

DILUTE ACID HYDROLYSIS OF BAGASSE AND FERMENTATION OF THE HYDROLYSATE TO PRODUCE SINGLE CELL PROTEIN

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Abstract

Details are presented on yields of fermentable components obtained by dilute acid hydrolysis of mill-run bagasse in a 100 litre pressure reactor together with results of fermentation studies using this hydrolysate and a specially selected yeast strain. A brief description is given of the pilot plant with a capacity of 2 ton mill-run bagasse per day, erected at Malelane sugar mill, for the collection of scale-up and economic data.

Introduction

It is known that modern sugar-cane mills can produce a surplus of bagasse, the extent of that surplus depending largely on the use of efficient evaporation and boiling schemes. It has been estimated (Reid and Rein⁸) that it is possible to achieve a surplus of between 35 and 45% of all bagasse produced. An average size sugar mill produces 600 000 t mill-run bagasse yearly and hence could provide 270 000 t for by-products if designed for maximum efficiency.

Sugar-cane bagasse has a multitude of possible uses. It is not the intention of the authors to give a full description of all these. An excellent summary of this topic is given by Paturau.⁷ The decision on which of these uses are exploited is largely an economic one. This paper concentrates on just one possible use which has particular relevance for the future food needs of South Africa's rapidly growing population, namely the production of large quantities of protein for use in animal feeds.

At present the major sources of protein supplements in animal feeds are fish meal and oil cakes. During the first six months of 1989 SA imported a total of 168 000 t of these sources at a value of R195 million. The corresponding local production amounted to 325 000 t (IDC: unpublished data). There has been a steady increase in imported quantities since 1981. The amounts imported at present represent one third of South Africa's requirements. Furthermore, these commodities have experienced higher price increases than many other imported goods. These facts emphasise that one is dealing with a strategic raw material. A possible way to reduce this deficit of raw protein for animal feeds lies in the production of Single Cell Protein (SCP).

Various sources can provide substrates for SCP production. These include molasses, sugar juice, petrochemical products, agricultural waste products such as maize cobs, corn straw, sugarcane bagasse, wood shavings and saw dust, and industrial effluents from, *inter alia*, oil-from-coal processes (SASOL), dairy processes and paper manufacture. Substrate preparation or procurement represents one of the major cost items in SCP production (Moo-Young⁵) and hence the choice of the raw material to provide a suitable substrate for SCP production is an important one. Sugar-cane bagasse has been identified as a potentially suitable raw material source (Pateron-Jones⁶).

Process and pilot plant description

A schematic outline of the process is given in Figure 1. The two key processes consist of the hydrolysis of bagasse and the subsequent fermentation of this hydrolysate.

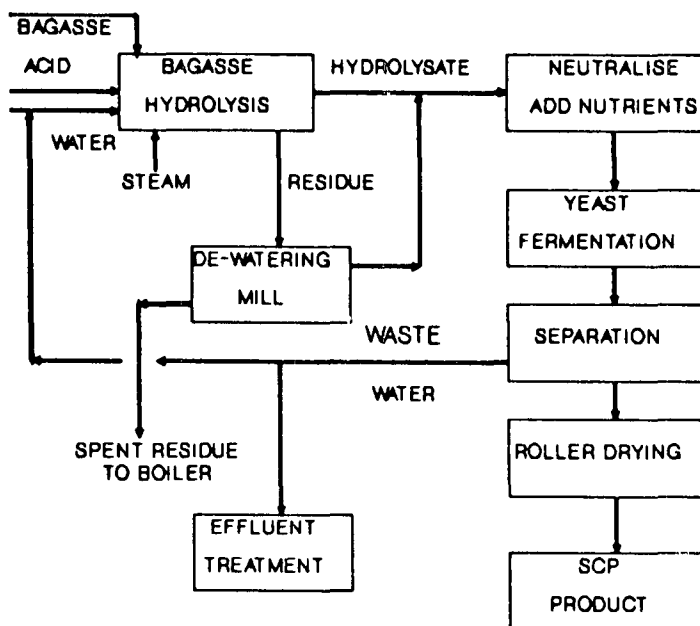


FIGURE 1 Schematic diagram of the SCP production process.

Hydrolysis

Mill-run bagasse contains 46-52% moisture. The major components and their relative quantities in dry bagasse are shown in Table 1.

Table 1
Major components of dry bagasse (Trickett and Neytzell-de Wilde¹⁰)

Component	Per cent
Cellulose	38
Hemicellulose	33
Lignin	22
Ash	3

Both the cellulose and the hemicellulose components can theoretically be broken down to produce a substrate that can be fermented by micro-organisms to produce SCP. Break-down of the cellulose fraction produces a solution rich in glucose, whereas the hemicellulose portion provides predominantly a xylose solution. Xylose is a five carbon sugar which is generally fermented by a different group of micro-organisms from those favouring glucose. It is far more difficult chemically (and hence more expensive) to extract the cellulose (Waugh¹²) compared to the hemicellulose. Therefore in this project it was decided to concentrate on using

the hemicellulose fraction to provide a fermentable substrate.

One of the most economical ways of breaking down hemicellulose is to use a dilute solution of sulphuric acid in which the bagasse is cooked (Trickett⁹). The theoretical yield of fermentable components, of which xylose is the major one, is given in Table 2.

Table 2

The chemical composition of bagasse hemicellulose hydrolysates (Trickett and Neytzell-de Wilde.¹⁰)

Component in hydrolysate	Quantity (percentage of original bagasse)
Arabinose	4,4
Glucose	4,0
Xylose	24,0
Acetic acid	5,5

From the mathematical model of xylose yield developed by Trickett⁹ it is clear that the variables that affect this hydrolysis process are: temperature, time, acid concentration and liquid:solid ratio.

Trickett's model was developed for xylose only, whereas from an industrial perspective all the fermentable components (including arabinose, glucose and acetic acid) are significant. Furthermore, the model describes the kinetics of xylose formation under ideal mass and heat transfer conditions. The development of a theoretical model incorporating all the fermentable components together with the heat and mass transfer characteristics of industrial-scale reactors is very complex. For this reason it was decided to build a 100 litre reactor which could provide data for the establishment of an empirical relationship, which would be useful in an industrial process.

A stainless steel pressure vessel which could hold approximately 12 kg of mill-run bagasse was designed and erected at the Sugar Milling Research Institute (SMRI). This vessel was equipped to monitor continuously pressure, temperature, in the bottom and top of the reactor, and weight. Heating was by live steam injected through four ports at the bottom of the reactor and the steam consumption could be determined from the increase in weight during hydrolysis. This vessel was used to determine optimum conditions for an intermediate scale operation.

One of the problems associated with the use of hot dilute sulphuric acid is corrosion. A comparison of the relative corrosion rates for a variety of commercially available stainless steels in dilute sulphuric acid as well as in vessels containing the typical contents of bagasse hydrolysis under identical exposure time and temperature conditions was made. The results are shown in Figure 2.

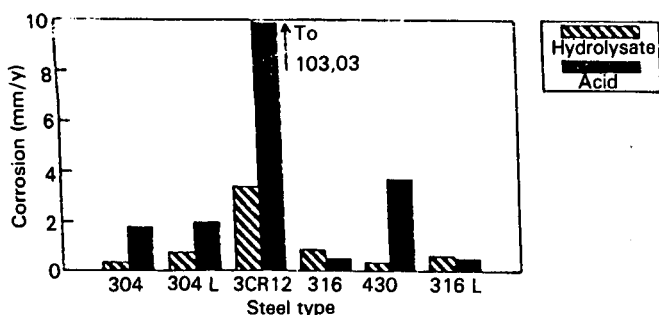


FIGURE 2 Penetration rates (mm per year) for different steels caused by hydrolysate and 0,5% sulphuric acid at 120°C.

The graphs in Figure 2 show that the corrosion rate of virtually all steels tested (with the exception of 316 and 316L) is significantly lower in the actual hydrolysis liquor than in fresh dilute sulphuric acid.

The rate of mass loss due to corrosion (Figure 3) shows that the corrosion rate for all steels tested, except 3CR12, decreases rapidly after five hours' exposure. Examination of the test coupons revealed that this appears to be due to the formation of a protective coating by the products of hydrolysis.

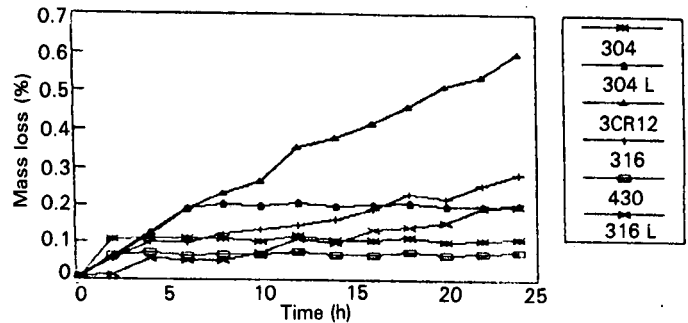


FIGURE 3 Percentage mass loss for different steels in hydrolysate at 120°C over a 24 h exposure period.

Figures 2 and 3 indicate that the most suitable construction materials for a hydrolysis reactor from the corrosion aspect are types 304 and 430.

Fermentation

While optimisation of the bagasse hydrolysis was being carried out at the SMRI, the Council for Scientific and Industrial Research (CSIR) directed research on identifying micro-organisms that are capable of using xylose and all the other fermentable constituents of bagasse hydrolysate. A new strain of *Candida* yeast was isolated and evaluated in terms of protein yield and other growth characteristics. This isolate showed the following advantages over an industrial reference strain, *Candida utilis* (Du Preez and Kilian³):

- It uses arabinose whereas *C. utilis* cannot
- It grows at temperatures as high as 44°C in contrast to *C. utilis* which grows at 35°C. This can substantially reduce cooling costs and assist in controlling contamination by other organisms
- It exhibits a much higher tolerance to furfural, an inevitable by-product of bagasse hydrolysis.

In addition, the strain grows at low pH values (3-4) which prevents bacterial contamination, and vitamin supplementation of the hydrolysate is not required.

Preliminary pathogenicity and toxicity tests (Johannsen⁴) revealed no harmful effects for animal feed use.

The strain was successfully grown on bagasse hydrolysate at the SMRI but optimisation tests for the fermentation are still necessary.

Pilot plant

To gather data for industrial scale-up and economic evaluation, a pilot plant capable of processing 2 ton mill-run bagasse per day is being built at Malelane on the site of the TSB sugar mill at an estimated cost of R250 000. Most of the process vessels were acquired second-hand and had to be modified for their specific functions. The hydrolysis is done as a batch process whereas the fermentation is to be continuous. The plant can hydrolyse 360 kg mill-run bagasse per batch which takes approximately three hours and produces 1 200 litres hydrolysate. Live exhaust steam at one bar (gauge) has been used for heating, but the steam pressure

has been rather erratic resulting in lower yields of fermentables than had been obtained at the SMRI. In the new season it is planned to use throttled 4,5 bar steam to provide a constant temperature and pressure.

Commissioning of the fermentation section of the pilot plant is scheduled near the beginning of the 1990 sugar milling season. The equipment for harvesting the SCP still has to be procured and installed.

Results and Discussion

The variables determining the yield of fermentable constituents from hydrolysis of the bagasse are: temperature, time, acid concentration and liquid:solid ratio. Optimisation studies using the 100 l reactor at the SMRI yielded the results shown in Figure 4 (a)–(d). The base conditions for these studies were 123°C, 2 h hydrolysis time, 0,7% acid concentration and a 6:1 liquid:solid ratio. These conditions were held constant except for the variable being investigated.

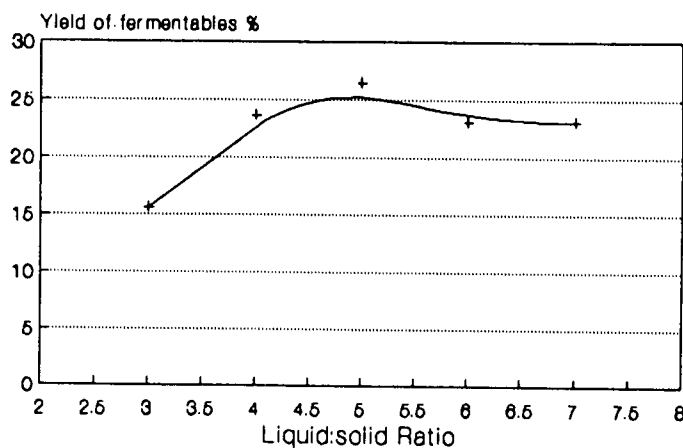
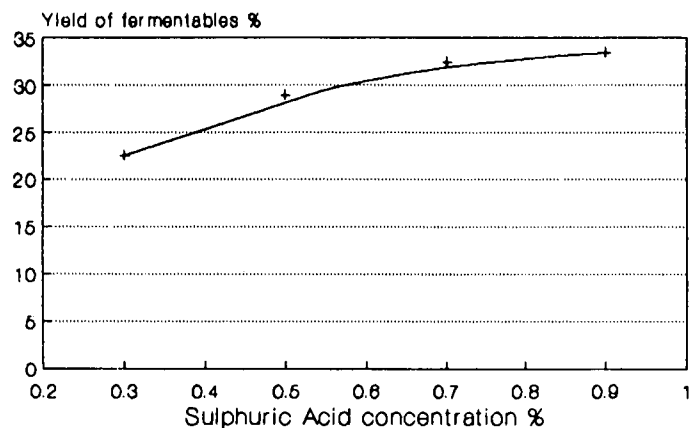
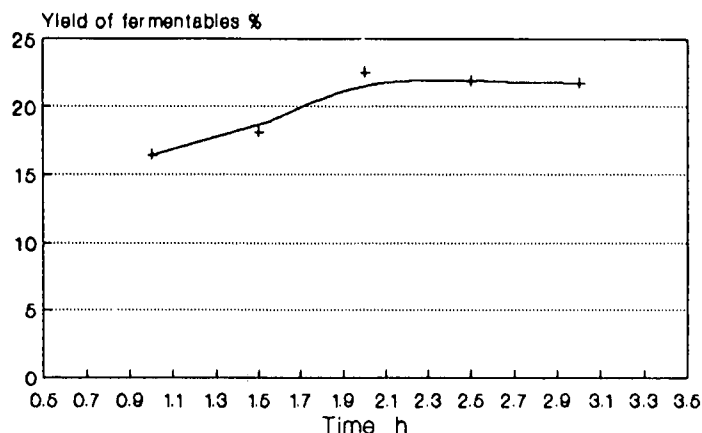
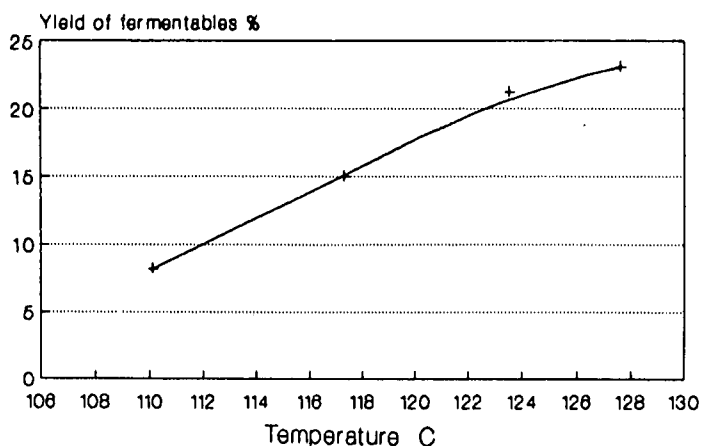


FIGURE 4 The effect of temperature, time, acid concentration and liquid:solid ratio on yield of fermentables.

The graphs show the following:

- The efficiency of the hydrolysis process is very sensitive to temperature, the higher the temperature the better the yield. There is however a limit imposed by increasing amounts of furfural (which is a degradation product of xylose and inhibits yeast growth) being formed at higher temperatures. The optimum temperature, taking acceptable levels of furfural formation into account, is 125°C
- The hydrolysis time which gives the best yield is 2 hours. The possibility of extracting more fermentables with increased cooking time is counteracted by the increased breakdown of xylose to furfural
- Acid concentrations higher than 0,7% appear to have a limited beneficial effect on production of fermentables. It is, of course, desirable to keep the acid concentration as low as possible so as to minimise the cost of chemicals
- The most effective liquid:solid ratio appears to be 5:1. This ratio represents the total mass of liquid per unit mass of dry bagasse. It is advantageous to work with as low a liquid:solid ratio as possible because a higher concentration of fermentables in the hydrolysate will result, in addition to saving on utilities.

The data summarised in Figure 4 were subjected to a quadratic curve-fitting technique (Caceci and Cacheris²) to obtain an empirical equation from which the yield of fermentables at different process conditions could be calculated. The equation which gave the best fit was:

$$Y = 97,5 + 0,317c + 2,89c^2 - 3,05 T + 0,0137 T^2 + 6,71t - 1,181t^2 + 8,683r - 0,616r^2 \dots (1)$$

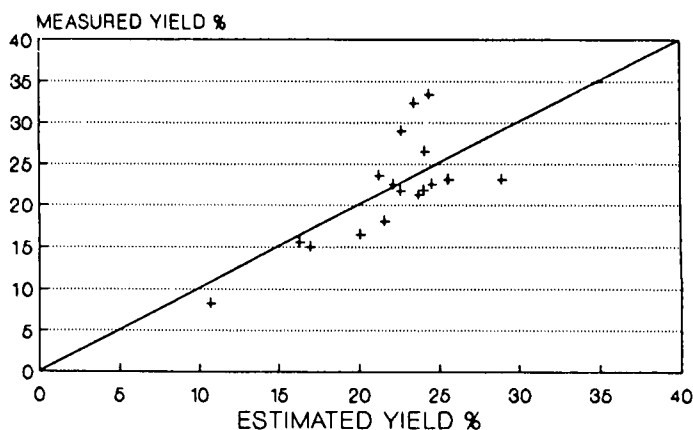


FIGURE 5 Comparison of measured yield against yield estimated from the regression equation (1).

where

Y = yield of fermentables (g/100g dry bagasse)

c = acid concentration (g/100ml)

T = temperature (°C)

t = time (h)

r = liquid:solid ratio (dimensionless)

This equation gave a standard error of the yield of 4,3% on 18 data points. A comparison between the yield estimated from this equation and that actually obtained in the experiments is shown in Figure 5.

The equation predicts a yield of 25% for the optimum conditions listed above. The maximum theoretical yield (see Table 1) is 33%.

The concentration of fermentable components achieved in the hydrolysate with the 100 l reactor under optimum conditions was 3-4%. This is adequate for fermentation, but higher concentrations (up to 10%) are desirable. To obtain a better concentration, hydrolysate was used for a second cooking on fresh bagasse (after acid replenishment) but the results have been disappointing. This is probably due to a significant decomposition of xylose in the second stage. Alternative methods of achieving higher concentrations will be investigated.

During the first series of hydrolysis tests at the Malelane pilot plant, the concentration of fermentables obtained ranged between 1,3 and 1,6%. This relatively low concentration can be ascribed to a fluctuating steam supply, which resulted in temperatures up to 15°C lower on average than those employed in the 100 l SMRI reactor. The high degree of temperature sensitivity has been mentioned above.

The SCP yield (g dry cells/g fermentable substrate) on bagasse hydrolysate obtained by continuous fermentation at the CSIR (Watson¹¹) was 0,31 representing a true protein yield of 14%.

Some economic data

Molasses is a possible competitor as substrate for yeast fermentation. The average SA price of molasses during the 1989/1990 season was R87/t. The mean content of fermentable sugars is 43%. This is equivalent to a cost of R202 per ton fermentables from molasses.

From results gathered from the 100 l reactor and current bulk prices of the relevant chemicals, the operating and raw material cost of producing one ton of fermentables from bagasse hydrolysis is calculated as R236/t. This figure takes into account the cost of bagasse (at coal replacement value), water, steam, acid and neutralisation. It may be argued that molasses would be a more suitable substrate because it has a higher concentration of nutrients than bagasse hydrolysate. This advantage is partly negated by the need for sterilisation in the case of molasses fermentation. One must also bear in mind that the supply of molasses is more limited than bagasse, and that it will probably increase in price at a faster rate than bagasse. Furthermore, since the steam cost represents 43% of the total production cost, considerable savings can be effected by energy recovery measures which have not been included in the calculation.

From the yields quoted above, it is calculated that one ton fermentables would produce 310 kg SCP, which at the current approximate price of R1 000/t would result in an income of R310.

If all of the available bagasse from an average SA sugar mill (600 000 t mill-run bagasse p.a.) were used, the quantity of SCP that could be produced is 23 000 ton, which would sell for approximately R23 million. In a feasibility study carried out in 1987 (Anon¹) it was estimated that the capital

cost for a plant producing 25 000t SCP per annum would be R33 million.

The data accumulated thus far seem to indicate the following:

- Since steam is one of the major cost items in the extraction process, the coal price applicable to a particular mill will significantly influence its profitability
- Substantial savings in energy, utilities and chemicals can be achieved by partial re-cycle of effluent and by other recovery schemes
- A reliable economic evaluation would have to include a fairly detailed process plant design
- The SCP process is likely to offer a better return on investment if the residue is processed further into a useful product instead of returning it to the boiler.

Conclusions

The tests done in the 100 litre SMRI hydrolysis reactor have shown that approximately 25% of the dry weight of bagasse can be extracted as a useful substrate for single cell protein production. The initial pilot plant studies at Malelane have indicated that there are no major processing problems, and that yields similar to those obtained on a small scale are achievable in industrial-size reactors. Raw material and operating costs to produce a fermentable substrate by this route are comparable to the cost of sugar in molasses.

Further pilot plant studies will concentrate on fermentation optimisation and on energy recovery measures. Full-scale animal feeding trials using the single cell protein are planned. The pilot plant will also be used to obtain estimates of the capital investment required for an industrial-scale process.

Acknowledgements

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REFERENCES

1. Anon (1987). Project Engineering Africa. Preliminary feasibility study - single cell protein - addendum to bagasse hydrolysis. *Report, Reference No 701*, March.
2. Caceci, MS and Cacheris, WP (1984). Fitting curves to data. *BYTE*, May: 340-362.
3. Du Preez, JC and Kilian, SG (1989). Selection and evaluation of yeasts for SCP production. In: Single cell protein production. *Progress Report C-Voed 231*, DFST, CSIR, Pretoria, Nov. Appendix I.
4. Johannsen, E (1989). Preliminary report on pathogenicity and toxicity tests involving *ESP94* and *Candida utilis* Y1044 (ATCC 9950). In: Single cell protein production. *Progress Report C-Voed 231*, DFST, CSIR, Pretoria, Nov. Appendix IV.
5. Moo-Young, M (1977). Economics of SCP production. *Process Biochemistry*, May: 6-10.
6. Paterson-Jones, JC (ed) (1989). The biological utilization of bagasse, a lignocellulose waste. *SA National Scientific Programmes Report No 149*, FRD, CSIR, Pretoria.
7. Paturau, JM (1989). *By-products of the cane sugar industry*. 3rd edition. Elsevier, Amsterdam 435 pp.
8. Reid, MJ and Rein, PW (1983). Steam balance for the new Felixton II mill. *Proc S Afr Sug Technol Ass* 57: 85-91.
9. Trickett, RC (1982). Utilisation of bagasse for the production of C5 and C6 sugars. *M.Sc. thesis*, Department of Chemical Engineering, University of Natal, Durban.
10. Trickett, RC and Neytzel-de Wilde, FG (1982). Dilute acid hydrolysis of bagasse hemicellulose. *Chemsa* 8: 11-15.
11. Watson, TG (1989). Task 6: Substrate handling and laboratory scale fermentation optimization. In: Single cell protein production. *Progress Report C-Voed 231*, DFST, CSIR, Pretoria, Nov.
12. Waugh, EJ (1989). Cellulose hydrolysis and fermentation. In: The biological utilization of bagasse, a lignocellulose waste. *SA National Scientific Programmes Report No 149*, FRD, CSIR, Pretoria, chapter 5:56-70.