UTILIZATION OF CELLULOSIC WASTES

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Abstract. Reduction in the particle size of bagasse and rice husk from 60-120 mesh resulted in an increase in the release of sugars from 0.6-6.0 mg/g. Heating of the samples at 125° C for 30-40 min released maximum amount of sugar 15.8 and 5.93 mg/g from bagasse and rice husk respectively. Soaking in 2N HCl and 2% NaOH for 1 hr at ambient temperature released 2.8 and 1.85 mg/g sugar from bagasse and rice husk respectively. Bagasse samples previously heated at 125° C for 40 min when refluxed with 2N HCl for 40 min gave maximum release of sugar, i.e. 282.33 mg/g.

About 2.5 million tons of cellulosic wastes, such as bagasse (954,609 tons), wheat straw (6,605,000 tons), rice straw (13,165,000 tons), and rice husk (4,388,000 tons) are available in Pakistan. Though wheat bran, beet pulp, cotton linters and paper wastes form a sizeable amount of the wastes but unfortunately the figures are not available presently. These wastes contain from 50 to 70% of carbohydrates which cannot be degraded easily into metabolizable sugars by animals. Recently, a large number of rumen and soil microorganisms have been found to possess the ability of degrading native cellulose I-5 in vitro, and converting it into a wide range of products from hydrocarbons to carbohydrates.

Whitaker⁷ reported that enzymic degradation of cellulose was by random hydrolysis; addition of urea either inhibited or stimulated this activity depending upon the pH of the medium.^{6,7} An increase in the susceptibility of cellulose to the action of the enzymes was observed with modification of cellulose by physical or chemical means.^{8–12} Present studies were aimed at establishing optimum conditions for biodegrading bagasse and rice husk into easily disgestible products.

Materials and Methods

Sample Preparation. Bagasse or rice husk was ground and samples of different particle sizes were prepared by sieving through 22, 60, 80, 100 and 120 mesh sieves. A ball mill was used for fine grinding.

Estimation of Sugar. One g sample of cellulosic material was extracted with 100 ml water or 2% NaOH or 2N HCl. Alkaline and acidic extracts were neutralized and reducing sugars present in the extract were determined by Fujita and Iwatake method.¹³

Cellulose Determination. Cellulosic sample $(\frac{1}{2}-2 \text{ g})$ was taken in 100 ml water and was homogenized in a Waring blender for 20 min. Ten ml homogenized sample was taken for estimation of cellulose.¹⁴

Estimation of Starch. Starch present in 0.5 g bagasse or rice husk was estimated by A.O.A.C. method.¹⁵

Results and Discussion

Effect of Reduction in the Particle Size on the Release of Sugars. An increase in the release of sugar

from 0.6 mg/g to 6.0 mg/g was noted when particle size of bagasse sample was reduced from 60 mesh to 120 mesh. This shows that reduction in particle size increased the surface area which improved extractability of sugars. Maximum sugar (6.0 mg/g) was releasd when particle size was 120 mesh. 5-8% increase in sugar was also observed by Ghose and Kostick¹⁶ when particle size of Solka Flock was reduced from 149 to 4.6 μ .

Effect of Dry Heat on the Release of Sugars. The release of sugar increased from 8.75 to 15.2 mg/g with an increase in time up to 60 min when the samples were heated at 100°C. Increase in temperature from 100 to 125°C resulted in a quicker release of sugar. Maximum sugar was released (15.8 mg/g) when the samples were kept at 125°C for 40 min. Further heating for 10 min did not show any increase in the release of sugar. A decrease in sugar content from 15.8 to 15.0 mg/g was noticed when the sample was heated for 60 min. Similar results were obtained when the samples were kept at 150° and 175°C.

An increase in sugar content from 0.81 to 5.93 mg/g occurred when rice husk samples were heated for 30 min at 100°C. Increase in heating time up to 45 min did not improve the release of sugar. Further increase in heating time up to 60 min resulted in a decrease (i.e. 0.56 mg/g) in the release of sugar. An increase in temperature (from 100° to 125° and 150°C) and time (30–60 min), showed a gradual decrease in sugar content (from 5.93 to 3.43 mg/g). Maximum sugar (5.93 mg/g) was released when a rice husk sample was heated for 30 min at 100°C.

Increase in sugar contents seems to be due to oxidative changes brought about by heat treatment and changes in crystalline character of the cellulosic material which renders it more susceptible to the action of cellulolytic enzymes. Ground and heated cellulose was considered a better substrate by Ghose¹⁷; and Katz and Reese,¹⁸ because it showed more susceptibility to cellulolytic enzymes than the unheated cellulose.

Effect of Acid or Alkali on the Release of Sugars. The sugar content decreased from 0.6 to 0.5 mg/g when bagasse samples were soaked in 2% NaOH solution up to 2 hr (Table 1). This decrease seems to be due to the exposure of alkali-treated cellulose to air, making the substrate more resistant to hydrolysis.

| TABLE 1. EFFECT | OF ALK | ALI AND | ACID ON THE |
|-----------------|--------|---------|-------------|
| RELEASE OF SUGA | AR CON | TENT IN | BAGASSE AND |
| RICE | HUSK | SAMPLE | s. |

| Sample | Particle size | Soaking | Time for treatment | Sugar concn (mg/g) |
|-----------|------------------|---------|--------------------|-----------------------|
| Bagasse | 60 mesh | | | 0.6 |
| ** | " | 2% NaOH | 1 hr | 0.52 |
| ,, | ,, | 2N HC1 | 2 ,, | 0.51 |
| ** | ,, | 2N HCI | 1 ,, | 2.8 |
| | " | ** | 2 ,, | 2.9 |
| Rice husk | 22 mesh | | | 0.81 |
| ** | ,, | 2% NaOH | 1 hr | 1.85 |
| ** | ,, / | ** | 2 ,, | 1.81 |
| ** | ,, | 2N HCl | 1 " | 0.9 |
| ,, | " | ,, | 2 ,, | 0.92 |

A decrease in the release of sugar was reported by Reese¹⁹ when the samples were treated with NaOH solution. This was attributed to the changes which occurred during the exposure of alkali-treated cellulose to air. The reduced susceptibility of the residual cellulose may also be due to the removal of more readily hydrolyzed (amorphous) areas by the formation of substituted cellulose derivatives. Sugar content increased from 0.6 to 2.9 mg/g when bagasse sample was soaked in 2N HCl up to 2 hr. This increase may be due to swelling or opening up of the larger molecules (polymeric structure of the cellulose) by depolymerization.

There was no significant increase (0.81-0.9, 0.92 mg/g) in the release of sugar when rice husk samples were soaked in 2N HCl for 1 and 2 hr. An increase in the release of sugar up to 1.04 mg/g was noted when the sample was soaked in 2% NaOH solution for 2 hr. This increase seems to be due to the removal of silica in the form of sodium silicate (as 48.46% loss in weight was observed after alkali treatment) from rice husk which resulted in the formation of easily breakable cellulose molecules.

Effect of Hydrolysis. There was a gradual increase in the release of sugar from 196.25 to 27.5 mg/g when bagasse preheated at 100°C was refluxed for 30–60 min. However, maximum release of sugar (282.33 mg/g) was observed in bagasse preheated at 125°C and refluxed for 40 min. Further increase in refluxing time resulted in a decrease in the release of sugar. Bagasse preheated at 150–175°C also showed the above pattern for the release of sugar.

Release of sugar in preheated rice husk at 100, 125, 150°C varied from 54.07 to 134. 38 mg/g. The increase in preheating temperature 100–125°C and refluxing time 30–60 min showed an increase in the release of sugar. Maximum amount of sugar was released when the sample was heated at 125°C and subsequently refluxed for 60 min. Rice husk preheated at 150°C and subsequently refluxed for 30–60 min did not show any increase in the release of sugar.

A comparison of these results with those obtained by subjecting bagasse and rice husk to dry heat only showed a significant increase in the release of sugar (8.75–282.33, 5.93–134.38 mg/g). The increase in sugar content might be due to the hydrolysis of polysaccharides in HCl, which deeply penetrates into the extended amorphous surface. Ghose¹⁷ also observed an increase in sugar content and interpreted his re-

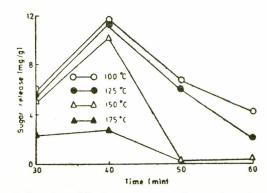


Fig. 1. Effect of dry heat on release of sugar from prerefluxed bagasse.

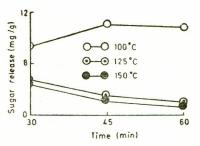


Fig. 2. Effect of dry heat on the release of sugar from prerefluxed rice husk.

results in this way when he soaked Solk Floc in 1N HCl.

Maximum sugar (11.6, 11.8 mg/g) was released when bagasse and rice husk was refluxed for 40 and 45 min respectively prior to heating at 100°C (Figs. 1 and 2). Further increase in refluxing period and heating period and heating temperature caused a decrease in the release of sugar in both the cases. It was observed that the amount of sugar released by refluxing prior to heating of bagasse and rice husk under different conditions of time and temperature was much less than those obtained by heating followed by refluxing. Ghose¹⁷ also preferred preheating while working with Solk Floc. This increase in the extractability of sugar indicated that heat treatment of cellulosic material with modified crystal structure increased its susceptibility towards saccharification.

References

- 1. H.O.W. Eggins and K. A. Malik, Antonie van Lecuwenhock, J. Microbiol. Serol., 35, 178 (1969).
- 2. H.O.W. Eggins, K.A. Malik and R.F. Sharp, Proc. Ist Intern. Biodeterioration Symp., 121, (1968).
- K.A. Malik and H.O.W. Eggins, Trans, Brit. Mycol. Soc., 54, 289 (1970).
- 4. M. Yrmossym, G. J. Weiszfeiler, Maria Somla and J. Hajdu, Proc. Microbiol. Res. Group, Hungarian Acad. Sci., 2, 97 (1968).
- 5. K.A. Malik and H.O.W. Eggins, Intern. Biodeterioration Bull., 5, 163 (1969).
- 6. D.R. Whitaker, Science, 116, 90 (1952).
- 7. P.K. Datta, K.R. Hason and D.R. Whitaker, Biochem. Biophys. Acta., 50, 113 (1961).
- 8. G. Halliwell, Biochem. J., 79, 185 (1961).

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9. G. Halliwell, Biochem. J., 85, 67 (1962).

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- 10. R.G.H. Siu, Microbial Decomposition of Cellulose with Special Reference to Cotton Textile (Reinhold, New York, 1951), p. 159
- 11. E.T. Reese, R.G.H. Siu and H.S. Levinsons, J. Bact., 59, 485 (1950).
- T.K. Ghose, Biotech. Bioengg., 11, 239 (1969).
 A. Fujita and D.C. Iwatake, Biochem. Z. 2, 243 (1931).

14. D.M. Updegraff, Anal. Biochem., 32, 420 (1969). 15. W.G. Pucher, C.S., Leavenworth, and H.B. Vickery, Anal. Chem., 20, 850 (1948). 16. T.K. Ghose and J.A. Kostick, Biotech. Bioengg. 12. 921 (1970). 17. T.K. Ghose, Biotech. Bioengg., 11, 239 (1969). 18. M. Katz, and E.T. Reese, Appl. Microbiol., **16**, 419 (1968). 19. E. T. Reese, Appl. Microbiol., 4, 39 (1956).