Table 1. Waste Papers, Summative Analysis and Ethanol Potential[a]

	Shredded white office paper	*Newspaper*	*Kraft paper*	*Milk carton*	*Corrugated carton*	*Paperboard*	*Mixed waste paper*[b]
Moisture (%)	6·6	8·9	5·5	5·9	6·0	6·8	6·3
Ash (%)	9·1	0·3	1·3	0·1	1·1		2·8
Carbohydrates as sugars (%)	88·0	70·9	81·3	77·2	69·1	77·6	78·3
Ethanol potential (litres/t)	568	457	524	498	446	500	508

[a] Ethanol potential $= \frac{\text{carbohydrates as sugars} \times 0{\cdot}51 \times 10}{0{\cdot}79}$ litres/t. Cellulose ethanol potential = 710 litres/t.

[b] Mixed waste paper used here is 25% white office paper, 25% newspaper, 20% kraft paper, 20% milk carton, 10% corrugated carton. Ethanol potential is calculated from this composition.

with an Aminex HPX 87P column using water at 65 °C as the eluant, a refractive index detector and 1·6% *n*-propanol as internal standard). Ethanol was determined by GC (Hewlett Packard 5700A, with an ionization flame detector, using 0·7% *n*-propanol as internal standard).

Saccharification and fermentation

None of these paper samples were given any pretreatment before saccharification. The paper was shredded and cut into squares 1–2 cm.

Saccharification was carried out in 250 ml shake flasks containing 100 ml saccharification medium, or in 2·5 litre fermenters designed for the processing of paper, as described below. The amount of paper was initially 2–20% solids. The amount of cellulase enzymes used was 8·6–70 IU/g paper, the enzyme being a mixture of 70% cellulase and 30% cellobiase. The pH was adjusted with lime to 4·8. Small amounts of Vitamin B_{12} and trace elements were added, along with a few drops of Triton X100. Saccharification in the 250 ml shake flasks was assisted by several small stainless steel balls. Incubation was at 45 °C in a rotary shaker at 150 rev/min.

Fermentation was begun either when saccharification had proceeded for 6 h, or after it was apparently complete. In either case, the temperature was reduced to 37 °C and the yeast added. This temperature was chosen to permit saccharification to continue during fermentation. Nutrients added for fermentation were either YMP (0·3% yeast extract, 0·3% malt extract, 0·5% peptone) or DAP (0·01% diammonium phosphate, 0·02% urea). Initial cell levels were 10–20 g/litre freeze dried weight. Anaerobic closures were used.

For larger scale saccharification and fermentation, conventional stirred tank fermenters proved unsatisfactory, since the paper clogged the internals even at low consistency. A simple, efficient bioreactor designed for paper saccharification and fermentation is shown in Fig. 1. The body of the bioreactor is a (recycled) plastic 2·5 litre chemical bottle, held horizontally in a reciprocal shaker water bath with temperature control. A 100 ml bottle is sealed to a hole in its side, the bioreactor being oriented so that this smaller bottle is vertical, serving to keep the gas outlet at its head free of paper. The gas outlet is equipped with an anaerobic closure. In operation, the bioreactor cycles about 120 times per minute, enough to keep the paper in suspension even at high consistency. For use in aerobic fermentations, such as for enzyme production, an air inlet and air flowmeter were added. Usually in this bioreactor 100 g of waste paper was processed suspended in 1 litre of medium, and after partial liquefaction a second 100 g of waste paper was added.

RESULTS AND DISCUSSION

Composition of waste papers

Table 1 presents the results of analysis of various waste papers. The carbohydrate content expressed as monomeric sugars ranged from 69·1% in corrugated paper to 88·0% in shredded white office paper (SWOP). Ash content was very low in milk carton and newspaper and high in office paper. The potential for ethanol production ranged from 446 litres/t for corrugated carton to 568 litres/t for SWOP. Pure cellulose upon saccharification and

Table 2. Saccharification of Shredded White Office Paper (SWOP), 100 ml Saccharification Medium in 250 ml Shake Flasks

[a]*E/SWOP (IU/g)*	*Solids (%)*	*(h)*	*Total sugars (% of SWOP)*	*Saccharification (%)*
8·6	5	48	57·2	65·0
		72	61·4	69·8
17·2	10	48	70·8	80·5
17·2	[b]10+10	72	47·6	54·1
34·4	10+10	72	43·6	49·5
34·4	10+10	72	48·8	55·5

[a] E is 70% cellulase (Multifect S-850), 30% cellobiase (Novozym 188).
[b] 10+10 represents 2 additions of SWOP 24 h apart.

Table 3. Simultaneous Saccharification and Fermentation of Shredded White Office Paper (SWOP)

[a]*E/SWOP (IU/g)*	*(h)*	*Ethanol*			
		g/litre	*% by vol*	*litre/t*	*g/g glucose*[b]
17·2	72	55·8	7·1	353	0·41
17·2	96	59·3	7·5	375	0·44
34·4	72	63·0	8·0	395	0·47
34·4	96	65·8	8·3	415	0·49

[a] SWOP composition, in %: moisture 1·06, acid insolubles 10·0, glucose 65·8, xylose 13·6, mannose+arabinose 3·8.
[b] The figures in the last column assume that all of the glucose and one-half the (mannose plus arabinose) are available for fermentation.

fermentation can theoretically yield 710 litres ethanol/t.

Ethanol from waste paper

Table 2 reports the results of saccharification of shredded white office paper (SWOP) by a 70:30 mixture of cellulase and cellobiase in 100 ml saccharification medium in 250 ml shake flasks at pH 4·8, incubated at 45 °C on a rotary shaker at 150 rev/min with the additives named in the section 'Saccharification and Fermentation' above. With 8·6 IU enzyme/g paper at 5% solids over a period of 72 h, 69·8% saccharification was obtained. When the ratio of enzyme to paper and the percentage of solids were both doubled, yields of sugars were 80·5% in 48 h.

Simultaneous saccharification and fermentation of SWOP gave the results shown in Table 3. This is not a truly *simultaneous* saccharification-fermentation: SWOP was treated with saccharification medium at 45 °C for 6 h, then cooled to 37 °C and yeast and YMP added. In this procedure, to 100 ml enzyme solution containing 240 IU cellulase and 27 IU cellobiase plus the additives listed above, 10 g SWOP was added, incubated on a rotary shaker at 45 °C for 6 h, then cooled to 37 °C, and yeast and nutrients were added for anaerobic fermentation. After 24 h, the same amount of SWOP and enzymes were added and the mixture incubated for an additional 48 h. Ethanol was produced at the rate of 353–415 litres/t SWOP, at concentrations from 7·1 to 8·3% ethanol by volume. Comparing the results of saccharification shown in Table 2 with those of simultaneous saccharification and fermentation shown in Table 3 it is apparent that saccharification proceeds more efficiently in the presence of yeast.

The results shown in Table 3 meet two of the targets set forth in the introduction: the ethanol yields are above 350 litres/t and the ethanol concentration is above 6% by volume. However, both the total processing time and the amount of enzyme acquired exceed our targets. Enzymes are now made from waste newspaper[7] or other inexpensive inducers,[8] so the amount of enzyme used may be tolerable. Processing time may be improved by adaptation of the yeast to this medium.[10]

Simultaneous saccharification-fermentation of mixed waste paper resulted in 306 litre ethanol/t at a concentration of 6·1% ethanol by volume in 72 h, about 87% of the value obtained with SWOP. Waste paperboard packaging yielded ethanol at the rate of 347 litres/t, at 1·7% ethanol by volume in a low density run.

Bioconversion process variations

Several variations on the above saccharification-fermentation procedure were investigated, with the objective of meeting all the targets set out in the Introduction.

Variation 1

SWOP (5 g) was added to 100 ml solution containing 43 IU mixed enzymes and incubated at 45 °C, and after 24 h incubation the sugar solution was removed by centrifugation. An additional 43 IU mixed enzymes was added to the residual paper, and after another 24 h incubation at 45 °C the new sugar solution was separated by centrifugation. The two sugar solutions were combined for analysis. Saccharification by this fed batch enzyme addition was complete, and fermentation of the combined sugar solution was very rapid, being complete in 6 h, to yield 438 litre ethanol/t, the best of any of the

procedures, but at very low ethanol concentration, 1·1 % by volume.

Variation 2
In an attempt to raise ethanol concentration, a run similar to Variation 1 was made beginning with 10 g SWOP in 100 ml enzyme solution containing 344 IU enzymes, and incubated at 45 °C for 24 h. Then the same amount of enzyme was added two more times at 24-h intervals.[a] Ethanol yield at 24 h was 411 litre/t, at 4·0 % ethanol by volume.

Variation 3
In this procedure, 20 % SWOP solids was obtained by adding the paper and enzymes in two equal portions with a 6-h interval, for a total of 1376 IU enzyme. Fermentation was slow, ethanol yields being 336 litre/t at 96 h, and ethanol concentration reaching 6·7 % by volume.

Variation 4
This is low solids, fed batch SWOP, 2 g SWOP plus 138 IU enzyme being added 10 times over 5 days. After 24 h fermentation, ethanol yield was 375 litres/t, at 6·8 % ethanol by volume.

None of these variations met all the performance targets. The results shown in Table 3 are the best so far in meeting these targets.

Bioconversion with laboratory-prepared cellulase
Enzyme solution was prepared according to the procedure of Chen & Wayman[7] in the 2·5 litre fermenter (Fig. 1) modified to provide a slow air flow over the fungal fermentation. The solution contained 1480 IU and 1·92 g protein/litre. To 100 ml enzyme preparation (a) after filtration or (b) without filtration, was added 5 g SWOP + 9·15 IU cellobiase. After adjustment of pH to 4·8 and incubation at 45 °C for 6 h, the suspension was cooled to 37 °C and 1 g bakers' yeast + YMP was added. Anaerobic fermentation yielded ethanol at the rates of (a) 292 and 359 litres/t SWOP in 24 and 48 h, and (b) 319 and 400 litres/t SWOP in the same periods. The laboratory-prepared enzyme is therefore effective, but there remains a requirement for cellobiase. It is more effective when not filtered, but used as the entire enzyme production mix, which may save added nutrients.

Bioconversion in the 2·5 litre fermenter
In an initial run with this fermenter, commercial enzyme was added as a fed batch. To 1000 ml enzyme solution containing 2408 IU cellulase and 275 IU cellobiase and the usual additives was added 100 g SWOP, the pH adjusted to 4·8 with lime, and the mixture incubated at 45 °C. At 24 and 48 h, fresh enzyme was added in the same amounts. After a total of 96 h at 45 °C and a total of 8049 IU enzyme, the mix was cooled to 37 °C, 20 g yeast and YMP were added. During 6 and 24 h of fermentation, ethanol was produced at the rate of 324 and 391 litres/t SWOP, and 3·2 and 3·9 % ethanol by volume. Samples taken at 72 and 96 h of saccharification showed no increase of sugar production, in both cases about 71 % of theory, suggesting that fermentation could have begun 24 h earlier.

Another run was made in which the 2·5 litre fermenter was used for both cellulase production[7] and for fermentation. The enzyme solution so made contained 1480 IU and 1·92 g protein/litre. To 1 litre of this enzyme solution was added 91·5 IU cellobiase and 50 g SWOP, plus a few drops of Triton X100, the pH was adjusted to 4·8 and the suspension was agitated in the reciprocal shaker at 45 °C for 6 h. It was then cooled to 37 °C and yeast and YMP were added. After 24 h fermentation, ethanol yield was 340 litres/t SWOP, at 1·7 % ethanol by volume. At this time, an additional 50 g SWOP was added plus an additional 91·5 IU cellobiase, and agitation was continued for another 24 h, resulting in an ethanol yield of 320 litres/t SWOP at an ethanol concentration of 3·2 % by volume. These yields, while somewhat lower than obtained in the initial run, are good considering that much less cellulase was used (1480 IU compared to 7224 IU) and less cellobiase (183 IU compared to 825 IU); the usual Vitamin B_{12} and trace elements were not added; and the total time was much shorter (54 h compared to 78 or 120 h). Cellulase usage of 14·8 IU/g waste paper is acceptable.

CONCLUSIONS

1. The questions asked in the last paragraph of the Introduction can now be answered, at least in part. Pretreatment before saccharification is not necessary for efficient bioconversion to ethanol of the papers we have studied. Enzymatic saccharification is to be preferred to acid hydrolysis because the yields are much higher and the conditions gentler. Cellulase enzymes can be made cheaply on site. The best relationship between time devoted to saccharification and that to fermentation in a modified