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MICROBIAL PRODUCTION OF ETHANOL FROM CELLULOSE

By

JANAKI ANOMA LELWELA GURUGE

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APPLIED SCIENCE, UNIVERSITY OF SRI JAYEWARDENEPURA,
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A B S T R A C T

3 strains of fungi & 4 strains of bacteria that showed cellulolytic activity were isolated from soil & 4 strains of bacteria were isolated aerobically from cow dung. 18 strains of bacteria were isolated anaerobically from the rumen contents of cattle. Major part of this investigation was carried out with anaerobes isolated from the rumen contents.

The cellulosic substrate used was microcrystalline cellulose (MCC), which is the crystalline portion of cellulose. Due to its insoluble nature however, carboxymethylcellulose (CMC) had to be substituted as the sole carbon source for some of the growth experiments. The applicability of most of the growth studies was tested using a strain of Ruminococcus albus which is known to require strict anaerobic conditions for growth and is excellent in cellulose digestibility & the production of ethanol.

The cellulolytic ability of the strains isolated from soil was tested by visual observation of cellulose degradation that included filter paper decay & the formation of cleared zones in agar medium & by the measurement of total carbohydrate content of the medium by the phenol-sulphuric acid colourimetric test. The isolates from cow dung were tested by the phenol-sulphuric acid test & chemical analysis of the culture broth. The strains isolated from the rumen contents were tested by the phenol sulphuric acid test.

Although isolated from cultures that showed cellulolytic activity only two soil isolates showed clearing on agar medium. Four strains showed significant cellulolytic activity by the phenol-sulphuric acid test.

Three isolates from cow dung showed filter paper activity while gas production was observed by only one isolate. Of the isolates from the rumen contents, only nine strains showed significant cellulolytic activity by the phenol - sulphuric acid test.

For analytical studies, the anaerobic condition of the medium was obtained by culturing yeast in the medium, prior to inoculation of the anaerobes. Experiments carried out using R. albus, & two isolates indicated that the presence of yeast in the medium did not affect growth & cellulose digestibility of the anaerobes.

Chemical analysis of the distillate of the culture broth of R. albus indicated positive results for ethanol & sugars. Quantitative analysis of ethanol was done by gas liquid chromatography. Sugars were separated by paper chromatography with isopropanol-pyridine-acetic acid - water & glucose was quantitatively analysed by the phenol-sulphuric acid test.

9.2 mg. ethanol per 100 ml. broth was produced by R. albus in 1% CMC medium at 10 day incubation at 37°C. The production of sugars increased upto a maximum of 30 mg. per 100 ml. broth at 6 days & decreased thereafter. Cell growth assessed by optical density measurements of the culture showed a maximum at 6 days. The maximum amounts of ethanol & sugars produced from 1% cellulose broth were 1.75 mg. & 15.48 mg. per 100 ml. broth respectively.

The effect of some environmental factors on the production of ethanol was studied using cultures of R.albus. Of these factors, maintenance of pH of the broth at 6.8, incubation under CO₂ gas & seed cultivation of bacteria in 0.03% cellobiose broth favoured production, while incubation at room temperature with shaking reduced production.

A study of growth curves of the isolates showed a harvest time of 10 days as suitable for obtaining samples for analysis for ethanol. The pattern of pH variation of each culture studied with the pattern of cell growth, indicates the enzymic activity of the relevant isolate.

The negligible amounts of ethanol produced by the isolates from cellulose have increased under favourable circumstances. Four isolates yielded ethanol from CMC while three others have yielded ethanol from cellulose. Glucose was detected in the culture broth of two isolates only. Cellobiose was detected in the cultures of four isolates. An unidentified trimer was produced by all isolates except one.

The production of ethanol was increased by mixed cultures, carried out using the combinations of ethanol producers with non-ethanol producers. A further increase in the production was observed using combinations of ethanol producers, coupled with the CI & Cx components. 56 mg. of ethanol per 100 ml. broth, the highest amount obtained in this study was produced by the combination of R. albus & an isolate. Although most of the combinations favoured production one combination did not produce ethanol.

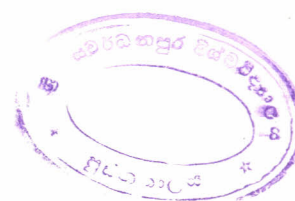
However, the cell yields & the production of ethanol & sugars obtained in this study are fairly low, partly due to the nature of the cellulosic substrate used. Hence, the results obtained are more of an academic interest rather than of economic value.

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