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Production of Acetone-Butanol-Ethanol from Corn Mash and Molasses in Batch Fermentation

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ABSTRACT

The production of solvents from corn mash and molasses in batch fermentation using *Clostridium acetobutylicum* P 262 was examined. The content of saccharose of beet molasses used in experiments is determined by using the gravimetric method (52.45% saccharose). The quantities of molasses that are used in the nutrient medium are calculated after doing the above determination. The samples of fermentation liquid are taken within a certain time, the determination of saccharose is done by using the same method, and all the saccharose is converted by the microorganism to organic end products. The quantitative and qualitative determination of acetone-butanol has been made by using gas chromatography. On the other hand, using the three isolation way, three different cultures are obtained, and with microscopic observations, the cultures obtained are of the C. *acetobutylicum* genus. According to the literature values, the concentration of maximum mixed solvent formed during fermentation is about 2%. This is seen in this experiment. There is only a slight difference from this value. This difference is caused by another organic product that is formed during fermentation.

Index Entries: Fermentation; corn mash; molasses; *Clostridium acetobutylicum*; acetone; butanol.

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INTRODUCTION

The production of ethanol and methane as fuel with organic solvents, such as alcoholic beverages, fermented food, acetone, and butanol, by anaerobic fermentation procedures from organic wastes has been carried out for years. Only the production of acetone-butanol by using species of the genus *Clostridium*, however, has attained historical importance. Nowadays, acetone, butanol, and ethanol production is carried out by fermentation.

Pasteur had conducted studies for the purpose of producing butanol bacteriologically. The production of butanol commercially was started in 1909 because of the raw material butadiene, which is used in the production of synthetic rubber. Weigmann discovered an organism that could ferment the starch in 1912, which he named *Clostridium acetobutylicum* (*1–3*).

There was much interest in production of acetone in this way during World War I, but the later development of the petrochemical industry decreased this interest. These days, the production of acetone, butanol, and ethanol by fermentation has become interesting again, since petrol is now an expensive raw material (*4,5*).

The cultures commonly used in the acetone-butanol fermentation of sugars and sugary mashes are members of the *Clostridium* genus. The saccharolytic microorganism are spore-forming rods. They are characterized by different fermentations of carbohydrates, different ratios of solvents, and different sugar concentrations utilizable. The isolation of *Clostridia* capable of utilizing saccharine may be accomplished in many ways (*6,7*).

Many different raw materials can be used by the saccharolytic acetone-butanol bacteria, but the classic materials are either inverted or black strap molasses. Invert or "high test" molasses is an evaporated sugar cane juice that contains all the original sugar of the juice, but most of it in an inverted form as a result of acid hydrolysis. Other saccharine materials that can be used are sucrose, glucose, beet molasses, sulfite waste liquor, inverted starches, or starch-containing grains. Some of the saccharolytic organisms are capable of fermenting starch directly under suitable conditions, producing almost full yields of solvents (*8*).

MATERIALS AND METHODS

Microorganism

The bacteria used for the production of acetone and butanol are a species of genus *Clostridium*. There are a number of cultures used in the sugar-medium fermentation. The bacteria has been given different names, and their morphological, cultural, physiological, and biochemical reactions are explained according to the list that has been defined by the American bacteriologists (*9*).

In this study, *C. acetobutylicum* is used as the bats that create spores, which are anaerobic, moving, and heat-resistant. Spores are 1–1.5 μ, and vegetative cells are approx 1–4 μ in length. The spore is fixed on top of the vegetative cell and becomes when the food material is finished in medium (*10*).

In the acetone and butanol fermentation, in addition to the acetone and butanol, some other side products form. These products form as a yellow fat consisting of isopropanol, ethanol formic acid, acetic acid, butyric acid, acetyl methyl carbinole, CO_2, H_2, and high alcohols, and a complex mixture of acids and esters (*11,12*).

The two species of *Clostridium* that are not very different from each other, and are appropriate for commercial production of acetone and butanol are named as *C. acetobutylicum* and *Clostridium saccharoacetobutylicum.*

The production of solvents by fermentation is of increasing interest worldwide. Various substrates are being considered for the process, including molasses, and several reports are now available describing the use of this raw material. The purpose of the present work was to investigate the ability of *C. acetobutylicum* P262 to produce solvent from molasses and corn mash.

In this study, lyophilized *C. acetobutylicum*, which was brought from NCIMB Ltd. Torry Research Station, P.O. Box 31, Scotland, was used. Production medium was chosen from the catalog of German collections of microorganism strains (*13*), and the spores of *C. acetobutylicum* were grown in meat bouillon and agar media.

Production Medium

Chopped meat medium;
Mince-meat from veal (500 g);
Distilled water (1000 cc); and
1*N* NaOH (25 cc).

This mixture was boiled for 1 h and filtered. After storing the filtrate over night in the refrigerator, the fat that accumulated on the upper portion was removed. Distilled water was added to the defatted mixture to 1L. To this mixture, Casitone (30.0 g), yeast extract (5.0 g), K_2HPO_4 (5.0 g), and Resazurine (1.0 mg) were added, boiled, and cooled. Then 0.4% glycose, 0.1% cellobiose, 0.1% maltose, and 0.1% soluble starch were added.

This mixture was liquefied, Cystein (0.5 g) was added and adjusted to pH 7. Some of the prepared mixture was used as nutrient broth for diluting, some of it was used as in the preparation of mince-meat bouillon with 1 vol mince-meat to make 4–5 vol of the mixture for the production bacteria, and some of it was used for a pure culture as agar medium to which 20 g of agar were added to the 1000 mL of the mixture.

Fermentation Medium

1. Corn medium: Corn flour (20 g) and distilled water (500 cc) were mixed, and the pH of this mixture was adjusted to 6.5–7 before sterilization.
2. Molasses medium:
   Sugar (molasses containing 48% saccharose) (22.2 g);
   $(NH_4)_2SO_4$ (5.0 g);
   $CaCO_3$ (6–7 g); and
   P_2O_5 (0.3 g).
   Distilled water (1000 cc) was mixed, and the pH was adjusted to 5.5–6.5 before sterilization.

Fermentation Conditions

In our study, the fermentation continued stage by stage. The bacteria that were sterilized and put to the heat-shock were added to fermentation media, which had been prepared in 50, 100, 150, and 500 cc. These media were incubated 20–24 h at 37°C in an anaerobic jar containing palladium-aluminat catalyst. The formation of acetone-butanol-ethanol and the change of the pH were observed. The quantitative and qualitative determination of acetone-butanol-ethanol were made using gas chromatography (*14*).

The samples that were obtained by intervals were centrifugated during the fermentation, filtrated, and analyzed by gas chromatography (Shimadzu, Model GC 6A) using a flame ionization detector (195°C) and a column of Carbowax 20M. The carrier gas (N_2) flow rate of 30 mL/min and the injector and column temperatures were 150 and 130°C, respectively. Samples were acidified using orthophosphoric acid. An internal standard of sec-butanol was used.

RESULTS AND DISCUSSION

It was observed that, *C. acetobutylicum* was effective in the corn medium, and its effectiveness was decreased in the molasses medium.

A batch study was conducted. During the opening and closing when contaminated, the microorganism's ability to produce the solvent decreased.

It was observed that the production of acetone increased up to 30 h in corn medium, and after this, it decreased. The butanol production reached a maximum level of 48 h (Fig. 1).

According to the time, acetone weight percentage increased to 42 h in molasses medium and suddenly then started to decrease (Fig. 2).

At the end of corn medium fermentation, the pH was 5.8, and in molasses medium, pH was 6.2 (Figs. 3 and 4).

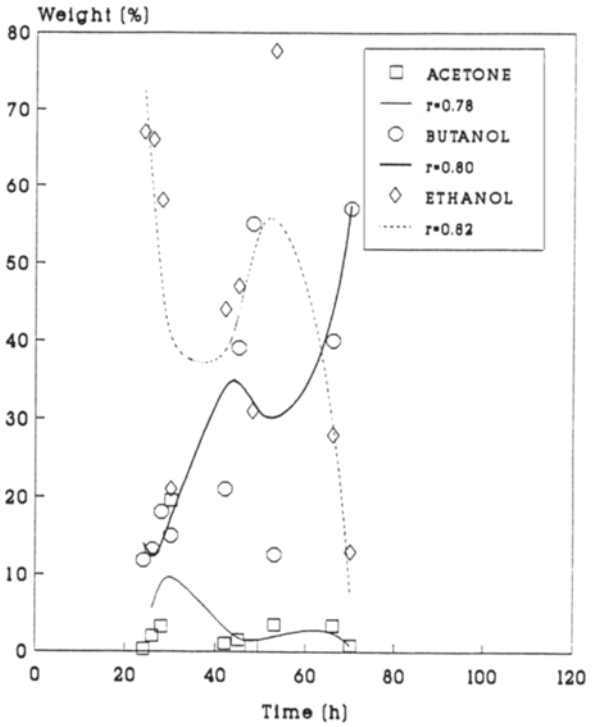

Fig. 1. Total amounts of products produced during batch fermentation from corn mash.

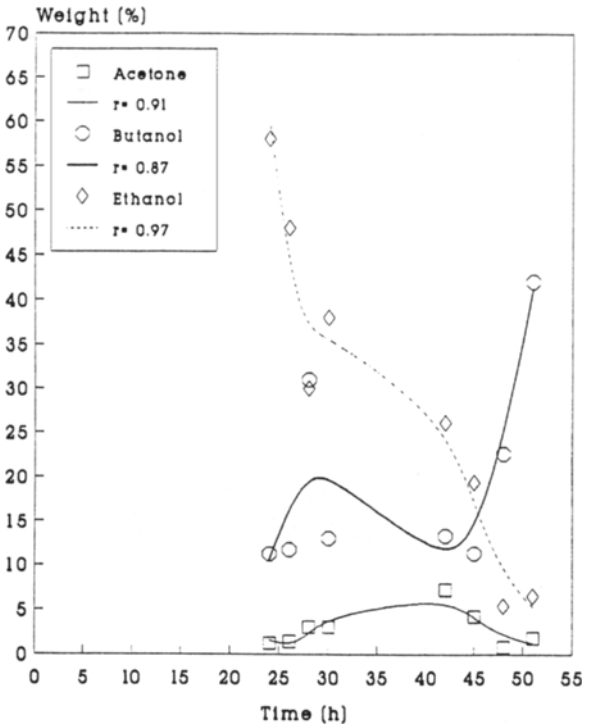

Fig. 2. Total amounts of products produced during batch fermentation from molasses.

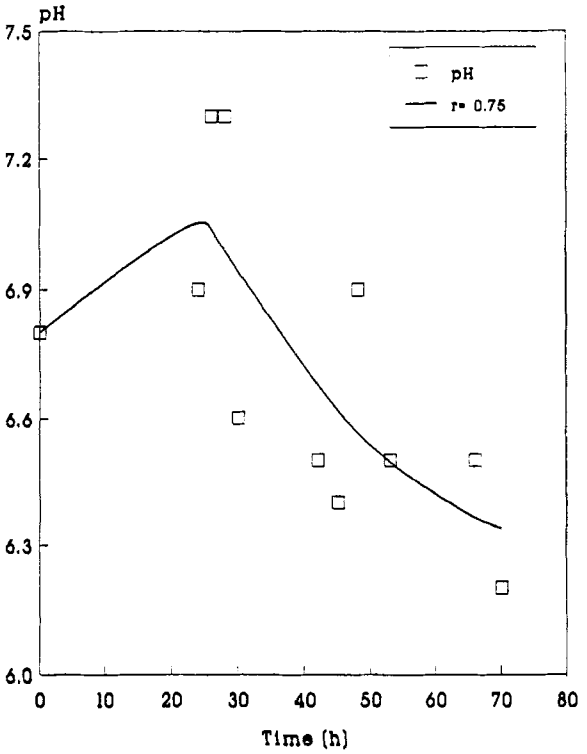

Fig. 3. pH Values during the fermentation from corn mash.

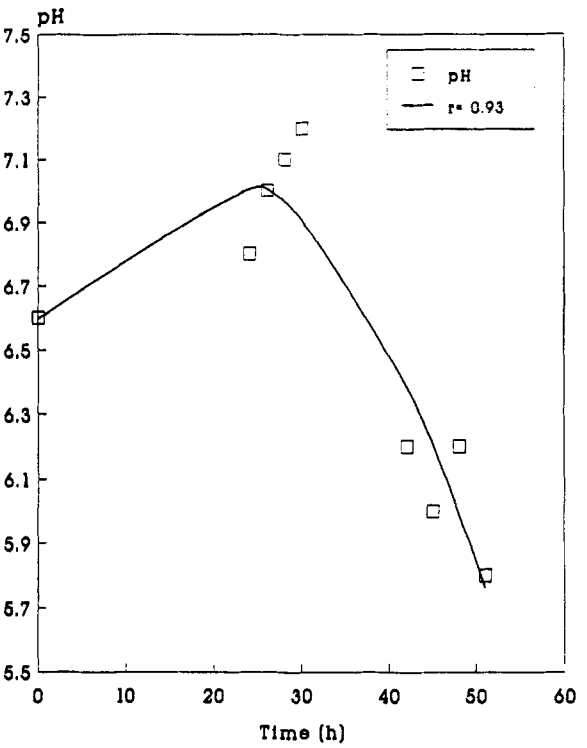

Fig. 4. pH Values during the fermentation from molasses.

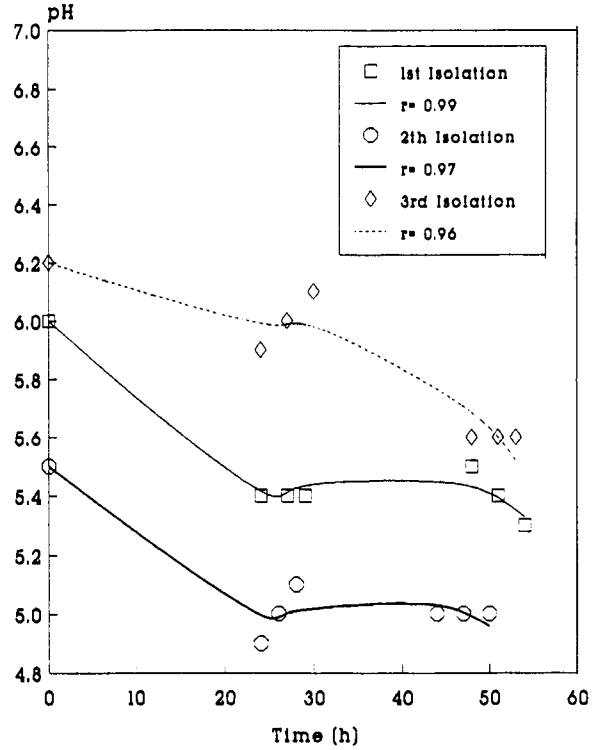

Fig. 5. pH-Time variations during the fermentation of acetone-butanol-ethanol from isolated culture of *C. acetobutylicum*.

It was observed that *C. acetobutylicum* from isolated was effective in the molasses medium. According to the time, pH variations in the molasses medium were observed (Fig. 5). According to the data given from the experiments by fermentation, as weight percentage, the acetone, butanol, and ethanol amounts in corn medium were 19.5, 15, and 21 and in molasses medium, 7.2, 13.3, and 26.1.

C. acetobutylicum was produced in 24 h. However, in agar culture, it was produced in 48 h.

The acetone-butanol fermentation is characterized by strong product inhibition. Butanol, the primary product of the fermentation of sugar or starch by *C. acetobutylicum*, inhibits its further production at concentrations above 10–15 g/L. This strong butanol inhibition adversely affects the economics of the acetone-butanol fermentation in three main ways. Butanol accumulation broth loves fermenter productivity, so that large fermenters are required; butanol inhibition limits the concentration of substrate that can be completely consumed, and thus, large volumes of waste water are produced; and product recovery is expensive owing to the low final product concentrations in the fermenter. The economic viability of the acetone-butanol fermentation depends on reducing the effects of butanol inhibition and thus overcoming these problems.

Previous studies have shown that the effects of product inhibition during the acetone-butanol fermentation can be reduced by batch fermentation. *C. acetobutylicum* has been used to reduce butanol inhibition.

Using the three isolation way, three different culture were obtained, and with microscopic observations, cultures obtained were of the *C. acetobutylicum* genus. The solvent-forming ability of cultures was tested. One way to improve the economics of the acetone-butanol production from molasses and corn mash is to carry out the fermentation in a batch process. In addition, it brings significantly higher productivies, which implies a minimal stability and longevity of the culture. This study, which investigated the optimal conditions for batch production of acetone-butanol, was mainly aimed at the longevity of *C. acetobutylicum* P262.

According to literature values, the concentration of maximum mixed solvent formed during fermentation is about 2%. This was demonstrated in the experiments. To be industrially attractive, this system must first yield high final concentration and conversion yields of solvents, which can be obtained in other published studies.

For this reason, this study would be advantageous for sugar industry. In Turkey, molasses from sugar industry waste is a traditional substrate. The amount of molasses is very high, and this substrate has not been used for anything. Thus, this work is continuing using laboratory-scale fermenters to improve and optimize the batch fermentation process.

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