PRODUCTION OF ETHYL ALCOHOL FROM MOLASSES USING CONTINUOUS PROCESS

GAFFA, T. KRAKWOWIAK, A.
Department of Science Technology
Federal Polytechnic, Bauchi,
P.m.b. 2031, Bauchi,
Bauchi State

ABSTRACT

Sugar cane molasses from Nigerian Sugar processing factory Bacita were used for ethanol production. A special ethanol resistant yeast strain *Saccharomyces cerevisiae* (DIST/IPF/90) employed as inoculum was propagated on clarified molasses. Fermentation of clarified molasses to ethanol was brought about at room temperature (27°C) in a three stage continuous process using the chemostat system of operation. The highest ethanol yield of 10.5% was obtained in the third stage of the fermentation cascade at the flow rate of 33ml/hr with a retention time of 30hr in each tank. The first and second stages of the process had alcohol yields of 4.4% and 6.3% respectively as their maximum. The pH of the fermenting medium maintained at 4.5 using sulphuric acid was found to be inhibitory to contaminants. The result obtained from this study shows that ethanol yield in continuous process increase with increase in the number of tanks.

INTRODUCTION

Ethanol also known as ethyl alcohol, grain alcohol and spirit is produced from a variety of substrates like tubers of cassava, potatoes, grains of all types and from molasses by the fermentation activities of yeasts. Its alcoholic content is usually increased above that of the original fermented mixture by distillation. The types of pretreatment a raw material receives depends on its nature but generally the fermentation process follows the general pattern of growth, harvest and delivery to a distillery, conversion of the raw materials to hydrolyzable substrate suitable for fermentation to ethanol, fermentation and purification by distillation. Faust *et al.*, (1983) reported molasses as being technically the most ideal raw material in ethanol production compared with other substrates giving the reason that they do not require additional preparation. The type of fermentation process practicsed in Nigeria for producing ethanol is the traditional batch technology process which has been described by Mairorella *et al.*, (1981) as antiquated and inefficient. Rosen (1978), Chaundhary *et al.*, (1989) and Chen (1990) have described the use of continuous fermentation process in the production of various products superiority of the process over batch technology system in terms of product yield, simplicity of operation and ease of automating the process among other benefits. No step has so far been taken by any of the industries in Nigeria to adopt this new method of ethanol production. Noting the importance of ethanol in various processes and the availability of molasses also in enough

quantities in Nigeria, this work was therefore designed to produce ethanol at the laboratory scale through the continuous system of operation. The contribution of the system in microbiologically based industries can not be overemphasized.

MATERIALS AND METHODS

Molasses from the Nigerian Sugar Company Bacita (Kwara State) Nigeria were collected from the process line in a clean rubber keg transported to the laboratory and kept in the refrigerator at 4°C for further processing.

A selected strain of the yeast *Saccharomyces cerevisiae* (DIST/IPF/90) was obtained from the Industrial Culture Collection Centre, Poland.

Preparation of Substrate:

Preparation of molasses consisted of its dilution with tap water in a 1:2 ration in a beaker. The diluted molasses were then clarified by acidifying to a pH of 4.5 with concentrated sulphuric acid followed by boiling on a hot plate for 30 minutes with constant stirring. This was left to cool, and poured into a measuring cylinder and covered. It was left overnight to settle some of the impurities such as peptides and proteins. The supernatant was decanted after settling and diluted with water to 20% sugar content, then salts, ammonium sulphate and ammonium hydrogen phosphate were added in the amount of 0.85% and 0.12% (W/V) respectively. The pH was again adjusted to 4.5 - 5.0, then sterilized at 150°C for 30 minutes and cooled to 27°C. The medium was now ready for inoculation with yeast for the fermentation.

A multistage continuous fermentation process of chemostat system was adopted, consisting of three fermentation tanks and a reservoir set out as shown in figure 1. A working volume of 1000mls of the production media was charged into the first fermentation tanks and yeast cells propagated on inoculum medium of 40mls quantity was added. The fermentation was allowed to take place for 24 hours and the medium reservoir was opened to allow fresh media to flow into the first tanks, then the second and subsequently the third tank based on the flow rate of 33 mls/hour. The fermentation was continued for 14 days at 27°C.

Analysis of alcohol content was by the method of Obtea and Krakowiak (1984). Ethanol was measured by oxidation using potassium dichromate. Excess potassium dichromate was assayed after addition of potassium iodide and starch solution; by titrating with sodium thiosulphate. All samples were diluted to 1:10 (V/V) for sharp end points. The figure obtained for the test sample was compared with that for the blank and the percentage alcohol read from a table of results.

Ethanol recovery from the broth was done through fractional distillation process. Recycling of the distillate was done to concentrate the alcohol.

RESULTS AND DISCUSSION

The first stage had its sugar content reduced from 20% to 16.0% on the first day, 14.0% on the second and the least level of 13.6% from the third day up to the fourteenth day. As the sugar level dropped, there was a corresponding rise in alcohol content of the

fermenting broth, starting from the first day with 3.05 value to 3.7% on the second up to seventh day. There was a slight increase to 3.8% in days 8 - 10. The remaining four days of the run had a constant alcohol value of 4.4%.

In the second stage of the process, the sugar was further utilised leaving only 10.20% content on the first day. This value decreased slightly to 9.80% in the second to the fifth day, then the valued remain constant at 9.60% for the rest of the days. The alcohol level was also increased from 3.8% from day 1 to 5.2% in day 2. On the third day, it rose to 6.3% and remained constant throughout the fermentation stages in the rest of the days. The gradual decrease in the level of sugar content at each of the stages with a final levelling off to a constant figure proves that even the continuous process starts like the batch process until a steady state is reached. This has already been mentioned by Okafor (1987). The marked decrease in sucrose from stage 1 and 2(6.0 - 10.2%) with no appreciable increase in alcohol content (3 - 3.8%) can be explained by the fact the tank in the second stage was empty and as the fermentation media began to drop inside, the yeast cells coming alongside with the media metabolised the substrate in aerobic condition giving rise to biomass rather than ethanol. This is also true of the situation from stages 2 to 3. This observation is in line with the report of Ryu *et al.* (1984). Immediately after the sharp drop when the tanks gets filled up, there is usually an increase in alcohol content. The high build up of biomass now acting on the substrate in anaerobic condition can not be unconnected with this observation.

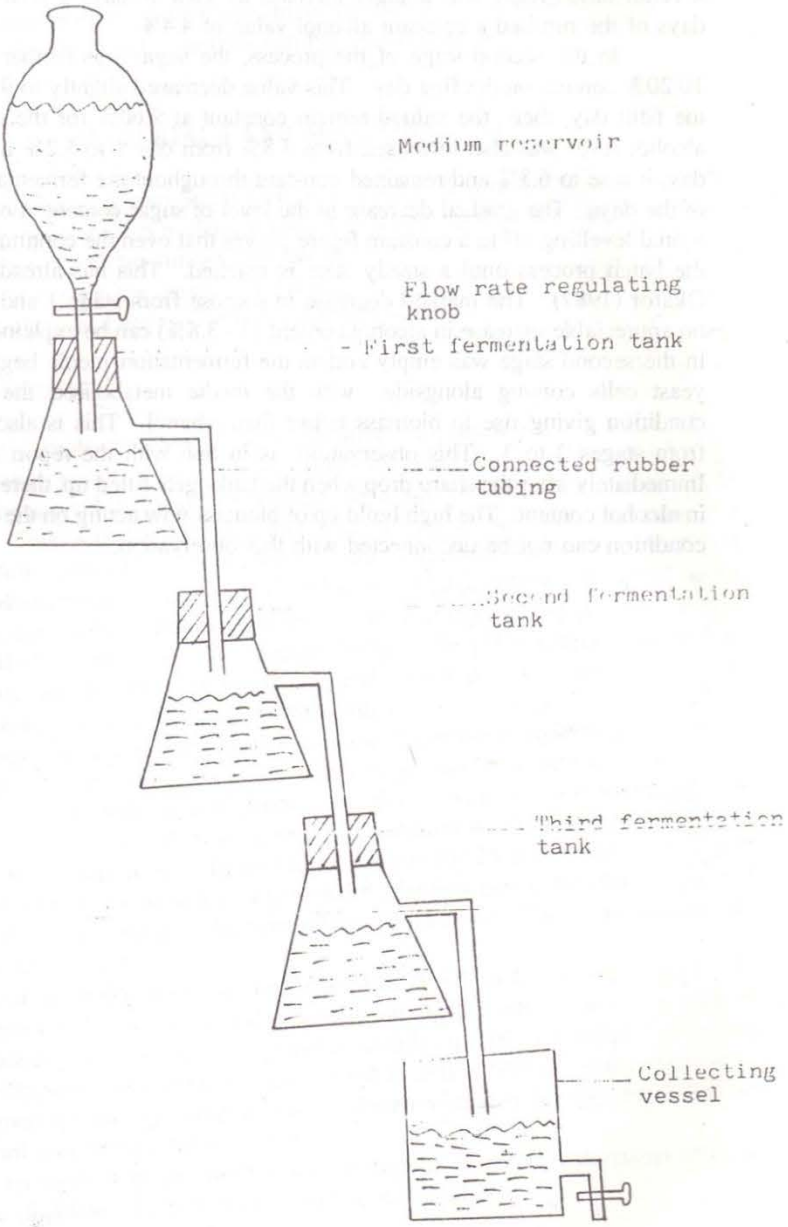

Fig. 1: A multistage fermentation process

Fermentation started in the third stage of the process with the sugar level being 10.20%. This dropped to 9.6% as the fermentation continued and reached a minimum value of 6.92%. The alcohol level was noted to have risen to 10.5% as the highest. The attainment of the highest value of alcohol at the third stage of the process can be explained that in the previous stages, fermentation has only been partial. The sugar was partially utilised in the first and second stages and the fermentation completed in the third stage. Ethanol inhibition of yeast activities was thus reduced. As the substrate was being utilised the more at each subsequent stage, the alcohol content also rose in the broth. These findings are in strong agreement with those of Parisi ai *et al.* (1986) who recorded ethanol productivity of 37.6g/L and 48.5g/L at the first and second stages respectively of a continuous process.

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