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Date Fruit as Carbon Source in RCM-Modified Medium to Produce Biobutanol by *Clostridium acetobutylicum* NCIMB 13357

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Abstract: Date fruit can be used as an organic carbohydrate source in modified fermentation media to produce biobutanol. Filtrate from date fruit contains high concentration of simple sugars ranging from 65 to 75 %. Reinforced Clostridial Medium (RCM) supplemented with filtrate of date fruit was investigated under anaerobic fermentation conditions using *Clostridium acetobutylicum* NCIMB 13357 for production of Acetone, Butanol and ethanol (ABE). The effects of temperature, pH and concentration of date fruit filtrate on the efficiency of *C. acetobutylicum* showed that 40 g L^{-1} date fruit filtrate at initial pH 7 and temperature 35°C were the optimal fermentation condition that gave the highest amount of butanol- 4.4 g L^{-1}. The yield and productivity of Biobutanol were 0. 3 g g^{-1} and 0.07 g L^{-1} h^{-1} respectively while the Yield and Productivity of ABE were 0.41 g g^{-1} and 0.09 g L^{-1} h^{-1}, respectively.

Key words: Date fruit (*Phoenix dactylifera* L.), *C. acetobutylicum* NCIMB 13357, RCM, biobutanol

INTRODUCTION

In recent years, the instability in the price of crude oil which often mostly results in increased price; greenhouse gases emissions and the increasing global warming phenomenon have resulted in the search for alternative renewable energy. The alternative energy should be renewable, suitable, available, ecological friendly and environmentally safe. Plant biomass is an abundant and renewable source of rich-energy carbohydrate which can effectively be converted by microorganism into biofuel.

The date Palm (*Phoenix dactylifera* L.) is widely planted across the globe. The fruit of date palm is a good source of simple sugars including monosaccharide and disaccharides, proteins, fat and minerals such as copper, sulphur, iron, magnesium and potassium (Ismail *et al*., 2006). The matured fruit of dry date varieties contain a low moisture percentage; 10-20% and high percentage of sugar; 65-75% as well as fibre, minerals and vitamins (El-Sharnouby *et al*., 2007). According to the World Food Agricultural Organization, there are 90 million date trees in the world and each tree can grow for more than 100 years. The mean annual production of date palm tree varies between 100-150 kg of date per tree per year (El-Sohaimy and Hafez, 2010). Besides its direct consumption, date fruit can also be used to prepare a wide range of different products such as date juice concentrates and fermented date products. In addition, dates that are unable to reach maturity and detached by external factors such as wind or rainfall due can be used as component of animal feed. In recent years, date fruit is considered as an excellent fermentable raw material source for the production of bioethanol by *Saccharomyces* (Gupta *et al*., 2009; Noor *et al*., 2003).

Clostridium acetobutylicum, an anaerobic, gram-positive and spore-forming microorganism, has the ability to produce butanol, acetone and ethanol as final products under anaerobic condition, using different carbohydrate sources including monosaccharides and polysaccharide. Many studies had reported that *Clostridium acetobutylicum* can metabolize glucose, sucrose, starch wheat; Palm Oil Mill Effluent (POME), molasses and corn in addition to converting them to acetone, butanol and ethanol during fermentation processes (Jones and Woods, 1986; Lee *et al*., 2008).

Butanol is an important industrial chemical. Half of the butanol produced is used in the form of butyl acrylate and methacrylate esters which are used in latex surface coating, enamels and lacquers (Kirschner, 2006; Lee *et al*., 2008). Other important derivatives of butanol are butyl

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glycol ether, butyl acetate and plasticizers. Butanol is also an excellent diluent for brake fluid formulations and solvents used for the manufacturing of antibiotics, vitamins and hormones. An important application of butanol receiving renewed interest is its usefulness as a direct replacement for 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.

There are many environmental parameters that affect Butanol production during ABE fermentation process by *Clostridium acetobutylicum*. The pH and temperature are the most important environmental parameters that play vital roles on the growth of this bacterium, its enzymes activity and its pathway during anaerobic fermentation process. Furthermore, the concentrations of substrate as well as the nature of the Carbon sources in the medium are other important factors determining fermentation pathway. In general, the amounts of acids and ABE produced by bacteria depend on the amount of carbohydrate consumed during fermentation process.

This study was carried out to investigate the effects of some environmental parameters including pH, temperature and substrate concentration on *C. acetobutylicum* NCIMB 13357 in the production of butanol and other solvents using RCM-Date medium by replacing the glucose with the rich-energy carbohydrate date fruit.

MATERIALS AND METHODS

Strain and culture media: *C. acetobutylicum* NCIMB 13357 was obtained from biotechnology laboratory, Department of Chemical and Process Engineering, UKM, in a frozen form and maintained in Reinforced Clostridium Medium (RCM) broth and agar (Shamsudin and Kalil, 2004). Fresh inoculum was prepared with RCM medium and incubated at 35°C for 24 h under anaerobic condition and directly used in the experiments 10% of the culture was used as inoculum to inoculate the fermentation medium.

Date medium preparation: Hundred gram of dry date was blended and made up to a final volume of 1000 mL with distilled water, it was then autoclaved at 121°C for 15 min and allowed to cool to room temperature. It was then filtered to remove solid particles and then mixed in known proportion with synthetic medium 2X. This procedure was repeated to obtain different concentrations i.e., 20, 30, 40 and 50 g L^{-1} of the filtrate of date fruit in the modified medium as respective final media concentrations. A volume of 150 mL of the prepared known concentration of 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.

Synthetic medium (2X): The reference synthetic RCM-medium used to test the effect of the environmental parameters factors, previously described (Elgadafi and Kalil, 2009), had the following composition; Yeast extract 3.0 g L^{-1}; "Lab-Lemco" Powder 10.0 g L^{-1}; Peptone 10.0 g L^{-1} Soluble starch 1.0 g L^{-1}; Cystine hydrochloride 0.5 g L^{-1}; Sodium hydrochloride 5.0 g L^{-1}; Sodium acetate 3.0 g L^{-1}; Agar 0.5 g L^{-1}.

2X concentration from the previous medium was prepared, an appropriate volume from 2X medium was then mixed with an equally appropriate volume from the fermentation medium of different concentrations (described above) to get the final date fruit concentrations 10, 20, 30, 40 and 50 g L^{-1}, as well 1X from the synthetic medium.

Analytical methods: Anthrone method of Frolund *et al.* (1996) was used to determine the amount of the Total carbohydrate consumed during the fermentation process. This involved the use of a UV-visible spectrophotometer to measure the amount of the total carbohydrate before and after the fermentation batch reaction. Solvents and Acids concentration were determined by gas chromatograph with capillary column (EquityTM-1 Supelco), previously described by Elgadafi and Kalil (2009).

ABE and acids (acetic and butyric) concentration were determined using a gas chromatograph (7890A GC-System, Agilent Technologies, Palo Alto, CA, USA) equipped with a Flame ionization detector and a 30 m capillary column (Equity 1; 30 m×0.32 mm×1.0 μm film thickness; Supelco Co, Bellefonate, PA, USA). The oven temperature was programmed to increase from 40 to 130 °C at a rate of 8°C min^{-1}. The injector and detector temperatures were set at 250 and 280°C, respectively. Helium, as the carrier gas, was set at a flow rate of 1.5 mL min^{-1}. The yield was calculated as the total ABE/butanol produced divided by the total carbohydrate utilized and is expressed as grams/gram. ABE/Butanol Productivity was calculated as the total ABE/Butanol (g L^{-1}) divided by fermentation time.

RESULTS

After conducting the batch fermentation experiment using various concentrations of date fruit ranging from 10 to 50 g L^{-1}, with *C. acetobutylicum* NCIMB13357 under anaerobic condition as the micro-organism, at different initial pH 7 and 6 and temperatures 30 and 35°C

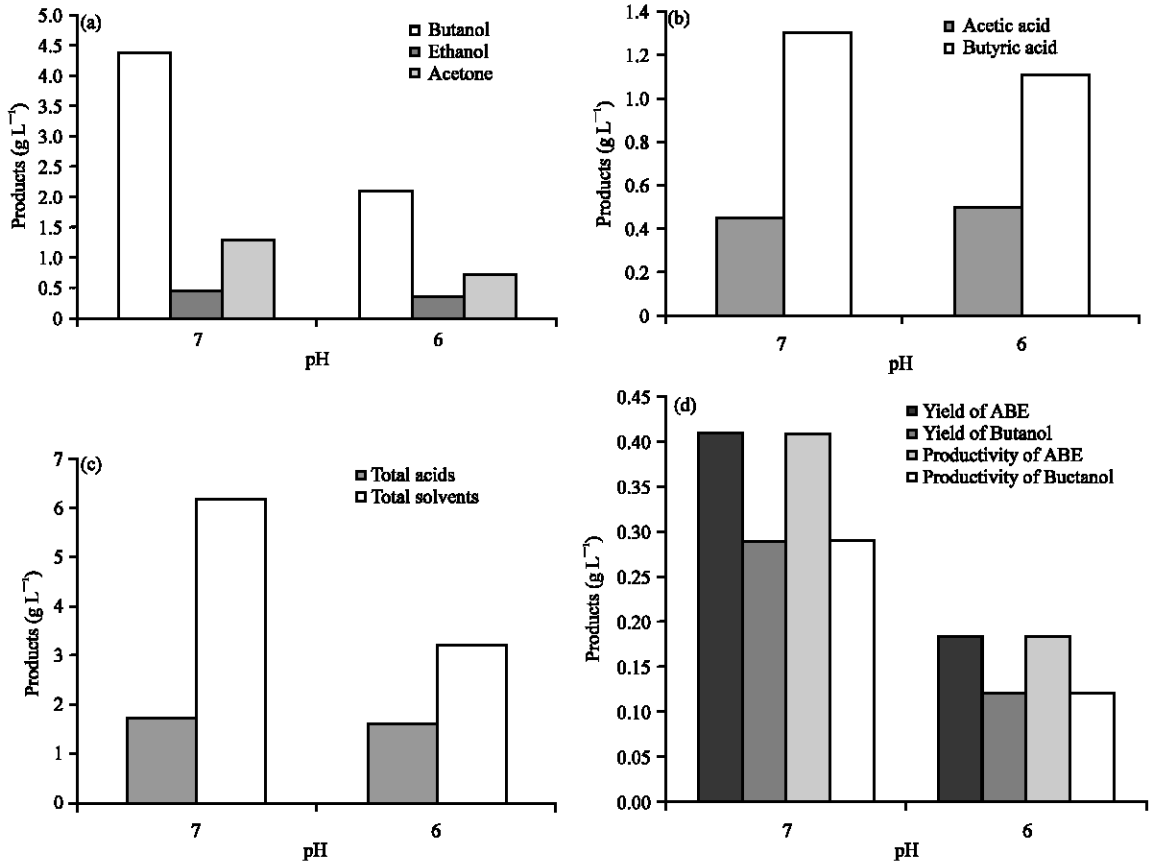

Fig. 1(a-d): Effect of pH on *C. acetobutylicum* NCIMB 13557: (a) Effect on butanol, ethanol and acetone production, (b) Effect on acetic acid and butyric acid production, (c) Effect on total acids and total solvents production, (d) Effect on yield and productivity of ABE and butanol, respectively

as incubation temperatures for 70 h, the final pH, total sugar consumption g L^{-1}, butanol production g L^{-1}, total ABE, total acids, yield of ABE, productivity of ABE g L^{-1} h^{-1}, yield of butanol and productivity of butanol were measured.

Effect of pH: The amount of butanol, ethanol and acetone produced during fermentation process at pH 7 was 4.4, 0.48 and 1.33 g L^{-1}, respectively, while on the other hand, the amount of butanol, ethanol and acetone produced during fermentation process at pH 6 were 2.1, 0.37 and 0.73 g L^{-1}, respectively as shown in Fig. 1a. In addition, the amount of acetic acid and butyric acid produced by *C. acetobutylicum* at pH 7 were 0.45 and 1.3 g L^{-1}, respectively while the amount of acetic acid and butyric acid produced by *C. acetobutylicum* at pH 6 were 0.51 and 1.1 g L^{-1}, respectively as show in Fig. 1b.

Total acids and total solvents produced are shown in Fig. 1c, at pH 7 and 6, they were 1.77 and 6.2 g L^{-1}, respectively. Total acids and total solvents at pH 6 were 1.62 and 3.2 g L^{-1}, respectively.

Effect of the temperature: The amount of butanol, ethanol and acetone produced during fermentation process at 35°C were 4.4, 0.48 and 1.33 g L^{-1}, respectively. However, the amount of butanol, ethanol and acetone produced during fermentation process at 30°C were 3.7, 0.44 and 1.1 g L^{-1}, respectively as shown in Fig. 2a. Furthermore, the amount of acetic acid and butyric acid produced by *C. acetobutylicum* at 35°C was 0.45 and 1.3 g L^{-1}, respectively. The amount of acetic acid and butyric acid produced by *C. acetobutylicum* at temperature of 30°C were 1.33 and 1.6 g L^{-1}, respectively as shown in Fig. 2b.

Total acids and total solvents are shown in Fig. 1c. At 35°C, they were 1.77 and 6.2 g L^{-1}, respectively they were 2.9 and 5.2, respectively at 30°C as shown in Fig. 2c.

Effect of date fruit concentrations: The amount of butanol, acetone and ethanol produced during fermentation process at date fruit concentration of 10 g L^{-1} were 0.7, 0.3 and 0.15 g L^{-1}, respectively, on the other hand, the amount of butanol, acetone and ethanol produced during fermentation process at date

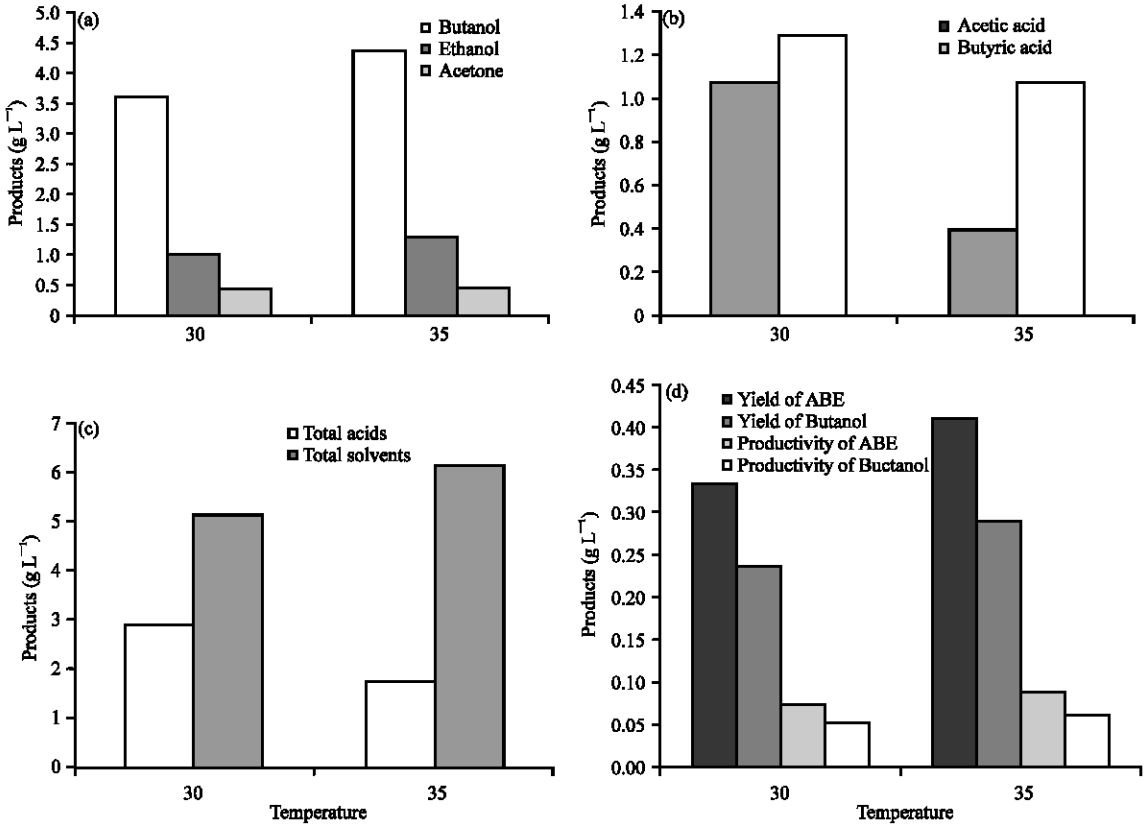

Fig. 2(a-d): Effect of Temperature on *C. acetobutylicum* NCIMB 13557: (a) Effect on butanol, ethanol and acetone production, (b) Effect on acetic acid and butyric acid production, (c) Effect on total acids and total solvents production, (d) Effect on yield and productivity of ABE and butanol, respectively

fruit concentration of 20 were 1.0, 0.4 and 0.18 g L^{-1}, respectively. The amount of butanol, acetone and ethanol produced during fermentation process at date fruit concentration of 30 were, 0.55 and 0.37 g L^{-1}, respectively. For concentration of 40 g L^{-1}, the amount of butanol, acetone and ethanol produced during fermentation process were 4.4, 1.33 and 0.48 g L^{-1}, respectively while the amount of butanol, acetone and ethanol produced during fermentation process at date fruit concentration of 50 g L^{-1} were 3.9, 1.0 and 0.45 g L^{-1}, respectively as shown in Fig. 3a.

The amount of acetic acid and butyric acid produced during the fermentation reaction using *C. acetobutylicum* at date fruit concentration of 10 g L^{-1} were 0.47 and 1.22 g L^{-1} respectively while for date fruit concentration of 20 g L^{-1}, the values were 0.43 and 1.32 g L^{-1}, respectively. The amount of acetic acid and butyric acid produced during the same process but with date fruit concentration of 30 were 0.55 and 1.0 g L^{-1}, respectively. For date fruit concentration of 40 g L^{-1}, the amounts of acetic and butyric acids were, respectively 0.45 and 1.3 g L^{-1}, whereas, at 50 g L^{-1}, the values were 0.47 and 1.16 g L^{-1}, respectively.

The total acids and total solvents produced at different concentrations of 10 g L^{-1} were 1.7 and 1.15 g L^{-1}, respectively; 20 g L^{-1}: 1.75 g L^{-1} and 1.6 g L^{-1}, respectively; 30 g L^{-1}: 1.53 g L^{-1} and 4.74 g L^{-1}, respectively; 40 g L^{-1}: 1.8 g L^{-1} and 6.2 g L^{-1}, respectively and 50 g L^{-1}:1.63 g L^{-1} and 5.33 g L^{-1}, respectively (Fig. 3c).

DISCUSSION

pH is one of the most important controlling factors of anaerobic fermentation processes. Jones and Woods (1986) reported that pH is considered as a key factor in determining the outcome of ABE fermentation. The optimum pH for *C. acetobutylicum* NCIMB 13557 has been reported in some recent studies. Elgadafi and Kalil (2009) stated that the optimum pH for the production of ABE using Reinforced Clostridial Medium (RCM) was 6. In addition, Alshiyab *et al.* (2008) reported pH 7 as the

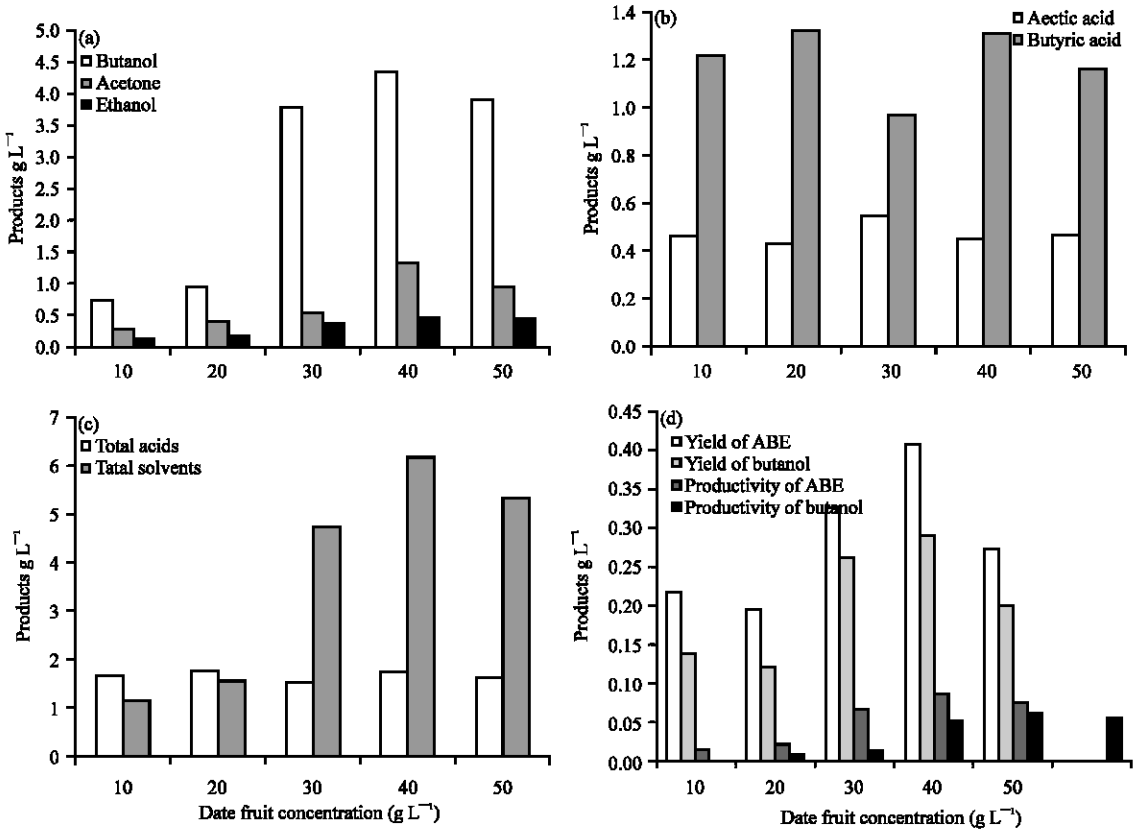

Fig. 3(a-d): Effect of Date Concentrations on *C. acetobutylicum* NCIMB 13557: (a) Effect on butanol, ethanol and acetone production, (b) Effect on acetic acid and butyric acid production, (c) Effect on total acids and total solvents production, (d) Effect on yield and productivity of ABE and butanol, respectively

optimum for the production of Hydrogen. This study illustrates the effect of initial pH (pH 6 and 7) on ABE/ Butanol production using modified RCM-date fruit medium by *C. acetobutylicum* NCIMB 13557.

It is evident from the results shown in Fig. 1 that the pH 7 is the optimum pH needed to produce maximum butanol and other solvents. Comparing the results of pH 6 and pH 7 at 35°C and an initial substrate concentration of 40 g L^{-1} of date fruit, it can be seen that the amount of butanol produced were 2.1 and 4.4 g L^{-1}, respectively. However, at pH 7, the yield and productivity of butanol were 0.3 and 0.07, respectively Fig. 1d.

Another important environmental factor which influences bacterial growth and solvent production is the operating temperature. *C. acetobutylicum* will lose its ability to produce solvents as well as acids at high or low temperature. The temperature affects 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 pathways (Jones and Woods, 1986). 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 as the most favourable for maximum butanol production as well as the yield of fermentation Fig. 2d. The temperature used with *C. acetobutylicum* NCIMB13357 has been reported (Shamsudin and Kalil, 2004).

The effect of date fruit concentration on ABE production was studied at various concentrations ranging from 10 to 50 g L^{-1}. Production of butanol, acetone and ethanol (ABE) in anaerobic fermentation process is accompanied by the conversion of the organic substrate from date fruit such as glucose, sucrose and fructose in the present study. The initial substrate concentration plays a key role in ABE production in the fermentation pathway. At a relatively low initial glucose concentration, the production of fermentation was low, according to the law of mass action (Fabiano and Perego, 2002). Figure 3 reveals that the maximum cumulative butanol production (4.4 g L^{-1}) was accomplished with 40 g L^{-1} of the date fruit concentration.

Butanol production noticeably increased from 10 g L^{-1} of date fruit concentration until 40 g L^{-1} of date concentration and decreased at 50 g L^{-1} of date fruit concentration. It has already been reported that substrate inhibition becomes more predominant at higher glucose or substrate concentration because this modifies the metabolic pathways (Jones and Woods, 1986; Oh *et al*., 2003). It was thus assumed that the high concentration of date fruit with RCM-Date used in this study inhibited the bacteria to produce butanol and other solvents.

CONCLUSION

This study shows that date fruit can be used as carbon source in fermentation of produce solvents and acids at different concentrations. The maximum Yield and Productivity of butanol obtained were 0.3 g g^{-1} and 0.07 g L^{-1} h^{-1}, respectively, using 40 g L^{-1} of date fruit concentration as the substrate in RCM-Date medium. An initial medium pH of 7±0.2 and a temperature of 35°C were found to be the most favourable condition for maximum butanol production.

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