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Production of Acetone, Butanol, and Ethanol in Repeated Batch Culture from Tryptone-Yeast Extract-Acetate Medium by *Clostridium acetobutylicum* YM1

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Abstract— **in this study, the use of a low-cost fermentation medium has a significant impact on the viability of most fermentation processes. A local strain of *Clostridium acetobutylicum* YM1 was used to repeatedly batch culture acetone-butanol-ethanol (ABE). Use of the tryptone-yeast extract-acetate (TYA) medium, which contains 30 g/L glucose, was made for batch and repeated batch cultures. The experiment discovered the highest acetone-butanol-ethanol (ABE) concentration in repeated batch culture. ABE production during repeated batch fermentation was examined for the effects of several parameters, including drain and fill volume of medium and drain and fill times. The findings of repeated batch fermentation demonstrated that the production was maximum at 50% (v/v) of drain and fill volume at 48 hours acetone, butanol, and ethanol (ABE). The second cycle had the highest concentrations of butanol, ethanol, and acetone at 7.12, 2.39, and 3.04 g/L, respectively. This study demonstrated that *Clostridium acetobutylicum* YM1 batch culture fermentation of acetone-butanol-ethanol (ABE) was improved by repeated batch fermentation. The TYA medium can be thought of as a viable low cost substrate for ABE fermentation, according to this study's findings.**

Index Terms: **Repeated batch fermentation; *Clostridium acetobutylicum* YM1, acetone-butanol-ethanol (ABE) fermentation; Biofuel.**

I. INTRODUCTION

One of the largest biotechnological processes ever known, the acetone-butanol-ethanol (ABE) fermentation by *Clostridium acetobutylicum* was the second-oldest industrial fermentation known (after ethanol). There may be certain benefits to a fermentation process that can be run in repeated batches. For instance, a batch of inoculum culture may be required in utilized tryptone-yeast extract-acetate (TYA) medium during the duration of the acetone-butanol-ethanol (ABE) production period. One of the recognized industrial fermentations was the acetone-butanol-ethanol (ABE) fermentation carried out by *Clostridium acetobutylicum* YM1 [1]. Using more advanced biotechnology, the ABE fermentation turns fermentable sugars into biobutanol using bacteria that produce solvents, particularly strains of Clostridium sp [2]. If acetone-butanol-ethanol (ABE) could be manufactured profitably from cheap biomass, it was predicted that the market's demand would rise sharply [3].

Hydrocarbons have been extensively converted to butanol using repeated-batch fermentation. There are a number of reasons why this advanced biotechnology, the ABE fermentation turns fermentable sugars into biobutanol using bacteria that produce solvents, particularly strains of Clostridium sp[4]. If acetone-butanol-ethanol (ABE) could be manufactured profitably from cheap biomass, it was predicted that the market's demand would rise sharply [5].

Hydrocarbons have been extensively converted to butanol using repeated-batch fermentation. There are a number of reasons why this methodology is preferred over traditional batch fermentation protocols. Long-term operations can benefit from it because it does not require a fresh inoculum for every batch [6].Furthermore, repeated-batch fermentation greatly reduces the amount of time needed for cleaning and sterilization, and, unlike continuous models, the technique enables simple operation control. However, portions of the fermented broth must be frequently removed from repeated-batch methods that use free Saccharomyces cerevisiae cells, with the leftovers being employed as an inoculum for the succeeding batch. Unfortunately, this can lead to noticeably lower yeast loads, which can reduce butanol production in subsequent batches [7]. A repeated-batch procedure using stationary fungal cells was recommended to avoid this from occurring. In comparison to free cell fermentation techniques, cell immobilization methods are connected with higher microbe densities, faster fermentation, simple cell recycling, and lower toxicity. They may also cut extraction expenses of product

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inhibition. Determine the most suitable time to remove and add fresh medium to the culture.

Because *C. acetobutylicum* is an anaerobic organism, numerous investigations have shown that the organism's cellular growth and DNA synthesis might be significantly reduced in the presence of oxygen [8]. However, *C. acetobutylicum* YM1 was discovered to be able to manufacture biobutanol effectively in weak anaerobic circumstances made possible by gas-flushing with oxygen-free nitrogen, in contrast to many of the Clostridium strains employed for ABE fermentation [9]. A local strain of *Clostridium acetobutylicum* YM1 was used in this investigation to construct the basic experiment to assess the production of acetone-butanol-ethanol (ABE).

II. METHODOLOGY

A. TYA Medium

The tryptone yeast extract-acetate (TYA) medium was used to prepare the pre-culture. Tryptone yeast extract - acetate medium was also used to prepare the inoculum and served as the primary medium in a number of tests. 1 L distilled water was used to dissolve 30 g glucose, 2 g yeast extract, 6 g tryptone, 3 g CH3COONH4, 0.3 g MgSO4.7H2O, 0.5 g KH2PO4, and 10 mg FeSO4.7H2O in the medium. The pH of the medium was adjusted to 6.0 0.2 by using 1 M NaOH and 1M HCl. Before use, the medium was autoclaved at 121°C for 15 min [10].

B. Inoculum

C. acetobutylicum YM1 inoculum was prepared in TYA medium. Microbial inoculum was prepared by transferring 1 mL of *C. acetobutylicum* YM1 spore suspension into mL of TYA medium, which was heated for 1 minute in boiling water, then cooled in ice water, and incubated for 1 to 2 days at 30°C under anaerobic conditions. This culture subculture in TYA medium for 18 hours before being used as a fresh inoculum source [11].

C. Repeated Batch Fermentation System

The fermentation was carried out in a 250 mL Duran bottle with a working volume of 100 mL TYA was used as a fermentation medium, and the fermentation conditions were similar to those used in batch fermentation. After the level of present glucose from 30 g/L to it was reduced to a predetermined level in repeated batch cycle 1 at the start to the end of run 4. The subsequent batch began by removing at 50% every 24,36 and 48 hours a portion of the operational volume, fermented broth, and immediately replacing it with 50% a fresh TYA medium every 24,36 and 48 hours. This is referred to as the fill and drain technique [12].

III. ANALYTICAL METHODS

The medium's acidity/alkalinity as measured by a pH meter (Metrohm, Switzerland). Prior to sampling, pH buffers standard solutions of pH 4 and pH 7 were used to calibrate the pH meter. Cell growth of *C. acetobutylicum* YM1 in TYA medium was measured using a Genesis 10 Scanning Spectrophotometry at 600 nm (Thermo Spectronic, Rochester, USA).

The concentration of glucose in the medium was determined using the 3,5 dinitrosalicylic acid (DNS) method[13].To measure, 1 mL and 2 mL of the sample and DNS reagent solution were transferred to a test tube and placed in boiling water for 10 minutes. The reagent mixture was allowed to cool before adding 7 mL of distillate water and reading absorbencies at 540 nm with a spectrophotometer. The absorbances at 540 nm were measured for all samples, and the glucose concentration was calculated using a standard curve.

Solvents (acetone, butanol, and ethanol) and acids (butyric acid and acetic acid) were identified using a gas chromatography system equipped with a flame ionization detector (Agilent Technologies, CA, USA) (FID). A 30-m Equity-1 capillary column (30 m 0.32 mm 1.0 m) was used (Supelco Co., PA, USA). A beginning oven temperature of 40°C was gradually increased to 130°C at an 8°C/min rate. The injector and detector were set to 250°C and 280°C, respectively. Helium was used as the carrier gas and was tried to introduce into the column at a rate of 1.5 mL/min.

IV. RESULTS AND DISCUSSIONS

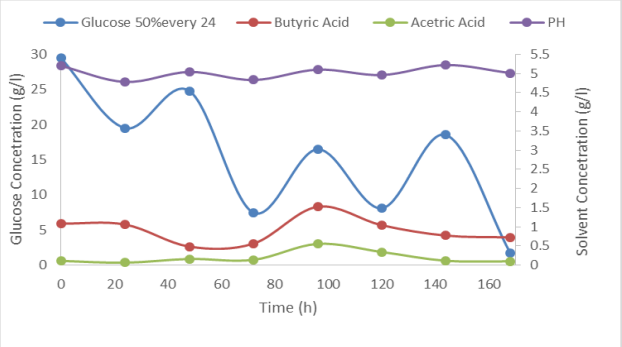

Figure 1. Profiles of bacterial growth, glucose concentration, PH in repeated batch culture by *C. acetobutylicum* YM1 for 50% DFV at 24h.

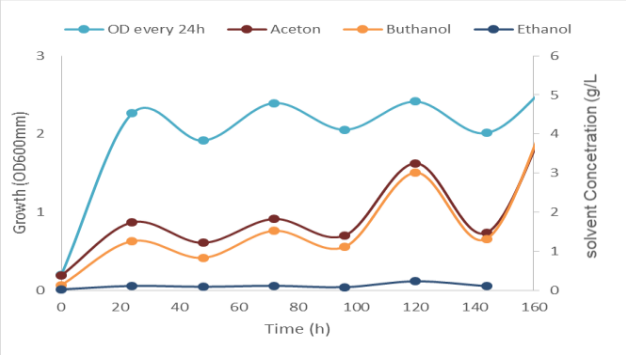

Figure 2. Profiles of butanol, ethanol, acetone production in repeated batch culture by *C. acetobutylicum* YM1 for 50% DFV at 24h.

The effect of medium time addition as a dump as well as fill volume (DFT) was investigated. The DFV was kept consistent at 50% and the time of addition (DFT) was changed at 24, 36, and 48 h periods all through repeated batch fermentation to determine the best time for butanol production [14]. The initial batch Repeated batch culture at 50% DFV has been tested every 24 hours for four runs; the pH in repeated batch cycle 1 ranged from

5.8 at the start to 5.01 at the end of run 4. The initial stage of the batch culture's glucose concentration was 30 g/L, which was higher than that tested in the repeated batches, which were 29.63, 25.79, 19.67, 16.77, and 15.47g/L "Figure 1" respectively. The remaining concentration of glucose in repeated batches 1 to run 4 were 19.49, 7.46, 8.10, and 1.74g/L, respectively, at the end of the experiment, indicating that glucose is the preferred sugar for clostridial cells in relation to the ABE process.. *C. acetobutylicum* YM1 grew from 0.2 after inoculum (0 h) to 2.25 at the end. In repeated batches 1– run 4, *C. acetobutylicum* YM1 cell population in TYA medium reported an increase in the production reading (OD at 600 nm) from 0.2 at 0 h to OD 2.26, 2.39, 2.42, and 2.74 at the end of the bacterial cell growth. Butanol concentrations in repeated batches 1 to 4 were 1.26, 1.52, 3.01, and 5.13g/L. The ethanol concentrations produced in repeated batches 1– run 4 were 0.12, 0.12, 0.24, 0.25, and g/L. In this experiment, the acetone concentrations were 0.87, 0.92, 1.63, and 2.45 g/L "Figure 2".

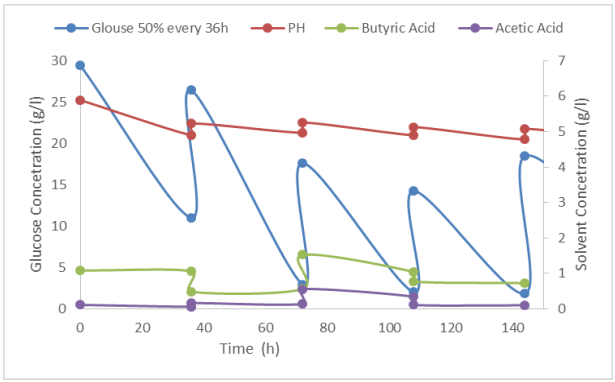

Figure 3. Profiles of bacterial growth, glucose concentration, PH in repeated batch culture by *C. acetobutylicum* YM1 for 50% DFV at 36h.

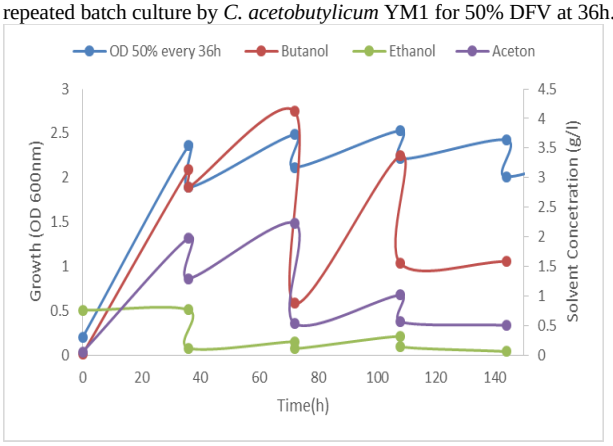

Figure 4. Profiles of butanol, ethanol, acetone production in repeated batch culture by *C. acetobutylicum* YM1 for 50% DFV at 36h.

The DFT was also investigated up to 36 hours. The pH in repeated batch cultures was 5.23, 5.26, 5.13, 5.08, and 5.11 at 36 h at the start of runs 1 to run 4, respectively "Figure 3". The glucose concentrations in repeated batches 1–run 4 were 29.46, 26.42, 17.64, and 18.49 g/L, respectively. At the end of the experiment, the concentration of glucose for repeated batches 1–run 4 were 11.01, 2.96, 2.03, and 7.19 g/L, respectively. The growth rates of *C. acetobutylicum* YM1 at DFT of 36 h were 2.36, 1.89, 2.49, and 2.38 for repeated batches 1 to 4 "Figure 4" [15]. The maximum concentrations of butanol, ethanol, and acetone were 4.12, 0.76, and 2.23 g/L, respectively. In a study similar to Khalifaa (2018), it was discovered that the minimum (ABE) support the desired was 50% 36h batch culture.

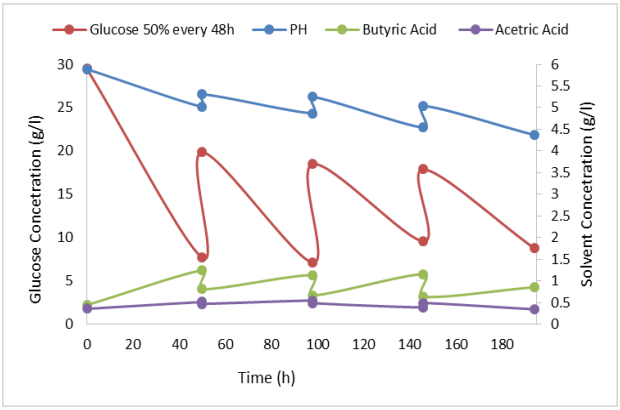

Figure 5. Profiles of bacterial growth, glucose concentration, PH in repeated batch culture by *C. acetobutylicum* YM1 for 50% DFV at 48h.

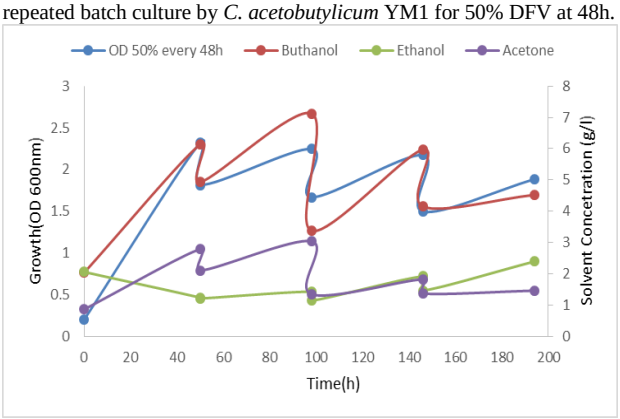

Figure 6. Profiles butanol, ethanol, and acetone production in repeated batch culture by *C. acetobutylicum* YM1 for 50% DFV at 48h.

Another result for DFT at 48h was discovered in a repeated batch cycle for the pH at the start was "Figure 5" 5.89 to 4.37 at the end of run 4. The initial concentration of glucose of the repeated batch culture was found to be 29.47, 19.87, 18.49, and 17.88 g/L in the repeated batches, respectively. The remaining concentration of glucose in repeated batches 1–4 were 7.68, 7.10, 9.56, and 8.77 g/L, respectively, at the end of the experiment. *C. acetobutylicum* YM1 grew from 0.2 after inoculum (0 h) to 2.25 at the end. Bacterial cell growth was estimated as OD in repeated batches 1–4. Decreased in repeated batches 1.81, 1.67, 1.50, and1.88 were the values from 1 to 4 "Figure 6". The concentrations of butanol produced in this experiment in repeated batches 1 to 4 were 6.14, 7.12, 5.97, and 4.52 g/L, respectively. The batch culture using *Clostridium acetobutylicum* YM1 from Treated group Palm Kernel Cake produced 5.24 g/L butanol and 8.24 g/L ABE during this time. It was noteworthy that, despite the low concentration of glucose in the mixture [16]. The ethanol concentrations in repeated batches 1 to 4 were 1.24, 2.39, 1.92, and 1.43 g/L, while the acetone concentrations were 2.79, 3.04,1.82, and 1.46 g/L. This is a study reported by Dashti and Abdeshahian (2016) who discovered that increasing the time from 12 h to 48 h resulted in a progressive increase in lipid production, indicating that increasing the time has a positive influence on the energy

metabolism of Cunninghamella bainieri 2A1 in the fermentation for laccase production.

V. CONCLUSIONS

TYA medium was found to be a suitable starting material for ABE fermentation in this study. According to the findings of this study, acetone-butanol-ethanol (ABE) was produced by repeated batch culture using a local strain of *Clostridium acetobutylicum* YM1. The drain and fill times in the 50 percent feeding strategy were varied between 24 hours, 36 hours, and 48 hours. The 48-hour drain and fill time produced high concentrations of butanol, ethanol, and acetone as 7.12, 2.39, and 3.04 g/L, respectively. More research is needed to improve the fed and continuous batch culture acetone-butanol-ethanol (ABE) fermentation process.

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