STUDIES ON THE UTILIZATION OF PALM OIL WASTES AS THE SUBSTRATES FOR BUTANOL FERMENTATION

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Studies were made on the feasibility of using palm oil wastes as feedstocks for the production of butanol and as a means of controlling pollution.

Of the various palm oil wastes, clarification sludge was found to be the best substrate for the fermentation by Clostridium acetobutylicum, *but the fermentation produced only acidic products due to the low fermentable sugar content of the sludge.*

About 74 mM of butanol was produced along with other products, from the fermentation of clarification sludge supplemented with 20 g/l of starch.

INTRODUCTION

The production of crude palm oil involves an essentially mechanical extraction process in which the fresh fruit bunches undergo sterilization, digestion, and extraction of the oil, which is then clarified; the process generates about 5.0 m^3 of palm oil mill effluent (POME) with an average biochemical oxygen demand (BOD) of 25 000 mg/l for every tonne of palm oil produced. In Malaysia, the palm oil industry contributes 83% of the industrial organic pollution load and ranks as the single largest polluter; the situation is probably similar in other palm oil producing countries (Aziz and Lee, 1974; Wang *et al.*, 1981).

At present few technologies are available to solve the problem while also exploiting the potential resource. The wastes might be used as fermentation substrates: palm oil sludge contains mixed sugars, starch, hemicellulose and other carbohydrates. Mixed sugars are used in few fermentation processes, but acetone-butanol fermentation by *Clostridium acetobutylicum* is among them.

The acetone-butanol fermentation has a long history as a successful industrial process and is

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currently being studied as a potential method for converting biomass to liquid fuel. Butanol has many characteristics which make it a better liquid fuel extender than ethanol, now used in the formulation of 'gasohol'. These include its low vapour pressure and its low miscibility with water, and the fact that butanol, unlike ethanol, is completely miscible with diesel fuel even at low temperatures.

The organism *Clostridium acetobutylicum* ferments virtually all the natural carbohydrates except cellulose to produce organic solvents and acids including butanol, acetone, ethanol, and butyric and acetic acids, as well as carbon dioxide and hydrogen. Butanol, acetone and ethanol are normally the principal products.

Economic analysis of butanol fermentation has shown that more than 70% of the cost of producing butanol is the cost of substrate when starch is used (Volesky *et al.*, 1981). Consequently attempts have been made to utilize organic wastes as substrates, including apple pomace (Voget *et al.*, 1985) and whey (Maddox, 1980). Although the carbohydrate content is low, palm oil sludge is an attractive organic waste for butanol fermentation, because it is generated throughout the year in vast amounts.

A major characteristic of the fermentation of sugars by *C. acetobutylicum* is the metabolic transition from an acidogenic growth phase to a solvent-producing phase. *C. acetobutylicum* catabolizes glucose to pyruvate by the glycolytic pathway and the pyruvate is oxidized to acetyl-CoA with the reduction of ferredoxin. During the acidogenic phase the reducing equivalent is used to produce gaseous hydrogen, and acetyl-CoA is converted to acetic and butyric acids, but in the solvent-producing phase acetyl-CoA is used as the electron acceptor, resulting in the formation of neutral organic solvents. The electron metabolism can be manipulated to increase the solvent yield with a decrease in the acid yield. The techniques include hydrogenase inhibition (Kim *et al.*, 1984), an electrochemical method (Kim and Kim, 1988), the use of artificial electron carriers such as viologens (Rao and Mutharasan, 1986) and increased hydrogen partial pressure (Yerushalmi *et al.*, 1985).

EXPERIMENTALS

Bacterial Strains and Culture Conditions

Clostridium acetobutylicum KCTC 1037 (ATCC 4259) was selected from over 100 butanol-producing strains maintained at the Korean Collection for Type Cultures (KCTC) and used throughout the study. A complex medium (CAB) was used, with the addition of 45 g/l glucose or sludge at the concentration indicated in the text. The CAB medium contained (in grams per litre of distilled water): yeast extract (Difco Laboratory Detroit) 4; tryptone (Difco) 1; $K_2HPO_4.3H_2O$ 1.5; $MgSO_4.7H_2O$ 0.1; $MnSO_4.7H_2O$ 0.1; $FeSO_4.7H_2O$ 0.015; and NaCl 0.1. One ml of 0.2% resazurin was added per litre of medium. The medium was prepared under nitrogen headspace and the pH was adjusted as indicated in the text before autoclaving.

A few grains of spore soil stock or 0.1 ml of spore suspension were inoculated into 10 ml of CAB medium (45 g/l glucose) in an anaerobic pressure tube (Bellco Glass, Inc. Vineland, N.J.) to develop inocula. The tubes were sealed with butyl rubber bungs and aluminum crimps before being heated at 85°C for five minutes. The heat-treated tubes were cultured at 35°C for 24 hours in a water bath, and their contents used for inocula.

Fresh inocula were made for each experiment. Soil cultures were prepared by the method of Walton and Martin (1979) and the spore suspension as described earlier (Kim *et al.*, 1984).

Substrates

The substrates used were supplied by the Palm Oil Research Institute of Malaysia (PORIM). They were:

1) Palm oil dried sludge (POS) and palm oil kernel meal (PKM), supplied as solids and ground to 10 mesh using a laboratory cutting mill before use.
2) Dewatered sludge, which was autoclaved at 121°C for 20 min and stored at 4°C until used.
3) Liquid palm oil mill effluent (POME), sterilizer condensate and clarification sludge, obtained in sterile cans.

Analytical Methods

Ethanol, acetone, acetic acid, butyric acid and butanol concentrations were analysed using a Varian 3300 gas chromatograph equipped with a flame ionization detector. One *ul* of each acidified sample was injected into a 6 ft x 1/8 inch glass column packed with Chromosorb

TABLE 1.
FERMENTATION PROFILES OF *C. ACETOBUTYLICUM* STRAINS FROM THE KOREAN COLLECTION FOR TYPE CULTURES (KCTC)

Substrates	KCTC Strains	Products (mM)					
		Acetate	Butyrate	Ethanol	Acetone	Butanol	Total (as glucose)
Kernel meal (PKM)	1037	30.0	29.0	2.4	17.8	7.1	70.1
	1669	18.3	24.0	1.8	6.9	14.1	55.1
	1724	16.3	41.0	2.1	4.4	4.8	59.5
	1788	23.8	22.1	0.5	3.1	3.2	40.6
Sludge (POS)	1037	50.9	51.0	8.5	30.0	5.1	115.9
	1669	31.3	25.0	5.3	8.7	6.8	58.9
	1724	19.6	30.0	4.6	4.4	5.1	51.6
	1788	43.3	30.0	1.4	10.5	4.1	67.0

WAW or Porapak Q. The temperatures of the columns were 120°C and 180°C, respectively. The injector and detector temperatures were both 210°C. The nitrogen carrier gas flow rate was 30 ml/minute.

Dilute acid hydrolysable polysaccharides were determined as reducing sugar after hydrolysis by 1 N -HCl at 100°C for 3 hr, reducing sugar being measured by Somogyi-Nelson method (Nelson, 1944; Somogyi, 1952).

Total nitrogen of palm oil wastes was measured by the Kjeldahl method using a Tecator Cyclotec Sample Mill (Sweden), and crude protein content was calculated by multiplying by 6.25.

RESULTS AND DISCUSSION

Strain Selection

Butanol-producing strains of *Clostridium* were inoculated to CAB-medium containing 180 g/l of POS or PKM as carbon source. After incubating at 35°C for a week the soluble fermentation products were analysed. *Table* 1 shows the results of fermentation using some of the more productive strains. As can be seen, KCTC 1037 gave the largest amount of fermentation products from POS and PKM, and POS was a better substrate than PKM for the butanol fermentation with this strain. This did not seem consistent with the carbohydrate analyses (*Table 4*), but a repeated fermentation showed similar results. It was decided to use POS as the substrate for further studies since it had given better fermentation results than PKM, which, in any case, has other uses and is more valuable.

A similar experiment was conducted using clarification sludge to which was added 2% glucose. High butanol producing strains were used in the fermentation and the results were compared with those obtained with *C. acetobutylicum* KCTC 1037 (*Table 2*).

KCTC 2071 showed a better fermentation profile in terms of butanol concentration and of total fermentation products than KCTC 1037, but in a repeated experiment the two strains showed similar results.

Fermentation of Palm Oil Sludges

A further fermentation experiment was done using CAB media to which had been added

TABLE 2.
COMPARISON OF FERMENTATION PRODUCTS USING CLARIFICATION SLUDGE MEDIUM WITH 2% GLUCOSE AND *C. ACETOBUTYLICUM* STRAINS FROM KCTC

KCTC strains	Products (mM)					
	Acetate	Butyrate	Ethanol	Acetone	Butanol	Total (as glucose)
1037	46.0	41.5	6.5	13.0	40.6	121.4
2063	40.3	47.7	8.6	13.3	39.2	124.7
2064	66.7	49.1	4.4	6.6	20.4	111.7
2068	72.9	45.0	4.6	5.7	25.9	115.4
2071	36.4	49.8	9.0	16.0	52.6	141.1
2086	39.6	58.2	7.3	9.9	24.4	116.0

Each strain was cultured in clarification sludge medium with 2% glucose, at 35°C for seven days. The initial pH was adjusted to 5.4.

TABLE 3.
FERMENTATION PRODUCTS FROM VARIOUS FORMS OF PALM OIL SLUDGE USING *C. ACETOBUTYLICUM* KCTC 1037

Substrates	Products (mM)					
	Acetate	Butyrate	Ethanol	Acetone	Butanol	Total (as glucose)
Glucose	13.3	1.5	13.8	39.1	88.2	142.4
Dry POS	45.0	36.2	1.4	10.7	4.4	74.5
Dewatered sludge	37.5	21.2	5.0	4.8	2.1	49.3
Palm Oil Mill Effluent (POME)	47.2	33.4	5.1	5.7	6.3	71.6
Clarification sludge	83.0	75.5	5.6	2.0	32.1	153.9

The concentrations of dry POS and dewatered sludge in the medium were 240 g/l and 720 g/l respectively. POME and clarification sludge were used without dilution.

TABLE 4.
THE COMPOSITION OF PALM OIL WASTES

(% by weight on a dry basis)

Constituents	Palm Oil Sludge	Palm Kernel Meal	Clarification Sludge
Polysaccharide hydrolysable by dilute HCl	10.2	29.8	17.5
Reducing sugar	0.2	0.7	ND[a)]
Crude protein	14.7	13.7	12.1
Ash	18.5	3.7	20.2
Moisture	5.0	4.7	95.5
N-free extractables	ND	43.0	25.5

ND; Not Determined

ND[a)]; The colour of the clarification sludge was too dark to use in the colourimetric method of the reducing sugar determination.

glucose, dry POS, dewatered sludge, POME or clarification sludge. As shown in *Table 3,* clarification sludge gave about 153 mM soluble fermentation products, a better result than with dry POS, dewatered sludge or POME.

The fermentation gave mainly acidic products with a low yield of solvent. Poor solventogenesis has been reported in fermentations with slow growth rate in a carbon-limited chemostat (Gottschalk and Bahl, 1981), a co-culture system (Petitdemange *et al.,* 1983) and other systems (Meyer *et al.,* 1986). The poor solventogenesis might, therefore, be due to the slow growth of bacterium on the waste.

Various factors associated with the waste could cause inhibition of the growth of the bacterium, including the nature and concentration of the fermentable sugar and unfavourable pH changes during fermentation.

Analyses of Palm Oil Residues

The general composition of palm oil wastes were determined analytically (*Table 4*). All the wastes analysed contain more than 12% of crude protein on a dry basis. As expected, it was difficult to determine the exact concentration of the fermentable sugar by a chemical method due to the nature of the wastes. PKM was found to have the highest sugar content by the chemical method, but PKM gave less fermentation products than POS (*Table 1*). A similar irregularity was observed with clarification sludge. The chemical analysis showed that it contained 25.5% N-free extractables on a dry basis (*Table 4*). This figure is equivalent to about 11.5 g of sugar per litre of the clarification sludge or to about 64 mM of glucose equivalent, but the fermentation of the clarification sludge gave over 150 mM of products (*Table 3*). These irregularities show that actual fermentation is better than the chemical method for determining the fermentable sugar content of the wastes. Reducing sugar content of the clarification sludge was not determined due to the technical difficulties associated with the dark nature of the waste. Differences in the fermentation results were also observed among different shipments of the clarification sludge (*Table 5*).

Fermentation of Clarification Sludge Supplemented with Glucose

In order to see if the poor solventogenesis from the wastes was due to the carbon limita-

TABLE 5.
FERMENTATION OF CLARIFICATION SLUDGE OF DIFFERENT SHIPMENT BY *C. ACETOBUTYLICUM* KCTC 1037

Substrates	Products (mM)						
	Acetate	Butyrate	Ethanol	Acetone	Butanol	Total (as glucose)	pH
1	71.3	76.7	7.2	20.4	38.8	175.2	4.67
2	71.3	59.4	3.9	14.3	15.6	127.0	4.53

1 ; Sludge shipped in 1988, 2; Sludge shipped in 1989

Sludge medium; Clarification sludge + Starch 20 g/l + Yeast extract 4 g/l + Tryptone 1 g/l + K_2HPO_4. $3H_2O$ 1.5 g/l + Salt solution 1 ml/l;

The initial pH was adjusted to 5.0.

TABLE 6.
ACETONE-BUTANOL FERMENTATION OF CLARIFICATION SLUDGE WITH ADDED GLUCOSE

Added glucose (%)	Products (mM)					
	Acetate	Butyrate	Ethanol	Acetone	Butanol	Total (as glucose)
0.0	83.0	75.5	5.5	2.0	32.1	153.9
0.5	97.1	85.2	5.6	5.3	33.9	175.8
1.0	105.1	95.2	7.4	14.3	50.7	216.6
2.0	92.2	84.6	7.3	17.7	58.7	210.8
3.0	90.0	88.8	7.0	16.3	55.1	208.7
4.0	87.9	84.6	6.3	12.1	48.5	192.4
5.0	87.9	85.8	5.0	12.5	42.7	187.5

TABLE 7.
BUTANOL FERMENTATION OF CLARIFICATION SLUDGE WITH CAB INGREDIENTS OR STARCH

Medium	Initial pH	Products (mM)						Final pH
		Acetate	Butyrate	Ethanol	Acetone	Butanol	Total (as glucose)	
1		30.8	3.3	5.3	6.7	3.8	31.9	4.33
2	4.0	35.1	1.8	6.7	6.2	4.3	33.3	4.27
3		38.9	4.1	1.0	5.6	4.5	34.2	4.27
1		26.1	20.4	7.6	1.4	20.4	59.1	5.26
2	4.5	31.5	29.5	6.7	3.2	17.5	69.4	4.95
3		58.5	40.8	7.6	12.1	51.9	138.0	4.76
1		29.4	28.1	6.6	9.2	20.8	76.1	5.44
2	5.0	35.5	48.8	10.9	15.4	56.3	143.8	4.86
3		60.7	44.6	10.6	13.3	52.6	146.2	4.87

1: Sludge + CAB ingredients (without glucose)
2: Sludge + 3% starch
3: 1 + 3% starch

tion, clarification sludge supplemented with increasing amounts of glucose was fermented and the products were analysed (*Table 6*). The highest yields of products were produced from the clarification sludge supplemented with 1% – 3% glucose. The addition of more than 4% of glucose resulted in less fermentation products than were obtained with 1% - 3% glucose. This suggests that with more than 4% glucose the osmotic pressure is raised to a level inhibitory to the organism. The culture without added glucose gave about 150 mM of total products, equivalent to 27 g/l hexose.

According to the literatures the maximum yield of butanol obtainable is about 120 mM with 60 mM acetone, 20 mM ethanol, and 30 mM acetate and 10 mM butyrate (Prescott and Dunn, 1959). About 220 mM of glucose or 40 g/l would need to be fermented to give these products. Because the clarification sludge contains about 27 g/l of fermentable sugars, enough sugar was present in the cultures supplemented with glucose. But acetate and butyrate were the main products in the fermentation with 2% added glucose.

The final pH of the culture was above 5.0, which is much higher than that of cultures made on CAB with glucose. The higher final pH in sludge fermentation suggests that the sludge has a high buffering capacity (*i.e. Table 8*).

Fermentation of Clarification Sludge without an Added Nitrogen Source

Clarification sludge was found to contain about 12% protein on a dry basis (*Table 4*), sufficient to support a successful butanol fermentation. In order to determine experimentally whether this protein could support the fermentation, media were made at different pH containing: 1) clarification sludge supplemented with CAB components, 2) clarification sludge with 3% starch, and 3) clarification sludge supplemented with CAB components and 3% starch. The results are shown in *Table*

TABLE 8.
BUTANOL FERMENTATION OF CLARIFICATION SLUDGE WITH ADDED CORN STEEP LIQUOR

	CSL concentration (%)	Products (mM)						Final pH
		Acetate	Butyrate	Ethanol	Acetone	Butanol	Total (as glucose)	
Clarification Sludge[a)]	1.0	53.1	49.6	4.0	11.6	28.4	118.2	4.37
	2.0	54.2	51.1	4.9	12.7	30.3	123.7	4.54
	3.0	59.0	50.1	6.1	16.4	37.6	136.7	4.69
	6.0	67.7	61.0	13.8	20.1	74.1	196.0	5.07
CAB[b)]	-	37.9	9.1	10.9	24.4	64.6	122.6	3.76
CAB[b)]	5.0	53.7	26.2	24.8	34.9	110.0	210.4	5.26

a) Sludge medium ; Clarification sludge + Starch 20 g/l + $K_2HPO_4.3H_2O$ 1 g/l + mineral salt solution 1 ml/l ;
The initial pH was adjusted to 5.0 and CSL added to the concentration shown.

b) CAB with glucose 45 g/l; The initial pH was adjusted to 5.4; in the second case 5.0% CSL was added.

7. With an initial pH of 5.0, there was little difference in the total products formed and in the ratio of the products between cultures on sludge supplemented with 3% starch, with or without the addition of yeast extract and tryptone. However with an initial pH of 4.5, yeast extract and tryptone stimulated the fermentation. These results indicate that the crude protein contained in the sludge can be utilized by *C. acetobutylicum* in the proper conditions. Within the pH range tested, higher pH gave better fermentation results, suggesting that acidic products at high concentration are toxic, even though the organism has an optimum initial pH of 4.5 and the ability to convert the acidic products to solvents (Shin and Kim, 1986).

Fermentation of Clarification Sludge with Added Corn Steep Liquor (CSL)

The main products of sludge fermentation with added sugar are acetate and butyrate, and as fermentation is not complete, a high level of residual sugars is left as calculated from the substrate used and the total fermentation products formed. The literature showed that corn steep liquor has been used for industrial butanol fermentation, and that fermentations were complete without residual sugar (Prescott and Dunn, 1959).

Fermentations were carried out with the sludge media with varying concentrations of CSL, using glucose media as the controls (*Table 8*). The culture with added starch and 6.0% CSL gave the highest yield of fermentation products: 196 mM with 74.1 mM butanol, and the final pH slightly increased from 5.0 to 5.07, whilst the pH of other cultures decreased.

These results indicate that complete sugar utilization was realized when CSL was added to the glucose medium, and that the inclusion of CSL in the sludge medium resulted in better solventogenesis. Even in the sludge fermentation with CSL the final acid concentration was too high. However, a butanol yield over 100 mM could be obtained with careful pH control during the fermentation.

Use of Electron Flow Modulators

There have been a few claims that solventogenesis can be enhanced by the alteration of electron flow during fermentation by *C. acetobutylicum*, as mentioned in the Introduction.

Electron flow modulators were tested to see whether the concentration of acidic products could be reduced during sludge fermentation (*Table 9*). Even though the addition of butyrate or methyl viologen increased the butanol yield, little change was observed in the acid

TABLE 9.
FERMENTATION OF CLARIFICATION SLUDGE BY *C. ACETOBUTYLICUM* KCTC 1037 WITH ELECTRON FLOW MODULATORS

Electron flow modulators		Products (mM)						Final pH
		Acetate	Butyrate	Ethanol	Acetone	Butanol	Total (as glucose)	
–		68.1	41.4	5.3	5.9	14.2	98.3	4.6
Acetate	15mM	58.4	45.4	5.0	5.6	11.2	93.9	4.6
Acetate	30mM	59.3	51.3	5.1	7.0	12.1	102.7	4.6
Butyrate	15mM	83.0	45.0	6.1	6.2	20.9	116.7	4.8
Butyrate	30mM	86.2	35.9	5.3	6.0	21.4	109.1	4.9
Butyrate	60mM	65.0	33.8	6.2	6.8	30.4	106.6	5.1
Methyl viologen	2mM	40.7	21.2	11.2	7.2	39.0	93.4	5.1
Carbon monoxide[a)]	5%	77.3	43.2	6.3	6.7	16.4	108.2	4.7
Neutral red	5mM	50.8	35.7	8.2	7.1	36.9	109.2	4.9

The added acids were subtracted from the measurements.
a) Carbon monoxide was added to the head space of the culture.

concentrations. This seems to have been due to the slow growth rate of the bacterium on the sludge.

BOD Balance in the Butanol Fermentation of POS with Added Starch

Clarification sludge is a waste which needs to be treated to control pollution. The addition of starch to the waste cannot be justified unless a net BOD removal is achieved by the fermentation. The BOD balance was calculated for the butanol fermentation of clarification sludge supplemented with 25 g/l of starch, assuming that a successful fermentation would convert about 80% of sugar to recoverable solvents (*Table 10*).

As shown in the table, from the initial BOD of about 50 000 ppm the amounts of BOD removed as hydrogen and solvents are 5600 ppm and 37 300 ppm, respectively, leaving distillery waste with a BOD value of about 7000 ppm.

If the solid from the distillery residue were removed and used as an animal supplement a further reduction of BOD could be achieved.

Economic Analysis of Clarification Sludge Fermentation

The economic feasibility of solvent production by fermentation is limited by the high cost of starch and sugar substrates (Lenz and Moreira, 1980). Without any doubt, the production cost would be reduced considerably by

TABLE 10.
BOD BALANCE IN THE BUTANOL FERMENTATION OF POS WITH STARCH

BOD in Substrate:

	ppm
POS (25 g/l sugar as glucose)	26 700
Starch (22 g/l glucose)	23 430
Total	50 130

BOD in Products

Product Yield	(M/M glucose)[a]	BOD
Butanol	0.56	28 100
Acetone	0.22	7 400
Ethanol	0.07	1 800
H_2	1.35	5 600
Total		42 900

BOD in Distillery Residue:
50 130 − 42 900 = 7230 ppm

a) Product yield was taken from Prescott and Dunn (1959) because the amount of hydrogen production was not determined in this study.

using a waste with negative value as the substrate.

A preliminary cost analysis of butanol fermentation with clarification sludge was made based on the calculations by Volesky *et al.* (1981), and with the following assumptions:

1. Clarification sludge contains 25 g/l fermentable carbohydrate and requires a supplement of 20 g/l carbohydrate to produce 15 g/l butanol.
2. Clarification sludge is supplied without charge.
3. The plant is constructed in a palm oil mill to avoid transportation of sludge.

Table 11 shows the cost analysis.

The total cost of production of butanol is about US$2.07/gallon or $0.31/lb. At present the price of butanol is $0.34/lb. This preliminary analysis shows that butanol production from clarification sludge is economically feasible when the assumptions mentioned above are satisfied.

CONCLUSIONS

Fermentation of various palm oil wastes has shown that the clarification sludge is the best substrate among those tested for butanol production.

The following observations were made:

1. *C. acetobutylicum* KCTC 1037 was selected from among KCTC strains for its high productivity and used in the fermentation of the clarification sludge.
2. Clarification sludge contains about 14.4-27.7 g/l of fermentable carbohydrate depending on the origin. About 20 g/l of carbohydrate should be added to make up a suitable medium for butanol fermenta-

TABLE 11.
BUTANOL PRODUCTION COST ANALYSIS

(US cents/gallon)

Item	POS-Starch	Starch
Labour		
operating ($10.00/hr)	2.45	2.45
maintenance	1.43	1.43
control laboratory	1.23	1.23
total labour	5.11	5.11
Material		
starch (4.2 kg/gal, at 28¢/kg)	117.60	265.25
minerals	1.50	1.50
maintenance	1.43	1.43
operating	0.25	0.25
total material	120.78	268.43
Utilities		
cooling water (3¢/1000 gal)	0.39	0.39
steam ($ 4/1000 lb)	50.19	50.19
process water (60¢/1000 gal)	0.47	0.47
electricity (3¢/kWh)	2.88	2.88
total utilities	53.93	53.93
Depreciation (10%/yr.)	7.90	7.90
Other indirect operating costs	19.16	19.16
Total production cost	206.88	354.53

tion.

3. The protein present in the sludge is less efficient than tryptone to support the satisfactory fermentation by *C. acetobutylicum*.
4. The clarification sludge fermentation is slower than those on glucose or starch, and results in mainly acidic products.
5. Clarification sludge contains a high concentration of solutes including inorganic compounds which contributes to the osmotic pressure in the medium which possibly explains the inhibition of the fermentation when more than 4% glucose is added.
6. Clarification sludge appears to have a high pH buffering capacity.

7. Corn steep liquor (CSL) could replace yeast extract and tryptone for the butanol fermentation.
8. A tentative cost analysis showed that butanol can be produced from the clarification sludge with 20 g/l of added starch. The fermentation reduces the BOD from 25 g/l to seven grams per litre.

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