rotating on a shaft at 6 rpm.

The procedure described above is repeated, but a catalytic agent is added, anthraquinone, at 0,05% based on the weight of the dry material.

For both Organosolv variants the following parameters are evaluated:

- pH of cooking liquors before and after the process
- total non-volatile solids content of fresh and spent liquors

The following pretreatments will be developed shortly:

1.2.2 Alkaline solid phase treatment

Various alkaline chemicals will be sprayed on a heap of the bagasse to be treated and the material will be left at ambient temperature for about one week, immediately followed by its further testing.

1.2.3 Gaseous treatment

SO_2 will be used using a similar technique as in 1.2.2.

1.2.4 Biological delignification

A series of white rot fungi have been ordered from culture collections. A technique will be developed to grow them in solid state culture.

The following pretreatments will be done by other laboratories with whom we have established communication and exchange of results:

1.2.5 Phenol treatment

BATTELLE-Geneva will treat our materials with their phenol-water patented pretreatment.

1.2.6 Steam-explosion

Forintek-Ottawa will treat our substrates in their small scale steam explosion gun.

1.3 Initial digestibility studies

1.3.1 Raw materials

. Sugarcane

Sugarcane was obtained from Ingenio Concepción, Escuintla, Guatemala (320 meters above sea level) during April (later part of crop). It was clean cane from the receiving storage, cut by hand and unburnt.

The canes were chipped in a pilot wood chipper (type 9-72, OY Santsalo). The chips were placed in 7000 l tanks, water was added to just cover them and 2% of commercial baker's yeast was mixed. The EX-FERM cycle took 48 hours converting the sucrose into ethanol (Rolz et al., 1979). The fermented chips were pressed in a continuous screwpress (model VP-6F, Vincent Corp.). Washed with water in an agitated tank and screwpressed again. Finally they were sun dried to a final moisture around 6-7% and packed in plastic bags.

. Lemon grass

Lemon grass bagasse was obtained from Finca Concepción, Coatepeque, Quezaltenango, Guatemala (420 meters above sea level) during June (early part of crop). It was collected as it came out of the essential oil steam distillery. It was sun dried to a final moisture around 6-7% and packed in plastic ventilated bags.

. Citronella

Citronella bagasse was obtained from finca Verapaz, Santa Bárbara, Suchitepequez, Guatemala (420 meters above sea level) during June (early part of crop). It was collected as it came out of the essential oil steam distillery. It was sun dried to a final moisture around 6-7% and packed in plastic ventilated bags.

. Substrates as such (SAS)

The SAS substrates were prepared by milling in a pilot Fitz Mill Comminutor (The Fitzpatrick Company) the dried raw materials, employing a 0.033" (0.84 mm) sieve. The final powder obtained was packed in plastic bags.

1.3.2 Chemical Analysis

The methodology for analyzing the raw materials was chosen taking into account the further processing to which they would be subjected and final end use. To these effect the methodology employed for evaluation of fibrous feeds and forages was chosen over the one employed in the cellulose, pulp and paper and textile industries. Although in general the latter methods give a better accuracy in determining the polymeric component distribution and some of its characteristics they usually are more time consuming. Moreover forages are evaluated for their eventual biodegradation in the rumen, one of the best natural continuous fermenter systems. As the objective of the project is to evaluate the biodegradation *in vitro* to organic acids, the former technique was more suitable as many feeds have been characterized that way.

The specific methodology employed was the so called "Detergent System" (Goering and van Soest, 1970; van Soest and Robertson, 1980) as depicted in Figure 1. The main polymeric components of the lignocellulosic solid matrix are recovered in the neutral-detergent residue (NDF). The acid-detergent fibrous residue recovers mainly cellulose and lignin. Hence the difference between NDF and ADF gives an approximate value for the hemicellulose fraction. Although cell wall proteins are also solubilized by acid-detergent, some of the protein might also be bound in crude lignin for example as Maillard products. Hence protein was Kjeldahl determined in a different sample, as were also moisture and total ash, by standard procedures. The NDF and ADF were determined employing as refluxing apparatus a Labconco Corp unit as suggested by the AOAC (1975). In this way crude values of cellulose, hemicellulose and lignin were determined. The results are shown in Table 1.

From these data the following points can be made between the three samples:

a) All of them have about the same amounts of lignin. Such value is about one half of what is commonly found in wood.

b) Protein and ash are higher in citronella and lemon grass bagasse than in sugarcane bagasse.

c) Cellulose and hence NDF and ADF are higher in sugarcane bagasse.

In Tables 2 and 3 similar analytical data found in the literature for sugarcane bagasse and the whole sugarcane plant have been summarized. Comparing the data the following points can be made:

a) Lignin, cellulose, ash and protein contents are within the reported values for cane from different parts of the world. The highest variation in both sets of data are found for cellulose.

b) The reported values for NDF and ADF are much higher than the data for samples from many countries. Although the ADF values show a large variation (see Table 2).

c) There is a possibility that we could have overestimated our ADF value. This is the cause of our low hemicellulose content (last column Table 1). This figure compares well with the values reported for whole sugarcane (last column Table 3), which on sugar removal approximately increase by two (last column Table 2).

d) Most of the data of Table 2 refers to sugarcane bagasse. This means that the whole sugarcane has been processed in the traditional way to extract sucrose from it, through the application of mechanical energy and heat and its combinations. Hence the data of Table 2 is not truly comparable to Table 1 which refers to EX-FERMented cane which has not been subjected to any high mechanical energy or heat levels. There are very few references on the damage to the sugarcane fiber during milling. One is the article by Gartside et al. (1982) which we have ordered and has not come as yet. So we will differ our comments to the next report.

e) As it was explained in section 1.1 the cane employed in the experiments came from late in the crop, presumably then it was more mature. As it has been shown by Banda y Valdez (1976) mature cane (16 months at harvest) has less NDF, ADF, cellulose and lignin than early cane (8 months at harvest). Swakon (1977) found differences in the weight distribution of tissues in the top (vegetative green material) from the bottom (mature) internodes of cane stalks. With increasing maturity there were greater weight percentages of ground tissue and rind and less of vascular bundles and epidermis. Sucrose increases with maturity and its internal movement was mainly towards the base of the plant, hence it was logical that the sucrose storing tissue, the parenchyma or ground tissue, would increase in amount in the bottom. The data shown on Table 2 as No. 7 was done for Guatemala cane planted around 1200 meters above sea level which usually stays in the fields from 18-20 months. If the cane used in these experiments was at harvest time between 10-18 months, then our results would coincide with those mentioned before in a sence that NDF and ADF do decrease with age. However much more analytical data is needed on this respect. We intend to take samples from the same plantation in the next cane crop and check its detergent analysis in order to confirm at least qualitatively this trend. Another sample of bagasse from the mill will also be taken in order to quantify any effects of milling on the distribution of the macrocomponents as separated by fiber analysis. An indication of the degree of dispersion found in sugarcane and the difficulties found in predicting any trends is easily seen by the comments of Donefer and Latrille (1980) who said that the U. of Florida study of 66 cane varieties of similar age indicated an average of cellular constituents of 47.3% with a surprising range of 32-57%.

No data were found in the literature for citronella and lemon grass bagasse. Only proximal analysis for crude fiber, ash and proteins were reported by INCAP (1968) as follows: 28.1, 8.4 and 10.7 and 34.1, 8.0 and 8.0 respectively for citronella and lemon grass bagasse. The values on Table 1 are similar except in protein which are lower. However this value varies markedly depending on the fertilization rate and plant age among other factors. We intend to take some samples from the same sources during the early part of the dry season in order to check any differences.

1.3.3 Digestibility prediction from chemical analysis

Many researchers have developed correlations between the so called digestibility (or biodegradability in certain specific conditions) and the analytical data obtained by the detergent system.

For the case of sugarcane Banda and Valdez (1976) provide four equations as given in Table 4 to predict the *in vivo* digestibility of whole sugarcane in the rumen. This provides an estimate of the biodegradability expected by the action of the complex microflora existing in the rumen and its conditions for temperature, pH, retention time and product removal. Only formulas one and four gave positive values and they predict a digestibility for cane of about 24 which is approximately half of what is predicted for citronella and lemon grass bagasse.

Chandler et al. (1980) obtained three formulas to predict total anaerobic biodegradation for lignocellulosic materials. This provides a figure equivalent to the lignocellulosic sequential conversion to monomeric sugars, organic acids and a gaseous mixture of carbon dioxide and methane. Two of the models have been applied to the materials of Tables 1 and 2 and appear in Table 5. These formulas usually predict the maximum conversion attainable under anaerobic conditions and total gasification obtained only with long residence times. Logically they predict a higher biodegradability

for sugarcane bagasse (an average between 46 to 50 of the organic matter), which is similar to the one predicted for lemon grass bagasse. Interestingly the values for citronella bagasse are definitely higher (60-69%).

1.3.4 Evaluation of in vitro digestibility

Many attempts have been made to design simple methodology to evaluate the digestibility of lignocellulosic feeds in the rumen. These methods have not been designed to duplicate perfectly the conditions found in the rumen, but rather to provide a final result that would predict *in vivo* values. The problem has been not only of estimating soluble cell contents and the distribution of the cell wall polymers but also the amount of digestible cell wall components and the degradation rates of each component. In this project we thought that this parameter, besides the data from the analysis by the detergent system, could help us comparing not only different raw materials but also the expected effect on biodegradability caused by the various planned pretreatments.

We do not intend to present a complete literature review on this methodology, rather we refer to the reader to two recent and key publications, the one by USDA scientists (Manten and Barnes, 1980) and the one by Dowman and Collins (1982). We choose to evaluate this parameter by two methods:

a) By using proteolytic and hollocellulolytic commercial enzymes, and

b) By employing a mixed culture inoculum of pure cultures of cellulolytic and non-cellulolytic bacteria

. The enzymatic method. In this method the material is treated sequentially with a proteolytic and a hollocellulolytic enzyme and the weight loss is recorded after a certain reaction time. The conditions employed were set after considering the following methods:

a) Jones and Hayward (1973, 1975) as modified by Dowman and Collins (1982)

b) Clark and Beard (1977) as modified by Dowman and Collins (1982)

c) Goto and Minson (1977) modifications of the Jones-Hayward (1973, 1975) technique

d) Roughan and Holland (1977) as modified by Dowman and Collins (1982)

e) Adegbola and Paladines (1977)

f) McLeod and Minson (1978, 1980)

Approximately 0.2 g of the powdered raw materials prepared as in section 1.4 were placed in a tared 60 ml plastic centrifuge tube. This was incubated at 40°C with 20 ml of 0.2% pepsin (Sigma Chemical Co.) in 0.1M HCl for 48 h. Manual shaking of the tube was done twice during the day. The supernatant was removed after centrifugation at 10 000 rpm, 4°C for 10 min. An equal volume of liquid was added, the contents mixed and the tube centrifuged again employing the same conditions as before. The supernatant was removed and 20 ml of 2.5% Onozuka cellulase (Yakult Honsha Co. Ltd.) in 0.1M acetate buffer pH 4.8 were added. The sample was incubated for another 48 h at 40°C shaking it manually twice a day. The residue was collected in a sintered glass crucible, washed once, dried and weighed. The dried material was ignited at 510°C for 3 h, cooled and weighed again. A control sample was included with no enzyme additions, the samples were done in duplicate.

Two parameters were calculated:

a) The <u>in vitro</u> dry matter digestibility (IVDMD) equal to:

IVDMD = 100 [1.0 - [(Dry weight after enzyme treatment) + (Amount solubilized in the control)] / (Sample dry weight)]

b) The <u>in vitro</u> organic matter digestibility (IVOMD) equal to:

IVOMD = 100 [1.0 - [(Dry weight after enzyme treatment) - (weight of ashes) + (Weight difference in ash between sample and control)] / (Sample organic dry weight)]

Results are presented in Table 6. Note that citronella bagasse gave the higher values, specially when compared employing an organic matter content base. The results for lemon grass were unexpectedly low. At this point is appropriate to remember that the citronella and lemon grass bagasse are raw materials which have been steam stripped of their essential oil. These oils are rather toxic substances for some microbes and their enzymes. Could it be that the lemon grass sample had still more residual oil than the citronella one? We will check residual oil content on our samples.

In Table 7 we have summarized some of the corresponding values of IVDMD for cane bagasse found in the literature. As explained in the footnotes any comparison among those values and in relation to the ones reported here must be approached with caution. Nevertheless it is seen that the values of Table 6 fall within the middle part of the range of span found in the data of Table 7 which for bagasse go from 9.2 to 38.3.

Swakon (1977) studied in detail the in vitro digestibility of different tissues. All tissues showed a significant decline in fiber digestion with maturity. The ground tissue fiber was the most digestible within a maturity. The difference in digestibility could be attribute not only to lignification but also to the physical orientation and structure of the cellulosic fibers as fibers in the rind were more digestible than fibers in the vascular bundles.

- Mixed culture inoculum of cellulolytic and non-cellulolytic bacteria

The methodology is being developed according to techniques described by Ikkovits et al. (1965), Stewart et al. (1979) and Wiegel (1982). The following pure cultures have been obtained:

- from DSM, Göttingen
  - a) Acetivibrio cellulolyticus ATCC 33288
  - b) Ruminococcus albus ATCC 27210
  - c) Clostridium thermocellum ATCC 27405
  - d) Clostridium thermosaccharolyticum ATCC 7956
- from NRCC, Canada
  - e) Acetivibrio cellulolyticus CD_2-NRC-2248
- from NIRD, Shinfield, Reading
  - f) Ruminococcus albus NCDO-2250
  - g) Ruminococcus flavefaciens NCDO-2213
  - h) Bacteroides succinogenes NCDO-2212
  - i) Bacteroides ruminicola subs. brevis NCDO-2212
  - j) Bacteroides ruminicola subs. ruminicola NCDO-2202
- from NCRL, Pretoria
  - k) Butyrivibrio fibrisolvens
  - l) Fusobacterium polysaccharolyticum

We are currently obtaining a sample of Clostridium thermoaceticum.

1.3.5 Prediction of in vivo digestibility

Working with 50 grass samples of tropical grasses, McLeod and Minson (1982) proposed the following equations to predict dry matter digestion in vivo (x) from IVDMD valves:

$$x = 0.7\ \text{IVDMD} + 18.2 \qquad r = 0.94\ (P < 0.01) \qquad \text{RSD} = \pm 2.6$$

Taking the IVDMD values of Table 6 we calculate the following figures for the actual in vivo anaerobic rumen digestibility:

a) Sugarcane EX-FERMented bagasse: 31.33%

b) Citronella bagasse: 31.95%

c) Lemon grass bagasse: 27.39%

The predicted value for cane bagasse falls in the upper bracket of the data tabulated in Table 7.

FIGURE 1. ANALYTICAL METHODOLOGY FOLLOWED FOR CHEMICAL EVALUATION

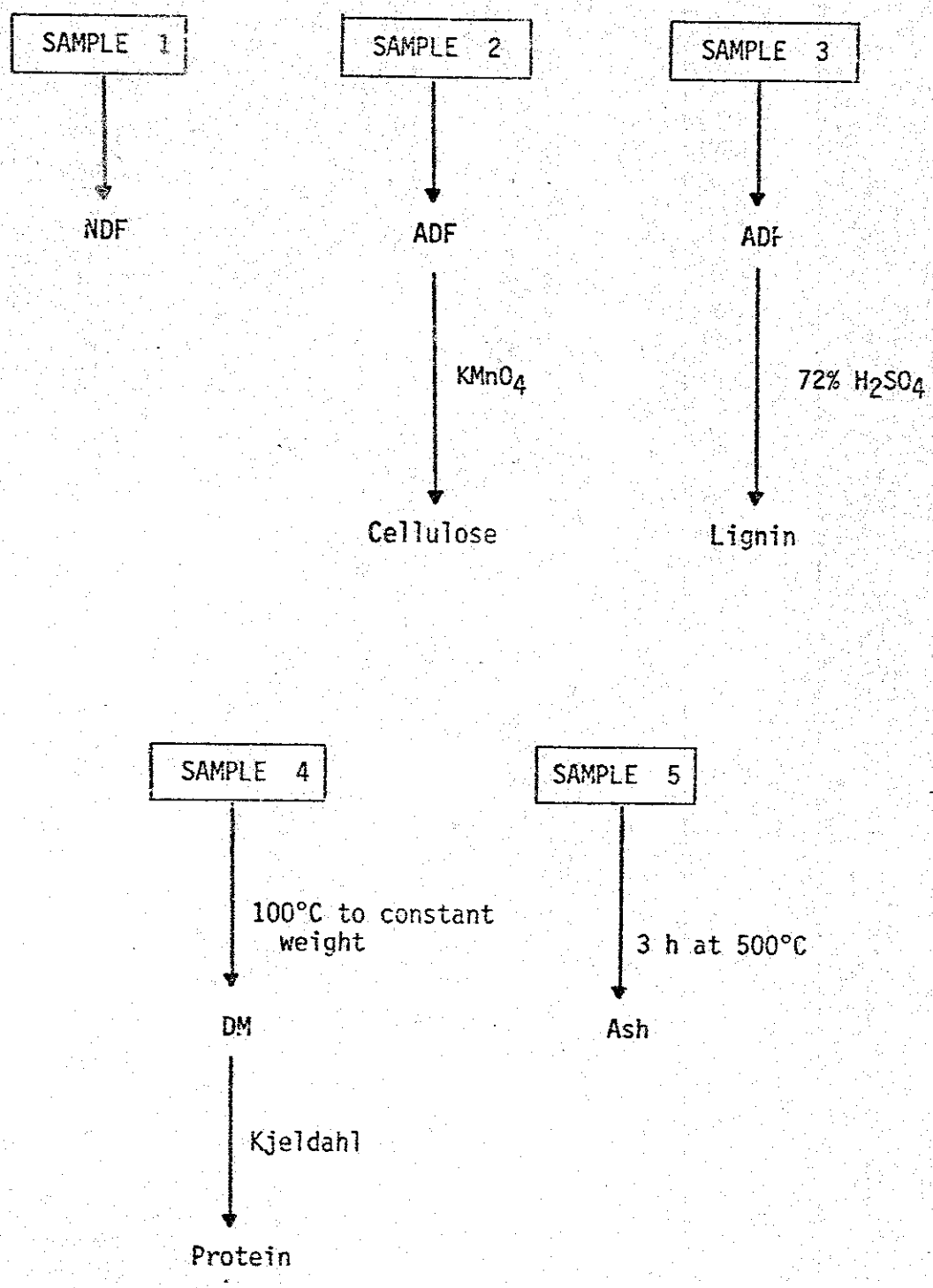

Table 1. ANALYTICAL RESULTS EMPLOYING THE DETERGENT SYSTEM.
UNDERLINED RESULTS GIVE THE AVERAGE

s = standard deviation

Raw material	Neutral detergent fiber residue (NDF)	Acid detergent fiber (ADF)	Lignine	Cellulose	Ash	Kjeldahl protein (Nx6.25)	Cellular constituents (100-NDF)	Hemicellulose (NDF-ADF)
1. Sugarcane EX-fermented bagasse	95.0 (95.00, 95.08)	78.0 (77.92, 78.13) s = 0.15	12.5 (12.24, 12.68)	48.5 (53.41, 42.94, 53.06, 53.17, 47.97, 53.87, 42.03, 41.21, 47.16, 50.21) s = 5.01	2.4 (2.40, 2.26, 2.43) s = 0.09	1.2 (1.17, 1.21)	5.0	17.0
2. Citronella bagasse	75.0 (75.63, 73.89)	59.5 (59.32, 59.64) s = 0.23	11.1 (11.18, 10.93)	30.2 (30.48, 29.50, 30.46, 30.22) s = 0.46	8.8 (8.90, 8.62, 8.72, 9.13, 8.60) s = 0.22	4.5 (4.56, 4.50)	25.0	15.5
3. Lemon grass bagasse	76.4 (76.26, 76.64)	66.6 (71.32, 64.57, 64.02) s = 4.07	11.0 (10.87, 11.29)	30.0 (32.31, 32.21, 30.64, 27.27, 27.71) s = 2.41	10.4 (10.15, 10.60, 10.40, 10.34) s = 0.19	5.1 (5.00, 5.03, 5.13)	23.6	9.8

TABLE 2. ANALYTICAL RESULTS FOR SUGARCANE BAGASSE EMPLOYING THE DETERGENT SYSTEM

Sugarcane bagasse	Neutral detergent fiber residue (NDF)	Acid detergent fiber (ADF)	Lignine	Cellulose	Ash	Kjeldahl protein (Nx6.25)	Cellular constituents (100-NDF)	Hemicellulose (NDF - ADF)
1. Randel (1972) Puerto Rico	84.2	58.0	11.3	-	5.4[a]	1.6	15.8	26.2
2. Jackson (1977) India	82.0[b]	53.0	13.0	40.0	2.0	-	18.0	29.0
3. Llamas et al. (1979) Mexico	84.6 - 85.7	68.0 - 68.3	15.4-15.5	48.8-48.9	-	-	14.3-15.4	16.6-17.5
4. Rexen (1979) Dinamarca	83.4	49.2	10.8	38.4	-	-	16.6	34.2
5. Ibrahim and Pearce (1980, 1983) Australia	88.1	60.2	11.8	46.6	5.6-5.9[a]	1.3	11.9	27.9
6. Han and Ciegler (1982)/Han et al (1983) USA	-	-	14.9	50.4	2.0	1.6	-	28.5
7. ICAITI (1982) Guatemala	85.3	45.5	14.9	44.0	1.3	-	14.7	39.8
Average values	84.7 s = 2.1	55.7 s = 8.2	13.2 s - 1.9	44.7 s = 4.8				30.0 s = 7.0

a = Soil contaminated due to mechanical harvest

b = Corrected for neutral detergent soluble silica

TABLE 3. ANALYTICAL RESULTS FOR WHOLE SUGARCANE EMPLOYING THE DETERGENT SYSTEM

Whole sugarcane	Neutral detergent fiber residue (NDF)	Acid detergent fiber (ADF)	Lignine	Cellulose	Ash	Kjeldahl protein (Nx6.25)	Cellular constituents (100-NDF)	Hemicellulose (NDF-ADF)
1. Banda y Valdez (1976) Mexico	54.1	33.4	5.4	26.2	1.1		45.9	20.7
2. Viana et al. (1978) (Mexico)	65.3	48.9	9.6	36.0	3.7	3.3	34.7	16.4

TABLE 4. FORMULAS OF BANDA Y VALDEZ (1976) TO PREDICT RUMEN DIGESTIBILITY OF WHOLE SUGARCANE

Y = digestibility in the rumen,%	1 Sugarcane EX-FERmented bagasse	2 Citronella bagasse	3 Lemon grass bagasse
Y1 = 120.4 - 1.0X; X = NDF	25.4	45.4	44.0
Y2 = 137.2 - 2.1X; X = ADF	-	12.3	-
Y3 = 123.8 - 10.4X; X = LIGNINE	-	8.4	9.4
Y4 = 114.2 - 1.9X; X = CELLULOSE	22.1	56.8	57.2

TABLE 5. FORMULAS OF CHANDLER ET AL. (1980) TO PREDICT TOTAL ANAEROBIC BIODEGRADATION

B1; B2 = Total anaerobic biodegradation, %

MODEL I

$B1 = -0.028X + 0.83$; X = lignin content as percent of organic matter

MODEL II

B2 = (Cellular constituents/Organic matter) + (NDF/100 (organic matter)) (-2.82 (X/NDF) + 104.61) - 0.192

Sample	X	X/NDF	B1	B2
1. Sugarcane EX-FERmented bagasse	12.81	13.48	47	51
2. Citronella bagasse	8.22	10.96	60	69
3. Lemon grass bagasse	12.28	16.07	49	57
4. Sugarcane bagasse, Randel (1972)	11.94	14.18	50	55
5. Sugarcane bagasse, Jackson (1972)	13.27	16.18	46	49
6. Sugarcane bagasse, Ibrahim and Pierce (1980, 1983)	12.52	14.21	48	54
7. Sugarcane bagasse, ICAITI (1982)	15.10	17.70	41	43
Average values for cane bagasse			46 s = 3.4	50 s = 4.8

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TABLE 6. IVDMD and IVOMD OF THE (SAS) MATERIALS

Sample	IVDMD, %	IVOMD, %
1. Sugarcane EX-FERmented bagasse	18.76 (18.17, 19.34)	17.65 (17.02, 18.28)
2. Citronella bagasse	19.64 (18.15, 21.13)	27.67 (28.98, 26.36)
3. Lemon grass bagasse	13.13 (12.61, 13.64)	16.42 (15.75, 17.09)

IVDMD = *In vitro* dry matter digestibility
IVOMD = *In vitro* organic matter digestibility

TABLE 7. LITERATURE VALUES FOR SUGARCANE BAGASSE IVDMD

	IVDMD,%[a]
1. Randel (1972) Puerto Rico	31.9
2. Dekker and Richards (1972/1973) Australia	23.6
3. Martin et al. (1974) Cuba	
. pith	3.8 - 20.5
. bagasse	9.2 - 11.4
4. Preston (1975) Mexico	20.0
5. Rexen (1979) Dinamarca	32.0
6. Cabello et al. (1981) Cuba	
. pith	21.0
. bagasse	11.2
7. Han et al. (1982/1983) USA	38.3
8. de Hernández (1983) Mexico	31.6[b]

a In some cases the enzymes and conditions employed in the analysis differ from those used here, also in other cases enzymes are substituted by the addition of rumen samples either through fistulated animals or employing the nylon bag technique.

b A corresponding value of 33.0 is reported for IVDMD. Ibrahim and Pearce (1980, 1982, 1983) give a value for IVOMD of 32.8.

1.4 Plans to quantify the lignocellulosic degradation by cellulolytic bacteria

From several culture collections, we obtained cellulolytic rumen bacteria as listed in section 1.3.4 (mixed culture inoculum of cellulolytic and non-cellulolytic bacteria). By using a defined medium with the following formula: trypticase 5 g/l; $(NH_4)_2SO_4$ .9 g/l; KH_2PO_4 .9 g/l; NaCl .9 g/l; $CaCl_2$ .02 g/l; $MgCl_2.6H_2O$ .02 g/l; $MnCL_2.4H_2O$ .01 g/l; $CaCl_2.6H_2O$ .001 g/l; $FeSO_4.7H_2O$ .1 g/l; Resazurin .33 g/l; rumen fluid .1 cc, acetic acid 1.7 cc; pH adjusted to 6.5. we intend to provide as the only carbon source for a bacterial mixture the following materials: lignocellulosic residues from EX-FERmented sugarcane, citronella bagasse and lemon grass.

Basically the dry weight of the substrate will be quantified to find if the bacteria have utilized the lignocellulosic material; also, acidity by volumetric analysis, pH, and total gas and composition of it.

Incubation temperature will be 35C and length of incubation probably around 72h.

All these tests will be done under strict anaerobiosis.

1.5 Maintenance of locally obtained mixed cultures

The cultures are maintained with semiweekly feeding and control of gas production. The cellulose substrate used is ground, untreated, EX-FERmented sugarcane bagasse. Diluted rumen extract is also added as a source of nitrogen and minerals.

The anaerobic systems have been set up for testing degradation of substrates with these materials. A gas scrubber furnace was built in the shop as well as a manifold. Gas flushing techniques have been established with O_2 free gases. The testing protocol has been established and the experiments will start momentarily.

1.6 Report of trips made for technical visits and consultation

1.6.1 Visit to the Bioengineering Research Center of the Department of Chemical Engineering, Lehigh University, Bethlehem, Penn.

Carlos Rolz made the visit on Wednesday July 6th and was received by Assistant Professor Janice Phillips. Of specific importance was to obtain their data, opinions and recent reports of their work on poplar wood chips delignification by employing ammonium hydroxide catalyzed solvent systems including information on the enzymatic susceptibility of the wood, employing butanol, ethanol and phenol as solvents.

1.6.2 Sheryl de Cabrera attended the course at MIT, Cambridge, Massachusetts "Biotechnology: microbial principles and processes for fuels, chemicals and ingredients" from August 1-5, 1983. Later, she visited the University of Georgia at Athens where she spent a day with Dr. Laura Carreira, who works with Drs. Ljungdahl and Wiegel on anaerobic fermentation with acid and ethanol production. The trip report is included in the appendix.

1.6.3 Visits made by Roberto de León. During the month of July two visits were made during a trip to the United States. The first visit on the 25th of July was done to the Northern Regional Research Laboratory of the USDA in Peoria, Illinois. Besides the general interest for the many activities of the NRRL, the main reason for visiting was to talk to Dr Robert Detroy who has been for the past years working at this center on the utilization of byproducts from hemicelluloses. The importance of their work relies on the utilization of pentoses by strains of yeasts; one of the collaborators, Dr. Bolen is presently doing some work in genetic engineering of the yeast Pachysolen tannophilus to enhance the use of xylan hydrolysates. Among the products of this metabolism, the more important is ethanol. The work of Dr. Detroy and colleagues involves the use of pretreatment steps by alkalies or by the use of high temperatures under steam pressure to obtain finally a xylose solution. The second visit was done to the Laboratory of Dr. M. Bryant at the

Animal Science Department, University of Illinois at Urbana-Champagne, Illinois. Dr. Bryant is widely recognized by his many contributions to the knowledge of rumen microbiology. The interest in this visit was to find from a first hand contact of his impressions about work in cellulolytic rumen microorganisms. Unfortunately Dr. Bryant was on a trip and his graduate students were assigned to accompany the visit. From the experience of the research done by these students, cellulose degradation has always been of great interest because of the role it plays in animal nutrition. This polymer has to be degraded into more simple compounds then converted into organic acids. However evaluation of cellulolytic capacity per se of the microorganisms involved is difficult because any data obtained from the activity on cellulose substrates cannot actually be extrapolated into what is really happening in a complex system like the rumen. From the experience of this laboratory work, enumeration procedures have evolved for liquids coming from the rumen and are mainly an application of a most probable number technique. Yet special performance of a particular strain can only be evaluated in an in vitro system using a very well defined growth medium, where the only source of carbon is cellulosic materials.

1.7 During this period the following purchase orders were processed:

Date	Purchase order N°	Supplier	Item	Cost
1982				
. Sept. 21	12302	A. Gallenkamp	Spare parts for lab. fermentor	132.00
1983				
. Jan. 17	12486	Fisher Scientific	Glassware and reagents	4 132.00
. Jan. 17	12487	Curtin Matheson	Glassware and reagents	2 948.25
. Jan. 18	12488	Pierce Chemical Co.	Glassware and reagents	796.64

.../

. Feb. 25	12585	Curtin Matheson	Laboratory centrifuge	8 272.25
. May 17	12719	Biospec	Reagents	366.00
. May 24	12741	Branson Sonic	Ultrasonic homogenizer	2 666.60
. May 24	12748	Fisher Scientific	Reagents	730.88
. July 20	12888	New Brunswick	Spare part for lab. shaker	255.00
Local supplies	-	-	-	1 101.11
TOTAL US$				21 400.74

2. Anticipated activities for the following six months

2.1 Pretreatment activities

Continuation of pretreatment trials to evaluate differences during the harvest seasons of the different grasses. Development of alkaline SO_2 and biological techniques. Writing of detailed report with the pretreatment procedures.

2.2 Biological evaluation of pretreated substrates and as such substrates

2.2.1 Continuation of digestibility evaluation as described in section 1.3

2.2.2 Evaluation with pure cultures as described in section 1.4

2.2.3 Evaluation with locally isolated mixed cultures with and without buffering system added at the 50 ml level. Effective conversion of lignocellulose substrate will be judged through loss of solids, gas production and composition, and in the case of non-buffered systems, acid production.

2.3 Optimization of selected substrate pretreatment - microorganism combinations

Acid profiles will be performed; ethanol and acetate production will be optimized.

2.4 Evaluation of pretreatment spent liquors for acid and ethanol production

Since the spent liquors may contain solubilized sugar polymers from degradation of the hemicellulose, as well as lignin, we will test them with selected yeasts from the ICAITI culture collection for ethanol production. The mixed flora from the digestors will be used for acid production trials.

3. FINANCIAL REPORT
PROYECT N°: AID 936-5542
ICAITI N°: 82/4.6.: 77
INNOVATIVE SCIENTIFIC RESEARCH (LIGNOCELLULOSICS)
PERIOD: September, 1982 through September, 1983

	Budget Category	Budget	Disbursed	(*) Commitments	Total
1.	Staff	119 000.00	27 390.13	-.-	27 390.13
2.	Travel and Perdiem	4 760.00	855.49	-.-	855.49
3.	Materials and supplies	45 700.00	13 564.62	7 836.12	21 400.74
	Total US$	169 460.00	41 810.24	7 836.12	49 646.36

(*) Purchase Orders issued, goods not yet received.

Prepared by:

Budget & Cost Control Division

4. References

Adegbola, A.A. and Paladines, O. (1977) Prediction of the digestibility of the dry matter of tropical forages from their solubility in fungal cellulase solutions. J. Sci. Food Agric. 28: 775

AOAC (1975) Official Methods of Analysis of the Association of Official Analytical Chemists, Chapter 7, sections 7.050-7.058, 12th edition, Washington

Banda, M. and Valdez, R.E. (1976) Effect of stage of maturity on nutritive value of sugarcane. Trop. Anim. Prod. 1: 94

Cabello, A., Conde, J. and Otero, M.A. (1981) Prediction of the degradability of sugarcane cellulosic residues by indirect methods. Biotechnol. Bioeng. 23: 2737

Chandler, J.A., Jewell, W.J., Gossett, J.M., van Soest, P.J. and Robertson, J.B. (1980) Predicting methane fermentation biodegradability. Biotechnol. Bioeng. Symp. 10: 98

Clark J. and Beard, J. (1977) Prediction of the digestibility of ruminant feeds from their solubility in enzyme solutions. Anim. Feed Sci. Technol. 2: 153

Dekker, R.F.H. and Richards, G.N. (1972) Effect of sulphate ion on the in vitro rumen digestion of polysaccharide constituents of bagasse. Aust. J. Biol. Sci. 25: 1377

Dekker, R.F.H. and Richards, G.N. (1973) Effect of delignification of the in vitro rumen digestion of polysaccharides of bagasse. J. Sci. Food Agric. 24: 375

de Hernández, I. (1983) Personnal communication (Jefe, Departamento de Análisis Instrumental, Instituto Nacional de Investigaciones Pecuarias, Palo Alto, D.F., México)

Donefer, E. & Latrille L. (1980) Description of sugarcane feeds: nomenclative and nutritional information. In: Standarization of Analytical Methodology for Feeds (Pigden, W.J., Balch, C.C. & Graham, M. eds.) IDRC-134e International Development Research Centre, pp 79-86, Ottawa

Dowman, M.G. and Collins, F.C. (1982) The use of enzymes to predict the digestibility of animal feeds. J. Sci. Food Agric. 33: 689

Gartside, G., Langfors, N.G. and Miller, K. (1982) An evaluation of the damage to sugarcane fibre during milling. Holzforsch 36: 23

Goering, H.K. and van Soest, P.J. (1970) Forage fiber analysis. Agricultural Handbook No. 379 ARS/USDA, p 20

Goto, I. and Minson, D.J. (1977) Prediction of the dry matter digestibility of tropical grasses using a pepsin-cellulase bioassay. Anim. Feed Science Technol. 2: 247

Han Y.W. and Ciegler, A. (1982) Use of nuclear wastes in utilization of lignocellulosic biomass. Process Biochem. 17 (1) 32

Han, Y.W., Catalano, E.A. and Ciegler, A. (1983) Chemical and physical properties of sugarcane bagasse irradiated with gama rays. J. Agric. Food Chem. 31: 34

Ibrahim, M.N.M. and Pearce, G.R. (1980) Effect of γ radiation on the composition and in vitro digestibility of crop byproducts. Agr. Wastes 2: 253

Ibrahim, M.N.M. and Pearce, G.R. (1982) The effect of boiling and of steaming under pressure on the chemical composition and in vitro digestibility of crop byproducts. Agr. Wastes 4: 443

Ibrahim, M.N.M. and Pearce, G.R. (1983) Effects of chemical pretreatments on the composition and in vitro digestibility of crop byproducts. Agr. Wastes 5: 135

ICAITI (1982) Unpublished results.

Ifkovits, R.W., Ragheb, H.S., Barnes, R.F. and Packett, L.V. (1965) A pure culture inoculum method for evaluation of forage cellulose digestibility. J. Anim. Sci. 24 (4) 1092-1099

INCAP (1968) Tabla de composición de pastos, forrajes y otros alimentos de Centroamérica y Panamá. Publicación INCAP E-440, Guatemala

Jackson, M.G. (1977) The alkali treatment of straws. Anim. Feed Science Technol. 2: 105

Jones, D.I.H. and Hayward, M.V. (1973) A cellulase digestion technique for predicting the dry matter digestibility of grasses. J. Sci. Food Agr. 24: 1419

Jones, D.I.H. and Hayward, M.V. (1975) The effect of pepsin pretreatment of herbage on the prediction of dry matter digestibility from solubility in fungal cellulase solutions. J. Sci. Food Agric. 26: 711

Llamas, G., Shimada, A., Castellanos S. and Merino, H. (1979) Estudio del valor alimenticio de subproductos de la caña de azúcar con bovinos en corral. Tec. Pec. Mex. 36: 59

Marten, G.C. and Barnes, R.F. (1980) Prediction of energy digestibility of forages with in vitro rumen fermentation and fungal enzyme systems. In: Standarization of Analytical Methodology for Feeds (Pigden, W.J., Balch, C. C. y Graham, M. eds.) IDRC-134e International Development Research Centre, 61-71 p, Ottawa

Martin, P.C., Cribeiro, T.C., Cabello, A. and Elías, A. (1974) The effect of sodium hydroxide and pressure on the dry matter digestibility of bagasse and bagasse pith. Cuban J. Agric. Sci. 8: 21

McLeod, M.N. and Minson, D.J. (1978) The accuracy of the pepsin-cellulase technique for estimating the dry matter digestibility in vivo of grasses and legumes. Anim. Feed Sci. Technol. 3: 277

McLeod, M.N. and Minson, D.J. (1980) A note on Onozuka 3S cellulase as a replacement for Onozuka SS (P1500) cellulase when estimating forage digestibility in vitro. Anim. Feed Sci. Technol. 5: 347

McLeod, M.N. and Minson, D.J. (1982) Accuracy of predicting digestibility by the cellulase technique; the effect of pretreatment of forage samples with neutral detergent or acid pepsin. Anim. Feed Sci. Technol. 7: 83

Preston, T.R. (1975) Sugarcane as the basis for intensive animal production in the tropics. In: Proc. Conference on Animal Feeds of Tropical and Subtropical Origin. Tropical Products Institute, pp 69-83, London

Randel, P.F. (1972) A comparison of the digestibility of two complete rations containing either raw or alkali-treated sugarcane bagasse. J. Agr. Univ. Puerto Rico 56: 18

Rexen, F. (1979) Low quality forages improve with alkali treatment. Feedstuffs 51: 33

Rolz, C., de Cabrera, S. and García, R. (1979) Ethanol from sugarcane: EXFERM concept. Biotechnol. Bioeng. 21: 2347-2349

Stewart, C.S., Dinsdale, D., Chang, E.J. and Paniagua, C. (1979) The digestion of straw in the rumen. In: Straw Decay and its Effect on Disposal and Utilization (Grossbard, ed.) p 123 John Wiley & Sons, Chichester

Swakon, D.H.D. (1977) Quantitative and qualitative digestion of different tissues in sugarcane stem by rumen microorganisms in vitro. M.S. Thesis, U. Florida, College of Agriculture, Gainsville

Viana, M., Shimada, A. and Calderon, F.M. (1978) Manipulación de la fermentación en ensilajes de caña de azúcar y su valor alimenticio para borregos. Tec. Pec. Mex. 35: 48

van Soest, P.J. and Robertson, J.B. (1980) Systems of analysis for evaluating fibrous feeds. In: Standarization of Analytical Methodology for Feeds (Pigden, W.J., Balch, C.C. y Graham, M. eds.) IDRC-134e International Development Research Centre, pp 49-60, Ottawa

Wiegel, J. (1982) Ethanol from cellulose. Experientia 38: 151-155

APPENDIX

1983 09 20

Lic. Francisco Aguirre B., Director

Lic. Sheryl de Cabrera, Jefe, Sección de Microbiología, División de Investigación Aplicada

Informe de viaje a los Estados Unidos del 31 de julio al 13 de agosto 1983

Ing. Carlos Rolz, Jefe, División de Investigación Aplicada

Del 1 al 5 de agosto, asistí al curso "Biotechnology: Microbial principles and processes for fuels, chemicals and ingredients" en el campus del Massachusetts Institute of Technology en Cambridge. Coordinado por el Dr. Anthony Sinskey, el curso trató de sistemas anaeróbicos principalmente, con énfasis en el metabolismo, la termodinámica y el manipuleo genético. El programa se encuentra adjunto al presente.

El 11 de agosto, visité el Departamento de Ciencia de Alimentos del Georgia Experiment Station, cerca de Griffin. El propósito de la visita había sido el de visitar a la Dra. Nancy Moon, quien había realizado muchos trabajos en el área de microbiología láctica y del rumen. La Dra. Moon había dejado la Estación Experimental unos meses antes para trabajar en la industria privada. Sin embargo, me atendió muy amablemente el Dr. Tommy Nakayama, Jefe del Departamento y varios empleados del centro. La Sra. Brenda V. Nail, técnico del Dr. Bechaut, me enseñó las técnicas que empleaban ellos en trabajar con las cepas de Aspergillus productoras de aflatoxinas, tanto en su enumeración como en la propagación e inoculación de granos.

El Departamento cuenta además con una planta piloto que colabora estrechamente con la industria privada en procesar lotes experimentales y lotes de productos enlatados especializados. Hay un buen laboratorio de análisis sensorial, equipo de secado, de cámaras de ambientes controlado para medir los parámetros asociados con el secado, etc.

El 12 pasé con la Dra. Laura Carreira en la Universidad de Georgia en Athens, quien con el Dr. Weigel ha estado trabajando con Clostridium thermoamyloliticum, un termofílico aislado de un nacimiento térmico de Islandia. Produce este microorganismo 2 moles de H_2 y 2 moles de CO_2, lactato y etanol de xilosa al 20%. En el mismo laboratorio se detecta la producción de ácidos orgánicos con una columna de Biorad en HPCL de Varian, modelo 5000, con detector de RI. El etanol lo detectan con un Cromatógrafo Perkin Elmer LC-75 con detector UV. También usan un Total Carbon Analyzer con el detector de Infrarrojo fijado para CO_2 para medir C. Hizo mucho hincapié en la importancia de observar técnicas anaeróbicas estrictas, con burbujeo de N_2, CO_2 o gas Argon por los medios,

jeringas, pipetas, viales, tubos, etc. antes del manipuleo de los microorganismos. Frecuentemente se les pierden los cultivos, y por lo tanto, mantienen congelados ejemplares, tomando alícuotas durante el período de crecimiento logarítmico, mezclándolo con igual cantidad de glicerol estéril anaeróbico y congelándolos a -20° por unos años.

Contaminación con C. thermohydrosulfuricum es común, y por lo tanto todo se esteriliza por 30 minutos. Azúcares se esterilizan por filtración.

Cada bacteria nueva que entra, se estudia con respecto al pH óptimo, temperatura óptima, perfil de substratos, perfil de productos, composición de pared celular, DNA, RNA. Las condiciones óptimas se determinan sacando les tiempos de replicación (duplicación) de las curvas de crecimiento.

En este laboratorio, se está empezando a estudiar la genética de estos anaeríbios estrictos, pero es un trabajo difícil. No habían tenido mucha suerte en encontrar ni los plasmids ni marcadores indicados o apropiados para tales esfuerzos.

Esta misma tarde, visité los laboratorios de J. LeGall en el Departamento de Bioquímica de la misma Universidad. Me atendió el Dr. Mohoussine Nacro, quien explicó los trabajos de este grupo en estudiar los sistemas enzimológicos para los metanogénicos.

El viaje fue de mucho provecho para las actividades del proyecto de lignocelulósicos (1777).

Atentamente,

SDEC/jldeg

BIOTECHNOLOGY: MICROBIAL PRINCIPLES AND PROCESSES FOR FUELS, CHEMICALS AND INGREDIENTS

DR. A. J. SINSKEY

DR. C. L. COONEY

DR. R. F. GOMEZ

DR. LARRY L. MCKAY

DR. R. K. THAUER

DR. G. A. WALKER

DR. D. L. WISE

DR. J. G. ZEIKUS

AUGUST 1 - 5, 1983

BIOTECHNOLOGY: MICROBIAL PRINCIPLES AND PROCESSES FOR FUELS CHEMICALS AND INGREDIENTS

Schedule of Lecture -- 8/1 - 8/5/83

Monday (8/1)	9:00 - 10:15	Introduction - (AJS) To review definition of biotechnology, outline microbial and engineering principles to be covered in course
	10:15 - 10:30	Break
	10:30 - 12:00	Biochemical Engineering Fundamentals - (CLC) An overview of the unit operations important to the development of fermentation and bioconversion processes fuels, chemicals and ingredients syntheses. Consideration will be given to selection and pretreatment of raw materials, bioreactor design and control, product separations, processes and recovery operations, examples of process flow sheets, and an introduction into process economics.
	12:00 - 1:30	Lunch
	1:30 - 3:15	Biochemical Engineering Principles Continued - (CLC)
	3:15 - 3:30	Break
	3:30 - 5:00	Introduction to basics of the microbial world, ecophysiology and laboratory techniques with special emphasis on anaerobic bacteria (JGZ)
	6:00 - 8:00	Cocktails

6th floor MIT Jact Club

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Tuesday (8/2)	9:00 - 10:15	Microbial Metabolism Thermodynamics and basic concepts of microbial metabolism (RT)
	10:15 - 10:30	Break
	10:30 - 12:00	Energy Metabolism in Acidogenic and Solventogenic Anaerobes (RT)
	12:00 - 1:30	Lunch
	1:30 - 3:15	Basic Mutagenic and Genetic Techniques for Strain Construction (GW) Emphasis of basic principles and application of advanced techniques of site-directed mutagenesis, etc.)
	3:15 - 3:30	Break
	3:30 - 5:00	Basic Mutagenic and Genetic Techniques for Strain Construction (GW)
Wednesday (8/3)	8:45 - 10:15	Energy Metabolism in Sulfidogenic and Methanogenic Bacteria (RT)
	10:15 - 10:30	Break
	10:30 - 12:00	Fuel and chemicals production from polymer transformations (JGZ)
	12:00 - 1:30	Lunch
	1:30 - 3:15	Fuel and chemicals production from single carbon transformations (JGZ)
	3:15 - 3:30	Break
	3:30 - 5:00	The Status of Genetic Systems in Strict Anaerobes (RFG)

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	7:00 - 9:00	Large-Scale Biologically Derived Methane Processes (DW)
Thursday (8/4)	9:00 - 10:15	Fundamental Biology of Dairy Starter Cultures (LM)
	10:15 - 10:30	Break
	10:30 - 12:00	Functional Role of Plasmids in Dairy Streptococci (LM)
	12:00 - 1:30	Lunch
	1:30 - 3:15	Genetic and Strain Construction Strategies for Dairy Streptococci (LM)
	3:15 - 3:30	Break
	3:30 - 5:00	rDNA Technology for Enzyme Engineering and Manipulation of Biochemical Pathways (RFG)
	6:00 -	Cocktails and Banquet
Friday (8/5)	9:00 - 10:15	Applications and Strategies for Manufacture of Fuels, Chemicals and Ingredients (AJS): A summarizing perspective
	10:15 - 10:30	Break
	10:30 - 12:00	(AJS) Continued

TECHNICAL ARTICLE

INSTITUTO CENTROAMERICANO DE
INVESTIGACION Y TECNOLOGIA INDUSTRIAL
(ICAITI)

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CASE STUDY

"BIOCONVERSION OF LIGNOCELLULOSE: AN APPROACH"

Carlos Rolz
Head, Applied Research Division

Presented at:

Biotechnology in the Americas:
Prospects for Developing Countries

3-6 May 1983
San José, Costa Rica

In the EX-FERM process ethanol is obtained by the extraction-fermentation of sucrose present in sugarcane by the action of suitable microorganisms (Rolz, et al., 1983). In one ton of cane (1000 kg) there are, on the average, around 130 kg of fermentable sugars and 125 kg of a fibrous residue. Usually this last product is employed as fuel to purify the ethanol produced.

The hypothesis discussed here deals with the existing alternatives to employ this lignocellulosic residue, separating it into its major macro-components by a series of treatments and employing the resulting products either as raw materials for producing more ethanol or as a source of energy for the overall process. A detailed energetic balance is beyond the scope of the presentation as very frequently new engineering schemes are presented that lower the energy demand to purify fermentation ethanol. However what is important is that no matter how small still a fraction of this residue must be used as process fuel.

The principal macrocomponents of the fibrous residue are cellulose, lignin and hemicellulose. Their distribution varies with cane variety and age, agricultural practice and ecological conditions. A sample of sugarcane from the Pacific coast of Guatemala taken in the later part of the 82/83 crop was EX-FERmented; the lignocellulosic residue was dewatered and washed in a screw press and sun dried. It was analyzed according to techniques used with fibrous feeds or forages (van Soest y Robertson, 1980) giving the following values: cellulose, 48%; lignin, 12% and hemicellulose, 17%. The hemicellulose content was much lower than other values found in samples previously analyzed, which gave the following average results: 44, 15 and 40% respectively. These

data are within the values for sugarcane bagasse reported in the literature (INIP, 1983; Jackson, 1977; Randel, 1972; Rexen, 1979; Han et al., 1983; Ibrahim and Pearce, 1980, 1982). The in vitro dry matter digestibility (IVDMD) of such product is relatively low due to its lignin content and structural characteristics. However the reported values vary widely, not only because of the factors mentioned above, but also due to analytical differences, for example the use of rumen liquor or commercial enzymes to carry out the hydrolysis. In the same EX-FERmented sample an IVDMD of 18.8% was determined (employing commercial enzymes). In the literature values between 9.2 and 31.6% are found (Martin et al., 1974; Cabello et al., 1981; Dekker and Richards, 1972; Preston, 1975; Randel, 1972).

During the design of strategies to separate the macrocomponents of lignocellulosic materials for their further biotransformations, it is necessary to realize that their rates of reaction must be increased and hence pretreatment operations must be an integral part of any process scheme. It is well to remember that there is no optimum general pretreatment, as this will depend on the process, the products, raw materials and microorganisms to be used subsequently.

One interesting alternative in cases where ethanol is the final product sought is the delignification "Organosolv" process (Kleinert, 1974, 1975; Bowers and April, 1977; April et al., 1979; Baumeister y Edel, 1980; Katzen et al., 1980; Sarhanen, 1980; April et al., 1982; Green & Sawyer, 1982; Hansen & April, 1982; Marton & Graizow, 1982; Averignos & Wang, 1983; Neilson et al., 1983). Basically in this process lignin is separated by mixtures of ethanol-water acting above atmospheric pressure values. Cellulose remains in the fibrous residue and usually hemicelluloses are hydrolyzed to pentoses. Lignin can be separated easily from the solvent. As the solvent ethanol is employed (which is also the main product of the process) and not a chemical derived from fossil resources, like phenol which has been used successfully for the same purpose in the BATTELLE-Geneve process (although phenol can eventually be obtained from the pyrolysis of the separated lignin). Hemicellulose is broken to fermentable sugars (pentoses) and cellulose, still as a polymer, has been transformed to a more reactive form. At ICAITI experiments have started employing ethanol-water alkaline mixtures with and without the addition of anthraquinone. With ethanol:water (60:40) at 175°C for 6 h a solids yield of 74% was obtained with a Kappa No. between 129 and 139. By adding 2% NaOH and reducing the reaction time to 4 h the figures obtained were 56% and 7 respectively. And with anthraquinone the values were 60% and 5.5.

The alkali action on lignocellulose causes a swelling of the solid matrix which allows solvents, chemical reagents or biological catalysts to penetrate further within and establish a much better contact with the internal structure. If the objective of the pretreatment does not imply a lignin separation or a hemicellulose hydrolysis, an adequate alternative is the solid phase alkaline process. In this case the lignocellulosic residue arranged in a pile is sprayed with a concentrated alkaline solution, followed by a storage at ambient temperature usually for one week. The dosis are between 3-5 g/100 g of dry matter (Capper et al., 1977; Jackson, 1977; Dobie & Walker, 1977; Wilkinson & González, 1978; Berger et al., 1979, 1980; Solaiman et al., 1979; Acock et al., 1979; Garret et al., 1979; Church & Champs, 1980; Willis et al., 1980; Lesoing et al., 1980; Berg & Mietz, 1980; Plasse et al., 1980; Benghedalia et al., 1980, 1981; Miron & Benghedalia, 1981; Chesson, 1981; Higgins, 1981; Jayasuriya & Perera, 1982). In this process there are no effluents and very little dry

matter is lost. Contrary when alkaline process employing suspensions are employed, liquid effluents are produced which need some kind of processing. The alkali effects are dramatic on the lignocellulosic residue increasing substantially their potential degree of hydrolysis. With 12% NaOH on sugarcane bagasse the IVDMD and enzymatic solubility increased by 582 and 280% (Cabello et al., 1981).

Sometimes the SO_2 treatment of the solid residue is more effective than alkali. Dosis are around 5 g/100 g dry matter, followed by a storage for three days at 70°C. Experimental data on wheat straw have been published by Ben-Ghedalia and Miron (1981).

Finally among solid state pretreatments the biological processes must be mentioned, either with bacteria (Deschamps et al., 1981; Odier et al., 1981; Janshekar & Fiechter, 1982) or employing basidiomycetes (Sing & Rajarathnam, 1977; Zadrazil, 1977, 1978, 1979, 1980a-d; Eger, 1978, 1979; Chang, 1978; Lindenfelser, et al., 1979; Rajarathnam et al., 1979; Li & Eger-Hummel, 1979; Wicklow et al., 1980; Platt et al., 1981; Chang et al., 1981). Usually the fungi fructify producing an excellent human food and at the same time a destruction of the lignocellulosic matrix takes place due to the intensive search for nitrogen by the fungi, which employ very efficiently their enzymatic machinery, one of the most potent found to date (Antheunisse, 1979, 1980, 1981). Dry matter biomass is lost by the action of the fungi; cellulose and hemicellulose are hydrolyzed, lignin is usually broken to smaller molecules which in principle can be further biodegraded. Under optimal conditions and with the proper fungi the digestibility of the residue can increase by 100%.

A pretreated lignocellulosic material is then more susceptible for its biotransformation to ethanol or other chemicals. The many alternatives proposed to date can be classified into two groups: indirect and direct processes. In the former, the basic objective is to hydrolyze the hollocellulose (residue that remains after lignin removal) to monomeric units employing chemicals, enzymes or both. Once this is done the monomers are later on transformed into ethanol (Rolz et al., 1983). Glucose from cellulose is readily metabolized by brewer's and distiller's yeast. Hemicellulose hydrolysis originate mainly pentoses and to date there are no microorganisms available for their rapid conversion to alcohol and in sufficient quantities. This is a disadvantage of this type of processes.

In the direct processes the conversion of hollocellulose into ethanol takes place in only one step avoiding the production and accumulation of monomers. Recent work in the technical literature describe experiments where anaerobic bacteria have been able to convert hollocellulose to a spectrum of primary metabolites among them ethanol (Su, 1978; Zeikus, 1979, 1980; Rosenberg, 1980; Wiegel & Ljungdahl, 1980; Flickinger, 1980; Avgerinos & Wang, 1980; Villet, 1981). Present research deals with the regulation and control of product distribution, genetic manipulations and selection and strain stability. One of the main problems found has been the lack of tolerance by the bacteria to high concentrations of the products, specially Clostridium thermocellum (Herrero & Gomez, 1980, 1981).

Another alternative which is being studied by ICAITI scientists is the so called "intermediate-acid". The idea is to transform hollocellulose to volatile organic acids, mainly acetic acid, as it is done in the rumen. The acids will then be biotransformed to ethanol employing alternate biochemical

pathways. Two industrial companies in the USA have recently studied the acidification stage: Dynatech (Sanderson et al., 1978; Levy et al., 1981) and Exxon Research (Datta, 1981a,b). The acidification takes place under anaerobic conditions under non-sterile conditions and its rate is increased by alkaline pretreatments of the lignocellulosic substrate. ~ water-soluble compounds and hemicelluloses are converted by 74-80% and cellulose by 37%. Two excellent advantages are the non-sterility requirement and the conversion of both hemicelluloses and celluloses to the same final product. Khan and collaborators at NRC, Ottawa (Khan et al., 1977, 1978, 1979, 1981; Pate et al., 1980; Saddler & Khan, 1981) have isolated a bacteria identified as Acetivibrio cellulolyticus which transforms with good yields cellulose to organic acids. These acids in principle can be transformed to ethanol. Lactic acid can be oxidized to pyruvic catalyzed by lactic dehydrogenase and then continue by known biochemical pathways to ethanol. Corry (1978) believes that this reaction takes place during putrefaction of living tissue. However lactate dehydrogenation with hydrogen as electron acceptor is thermodynamically unfavorable and could take place under very low hydrogen pressures and without ATP synthesis (Thauer et al., 1977). Conversion of acetate to ethanol is thermodynamically more favorable and possibly have acetyl coenzymeA as intermediate.

The first reaction in this chain of bioevents still is the hydrolysis of hollocellulose. The most studied anaerobic cellulolytic bacteria are the ones present in the rumen: Ruminococcus albus, R. flavefaciens, Bacteroides succinogenes, Butyrivibrio fibrisolvens and Fusobacterium polysaccharolyticum. The enzymatic complex of the first three has been recently studied (Halliwell & Bryant, 1963; Leatherwood, 1965; Walker, 1967; Krishnamurthi & Kitts, 1969; Smith et al., 1973; Francis et al., 1978; Gawthorne, 1979; Yu & Hungate, 1979; Pettipher & Latham, 1979; Groleau & Forsberg, 1981; Forsberg et al., 1981). Other cellulolytic anaerobes have also been studied: Acetivibrio cellulolyticus, Clostridium thermocellum and Cl. acetobutylicum (Lee & Blackburn, 1975; Gordon et al., 1978; Shinmyo et al., 1979; Ait et al., 1979; Ng et al., 1979; Rahmatullah et al., 1979; Khan, 1980; Saddler & Khan, 1980, 1981; Garcia et al., 1980; Khan et al., 1981; Allcock & Woods, 1981; Ng & Zeikus, 1981; Johnson et al., 1982; Mackenzie & Bilou, 1982). In all cases the existance of a complete extra and intracellular cellulase complex has been demonstrated as also the presence of hemicellulases. The endo and exo glucanases are extracellular and include celo- and xilo-biohydrolases. Beta glucosidase usually is cell wall associated. In these microorganisms there is a strong interaction between bacteria and fiber. Several mechanisms have been experimentally found: a) strong surface attachment of the bacteria to the fiber, b) existance of enzymes packed in vesicles or satellites detached from the cell, and c) polymeric gel that retains the enzymes between bacteria and fiber (Leatherwood, 1973; Costerton et al., 1974a,b; Akin et al., 1974; Dinsdale et al., 1978; Morris & van Gylswyk, 1980; Forsberg et al., 1981). There is no need for aereation as is required with fungi and high levels of mixing are not required as they seem unfavorable for bacteria-fiber interactions.

Summarizing, then, the "acid-intermediate" alternative offers new elements for the conversion of hollocellulose to ethanol (or other products) through organic volatile acids (acetic). However the ideas still need to be explored in the laboratory as also the pretreatment optimization. It is believed that direct processes will eventually be used in the future.

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References

Acock, C.W.; Ward, J.K.; Rush, I.G. y Klopfenstein, T.J. (1979) Wheat straw and sodium hydroxide treatment in beef cow rations. J. Anim. Sci. 49: 354

Ait, N.; Creuzet, N. y Forget, P. (1979) Partial purification of cellulase from C. thermocellum. J. Gen. Microbiol. 113: 399

Akin, D.E.; Burdick, D. y Michaels, G.E. (1974) Rumen bacterial relationship with plant tissue during degradation revealed by transmission electron microscopy. Appl. Microbiol. 27: 1149

Allcock, E.R. y Woods, D.R. (1981) CMC and cellobiase production by Clostridium acetobutylicum in an industrial fermentation medium. Appl. Environ. Microbiol. 41: 539

Antheunisse, J. (1979) Observations on the decomposition of coconut fibres. J. Gen. Appl. Microbiol. 25: 273

Antheunisse, J. (1980) The breakdown of coconut fibres by Trametes versicolor and Stereum rugosum. J. Gen. Appl. Microbiol. 26: 167

Antheunisse, J. (1981) Decay of coconut fibres by soil microorganisms. J. Gen. Appl. Microbiol. 27: 435

April, G.C.; Kamal, M.M.; Reddy, J.A.; Bowers, G.H. y Hansen, S.M. (1979) Delignification with aqueous-organic solvents. TAPPI 62 (5) 83

April, G.C.; Bharoocha, R.; Sheng, J. y Hansen, S. (1982) Prehydrolysis achieves higher organosolv delignification. TAPPI 65 (2) 41

Averignos, G.C. y Wang, D.C. (1980) Direct microbiological conversion of cellulosics to ethanol. Ann. Reports Ferment. Processes 4: 165

Averignos, G.C. y Wang, D.I.C. (1983) Selective solvent delignification for fermentation enhancement. Biotechnol. Bioeng. 25: 67

Baumeister, M. y Edel, E. (1980) Athanol Wasser Aufschluss. Das Papier 34 (10A) V9

Ben-Ghedalia, D. y Miron, J. (1981) Effect of sodium hydroxide, ozone and sulfur dioxide on the composition and in vitro digestibility of wheat straw. J. Sci. Food Agric. 72: 224

Ben-Ghedalia, B.; Shelef, G. y Miron, J. (1980) Effect of ozone and ammonium hydroxide treatments on the composition and in vitro digestibility of cotton straw. J. Sci. Food Agric. 31: 1337

Berg, F. y Mietz, M. (1980) Okonomische wertung der futter werterhohung von chemisch behandeltem stroh. Arch. Tierenahrung 30: 311

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Berger, L.; Klopfenstein, T. y Britton, R. (1979) Effect of sodium hydroxide on efficiency of rumen digestion. J. Anim. Sci. 49: 1317

Berger, L.; Klopfenstein, T. y Britton, R. (1980) Effect of sodium hydroxide treatment on rate of passage and rate of ruminal fibre digestion. J. Anim. Sci. 50: 745

Bowers, G.H. y April, G.C. (1977) Aqueous n-butanol delignification of southern yellow pine. TAPPI 60 (8) 102

Cabello, A.; Conde, J. y Otero, M.A. (1981) Prediction of the degradability of sugarcane cellulosic residues by indirect methods. Biotechnol. Bioeng. 23: 2737

Capper, B.S.; Morgan, D.J. y Parr, W.H. (1973) Alkali treated roughages for feeding ruminants: a review. Trop. Science 19: 73

Corry, J.E.L. (1978) A review. Possible sources of ethanol ante- and post-mortem: its relationship to the biochemistry and microbiology of decomposition. J. Appl. Bacteriol. 44: 1

Chang, S.T. (1978) Volvariella volvacea en "The Biology and Cultivation of Edible Mushrooms" (Chang, S.T. y Hayes, W.A., eds.) p 573, Academic Press, Nueva York

Chang, S.T.; Lau, O.W. y Cho, K.Y. (1981) The cultivation and nutritional value of Pleurotus sajor-caju. Eur. J. Appl. Microbiol. Bacteriol. 12: 58

Chesson, A. (1981) Effects of sodium hydroxide on cereal straws in relation to the enhanced degradation of structural polysaccharides by rumen microorganisms. J. Sci. Food Agric. 32: 745

Church, D.C. y Champs, K.A. (1980) Digestibility of untreated and hydroxide-treated animal ryegrass straw. J. Anim. Sci. 51: 20

Costerton, J.W.; Ingram, J.M. y Cheng, H.J. (1974a) Structure and functions of the cell envelope of gram negative bacteria. Bacteriol. Rev. 38: 87

Costerton, J.W.; Damgaard, H.N. y Cheng, H.J. (1946b) Cell envelope morphology of rumen bacteria. J. Bacteriol. 118: 1132

Datta, R. (1981a) Acidogenic fermentation of corn stover. Biotechnol. Bioeng. 23: 61

Datta, R. (1981b) Acidogenic fermentation of lignocellulose-acid yield and conservation of components. Biotechnol. Bioeng. 23: 2167

Dekker, R.F.H. y Richards, G.N. (1972) Effects of sulphate ion on the in vitro rumen digestion of polysaccharide constituents of bagasse. Aust. J. Biol. Sci. 25: 1377

Deschamps, A.M.; Gillie, J.P. y Lebeault, J.M. (1981) Direct delignification of untreated bark chips with mixed cultures of bacteria. Eur. J. Appl. Microbiol. Biotechnol. 13: 225

Dinsdale, D.; Morris, E.J. y Bacon, J.S. (1978) Electron microscopy of the microbial populations present and their modes of attack on various cellulosic substrates undergoing digestion in sheep rumen. Appl. Environ. Microbiol. 36: 160

Dobie, J.B. y Walker, H.G. (1977) Effects of sodium hydroxide and ammonia on cubability and digestibility of rice straw. Trans. Am. Soc. Agr. Eng. 20: 1016

Eger, G. (1978) Biology and breeding of Pleurotus en "The Biology and Cultivation of Edible Mushrooms" (Chang, S.T. y Hayes, W.A., eds.) p 497, Academic Press, Nueva York

Eger, G.; Li. S.F. y Leal, H. (1979) Contribution to the discussion on the species concept in the Pleurotus ostreatus complex. Mycol. 71: 577

Flickinger, M.C. (1980) Current biological research conversion of cellulosic carbohydrates into liquid fuels. Biotechnol. Bioeng. 22, Suppl. 1: 27

Forsberg, C.W.; Beveridge, T.J. y Hellstrom, A. (1981) Cellulase and xylanase release from Bacteroides succinogenes and its importance in the rumen environment. Appl. Environ. Microbiol. 42: 886

Francis, G.L.; Gawthorne, J.M. y Stover, G.B. (1978) Factors affecting the activity of cellulases isolated from the rumen digesta of sheep. Appl. Environ. Microbiol. 36: 643

García, D.V.; Shinmyo, A.; Madia, A. y Demain, A.L. (1980) Studies on cellulase production by Clostridium thermocellum. Eur. J. Appl. Microbiol. Biotechnol. 9: 189

Garrett, W.N.; Walker, H.G.; Kobber, G.O. y Hart, M.R. (1979) Response of ruminant to diets containing sodium hydroxide or ammonium treated rice straw. J. Anim. Sci. 48: 92

Gawthorne, J.M. (1979) Extracellular carbohydrase complex from rumen contents. Ann. Rech. Vet. 10: 249

Gordon, J.; Jimenez, M.; Cooney, C.L. y Wang, D.I.C. (1978) Sugar accumulation during enzyme hydrolysis and fermentation of cellulose. Am. Inst. Chem. Eng. Symp. 74 (181): 91

Green, J. y Sawyer, N. (1982) Alkaline pulping in aqueous alcohols and amines. TAPPI 65 (5) 133

Groteau, D. y Forsberg, C.W. (1981) Cellulolytic activity of the rumen bacteria Bacteroides succinogenes. Can. J. Microbiol. 27: 517; 27: 1132

Halliwell, G. y Bryant, M.P. (1963) The cellulolytic activity of pure strains of bacteria from the rumen of cattle. J. Gen. Microbiol. 32: 441

Han, Y.W.; Catalano, E.A. y Ciegler, A. (1983) Chemical and physical properties of sugarcane bagasse irradiated with gama rays. J. Agric. Food Chem. 31: 34

Hansen, S.M. y April, G.C. (1982) Prediction of solvent effects in aqueous-organic solvent delignification. Ind. Eng. Chem. Prod. Res. Dev. 21: 621

Herrero, A.A. y Gomez, R.F. (1980) Development of ethanol tolerance in Clostridium thermocellum: effect of growth temperature. Appl. Environ. Microbiol. 40: 472

Herrero, A.A. y Gomez, R.F. (1981) Inhibition of Clostridium thermocellum. en "Advances in Biotechnology" (Moo-Young, M. y Robinson, C.W., eds.) Vol. II, p 213, Pergamon Press, Toronto

Higgins, A.J. (1981) The effect of a sodium hydroxide spray treatment on digestibility of barley straw in sheep and goats. Agr. Wastes 3: 145

Ibrahim, M.N.M. y Pearce, G.R. (1980) Effects of gama radiation on the composition and in vitro digestibility of crop byproducts. Agr.Wastes 2: 253

Ibrahim, M.N.M. y Pearce, G.R. (1983) Effects of chemical pretreatments on the composition and in vitro digestibility of crop byproducts. Agr. Wastes 5: 135

INIP (1983) Comunicación personal (QFB Irma Tejada de Hernández, Jefe, Departamento de Análisis Instrumental, Instituto Nacional de Investigaciones Pecuarias, Palo Alto, D.F., México)

Jackson, M.G. (1977) The alkali treatment of straw. Anim. Feed Sci. Technol. 2: 105

Janshekar, H. y Fiechter, A. (1982) On the bacterial degradation of lignin. Eur. J. Appl. Microbiol. Biotechnol. 14: 47

Jayasuriya, M.C.N. y Perera, H.G.D. (1982) Urea-ammonia treatment of rice straw to improve its nutritive value for ruminants. Agr. Wastes 4: 143

Johnson, E.A.; Sakajoh, M.; Halliwell, G.; Madia, A. y Demain, A.L. (1982) Saccharification of complex cellulose substrates by the cellulase system from Clostridium thermocellum. Appl. Env. Microbiol. 43: 1125

Katzen, R.; Frederickson, R. y Brush, B.F. (1980) The alcohol pulping and recovery process. Chem. Eng. Prog. 76 (2) 62

Khan, A.W. (1977) Anaerobic degradation of cellulose by mixed cultures. Can. J. Microbiol. 23: 1700

Khan, A.W. y Trottier, T.M. (1978) Effect of sulfur-containing compounds on anaerobic degradation of cellulose to methane by mixed cultures obtained from sewage sludge. Appl. Environ. Microbiol. 35: 1027

Khan, A.W.; Trottier, T.M.; Patel, G.B. y Martin, S.M. (1979) Nutrient requirement for the degradation of cellulose to methane by a mixed population of anaerobes. J. Gen. Microbiol. 112: 365

Khan, A.W. (1980) Degradation of cellulose to methane by a coculture of Acetivibrio cellulolyticus and Methanosarcina barkeri. FEMS Microbiol. Letters 9: 233

Khan, A.W.; Wall, D. y van den Berg, L. (1981) Fermentative conversion of cellulose to acetic acid and cellulolytic enzyme production by a bacterial mixed culture obtained from sewage sludge. Appl. Environ. Microbiol. 41: 1214

Khan, A.W.; Wall, D. y van den Berg, L. (1981) Fermentative conversion of cellulose to acetic acid and cellulolytic enzyme production by a bacterial mixed culture obtained from sewage sludge. Appl. Environ. Microbiol. 41: 1214

Kleinert, T. (1975) Ethanol-water delignification of wood-rate contents and activation energy. TAPPI 58 (8) 170

Kleinert, T. (1974) Organosolv pulping with aqueous alcohol. TAPPI 57 (8) 99

Krishnamurti, C.R. y Kitts, W.D. (1969) Preparation and properties of cellulases from rumen microorganisms. Can. J. Microbiol. 15: 1373

Leatherwood, J.M. (1965) Cellulase from R. albus and mixed rumen microorganisms. Appl. Microbiol. 13: 771

Leatherwood, J.M. (1973) Cellulose degradation by Ruminococcus. Fed. Proc. 32: 1814

Lee, B.H. y Blackburn, T.H. (1975) Cellulase production by a thermophilic Clostridium species. Appl. Microbiol. 30: 346

Lesoing, G.; Klopfenstein, T.; Rush, I. y Ward, J. (1980) Chemical treatment of wheat straw. J. Anim. Sci. 51: 263

Levy, P.F.; Sanderson, J.E.; Kispert, R.G. y Wise, D.L. (1981) Biorefining of biomass to liquid fuels and organic chemicals. Enzyme Microbiol. Technol. 3: 207

Li, S.F. y Eger-Hummel, G. (1981) Genetic control of dikaryotic fruiting in the basidiomycete Pleurotus ostreatus. en "Advances in Biotechnology" (Moo-Young, M.; C.W. Robsinon and C. Vezina, eds.) Vol. I, p 101, Pergamon Press, Toronto

Lindenfelser, L.A.; Detroy, R.W.; Ramstak, I.M. y Worden, K.A. (1979) Biological modification of the lignin and cellulose components of wheat straw by Pleurotus ostreatus. Dev. Ind. Microbiol. 20: 541

McKenzie, C.R. y Bilous, D. (1982) Location and kinetic properties of the cellulase system of Acetivibrio cellulolyticus. Can. J. Microbiol. 28: 1153

Martin, P.C.; Cribeiro, T.C.; Cabello, A. y Elias, A. (1974) The effect of sodium hydroxide and pressure on the dry matter digestibility of bagasse and bagasse pith. Cuban J. Agric. Science 8: 21

Morton, R. y Granzow, S. (1982) Ethanol-alkali pulping. TAPPI 65 (6) 103

Morris, E.J. y van Gylswyk, N.D. (1980) Comparison of the action of rumen bacteria on cell walls from Eragrotistef. J. Agric. Sci. 95: 313

Neilson, M.J.; Shafizadeh, F.; Aziz, S. y Sarhanen, K.W. (1983) Evaluation of organosolv pulp as a suitable substrate for rapid enzymatic hydrolysis. Biotechnol. Bioeng. 25: 609

Ng, T.K.; Weimer, P.J. y Zeikus, J.G. (1977) Cellulolytic and physiological properties of Clostridium thermocellum. Arch. Microbiol. 114: 1

Ng, T.K. y Zeikus, J.G. (1981) Comparison of extracellular cellulose activities of Clostridium thermocellum LQRI and Trichoderma resei QM9414. Appl. Env. Microbiol. 42: 231

Odier, E.; Janin, G. y Monties, B. (1981) Poplar lignin decomposition by gram-aerobic bacteria. Appl. Environ. Microbiol. 41: 337

Patel, G.B.; Khan, A.W.; Agnew, B.J. y Calvin, J.R. (1980) Isolation and characterization of an anaerobic, cellulolytic microorganism, Acetivibrio cellulolyticus gen nov sp nove. Int. J. Syst. Bacteriol. 30: 179

Pettipher, G.L. y Latham, M.J. (1979) Characteristics of enzymes produced by Ruminococcus flavefaciens which degrade plant cell walls. J. Gen. Microbiol. 110: 21; 110: 29

Plasse, F.; Fardeau, M.L. y Belaich, J.P. (1980) Microcolorimetric evaluation of the efficacy of the alkaline treatment on the fermentation of straw. Bacteriol. Lett. 2: 11

Platt, M.W.; Chet, I y Henis, Y. (1981) Lignocellulose degradation during growth of the mushroom Pleurotus sp "Florida" on cotton straw. Eur. J. Appl. Microbiol. Biotechnol. 13: 194

Preston, T.R. (1975) Sugarcane as the basis for intensive animal production in the tropics. Proc. Conf. on Anim. Feeds of Tropical and Subtropical Origin, TPI, London, p 69

Rahmatullah, M.; Rahmau, A.; Chowdhurg, A.A. y Rashid, M.A. (1979) Anaerobic digestion of jute-stick powder by a mesophilic cellulolytic Clostridium species. J. Ferment. Technol. 57: 117

Rajarathnam, S.; Wankhede, D.B. y Patwardhan, M.V. (1979) Some chemical and biochemical changes in straw constituents during growth of Pleurotus flabellatus(Berk & Br) Sacc. Eur. J. Appl. Microbiol. 8: 125

Randel, P.F. (1972) A comparison of the digestibility of two complete rations containing either raw or alkali-treated sugarcane bagasse. J. Agr. Univ. Puerto Rico 56: 18

Rexen, F. (1979) Low quality forages improve with alkali treatment. Feedstuffs 51 (42) 33

Rolz, C.; de Cabrera, S.; Calzada, F.; García, R.; de León, R.; de Arriola, M.C.; de Micheo, F. y Morales, E. (1983) Concepts in the biotransformation of carbohydrates to ethanol. Adv. Biotechnol. Proc. 1: 97

Rosenberg, S.L. (1980) Fermentation of pentose sugars to ethanol and other neutral products by microorganisms. Enzyme Microb. Technol. 2: 185

Saddler, J.N. y Khan, A.W. (1980) Cellulose production by Acetivibrio cellulolyticus. Can. J. Microbiol. 26: 760

Saddler, J.N. y Khan, A.W. (1981) Cellulolytic enzyme system of Acetivibrio cellulolyticus. Can. J. Microbiol. 27: 258

Sanderson, J.E.; Wise, D.L. y Augenstein, D.C. (1978) Organic chemicals and liquid fuels from algal biomass. Biotechnol. Bioeng. Symp. 8: 131

Sarhanen, K.V. (1980) Acid catalyzed delignification of lignocellulosics in organic solvents. Prog. Biomass Conv. 2: 128

Shinmyo, A.; García, D.V. y Demain, A.L. (1979) Studies on the extracellular cellulolytic enzyme complex by Clostridium thermocellum. J. Appl. Biochem. 1: 202

Singh, N.S. y Rajarathnam, S. (1977) Pleurotus eous (Berk) Sacc.: a new cultivated mushroom. Current Science 46: 617

Smith, W.R.; Yu, I. y Hungate,R.L.(1973) Factors affecting cellulolysis by R. albus. J. Bacteriol. 114: 729

Solaiman, S.G.; Horn, G.W. y Owens, F.N. (1979) Ammonium hydroxide treatment of wheat straw. J. Anim. Sci. 49: 802

Su, T.M. (1978) Bioconversion of plant biomass to ethanol. Am. Inst. Chem. Eng. Symp. Series 74 (181): 75

Thauer, R.K.; Jungerman, K. y Deker, K. (1977) Energy conservation in chemotrophic anaerobic bacteria. Bacteriol. Reviews 41: 100

van Soest, P.J. y Robertson, J.B. (1980) Systems of analysis for evaluating fibrous feeds. en "Standarization of Analytical Methodology for Feeds" (Pidgen, W.J.; Balch, C.C. y Graham, M. eds.) International Development Research Centre, IRDR-134e, Ottawa

Villet, R. (1981) Biotechnological research and development for biomass conversion to chemicals and fuels. Dev. Ind. Microbiol. 22: 97

Walker, D.J. (1967) Some properties of xylanase and xelobiose from mixed rumen microorganisms. Aust. J. Biol. Science 20: 799

Wicklow, D.T.; Detroy, R.W. y Jessee, B.A. (1980) Decomposition of lignocellulose by Cyathus stercoreus (Sch) de Toni NRRL 6473, a white rot fungus from cattle dung. Appl. Environ. Microbiol. 40: 169

Wiegel, J. y Ljungdahl, L.G. (1980) Ethanol as fermentation product of extreme thermophilic anaerobic bacteria. 4th Symp. Tech. Microbiol. (Berlin) 117

Wilkinson, J.M. y Gonzalez, R. (1978) Ensiled alkali treated straw. I. Effect of level and type of alkali on the composition and digestibility in vitro of ensiled barley straw. Anim. Feed Science Technol. 3: 117

Willis, C.M.; Stallup, O.T. y Kreider, D.L. (1980) Influence of sodium hydroxide and enzyme additions on nutritive value of rice straw. J. Anim. Sci. 50: 303

Yu, I. y Hungate, R.E. (1979) The extracellular cellulases of R. albus. Ann. Rech. Vet. 10: 251

Zadrazil, F. (1977) The conversion of straw into feed by basidiomycetes. Eur. J. Appl. Microbiol. 4: 273

Zadrazil, F. (1978) Cultivation of Pleurotus. en "The Biology and Cultivation of Edible Mushrooms" (Chang, S.T. y Hayes, W.A., eds.) p 521, Academic Press, Nueva York

Zadrazil, F. (1979) Screening of basidiomycetes for optimal utilization of straw. en "Straw Decay and its Effect on Disposal and Utilization" (E. Grossbard, ed.) p 139, John Wiley & Sons, Chichester

Zadrazil, F. (1980a) Influence of ammonium nitrate and organic supplements on the yield of Pleurotus sajor caju (Fr) Sing. Eur. J. Appl. Microbiol. Biotechnol. 9: 31

Zadrazil, F. (1980b) The influence of ammonium nitrate supplementation on degradation and in vitro digestibility of straw colonized by higher fungi. Eur. J. Appl. Microbiol. Biotechnol. 9: 37

Zadrazil, F. (1980c) Conversion of different plant waste into feed by Basidiomycetes 9: 243

Zadrazil, F. (1980d) Factors determining lignin decomposition and in vitro digestibility of lignocellulose during solid state fermentation. en "Advances in Biotechnology" (Moo-Young, M. y Robinson, C.W., eds.) Vol. II, p 369, Pergamon Press, Toronto

Zeikus, J.G. (1979) Thermophilic bacteria: ecology, physiology and technology. Enzyme Microb. Technol. 1: 243

Zeikus, J.G. (1980) Chemical and fuel production by anaerobic bacteria. Ann. Rev. Microbiol. 34: 423