Biobutanol Production by *Clostridium acetobutylicum* NCIMB 13357 in Modified Medium using Date Fruit as a Carbon Source

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Abstract: Biofuel is an alternative energy that is conceived being the future energy source; it can be produced from natural and renewable agricultural raw materials by microbes. *Clostridium acetobutylicum* has the ability to ferment variety of compounds to produce solvents such as: Acetone, Butanol, and Ethanol (ABE). This study was designed to investigate the ability of *C. acetobutylicum* to produce biobutanol or any other possible solvents using date fruit as the organic carbon source under anaerobic condition. The fermentation process was carried out under the following conditions: initial pH 6 and 7, incubation temperatures 30 ^{o}C and 35 ^{o}C for 70 hours. The effect of date fruit concentrations on *C. acetobutylicum* was studied 10 -50 g/L. Results showed that 40 g/ L of date fruit under initial pH 7 at 35 ^{o}C were the optimum conditions for fermentation process to produce 5.31 g/ L and 8.42 g/ L as biobutanol and total solvents respectively. The yield and productivity of ABE was 0.52 and 0.12 g/(L.h), respectively.

Keywords: *Biobutanol, Date fruit, Clostridium acetobutylicum. .*

1. Introduction

In recent years, government, organization and people around the world are concerned about reflection in crude oil price, global warming, climate change and renewed interested in renewable alternative energy based on biomass by biotechnology processes. Butanol, acetone and ethanol (ABE) are naturally produced by *Clostridium acetobutylicum*, and it is able to metabolize a vast variety of carbon sources including monosaccharides and polysaccharides.

Clostridium acetobutylicum is an aerobic, gram-positive, and spore-forming microorganism, has an ability to produce butanol, acetone, ethanol, as a final product under anaerobic condition, using different carbohydrate sources including monosaccharides, and polysaccharide. Many studies had been reported that, *Clostridium acetobutylicum* can metabolize glucose, sucrose, starch wheat; Palm Oil Mill Effluent (POME), molasses, and corn [1,2].

The date Palm (*Phoenix dactylifera L.*) is widely planted in the world. The fruit of date is a good source for sugars including monosaccharides, disaccharides, proteins, fat, minerals including copper, sulphur, iron, magnesium and potassium [3]. The dry date varieties (El-Tamr), matured fruit of date contain a low moisture percentage 10-20% and high percentage of sugar 65-75%, fiber, minerals, and vitamins [4]. According to the World Food Agricultural Organization (FAO) [5], there are 90 million date palms in the world and each tree can grow for more than 100 years. 64 million trees were planted in Arab countries. The mean annual production of date tree varies between 100-150 kg of date per tree per year. [6]. Beside the direct consumption, date fruit can be used to prepare a wide range of different products such as date juice concentrates (spread, syrup and liquid sugar), fermented date products (wine, alcohol, vinegar, organic acids) and date pastes for different uses (e.g. bakery and

confectionery). Also dates falling down from palms before maturity can be used as animal feeding. In recent years, date fruit is considered as an excellent fermentable raw material source to produce Bioethanol by Saccharomyces [7, 8].

Butanol is an important industrial chemical. Half of the butanol production is used in the form of butyl acrylate and methacrylate esters used in latex surface coating, enamels and lacquers [9]. Other important derivatives of butanol are butyl glycol ether, butyl acetate and plasticizers. Butanol is also an excellent diluent for brake fluid formulations and solvent for the manufacturing of antibiotics, vitamins and hormones.

An important application of butanol receiving renewed interest is its can be used as a direct replacement of gasoline or as a fuel additive. Butanol has sufficiently similar characteristics to gasoline therefore it can be used directly in any gasoline engine without modification and/or substitution (Table I). Butanol is superior to ethanol as a fuel additive in many regards: higher energy content, lower volatility, less hydroscopic (thus does not pick up water), and less corrosive [10]. Also, branched chain 4-carbon alcohols including isobutanol, 2- methyl-1-butanol and 3-methyl-1-butanol have higher octane numbers compared with n-butanol [11], and thus are good candidates as fuel additives.

Table 1. Properties of butanol and other fuels.

	Butanol	Gasoline	Ethanol	Methanol
Energy content (MJ/L)	29.2	32	19.6	16
Air-Fuel Ratio	11.2	14.6	9	6.5
Heat of Vaporization (MJ/L)	0.43	0.36	0.92	1.2
Research Octane Number	96	91–99	129	136
Motor Octane Number	78	81–89	102	104

In recent years, high crude oil price and increasing concerns over global warming have renewed interest in biotechnological production of butanol, not only as a chemical but also as an alternative fuel. Reflecting this, a number of companies are developing bio-butanol processes. BP and DuPont, for example, recently announced a joint effort to develop a fermentative butanol process [2].

There are many environmental parameters affected on Butanol production during ABE fermentation process by Clostridium acetobutylicum. The pH and temperature are the most important environmental parameters play vital role on this bacteria growth, its enzymes activity, and its pathway during anaerobic fermentation process. On the other hand, the concentration of substrate and amount of Carbon sources in medium are important factor during fermentation pathway, amount of ABE produced by bacteria depend on the amount of carbohydrate consumed.

This study was carried out to study the effect of some environmental parameters including pH, temperature and substrate concentration on *C. acetobutylicum* NCIMB 13357, to produce butanol and other solvents using modified synthetic medium by replacing the carbon source with the date fruit.

2. Methodology

2.1. Strain and Culture Media

C. acetobutylicum NCIMB 13357 was obtained from a biotechnology laboratory, Department of Chemical and Process Engineering, UKM, in freeze form and maintained on Reinforced Clostridium Medium (RCM) broth and agar [12]. Fresh inoculum was prepared on RCM medium and incubated at 35 ^{o}C for 24 hours under anaerobic condition and directly used in the experiments. 10% of that culture used as inoculum to inoculate with experiment medium.

2.2. Fresh Date Medium

A weight of 100 g of dry date (Tamer stage) was taken and blended with distilled water to get final volume up to 1000 ml, autoclaved at 121 ^{o}C for 15 minutes, filtered it to remove sold particular and mixed with 2x synthetic medium to prepare 10 g/l, 20 g/l, 30 g/l, 40 g/l and 50 g/l of date fruit in modified medium as a final media concentrations. A volume of 150 ml of those media were transferred to 250 ml Duran bottle. The pH of the medium was adjusted by using 0.2 M NaOH and 0.1 M HCl, autoclaved again to use directly in the next set of experiments.

2.3. Synthetic Medium

The reference synthetic medium used to tested the effected of the environmental parameters factors, previously described [13], had the following composition; KH_2PO_4, 0.5g/l; K_2HPO_4, 0.5 g/l; $MgSO_4$.7H2O, 0.1 g/l; $MnSO_2.H_2O$, 0.01 g/liter; $FeSO_4$. $7H_2O$, 0.01 g/liter; NaCl, 0.01; ammonium acetate, 2.2g/ litter; *P*-aminobenzoic acid, 0.001g/llitter; and biotin, 0.00001 g/litter. A 2X concentration from the previous medium was prepared, appropriate volume from 2X medium mixed with appropriate volume from 100 g/l of date fruit to get final date fruit concentration 10g/l, 20 g/l, 30 g/l, 40 g/l and 50 g/l, as well 1X from the synthetic medium.

2.4. Analytical Methods

Total carbohydrate was measured at the beginning and end of each batch experiments to calculate the total carbohydrate consumed during fermentation process by using Anthrone method [14], using UV-visible spectrophotometer. Solvents and Acids concentration was determined by gas chromatograph with capillary column (EquityTM-1 Supelco), previously described [12].

3. Results and Discussion

This experiment was carried out to study the effect of some environmental parameters on *C. acetobutylicum* growth, fermentation pathway, outcome during fermentation process, and substrate consumption by *Clostridium acetobutylicum* using date fruit as an organic carbohydrate source.

3.1. Effect of pH

pH is one of the important controlling factors of anaerobic fermentation processes. Jones, and Woods, 1986, reported that, the pH consider as a key factor to determine the outcome of ABE fermentation. The optimum pH to *C. acetobutylicum* NCIMB 13557 was reported in number of recent studies. For example Saleha S. and Mohd S. [15], reported that, the initial pH used to produce ABE using immobilized cells of *C. acetobutylicum* was 6.2 and it was drop to 4.82 after inoculation. Furthermore, Elgadafi et al. [10] reported that, the optimum pH to produce

ABE using Reinforced Clostridial Medium (RCM) was 6. On the other hand, Alshiyab et al. [16] reported that, the optimum pH to produce Hydrogen was 7. This study is illustrating the effect of initial pH (pH 6 and 7) on ABE production using modified date fruit medium by *C. acetobutylicum* NCIMB 13557.

It is evidence from the results Fig. 1. that, the pH of was the optimum pH to produce maximum butanol and other solvents. When comparing between pH 6 and pH 7 at 35 ^{o}C and a 40 g/l of date fruit concentration, the butanol production was4.36 and 5.31 g/l, respectively. However, the yield and productivity of the butanol at pH 6 was 0.308 g/l/h and0.062 respectively. Besides that, at pH 7 the yield and butanol productivity was 0.328 g/l/h and 0.076, respectively. On the other hand, total carbohydrate consumed was 16.2 g/l at pH 7 and 14.2 g/l at pH 6.

3.2 Effect of the Temperature

The second important environmental factor which influence on bacterial growth and solvent production is a temperature. *C. acetobutylicum* will lose the ability to produce solvents as well acids at high or low temperature. The temperature affect on the enzymatic pathway of the C. acetobutylicum and this lead to loss of its ability to produce or to convert the substrate to acids and from acids to solvent in acidogenesis/ solventogenesis [1]. The effect of temperature of the fermentation medium on the ABE production is depicted in Fig. 2. It was observed that the butanol production increased with an increase in temperature and 35 °C was found most favorable for a maximum butanol production. The temperature used with *C. acetobutylicum* NCIMB13357 has been reported [15].

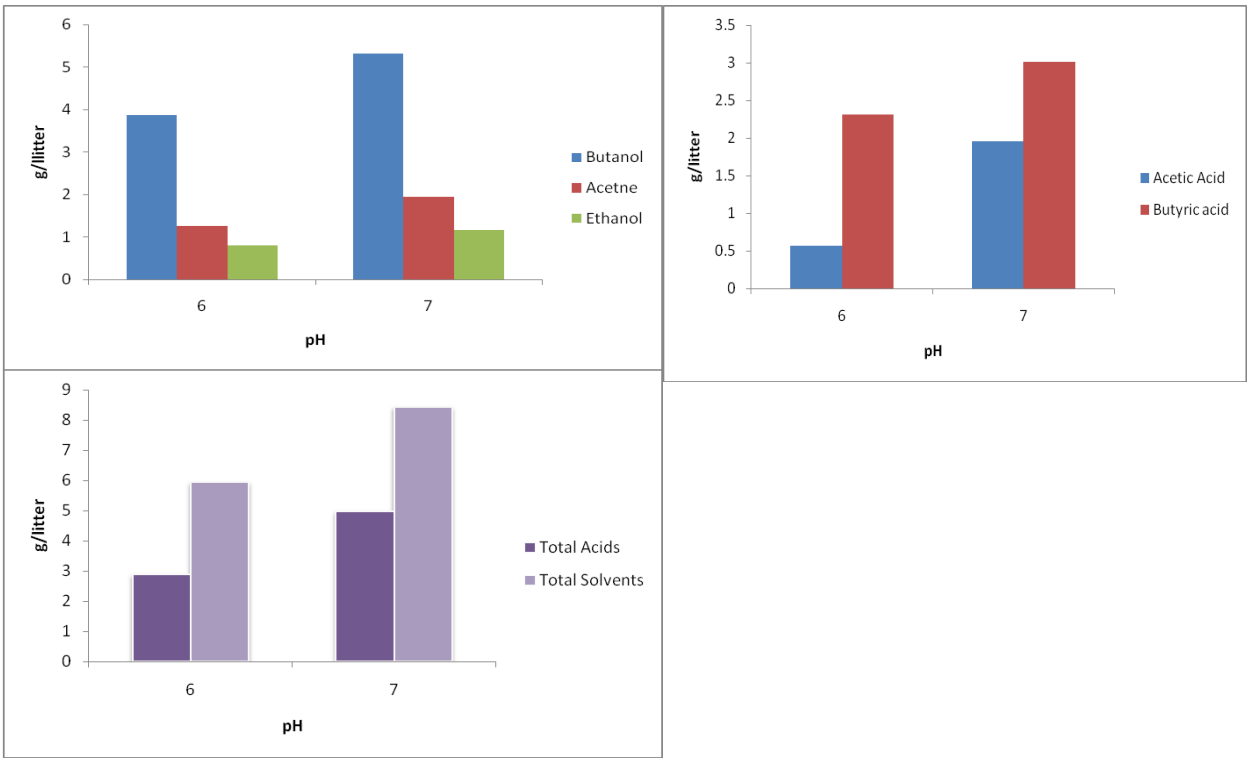

Fig. 1. Effect of pH on Butanol, Total acids and Total Solvents production using Date juice as carbohydrate source by C. acetobutylicum NCIMB 13557

3.3. Effect of Date Fruit concentration

The effect of date fruit concentration on the ABE production was studied at concentrations varying from 10g/l to 50 g/l. Production of butanol, Acetone and Ethanol (ABE) in anaerobic fermentation process is accompanied by the conversion of an organic substrate from date fruit like glucose, sucrose, and fructose in the present studies. The initial substrate concentration plays a key role in ABE production during the pathway of fermentation. At a relatively low initial glucose concentration, the production of fermentation was also low, according to the law of mass action [17]. Fig. 1 reveals that the maximum cumulative butanol production (5.31 g/l) was accomplished with 40 g/l of the date fruit concentration.

Butanol production noticeably increased from 10 g/l of date fruit concentration until 40 g/l of date concentration, and decreased at 50 g/l of date fruit concentration. It has already been reported that substrate inhibition gets predominant at higher glucose concentration because this modifies the metabolic pathways [1, 18]. So that, we suggest that the high concentration of date fruit with modified medium used in this study inhibit the bacteria to produce butanol and other solvent. On the other hand, the concentration of acids increased at 50 g/l concentration of the date more than 40 g/l, that suggest; at high concentration of date fruit the fermentation pathway lean to produce acids rather than solvents.

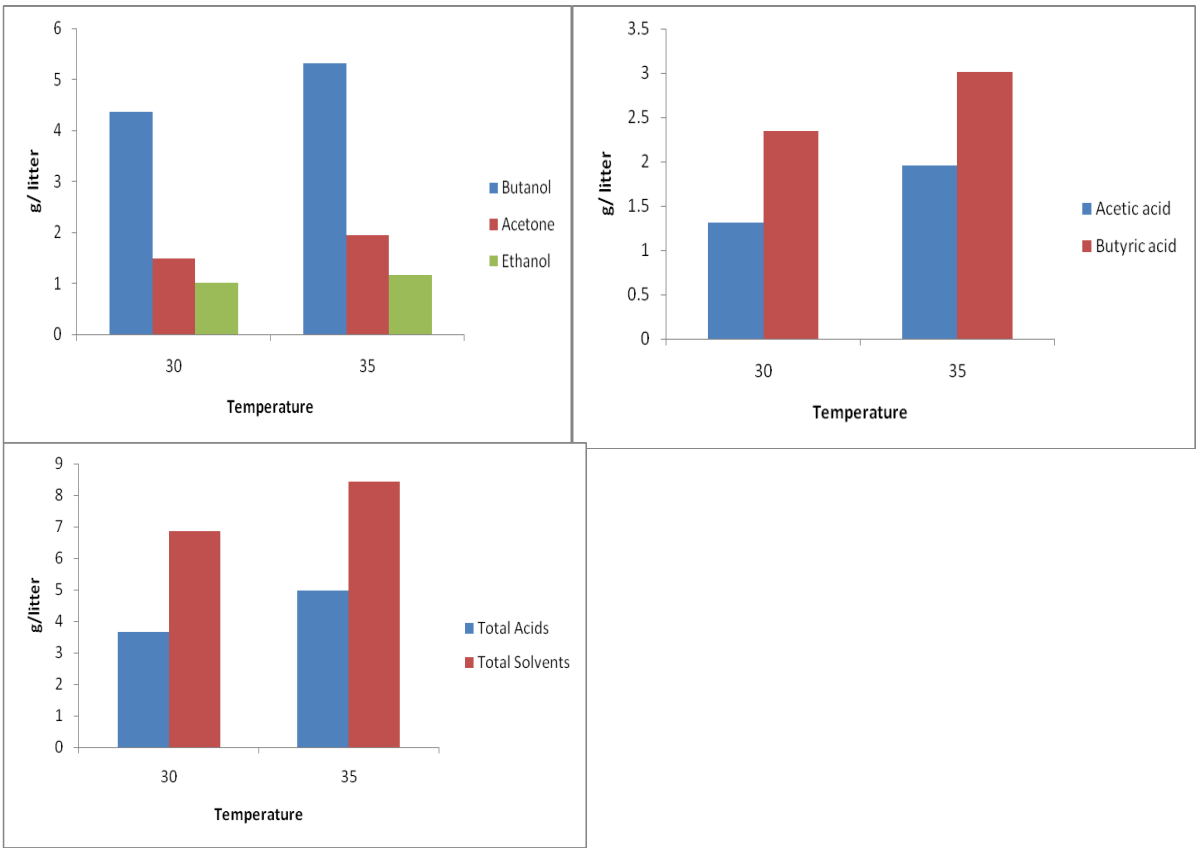

Fig. 2. Effect of Temperature on Butanol, Total acids and Total Solvents production using Date juice as carbohydrate source by C. acetobutylicum NCIMB 13557

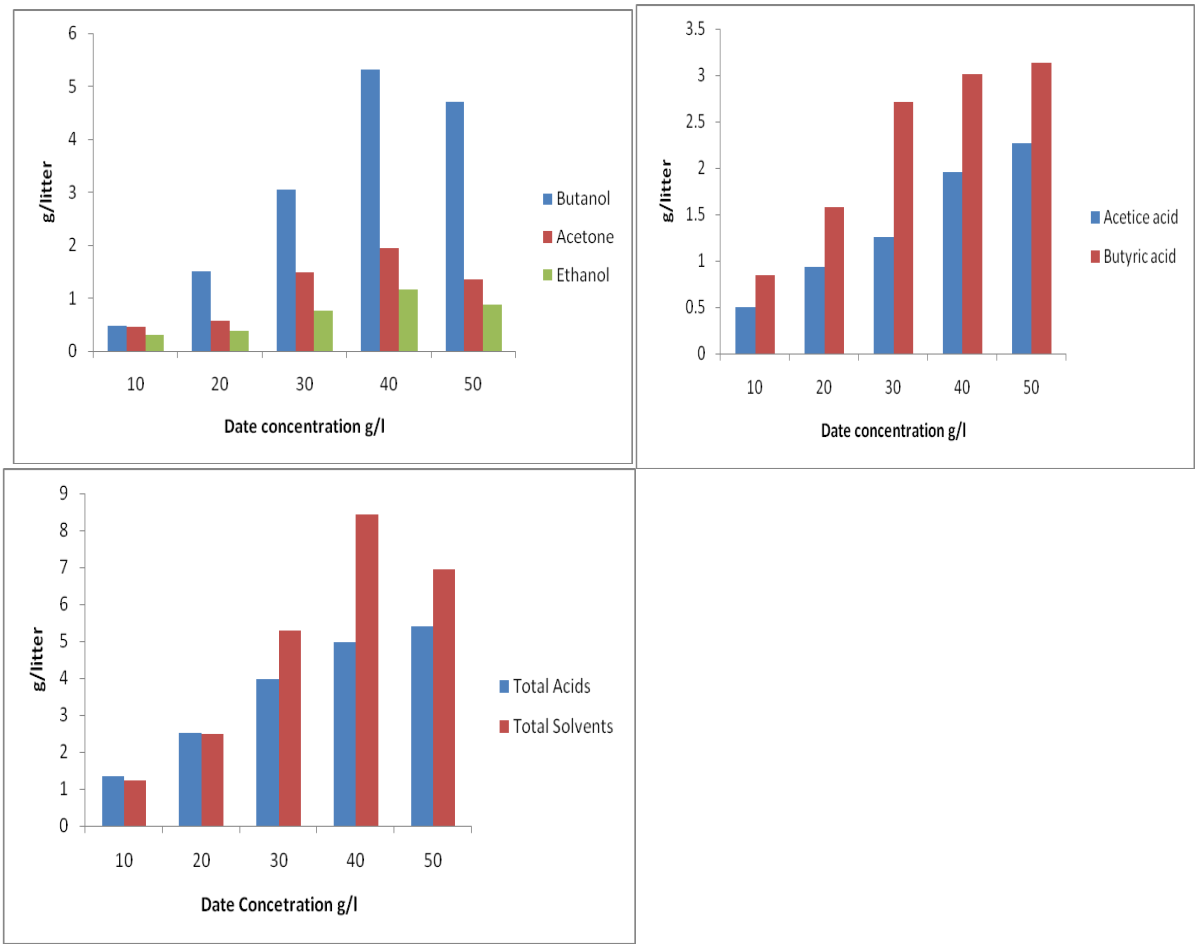

Fig. 3. Effect of Date concentration on Butanol, Total acids and Total Solvents production using Date juice as carbohydrate source by C. acetobutylicum NCIMB 13557

Total acids and total solvents at 40 g/l were 5 g/l and 8.43 g/l respectively. In addition, total acids and solvents at 50 g/l of date concentration was 5.4 g/l and 7 g/l respectively (Fig. 3. Table 2)

Table 2. Effect of substrate concentrations on Yield and productivity of Butanol and ABE

Date Concentration g/l	Total Carb. Consumed g/l	Final Butanol concentration g/l	Yield of Butanol	Productivity of Butanol g/l/h	Yield of ABE	Productivity of ABE g/l/h
10	4.5	0.48	0.11	0.007	0.27	0.018
20	7.3	1.51	0.21	0.022	0.34	0.035
30	11.5	3.04	0.26	0.044	0.46	0.075
40	16.2	5.31	0.33	0.076	0.52	0.12
50	22.9	4.71	0.21	0.067	0.30	0.10

4. Conclusions

This study revealed the facts that abundance of wastage of date fruit can be avoided by using it as a raw material for the production of solvents. The fermentative butanol production by *Clostridium acetobutylicum* NCIMB13357 in a batch culture was performed. The maximum productivity of butanol was obtained 0.328 g/l/h using 40 g/l of date fruit concentration as a substrate in synthetic media. An initial medium pH of 7 ± 0.2 and a reaction temperature of 35 °C were found to be the most favorable for maximum butanol production.

5. Acknowledgements

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