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ETHANOL FERMENTATION OF RAW STARCH

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Ethanol fermentation of raw starch was carried out at 30°, with and without shaking, in a 250 ml conical flask. 90% (v/v) ethanol was produced under shaking conditions while 6.5% (v/v) was produced under static conditions, after 96 hrs, proving agitation to be stimulative for ethanol production. The ethanol yield was further improved to 10% (v/v) when an aerobically cultivated yeast cells or mold mycelium was added to the fermentation broth.

Key words: Yeast cells, Anaerobic, Agitation.

Introduction

In the conventional alcoholic fermentation of starch, cooking is first necessary to liquefy the starch and to sterilize the broth. This process requires a large amount of heat, which accounts for 30 - 40% of the total energy input. In order to reduce the cost of this processing and cooking extensive reserch work is being carried out on the production of ethanol from starchy materials through a non-cookign systems. Raw starch hydrolyzing amylolytic enzymes are being extensively used for this purpose [1-8]. These enzymes are capable of hydrolysing starch to fermentable sugars, at ambient temperatures. The sugars being subsequently used in the manufacture of ethanol by yeast *Saccharomyces cerevisiae*.

Ueda *et al.* [4] reported the use of *Aspergillus niger* and *Aspergillus awamori* for hydrolysis of raw starches. Park and Rivera [9] conducted a comparative study on the alcohol production from various enzyme converted starches without cooking. Yamamoto *et al.* [10] observed that hydrolysis of Potato tubers was enhanced by the addition of Pectin depolymerase. The present paper describes optimum reaction conditions for the ethanol production from raw starch by simultaneous saccharification and fermentation process. This process combines the unit operations of liquification, saccharification, yeast-fermentation, cooking and autoclaving into a single step, thus making the production of ethanol more cheap and economical.

Materials and Methods

Materials. Corn starch was of commercial grade, peptone, yeast extract and malt extract were from Difco Laboratories, England. All other chemicals were of analytical grade.

Micro-organisms. A strain of *Aspergillus niger* PCSIR - 10 maintained on Potato - dextrose agar medium was used for the ethanol production of amyloglucosidase by solid substrate fermentation.

Saccharomyces cerevisiae was used for the ethanol production. The organism was maintained on MYPG - medium (malt-extract 0.5%, yeast-extract 0.3%, peptone 0.3%, glucose 1.0%, agar-agar 2.0%).

Enzyme synthesis and assay. Amyloglucosidase was synthesized by solid substrate fermentation of wheat bran as reported previously [1]. The enzyme assay was carried out by a modified method of Kainuma *et al.* [8].

Inoculum preparation. Twenty four hours old yeast cells were transferred from the slants to inoculate 50 ml of sterilised MYPG - medium in a 250 ml Erlenmeyer flask and incubated at 30° on a rotary shaker with 120 rpm. The 24 hrs old inoculum was used for ethanol fermentation of raw starch.

For anaerobic cultivation of yeast cells the inoculum was placed under anaerobic conditions at 30°, without shaking.

Fermentation technique. Twenty grams of raw corn starch (based on reducing value), 120 IU of amyloglucosidase, 50 ml of distilled water and 10 ml of 24 hrs old yeast inoculum were added simultaneously into 250 ml flask. pH of the medium was adjusted to 3.5 with 0.1N H_2SO_4. The fermentation was allowed to proceed at 30±2° with and without shaking.

Samples for the estimation of reducing sugars, pH and ethanol were taken out after 24 hrs. interval.

Analytical methods. Reducing sugars were determined by Somogyi-Nelson method [11] with glucose as standard. Ethanol was measured by the dichromate method of Barnard and Karayannis [12] and pH with a pH meter.

Results and Discussions

Effect of enzyme concentration. Three concentrations of enzyme i.e. 120, 90 and 60 IU were studied for their effect on the hydrolysis and subsequent ethanol fermentation of raw starch (Fig. 1). The maximum quantity of ethanol (4.0% v/v) was produced, after 72 hrs, when 120 IU of enzyme were

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added to the fermentation broth. Further increase in the enzyme concentration did not improve the rate of ethanol production.

Effect of substrate concentration and agitation. The effect of substrate concentration and agitation on ethanol production is depicted in Fig. 2. Three different quantities of starch i.e. 10, 20 and 30gm were tested for finding out optimum amount required for ethanol formation. Maximum production of ethanol was recorded when 20 gm of corn starch were added to the fermentation medium.

The production of ethanol increased on agitating the broth. This may be attributed to the fact that contact of amylase with raw starch was more on agitation, which produced more reducing sugars that was available for ethanol production.

Effect of pH, temperature and inoculation size on ethanol fermentation. The effect of initial pH, temperature and inoculum size on the production of ethanol is given in Fig. 3. Both hydrolysis of starch and ethanol fermentation were favoured at acidic pH. The maximum yield of ethanol recorded at pH 3.5 was 8.5% (v/v). The low pH not only favoured hydrolysis of raw starch by amylase but also minimized the chances of contamination with various microbes.

Similarly maximum production of ethanol (8.7% v/v) was recorded at 30°, after incubation for 72 hrs. At temperatures above 40° a fast leveling off in the ethanol concentration was observed. This could be due to partial inactivation of yeast cells as well as amylases. The results are in accordance with those of Yamashiro *et al.* [14].

The optimum inoculum size required for maximum ethanol production i.e. 9.0% (v/v) was 10% (v/v). Further increase in the size of inoculum did not improve the rate of ethanol production.

Use of fermenting yeast cells and mold mycelium. Ethanol production increased to 9.5% (v/v) when anaerobically cultivated yeast cells were used in place of those obtained by acrobic cultivation (Table 1). Yamashiro [14] reported that yeast cells produced anaerobically had tolerance against high alcohol concentration.

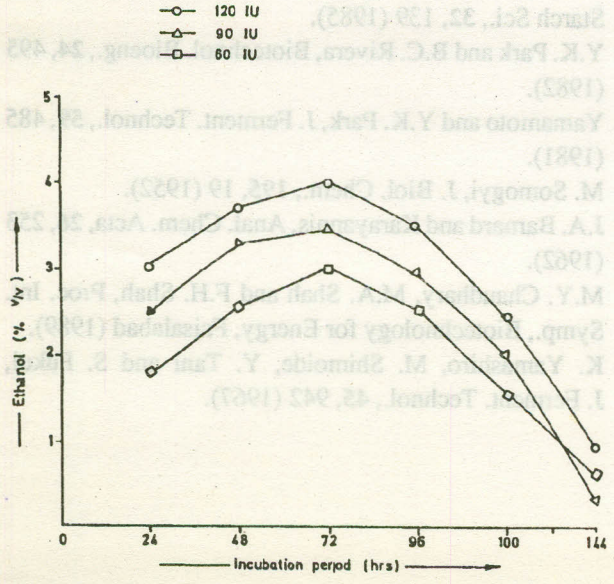

Fig. 1. Effect of different concentrations of glucoamylase on ethanol production of raw strach. Starch = 10gm, Temperature = 30°, pH = 3.5

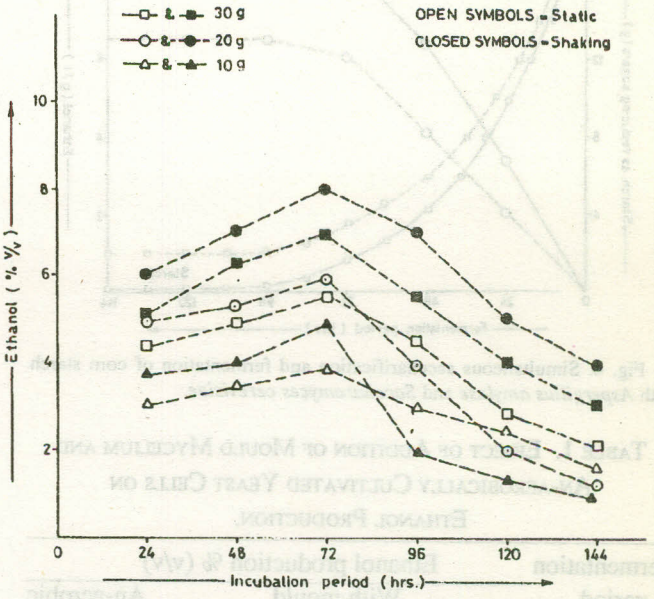

Fig. 2.Effect of different concentrations of corn starch on ethanol production. Enzyme =120 IU, Temperature = 30°, pH = 3.5

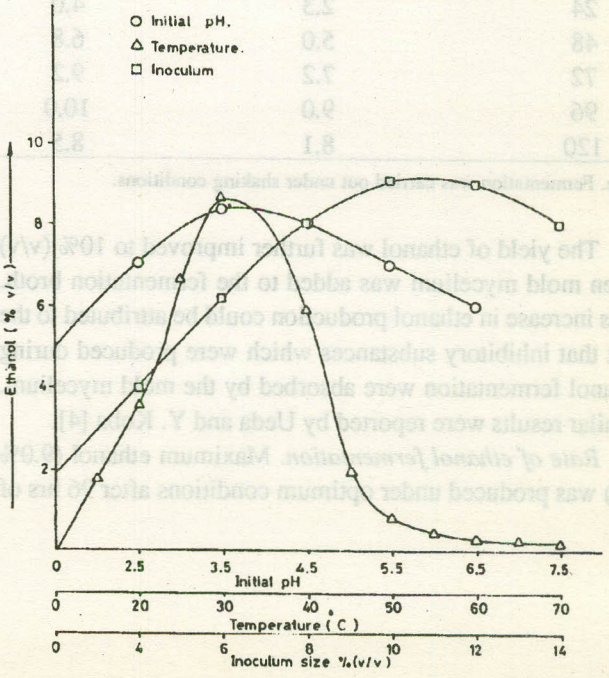

Fig. 3. Effect of temperature, inoculum size and pH on ethanol production.

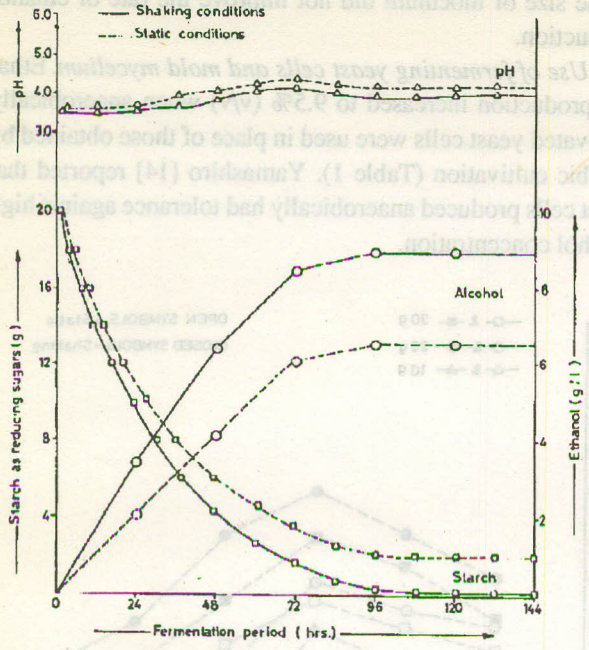

Fig. 4. Simultaneous saccharification and fermentation of corn starch with *Aspergillus amylase* and *Saccharomyces cerevisiae*.

TABLE 1. EFFECT OF ADDITION OF MOULD MYCELIUM AND AN-AEROBICALLY CULTIVATED YEAST CELLS ON ETHANOL PRODUCTION.

Fermentation period (hrs)	Ethanol production % (v/v)	
	With mould mycelium	An-aerobic yeast cells
24	2.3	4.0
48	5.0	6.8
72	7.2	9.2
96	9.0	10.0
120	8.1	8.5

Note. Fermentation was carried out under shaking conditions.

The yield of ethanol was further improved to 10% (v/v) when mold mycelium was added to the fermentation broth. This increase in ethanol production could be attributed to the fact that inhibitory substances which were produced during ethanol fermentation were absorbed by the mold mycelium. Similar results were reported by Ueda and Y. Koba [4].

Rate of ethanol fermentation. Maximum ethanol (9.0% v/v) was produced under optimum conditions after 96 hrs of shaking when 80 - 85% of the total reducing sugars were consumed (Fig. 4). The amount of ethanol produced under static conditions was 6.5% (v/v) and only 60 - 65% of the total reducing sugars were consumed.

Therefore shaking proved to be stimulative for ethanol production. This might be due to the fact that agitation causes greater contact between the raw starch granules and the amylolytic enzymes, thus making the release of reducing sugars more rapid and their subsequent utilisation by the yeast cells more adequate, resulting in an overall increase in the rate of ethanol fermentation.

The lower rate of ethanol production under static conditions could be due to lack of contact between the enzyme and the substrate. pH of the medium changed from 3.5-4.0 under shaking and to 4.2 under static conditions.

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