ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 10 (3): 280-285, 2011
ISSN 1682-296X / DOI: 10.3923/biotech.2011.280.285

The Effect of Different Carbon Sources on Biobutanol Production using *Clostridium saccharoperbutylacetonicum* N1-4

[2]Najeeb Kaid Nasser Al-Shorgani, [1]Mohd Sahaid Kalil and [2]Wan Mohtar Wan Yusoff
[1]Department of Chemical and Process Engineering, Faculty of Engineering,
Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia
[2]School of Bioscience and Biotechnology, Faculty of Science and Technology,
Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia

Abstract: The aim of this study was to investigate the influence of various carbohydrate sources on the production of acetone-butanol-ethanol (ABE) via microbial fermentation using *Clostridium saccharoperbutylacetonicum* N1-4 (ATCC 13564). The anaerobic batch fermentation was conducted in 250 m L^{-1} Schott (Duran) bottles with working volume of 150 m L^{-1} using different carbon sources supplemented with P2 medium or tryptone-yeast extract-acetate medium (TYA). The results showed that *C. saccharoperbutylacetonicum* N1-4 could utilize various carbohydrates. Out of five carbon sources tested, glucose showed the best results. A concentration of 50 g L^{-1} of glucose in the medium produced 13.61 g L^{-1} of ABE containing 8.69 g L^{-1} of biobutanol (63.8%). Regardless of the carbon source, P2 medium produced higher biobutanol concentration compared to TYA medium. A 15 g L^{-1} addition of butanol in the medium totally inhibited the growth of *C. saccharoperbutylacetonicum* N1-4 indicating a critical concentration of biobutanol achievable is 15 g L^{-1}. The ability of *C. saccharoperbutylacetonicum* N1-4 to utilize various carbohydrates makes agro-industrial wastes to be a cheap substrate for biobutanol production.

Key words: Biobutanol, renewable energy, anaerobic fermentation, *Clostridium saccharoperbutylacetonicum* N1-4, carbohydrates

INTRODUCTION

Acetone-Butanol-Ethanol (ABE) fermentation by clostridia was widely carried out industrially during the first half of the last century but later it could not compete economically with petrochemical synthesis due to the cost of substrate, the development in petrochemical industry and the low yield of butanol due to its hetero-fermentative characteristic (Lee *et al*., 2008).

There has been a growing interest in the bioconversion of agricultural biomass into biofuels and chemical feedstock. That was not only because of the limiting nature of the source of petroleum and fossil fuels but also because of the global increase in fossil fuel-derived oil price as well as the environmental problems resulting from waste accumulation. The cost of substrate is an important factor in butanol fermentation. Butanol can be produced from various raw materials or renewable agricultural crops including sago starch (Madihah *et al*., 2001) corn (Qureshi and Blaschek, 2001) molasses (Qureshi *et al*., 2001; Syed *et al*., 2008) and whey permeate (Ennis and Maddox, 1985) among others. Butanol has a lower vapor pressure but higher energy content than ethanol which makes the former safer for blending with gasoline as well as offering better fuel economy than ethanol-gasoline blends (Hipolito *et al*., 2008).

In addition, butanol has a higher tolerance to water contamination in gasoline blends and therefore butanol-gasoline blends are less susceptible to separation and this facilitates its use in the existing gasoline supply and distribution channels (Durre, 2007; Shamsudin *et al*., 2006). While branched chain 4-carbon alcohols including isobutanol 2-methyl-1-butanol and 3-methyl-1-butanol have higher octane numbers compared with n-butanol (Atsumi *et al*., 2008).

Furthermore, Butanol has sufficiently similar characteristics to gasoline when used directly or it can be blended with gasoline at higher concentrations than ethanol in any gasoline engine without any modification (Kalil *et al*., 2003). Consequently, butanol is regarded as the most promising solvent compared to ethanol and acetone. Subsequently, ABE fermentation should be optimized to enhance butanol production over ethanol since butanol appears to be of more value commercially and technologically (Al-Shorgani *et al*., 2011).

Corresponding Author: Najeeb Kaid Nasser Al-Shorgani, School of Bioscience and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia

A typical feature of the clostridial solvent production is biphasic fermentation. The first phase is the acidogenic phase during which the acids forming pathways are activated. Acetate, butyrate, hydrogen and carbon dioxide are the major products of this phase. This acidogenic phase usually occurs during the exponential growth phase. The second phase is the solventogenic phase during which acids are reassimilated and used in the production of acetone, butanol and ethanol (or isopropanol instead of acetone in some *C. beijerinckii* strains) (Jojima *et al*., 2008). The present study was carried out to investigate the ability of *C. saccharoperbutylacetonicum* N1-4 to utilize various carbon sources as substrates and the butanol tolerance in anaerobic batch fermentation.

MATERIALS AND METHODS

Microorganism and inoculum preparation: *Clostridium saccharoperbutylacetonicum* N1-4 was provided by the Biotechnology Lab, Chemical Engineering and Bioprocess Department at Universiti Kebangsaan Malaysia where this study was carried out in year 2010. It was kept at 4°C as a suspension of spores in a potato glucose medium (PG medium) [150 g potato, 10 g glucose 0.5 g $(NH_4)_2SO_4$, 3 g $CaCO_3$] as a stock culture. The inoculum was prepared by transferring the suspension of spore 1 mL to 10 mL of 15% potato-glucose medium with subsequent heat shock for 1 min in boiling water and thereafter, cooled in iced water (Mun *et al*., 1995) and incubated for 1-2 days at 30°C under anaerobic conditions. The inoculum was checked by the colony morphology characteristics and Gram-stain technique to ensure that the culture was still pure. This culture was then transferred to TYA medium (TYA medium components per liter of deionized water to constitute a 20 g glucose, tryptone , 6 g; yeast extract, 2 g; ammonium acetate 3 g; KH_2PO_4, 0.5 g; $MgSO_4.7H_2O$, 0.3 g and $FeSO_4.7H_2O$, 10 mg) and was incubated for 15-18 h and used as the inoculum.

Preparation of medium: The fresh potato medium (PG medium) contained the following substances per liter of distilled water; 150 g grated fresh potato, 10 g glucose, 0.5 g $(NH_4)_2SO_4$ and 3 g $CaCO_3$. After mixing the above substances the medium was incubated in boiling water for 1 h and stirred for thorough mixing at 10-min intervals. After that, the medium was filtered through gauze and sterilized at 121°C for 15 min.

Tryptone-yeast extract acetate medium (TYA medium) was used for the pre-culture. The medium per liter of distilled water consisted of 20 g glucose, 2 yeast extract, 6 g tryptone, 3 g CH_3COONH_4, 0.3 g $MgSO_4•7H_2O$, 0.05 g, 0.5 g KH_2PO_4 and 10 mg $FeSO_4•7H_2O$. The medium was sterilized at 121°C for 15 min.

The P2 medium consisted of (g L^{-1}) KH_2PO_4 0.5, K_2HPO_4 0.5, $MgSO_4.7H_2O$ 0.4, $MnSO_4.4H_2O$ 0.01, $FeSO_4.5H_20$ 0.01 yeast extract 1.0 cysteine 0.5 and gelatinized starch. One milliliter of a solution containing 1 mg L^{-1} 4-aminobenzoic acid; 80 μg L^{-1} biotin was added to 1 L of the medium. The main fermentation medium contained various glucose concentrations (from 10 to 60 g L^{-1}) and other carbon sources such as sago starch xylose xylan and cellulose (CMC). The medium was then autoclaved at 121°C for 15 min. After autoclaving the fermentation broth was sparged with oxygen-free Nitrogen to achieve an anaerobic condition. Starch was gelatinized by heating slightly slurry at 80°C for 30 min. TYA medium was initially supplemented with various concentrations of butanol ranging between 0-35 g L^{-1} in order to investigate the effect of butanol on *C. saccharoperbutylacetonicum* N1-4.

Analytical procedures: Samples were centrifuged at 10,000 rpm for 5 min. The supernatant was used for determining the concentration of solvent (ABE), sugars and organic acids (butyric and acetic acids).

ABE and acids were measured 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 (Equity1; 30 m×0.32 mm×1.0 μm film thickness Supelco Co, Bellefonate, PA, USA). 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 glucose concentration in the fermentation broth was determined with a Biochemistry Analyzer (YSI 2700D, YSI Inc. Life Sciences Yellow Springs, OH, USA).

Reducing sugars concentration in the medium were measured using the 3, 5 Dinitrosalicylic Acid (DNS) assay according to the method of Miller (1959). The absorbance at OD 575 nm for all samples were recorded and the reduced sugars concentration was calculated from a standard curve (Miller, 1959).

RESULTS AND DISCUSSION

Effect of different carbon sources on biobutanol concentration: In order to investigate the ability of *C. saccharoperbutylacetonicum* N1-4 to utilize various carbon sources, batch culture was carried out using, glucose, xylose, xylan, cellulose (CMC) and starch in TYA medium or P2 medium.

Table 1: Biobutanol production from different carbohydrates in batch fermentation

Medium	Bacteria	($g L^{-1}$)	Production ($g L^{-1}$)				Productivity (g/L.h)		Fermentation mode
			Acetone	Butanol	Ethanol	ABE	ABE	Butanol	
P2	C.S N1-4	30 glucose	0.56	4.86	0.85	6.27	0.087	0.068	Batch (flask)
P2	CA	30 glucose	1.35	5.36	0.86	7.57	0.105	0.074	Batch (flask)
P2	C.S N1-4 + C.A	30 glucose	1.74	4.34	0.90	6.98	0.097	0.060	Batch (flask)
P2	C.A	30 glucose	1.60	6.12	0.92	8.65	0.180	0.128	Batch (3 L fermentor)
TYA	C.S N1-4	30 glucose	1.34	4.33	0.85	6.52	0.091	0.060	Batch (flask)
TYA	C.S N1-4	20 xylose	0.12	0.74	0.77	1.63	0.023	0.010	Batch (flask)
TYA	C.S N1-4	30 xylose	0.84	1.23	0.15	2.22	0.031	0.017	Batch(flask)
TYA	C.S N1-4	10 xylan	0.10	0.04	0.03	0.17	0.002	0.001	Batch (flask)
TYA	C.S N1-4	10 CMC	0.09	0.47	0.00	0.57	0.008	0.007	Batch (flask)
TYA	C.S N1-4	20 CMC	0.11	0.25	0.04	0.40	0.006	0.003	Batch (flask)
TYA	C.S N1-4	30 sago starch	1.37	3.02	0.78	5.17	0.108	0.063	Batch (flask)
P2	C.S N1-4	30 sago starch	1.58	5.24	0.90	7.72	0.161	0.109	Batch(flask)

C.S N1-4: *C. saccharoperbutylacetonicum* N1-4; C.A: *C. acetobutylicum* NCIMB 13357; CMC: Carboxymethyl cellulose

In an experiment using P2 medium containing 30 g L^{-1} glucose, *C. saccharoperbutylacetonicum* N1-4 produced 6.27 g L^{-1} of ABE containing 4.86 g L^{-1} butanol whereas the total ABE and butanol concentrations obtained from *C. acetobutylicum* were higher i.e., 7.57 and 5.36 g L^{-1}, respectively. On the other hand, when an equivalent inoculum from both *C. saccharoperbutylacetonicum* N1-4 and *C. acetobutylicum* (5% of each) was subjected to P2 medium containing 30 g L^{-1}, glucose the ABE and butanol concentrations were 6.98 and 4.34 g L^{-1}, respectively (Table 1). It was found that 6.98 g L as ABE concentration is the average between 6.27 and 7.57 g L^{-1} as the ABE produced by *C. saccharoperbutylacetonicum* N1-4 and *C. acetobutylicum*, respectively. These results showed no relationship between the two strains, each fermented the glucose individually.

The butanol production from the mixed culture was lower than that produced from the individual fermentation. *C. acetobutylicum* produced higher ABE and butanol compared to *C. saccharoperbutylacetonicum* N1-4 in batch culture by flask. ABE and butanol production using *C. acetobutylicum* improved when batch culture fermentation was carried out using 3 L fermentor with working volume of 1.5 L as 8.65 g L^{-1} and 6.12 g L^{-1} with productivity of 0.18 and 0.128 g/L.h, respectively (Table 1). This may be ascribed to the suitable fermentation conditions which were provided by the fermentor. The P2 medium showed higher ABE and butanol concentrations compared to TYA medium when 30 g L^{-1} of sago starch was used as a carbon source (7.72 and 5.24 g L^{-1} vs. 5.17 and 3.02 g L^{-1}, respectively).

C. saccharoperbutylacetonicum N1-4 showed the ability to utilize various carbohydrates including glucose, starch, xylose, xylan and CMC. However, the utilization of xylose, xylan and CMC was partly while on the other hand P2 medium produced higher butanol concentration compared to TYA medium regardless of the carbon source. Butanol production from 20 g L^{-1} CMC was lower than that produced from 10 g L^{-1} of CMC. This may be attributed to the viscosity of 20 g L^{-1} of CMC, however, solubility was observed after 24 h from the cultivation. Previously, it was reported that solvent-producing Clostridia can utilize some carbohydrates fully such as, glucose, fructose, sucrose, lactose, mannose and dextrin while xylose, arabinose, raffinose, galactose, inulin meletose and mannitol are partially consumed (Jones and Woods, 1986). *C. saccharoperbutylacetonicum* N1-4 exhibited a relatively good production of butanol with glucose and starch. Butanol production was also observed in case of xylose, xylan and CMC as substrate but with comparatively lower yields. These observations indicate that the presence of various types of carbon in the agro-industrial wastes could be utilized for butanol production and this will greatly help in waste recycling. This indicates the potentiality of *C. saccharoperbutylacetonicum* N1-4 in using mixed carbon sources which is actually a means of exploring the possibility of using cheaper and renewable raw materials.

Effect of glucose concentration on butanol production using *C. saccharoperbutylacetonicum* N1-4: Batch culture experiments in TYA medium using *C. saccharoperbutylacetonicum* N1-4 to investigate the effect of glucose concentration in the culture media on butanol fermentation were carried out by varying the initial glucose concentration between 10 and 60 g L^{-1} at fixed conditions of incubation temperature 30°C, initial medium pH of 6.0±0.2, anaerobic conditions and without shaking. The production of butanol and other solvent were determined and presented in Table 2.

Biobutanol production for 10, 20, 30, 40, 50 and 60 g L^{-1}, glucose was determined as 0.49, 2.11, 4.33, 6.40, 8.69 and 7.99 g L^{-1}, respectively. The highest ABE and butanol productivity of 0.142 and 0.091 g/L.h, respectively were obtained when 50 g L^{-1} of glucose concentration

Table 2: Effect of glucose concentration on butanol production by *C. saccharoperbutylacetonicum* N1-4

	Production (g L^{-1})				Productivity (g/L.h)	
Glucose-concentration (g L^{-1})	Acetone	Butanol	Ethanol	ABE	ABE	Butanol
10	0.28	0.49	0.00	0.77	0.016	0.010
20	0.90	2.11	0.02	3.03	0.042	0.029
30	1.34	4.33	0.85	6.52	0.091	0.060
40	2.65	6.40	0.27	9.32	0.129	0.089
50	4.81	8.69	0.11	13.61	0.142	0.091
60	3.54	7.99	0.40	11.92	0.124	0.083

was used. The results showed that increasing glucose concentration from 10 to 50 g L^{-1} tends to enhance the production of ABE and butanol and the relationship between glucose concentrations and butanol production is linear. Increasing the glucose concentration beyond 50 to 60 g L^{-1} decreased the ABE production (Table 2). Results indicated that increasing the initial glucose concentration above 50 g L^{-1} decreased the butanol and total ABE production due to substrate inhibition as reported earlier by Kumar and Das (2000), it was also found out that at high substrate concentrations, microbial growth rate is inhibited by the substrate. Though the effect of substrate concentration such as glucose and xylose had been reported by Ounine *et al.* (1985). It was shown that increasing the glucose and xylose concentration over the optimal concentration caused a decrease in the activities of the glucose and xylose transport systems.

Effect of nutrient limitation on cell growth and ABE production had been well studied and recognized. When carbon source is limited in the medium, only acids are produced. At least 10 g L^{-1} glucose can induce the culture of *C. saccharobutylicum* NCP 262 to solvent production (Long *et al*., 1984).

Fond *et al.* (1985) reported that presence of less than 10 g L^{-1} glucose in batch cultures and 4 g L^{-1} per day in fed-batch cultures of *C. acetobutylicum* produced only acids and no shift to ABE production occurs. The shortage of ABE production in glucose-limited cultures was ascribed to the failure to accumulate the threshold concentrations of acid end-products.

In continuous fermentation of ABE under conditions of carbon source limitation, no sufficient acid concentrations required to stimulate solvent production were produced (Bahl and Gottschalk, 1984; Haggstrom, 1985; Petitdemange *et al*., 1984).

In contrast, ABE were produced by cultures grown in phosphate- or sulfate-limited media (Bahl *et al*., 1982; Bahl and Gottschalk, 1984). However, despite these notes no distinct growth-limiting nutrient that specifically induces solvent production has been identified. So far despite a lot of intensive studies on solvent production, none of the growth nutrients that limit the production of solvents stimulants has been identified nor isolated.

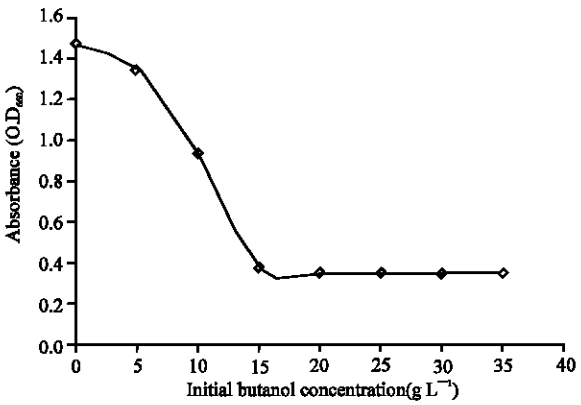

Fig. 1: Effect of butanol addition on the growth of *C. saccharoperbutylacetonicum* N1-4 (the growth was measured after 24 h of cultivation).

Effect of butanol on *C. saccharoperbutylacetonicum* N1-4 growth: The purpose of this experiment was to know what concentration of butanol can be tolerated by *C. saccharoperbutylacetonicum* N1-4. The test was carried out in 20 mL test tubes with 15 mL working volume. Various concentrations of butanol ranging between 0-35 g L^{-1} were initially added to TYA medium. The medium was inoculated with 10% of fresh *C. saccharoperbutylacetonicum* N1-4 and incubated at 30°C in anaerobic conditions. The growth was estimated after 24 h by optical density at 660 nm using spectrophotometer. It was found that increasing the concentration of butanol decreased the growth of this bacterium and no growth was observed at butanol concentration between 10-15 g L^{-1} (Fig 1). Butanol showed a toxic effect on growth of *C. saccharoperbutylacetonicum* N1-4 at concentration of 5 g L^{-1} or higher. When 15 g L^{-1} butanol was added to the culture the growth was inhibited by 99.7 %. These results are in agreement with what was reported by Soni *et al.* (1987). Earlier study conducted by Soni *et al.* (1987) reported that 13 g L butanol inhibited the growth of *C. saccharoperbutylacetonicum* (ATCC 27022). In addition Ounine *et al.* (1985) reported that at butanol concentration of 14 g L^{-1}, growth of *C. acetobutylicum* was totally inhibited.

The effect of butanol on the bacterial cell is known as a chaotropic. This happens when the functions and fluidity of cell membrane are disrupted (Bowles and Ellefson, 1985) or rather the response of cells against butanol effect in which there is increase in saturated-unsaturated fatty acid ratio in the membrane (Sullivan *et al*., 1979). It has been reported that with *C. acetobutylicum* butanol disrupts the transmembrane electrical gradients and pH lowers concentration of ATP and stops glucose uptake (Bowles and Ellefson, 1985; Terracciano and Kashket, 1986). It was also observed that 14-15 g L^{-1} butanol completely inhibited growth and maintenance of the pH gradient (Bowles and Ellefson, 1985; Ounine *et al*. (1985). This inhibitory action explains the limitation of the production of solvents by *C. saccharoperbutylacetonicum* N1-4 beyond 15 g L^{-1} concentration of butanol.

CONCLUSIONS

Clostridium saccharoperbutylacetonicum N1-4 showed the ability to utilize various carbohydrates including glucose, starch, xylose, xylan and CMC for the production of ABE. With partial utilization of xylose, xylan and CMC, P2 medium produced higher butanol concentration compared to TYA medium regardless of the carbon source. The critical concentration of butanol achievable is 15 g L^{-1}.

ACKNOWLEDGMENT

We would like to thank Prof. Dr Yoshino Sadazo, Kyushu University, Japan, who provided us with *C. saccharoperbutylacetonicum* N1-4. This research was supported by the UKM-GUP-KPB-08-32-128 grant.

REFERENCES

Al-Shorgani, N.K.N., E. Ali, M.S. Kalil and W.M.W. Yusoff, 2011. Bioconversion of butyric acid to butanol by *Clostridium saccharoperbutylacetonicum* N1-4 (ATCC 13564) in a limited nutrient medium. BioEnergy Res., 10.1007/s12155-011-9126-6 http:// www.springerlink.com/content/ 3061354753051528/

Atsumi, S., T. Hanai and J.C. Liao, 2008. Non-fermentative pathways for synthesis of branched-chain higher alcohols as biofuels. Nature, 451: 86-89.

Bahl, H. and G. Gottschalk, 1984. Parameters affecting solvent production by *Clostridium acetobutylicum* in continuous culture. Biotechnol. Bioeng. Symp., 14: 215-223.

Bahl, H., W. Andersch, K. Braun and G. Gottschalk, 1982. Effect of pH and butyrate concentration on the production of acetone and butanol by *Clostridium acetobutylicum* grown in continuous culture. Applied Microbiol. Biotechnol., 14: 17-20.

Bowles, L.K. and W.L. Ellefson, 1985. Effects of butanol on *Clostridium acetobutylicum*. Applied Environ. Microbiol., 50: 1165-1170.

Durre, P., 2007. Biobutanol: An attractive biofuel. Biotechnol. J., 2: 1525-1534.

Ennis, B.M. and I.S. Maddox, 1985. Use of *Clostridium acetobutylicum* P262 for production of solvents from whey permeate. Biotechnol. Lett., 7: 601-606.

Fond, O., G. Matta-Ammouri, H. Petitdemange and J.M. Engasser, 1985. The role of acids on the production of acetone and butanol by *Clostridium acetobutylicum*. Applied Microbiol. Biotechnol., 22: 195-200.

Haggstrom, L., 1985. Acetone-butanol fermentation and its variants. Biotechnol. Adv., 3: 13-28.

Hipolito, C.N., E. Crabbe, C.M. Badillo, O.C. Zarrabal and M.A.M. Mora *et al*., 2008. Bioconversion of industrial wastewater from palm oil processing to butanol by *Clostridium saccharoperbutylacetonicum* N1-4 (ATCC 13564). J. Cleaner Prod., 16: 632-638.

Jojima, T., M. Inui and H. Yukawa, 2008. Production of isopropanol by metabolically engineered *Escherichia coli*. Applied Microbiol. Biotechnol., 77: 1219-1224.

Jones, D.T. and D.R. Woods, 1986. Acetone-butanol fermentation revisited. Microbiol. Mol. Biol. Rev., 50: 484-524.

Kalil, M.S., P.W. Kit, W.M.W. Yusoff, Y. Sadazo and R.A. Rahman, 2003. Direct fermentation of palm oil mill effluent to acetone-butanol-ethanol by solvent producing clostridia. Pak. J. Biol. Sci., 6: 1273-1275.

Kumar, N. and D. Das, 2000. Enhancement of hydrogen production by *Enterobacter cloaca* IIT-BT 08. Process Biochem., 35: 589-593.

Lee, S.Y., J.H. Park, S.H. Jang, L.K. Nielsen, J. Kim and K.S. Jung, 2008. Fermentative butanol production by clostridia. Biotechnol. Bioeng., 101: 209-228.

Long, S., D.T. Jones and D.R. Woods, 1984. Initiation of solvent production, clostridial stage and endospore formation in *Clostridium acetobutylicum* P262. Applied Microbiol. Biotechnol., 20: 256-261.

Madihah, M.S., A.B. Ariff, K.M. Sahaid, A.A. Suraini and M.I.A. Karim, 2001. Direct fermentation of gelatinized sago starch to acetone-butanol-ethanol by *Clostridium acetobutylicum*. World J. Microbiol. Biotechnol., 17: 567-576.

Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem., 31: 426-428.

Mun, L.T., A. Ishizaki, S. Yoshino and K. Furukawa, 1995. Production of acetone, butanol and ethanol from palm oil waste by *Clostridium saccharoperbutylacetonicum* N1-4. Biotechnol. Lett., 17: 649-654.

Ounine, K., H. Petitdemange, G. Raval and R. Gay, 1985. Regulation and butanol inhibition of D-xylose and D-glucose uptake in *Clostridium acetobutylicum*. Appl. Environ. Microbiol., 49: 874-878.

Petitdemange, E., F. Caillet, J. Giallo and C. Gaudin, 1984. *Clostridium cellulolyticum* sp. nov. a cellulolytic, mesophilic: Species from decayed grass. Int. J. Syst. Bacteriol., 34: 155-159.

Qureshi, N. and H.P. Blaschek, 2001. ABE production from corn: A recent economic evaluation. J. Ind. Microbiol. Biotechnol., 27: 292-297.

Qureshi, N., A. Lolas and H.P. Blaschek, 2001. Soy molasses as fermentation substrate for production of butanol using *Clostridium beijerinckii* BA 101. J. Ind. Microbiol. Biotechnol., 26: 290-295.

Shamsudin, S., M.S. Hj Kalil and W.M. Wan Yusoff, 2006. Production of Acetone, Butanol and Ethanol (ABE) by *Clostridium saccharoperbutylacetonicum* N1-4 with different immobilization systems. Pak. J. Biol. Sci., 9: 1923-1928.

Soni, B.K., K. Das and T.K. Ghose, 1987. Inhibitory factors involved in acetone-butanol fermentation by *Clostridium saccharoperbutylacetonicum*. Curr. Microbiol., 16: 61-67.

Sullivan, K.H., G.D. Hegeman and E.H. Cordes, 1979. Alteration of the fatty acid composition of *Escherichia coli* by growth in the presence of normal alcohols. J. Bacteriol., 138: 133-138.

Syed, Q.A., M. Nadeem and R. Nelofer, 2008. Enhanced butanol production by mutant strains of *Clostridium acetobutylicum* in molasses medium. Turk J. Biochem., 33: 25-30.

Terracciano, J.S. and E.R. Kashket, 1986. Intracellular conditions required for initiation of solvent production by *Clostridium acetobutylicum*. Applied Environ. Microbiol., 52: 86-91.