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COMPARATIVE EVALUATION OF *ESCHERICHIA COLI* AND *BACILLUS CEREUS* PRODUCTION POTENTIALS OF ACETIC ACID UNDER OPTIMIZED FERMENTATION CONDITIONS

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ABSTRACT

Escherichia coli remains of the best-established production organism in industrial biotechnology. *Bacillus Cereus* is a recognized human pathogenic bacterium, which can colonize in the human intestine and frequently implicated in cases of foodborne poisoning. The goal of this work focused on production of acetic acid for it's important and benefit uses in different industrial products. The aerobic fermentation was run on media containing glucose and supplied with some minerals to increase the growth rate. The uptake of glucose was converted into biomass and acetic acid. The results of this work indicated that, the using of glucose and minerals increased the growth rate. The dry cell weight was 3.5 g/l , 2.9 g/l and the acetic acid concentration was 30 mg/ml , 4.33 mg/ml in case of *E. coli* and *B. Cereus* respectively. These data indicated that the fermentation culture of *E. coli* can be industrially used for production of a high yield of acetic acid than *B. Cereus*.

INTRODUCTION

Escherichia coli was the first and is still one of the most commonly used production organisms in industrial biotechnology. The Enterobacteriaceae, such as *E. coli*, *Salmonella* and *Shigella*, could decompose hexose into ethanol, succinic acid, 2,3-butanediol, lactic acid, acetic acid, formic acid and carbon dioxide. Although these bacteria are gram-negative, non-spore formers, their associated fermentation pathways are similar to those of *B. cereus*. Aerobic high cell density cultures of *E. coli* are most frequently used to arrive at high biomass yields and high metabolite concentrations (Vandamme and Soetaert, 2007). A major problem encountered with *E. coli* cultures is acetate production, which can retard growth and product formation (Luli and Strohl, 1990). Acetic acid produced in high cell density *E. coli* fermentation inhibits cell growth and product synthesis. *Bacillus cereus* can metabolize a variety of compounds including carbohydrates, proteins, peptides and amino acids for growth and energy. Some of the major product produced from carbon sources such as sucrose or glucose include L- lactate, acetate, formate, succinate and ethanol (de Been *et al.,* 2007). *Bacillus cereus* requires glucose for growth and consequently to form organic acids, glucose was used as the only carbon source for the fermentation. The metabolic characteristics of the bacterium and the production of its final products including volatile metabolites. The major products under aerobic condition were 2,3- butanediol and carbon dioxide, and the minor products were acetic acid, lactic acid, glycerol, ethanol, formic acid and succinic acid (Wang and Wang, 2002).

MATERIALS AND METHODS

Organism and inoculum preparation

Both *Escherichia coli* (code NRRN 3008) and *Bacillus Cereus* were kindly supplied from National research center. Then, the strains were reconstituted and streaked on trypticase soya agar media. A loopful from each of the overnight growth of *B. Cereus* and *E. coli* were transferred to 300 ml tryptone soya broth medium and incubated at 37°C with vigorous shaking 200 rpm for 18 h; cell growth was checked by measuring optical density (OD) of the culture at absorbance at 600 nm and purity was checked by Gram stain.

Culture

A 5% of the primary culture was transferred to three litters of base fermentation medium (40 g casein hydrolysate, 7.5 g potassium dihydrogen phosphate, 7.5 g disodium hydrogen phosphate, 0.85g dipotassium sulphate) were dissolved in distilled water to make the volume up to 1000 ml and steam sterilized at 121 C for 20 min and incubated for 24 h according to Kleman *et al.,* 1991.

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Glucose-salts solution

This solution contained 0.17 g of $MgSO_4$.$7H_2O$, 0.5 g glucose and 0.8 ml /l trace elements 100 ml distilled water were sterilized as previous and added the fermentation base medium at room temperature.

Trace elements

The trace elements solution was prepared according to Pan *et al.,* 1987 with little modified (40$MgSO_4$.7 H2O, 10 g $MnSO_4$. H_20, 28.25g $AlSO_4.18H_2O$, 2g $ZnSO_4$. $7H_2O$, 1g $CuCl_2.2H_2O$ and 0.5g H_3BO_4) dissolved in one litter 5N HCl. The final volume of the initial medium was 3.3 liters (3.0 L of base medium, 0.1 liter of glucose-salts solution, and 0.2 liter of inoculum).

Fermentation

Fermentation conditions were according to Kleman *et al.,* 1991shown in table (1).

Table 1 Fermentation Data Sheet table

Item	Culture
Seed Volume	200 ml
Agitation	500 rpm
Temperature	37 C
Air Flow	5 L /min
Dissolved Oxygen (DO)	Air saturation by automatic adjustment of the impeller speed and air flow.
pH	7.0 was adjusted by using 25% ammonia solution
Antifoam	0.5 ml/l was added of antifoam.

Inoculation

The plug was removed from the inoculation port. The inoculum was removed aseptically from its flask using inoculation syringe. The inoculum was injected through the septum in the inoculation port. The plug was reinstalled in the port. When the whole lot of the liquid media is ready about three L for each fermentor, i.e., the pH adjusted to 7.0 and add a 5% inoculum. Purity testing of the inoculum was confirmed by gram stain. The growth of the cultures takes place at 37°C with shaking incubator 500 rpm for each fermentor for 24 hrs and sampling was collected at 2 h intervals.

Harvesting

The culture had been harvested from late stationary phase culture grown for 24h at 37°C. The cultures were harvested after purity testing of the inoculum is examined and centrifuged at 3000 for 30 minutes for clarification. The supernatant was collected and sterilized through Millipore 0.22 µm stericup USA. The cells, on the other hand, were pooled and kept frozen (Collier and Kandel, 1971).

Estimation of dry cell mass

Cell concentration was expressed as dry biomass weight per liter (g/l) after centrifugation of 3 L culture, followed by pellet drying at 60 °C for 48 h (Barugue-Ramos *et al.,* 2005).

Estimation of acetic acid

HPLC conditions of acetic acid

The filtrate was subjected to separation by using HPLC with the following condition: the mobile phase at flow rate 1 ml/min; was Methanol to water 80:20. Agilent 1100 series (Waldborn, Germany), quaternary pump (G1311A), Degasser (G1322A), Thermostated Autosamples (G1329A), variable wave length detector (G1314A); and column: Zorbax 300SB C_{18} column (4.5 X 250 mm) (Agilent Technologies, USA). Injection was carried out at wave lengths 210 nm for separation.

RESULTS

The Gram stained smear of each *E. coli* and *B. Cereus* was utilized as indicator for the purity of the culture and demonstrated the morphological characteristic. *E. coli* was Gram negative bacilli and *B. Cereus* was Gram positive bacilli. The summary of results culture batch of *E. coli* and *B. Cereus* were described in table (2) below.

Table 2 Summary of experimental results obtained with cultures batches.

	Batch *E. coli*	Batch *B. cereus*
Dry cell mass g/L	3.5 g/L	2.9 g/L
Supernatant of culture	2400 ml	2250 ml
Optical Density	2.44	1.857

Estimation of bacterial growth

As shown in figure (1) and figure (2), Bacterial growth for the batch fermentation of ***E. coli*** and ***B. cereus*** were estimated, and were drawn using optical density versus time.

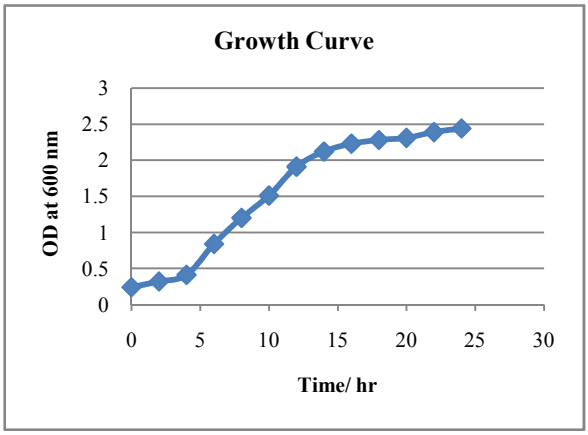

Figure 1 Growth curve of *E. coli* fermentation

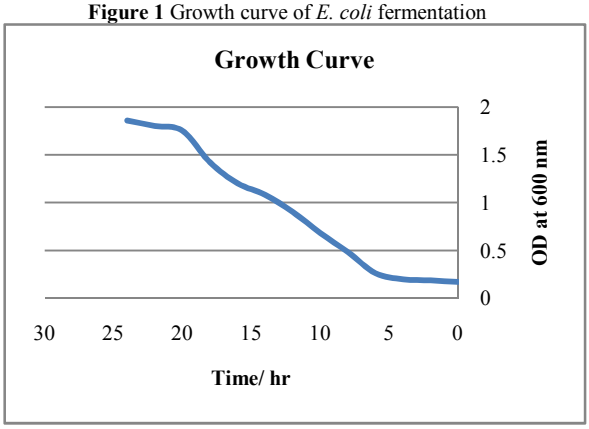

Figure 2 Growth curve of *B. Cereus* fermentation.

Estimation of acetic acid

The quantity of acetic acid was determined for the supernatant of each *E. coli* and B. *Cereus* using HPLC, the concentration of

acetic acid for *E. coli* was 30 mg/ml and concentration of acetic acid for *B. cereus* was 4.33 mg/ml, the data represented in fig.(3), (4) and fig. (5).

Kleman (Kleman *et al.,* 1991,). The dry cell mass for *E. coli* was 3.5 g/L while dry cell mass for *B. cereus* was 2.9 g/L.

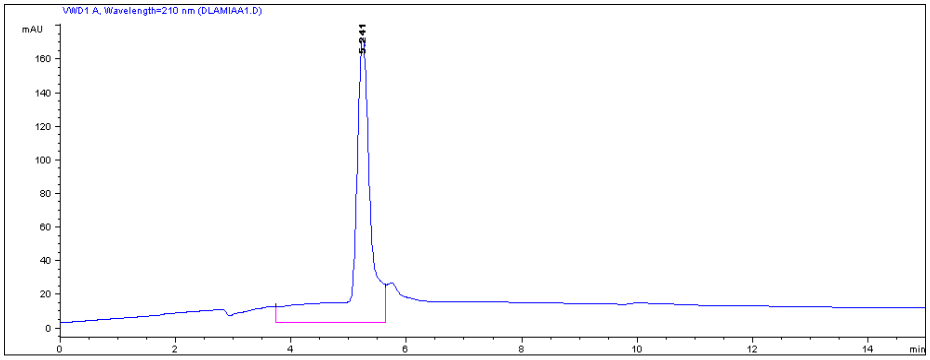

Figure 3 Elution profile of standard acetic acid was 5.241.

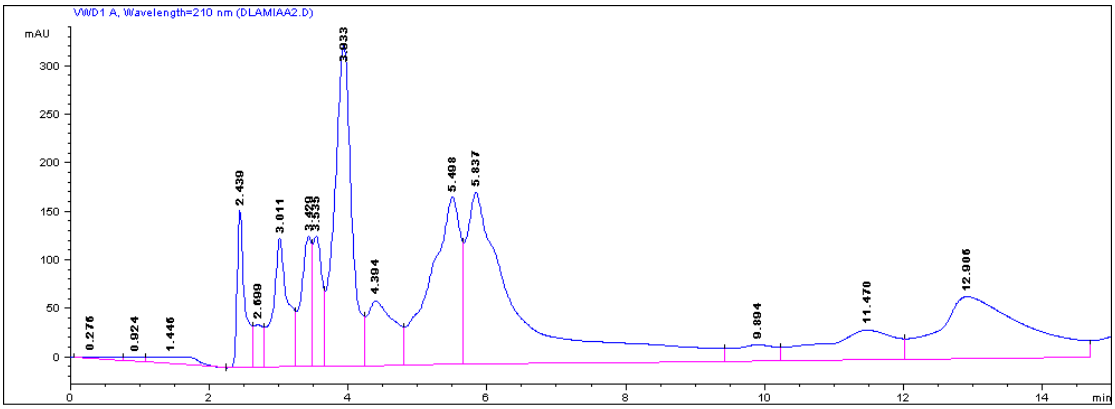

Figure 4 Elution profile of acetic acid in supernatant of *E. coli* was 5.408.

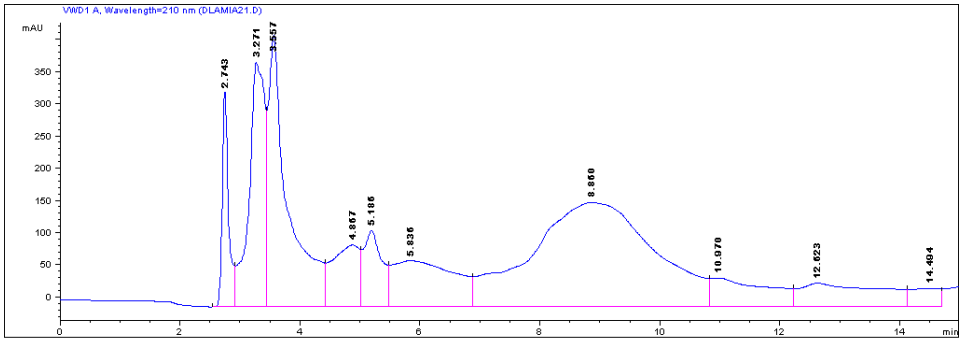

Figure 5 Elution profile of acetic acid in supernatant of *B. cereus* was 5.186.

DISCUSSION

Escherichia coli was one of the most commonly used production organisms in industrial biotechnology. The goal of this work focused on production of acetic acid for it's important and benefit uses in different industrial products. Formation of acetate in *E. coli* cultures under fully aerobic conditions can be caused by two phenomena. On the other hand, a lack of dissolved oxygen activates the fermentation pathways causing acetate excretion. This referred to as mixed-acid fermentation. On the other hand, this acetate excretion is also due to a metabolic overflow mechanism, caused by an imbalance between the rapid uptake of glucose and its conversion into biomass and products, diverting acetyl-CoA from the TCA-cycle toward acetate (Akesson *et al.,* 1999; Soetaert and Vandamme 2007). In the present study, we have the inoculum of each *E. coli* and *B. cereus* was prepared according to

The growth and dry cell mass of *E. coli* was obtained high than *B. cereus*; these results were agreement with. On the other hand, the concentration of acetic acid in *E. coli* was 30 mg/ml these results agreement with (Kleman *et al.,* 1991; Luli and Strohl, 1990) who reported that the concentration of acetic acid reached approximately 1.5 to 3.1 g/l. On the other hand, the concentration of acetic acid in *B. cereus* was 4.33 mg/ml these result agreement with (Caldwell, 1995;Wang and Wang, 2002) who reported that the concentration of acetic acid was minor product. These data indicated that the fermentation culture of *E. coli* can be industrially used for production of a high yield of acetic acid than *B. Cereus*.

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