ABSTRACT

A Biochemical Audit of the Fermentation of Sugarcane Molasses to Ethanol

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A biochemical audit during the manufacturing process for rum and alcohol, involving the batch fermentation of sugarcane molasses by *Saccharomyces cerevisiae* followed by distillation was studied by analysing samples of media taken at five different stages in the process. Where the samples were separated into solid and supernatant fractions before analysis, centrifugation proved to be more efficient than filtration at achieving the separation and for obtaining dried solids, freeze-drying was applied.

For some of the parameters measured, different analytical methods were compared. Measurements of dissolved solid content by freeze-drying and by hydrometry proved equally reproducible under fixed conditions, but yield different results; protein content was best estimated by the Bradford method once the yeast cells had been suitably ruptured and the proteins solubilized. This method yielded better

correlation with the levels of amino acids detected after acid hydrolysis than did the method of Lowry *et al.*, which was possibly due to interference by other substances.

The results of BOD and COD measurements suggest that the five day incubation period of the Standard Method for BOD determination may be inadequate for complex media obtained from the fermenter and that any attempts to establish a correlation between BOD and COD levels would require that each stage be treated separately and be precisely defined because of the dynamic nature of the fermentation process.

Fermentation occurs under moderately acidic conditions which is maintained by the buffering action of the phosphate present in the media and is characterised in the early stages by high levels of biosynthetic activity. The levels of amino acids were markedly increased, approximately two-thirds of the amount being incorporated into proteins and the levels of fatty acids and RNA were also increased. This is consistent with the increase seen in yeast cell content during fermentation. By the end of fermentation, the levels of these components were reduced, with the protein

levels showing the most significant decline and that of amino acid being only slight.

The metabolic processes leading to the synthesis of these compounds along with ethyl alcohol result in a decline in the levels of sucrose from 203 g/L to 48 g/L, as measured by hydrometry.

During the distillation process, in which the required metabolic product, alcohol, is extracted, there is also the reduction in the levels of solid, whole cells, biochemical and chemical oxygen demand, and amino acids, and an increase in fatty acid content.

Analysis of the distillery waste: fermenter bottoms and dunder, indicate that 327 kg of protein, more than 400 kg of amino acids 124 kg of fatty acids and a variety of other components are emitted daily from the distillery in wastewater.