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Variations of Solvent Yield in Acetone-Butanol Fermentation

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Summary. Calculation of a "theoretical yield" of microbial process is often a subject of controversy. Theoretical yield values of the solvents (butanol-acetone-ethanol) produced in the cultivation of *Clostridium acetobutylicum* on glucose have been calculated for 30 different culture conditions. Two different approaches were taken based on expressing the stoichiometric relationship between the substrate and the products of the process. The maximum theoretical yield under acceptable conditions was established ranging from 38.6% to 39.9%. It was considered for an ideal biosynthetic situation when no intermediate acids were left over in the system and no carbon was utilized in the production of biomass. The values of the solvent yield are dependent on the ratio between the solvent products. The coefficients of the process stoichiometric relationship and the ratios between hydrogen gas and butanol are presented for each set of process conditions. A three-dimensional plot of the yield versus the weight fractions of butanol and ethanol in the system has been developed reflecting the continuous variations of this parameter with the solvent ratio.

Introduction

Production of butanol and acetone by fermentation processes is one of the oldest industrial fermentations. A strain of the anaerobic bacterium *Clostridium acetobutylicum* is usually used in the production of the solvents. Various sugars of the mono-, di- and polysaccharide groups can serve as the carbon and energy source. The mechanism of glucose utilization by *C. acetobutylicum* and the biological reactions involved in the production of the solvents and acids have been extensively studied and frequently reported (Prescott and Dunn 1959; Doelle 1975). Butanol and acetone are the major products of the fermentative process. The other common products are ethanol, acetic acid, butyric acid, carbon dioxide, and hydrogen gas.

An economic analysis of the process shows that more than 60% of the total production cost is in the raw material (Lenz and Moreira 1980). The substrate to solvents conversion yield is therefore the most important process parameter. Butanol and acetone are biochemically synthesized from glucose with a yield of approximately 30% based on weight. This value has been reported to be very close to the maximum achievable theoretical yield (Leung and Wang 1981).

In order to evaluate the efficiency of the biological system, it is essential to establish the theoretical yield value for the total neutral volatile products (TNVP).

A linear stoichiometric relationship has been developed and is presented in this communication between the substrate and the products at each biosynthesis condition considered in the system. The value of the TNVP yield is calculated at 30 different conditions by making certain assumptions based on experimental findings.

Process Analysis

Calculation of the theoretical yield is based on the information obtained from the fermentation experiments with *C. acetobutylicum* on glucose in a batch culture. For a glucose fermentation the experimental division of the substrate carbon and energy and the available electrons among the fermentation products are presented in Table 1. Almost 99% of the glucose carbon was recovered in the products and the energy and electron balances closed at the total of 102%. The heat of fermentation was neglected since it

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Table 1. Experimental batch acetone-butanol fermentation. Division of the glucose carbon, energy, and electrons among the fermentation products

Fermentation product	Recovered in the product		
	% total carbon	% total energy	% total available
Butanol	34.0	48.7	51.0
Acetone	15.4	19.6	21.3
Ethanol	2.6	3.9	4.1
Acetic acid	4.5	4.3	4.6
Butyric acid	0.5	0.6	0.6
Carbon dioxide	33.1	–	–
Hydrogen	–	14.9	12.0
Biomass	8.4	10.2	8.9
Total	98.5	102.2	102.5

accounted for less than 5% of the total energy input. The electron balance was attempted by using the concept of the reductance degrees for the reactants and the products developed by Erickson (1979) and applied by Yerushalmi and Volesky (1981). Butanol and acetone accounted for 49.4% of the glucose carbon, 68.3% of its energy and 72.3% of the available electrons in the system. Considering ethanol, the solvents accounted for almost 52% of the glucose carbon, 72.2% of its energy, and 76.4% of the available electrons.

By incorporating this information, a stoichiometric equation can be written to relate the total glucose consumed to the total solvents, acids and the gases produced.

Based on one mole of glucose consumed, the equation would be:

$$\overset{\text{Glucose}}{C_6H_{12}O_6} \rightarrow 0.51\ \overset{\text{Butanol}}{C_4H_9OH} + 0.31\ \overset{\text{Acetone}}{CH_3\text{-}CO\text{-}CH_3}$$

$$+\ 0.08\ \overset{\text{Ethanol}}{C_2H_5OH} + 0.12\ \overset{\text{Acetic acid}}{CH_3\text{-}COOH}$$

$$+\ 0.007\ \overset{\text{Butyric acid}}{C_3H_7\text{-}COOH} + 1.98\ CO_2 + 1.43\ H_2$$

$$+\ (\text{C, H, O incorporated in the cell structure}). \quad (1)$$

An average of almost 9% of glucose carbon was incorporated into the cell structure. The ratios between butanol, acetone and ethanol were 64 : 30 : 6. TNVP actual yield was 33% (by weight) and total hydrogen to total butanol gave a ratio of 2.8 mole H_2/mole Butanol.

Analytical Methods

Two basically different approaches were taken in expressing the process stoichiometry for the calculation of the theoretical yield.

1) The first approach towards estimation of the theoretical TNVP yield was made by using the stoichiometric relationships expressing the formation of a definite product from glucose (Johnson et al. 1931). The set of equations was as follows:

$$C_6H_{12}O_6 \rightarrow C_4H_9OH + 2\ CO_2 + H_2O \quad (2)$$

$$C_6H_{12}O_6 + H_2O \rightarrow CH_3\text{-}CO\text{-}CH_3 + 3\ CO_2 + 4\ H_2. \quad (3)$$

$$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2 \quad (4)$$

$$C_6H_{12}O_6 + 2\ H_2O \rightarrow 2\ CH_3\text{-}COOH + 2\ CO_2 + 4\ H_2. \quad (5)$$

$$C_6H_{12}O_6 \rightarrow C_3H_7\text{-}COOH + 2\ CO_2 + 2\ H_2. \quad (6)$$

These equations however, do not reflect the fermentation process reality well; the underlying metabolic pathway is branched rather than linear and fermentation of the end products follows a dynamic equilibrium rather than a numerical relationship. This set of equations, nevertheless, gives quantitative relationships between the glucose consumed, gases evolved and solvents and acids produced.

In Approach I three different biosynthesis conditions were examined and considered in deriving more meaningful quantitative stoichiometric relationships:

A. Butanol and acetone are the only final products in the liquid phase (i.e., no biomass, ethanol or acids are present).

B. Butanol, acetone and ethanol are the only final products in the liquid phase (i.e., no biomass or acids are present).

C. Butanol, acetone, ethanol, acetic acid, and butyric acid are the final products in the liquid phase (i.e., no biomass is present).

The ensuing respective calculations were always based on one mole of glucose consumed and known ratios of butanol : acetone : ethanol in the system.

2) In the second approach a single linear general equation was assumed to relate the consumption of glucose to the formation of all the products as follows:

$$C_6H_{12}O_6 \rightarrow b\ C_4H_9OH + c\ CH_3\text{-}CO\text{-}CH_3 + d\ C_2H_5OH + e\ CH_3\text{-}COOH + f\ C_3H_7\text{-}COOH + g\ CO_2 + h\ H_2 + z\ H_2O \quad (7)$$

Biomass can also be considered to be stoichiometrically related to the substrate conversion with ammonia providing the nitrogen requirements of the cell. A simple molecular formula of $C_4H_8O_2N$ calculated by Mayberry et al. (1968) for a wide range of bacteria has been used in the present study to represent the biomass composition. Upon inclusion of biomass, Eq. (7) will then change to the following form:

$$C_6H_{12}O_6 + x\ NH_3 \rightarrow b\ C_4H_9OH + c\ CH_3\text{-}CO\text{-}CH_3 + d\ C_2H_5OH + e\ CH_3\text{-}COOH + f\ C_3H_7\text{-}COOH + g\ CO_2 + h\ H_2 + x\ C_4H_8O_2N + z\ H_2O. \quad (8)$$

Table 2 lists the unknown variables of Eq. (8).

Three independent equations can be written reflecting balances on carbon, energy, and oxygen for the calculation of the unknowns in Eq. (8).

$$\Sigma C = 0 \Rightarrow 4b + 3c + 2d + 2e + 4f + g + 4x = 6. \quad (9)$$

$$\Sigma H = 0 \Rightarrow 10b + 6c + 6d + 4e + 8f + 2h + 5X + 2z = 12. \quad (10)$$

$$\Sigma O = 0 \Rightarrow b + c + d + 2e + 2f + 2g + 2X + z = 6. \quad (11)$$

Table 2. Unknown variables of Eq (8) (per mole of glucose consumed)

b = moles of butanol produced; c = moles of acetone produced; d = moles of ethanol produced; e = moles of acetic acid produced; f = moles of butyric acid produced; g = moles of carbon dioxide produced; h = moles of hydrogen produced; x = moles of biomass produced; z = moles of water produced

Table 3. Equations representing model 1, 2, and 3 for the solution of Eq (8); butanol : acetone : ethanol = 64 : 30 : 6

Eq. no.	Model 1	Model 2	Model 3
9	Σ carbon = 0	Σ carbon = 0	Σ carbon = 0
10	$\Sigma H = 0$	$\Sigma H = 0$	$\Sigma H = 0$
11	$\Sigma O = 0$	$\Sigma O = 0$	$\Sigma O = 0$
12	$b = 1.67c$	$b = 1.67c$	$b = 1.67c$
13	$b = 6.37d$	$b = 6.37d$	$b = 6.37d$
14	$b = 3.76e$	$b = 3.76e$	$b = 3.76e$
15	$b = 65.45f$	$b = 65.45f$	$b = 65.45f$
16	$b = 4x$	$b = 4x$	$b = 4x$
17	$b = 0.26g$	$g = 1.39h$	$g = 1.98$

Six more independent equations or known values are required in order to solve Eq. (8) for all the unknowns. Three different sets of equations representing three different models can be used. These equations are based on the information obtained from the batch experiments. Model 1 utilizes the real experimentally observed ratios between butanol and acetone, ethanol, acetic acid, butyric acid, biomass, and carbon dioxide respectively. Model 2 uses the ratio between carbon dioxide and hydrogen instead of the butanol/CO_2 ratio. The fraction of the glucose carbon incorporated in carbon dioxide was considered to be fixed in Model 3. The summary of the Models is presented in Table 3. A computer program utilizing a "Gauss-Jordan" method for solving a system of equations was used in computation of the unknown variables. The reason for choosing the fixed ratios between butanol and the other products as the basis for computations was the interrelated mechanism in the production of all the products in the process and the significance of butanol production in the metabolic pathways. Other equations could also be used in the computations such as the equations based on the ratios between acetone and the other compounds.

Two more biosynthesis conditions are considered in Approach II in addition to those used in Approach I. These conditions are as follows:

D. Butanol, acetone, ethanol, and biomass are the only products in the liquid phase (i.e., no acids are present).

E. Butanol, acetone, ethanol, acetic acid, butyric acid, and biomass are present in the liquid phase (i.e., a true system is considered).

Depending on the condition considered in the study, a corresponding number of equations were used. For instance, when butanol, acetone, and ethanol were the only products considered to be present in the system, Eq. (8) was reduced to the following form:

$$C_6H_{12}O_6 \rightarrow b\ C_4H_9OH + c\ CH_3\text{-}CO\text{-}CH_3 + d\ C_2H_5OH$$
$$+ g\ CO_2 + h\ H_2 + z\ H_2O$$

There are six unknowns, b, c, d, g, h, and z, in this equation. Therefore Eqs. (9), (10), (11), (12), (13), and (17) were to be solved for the unknown variables (see Tables 3 and 8).

In addition to the foregoing computations, a three-dimensional plot has been developed for each model to reflect the continuous variations of the TNVP yield in response to the variations in the ratios between the solvents. The plots were generated by using the Statistical Analysis System (SAS) facilities of the Computing Center of McGill University (Montreal).

Results

The results of the calculations based on Approach I are tabulated in Table 4. Since the production of biomass is not related to the glucose consumption by a separate equation, its formation is not included in the calculations.

Tables 5, 6, and 7 present the stoichiometric relationships, the theoretical TNVP yield and the mole H_2/mole Butanol ratio when Approach II and Models 1, 2, and 3 were used in the computations, respectively. When Eq. (8) was solved for Condition A, negative values for the gases or unrealistically low values for the solvents resulted. These data are not included here. The value of the TNVP yield changed from 31.9% when a fixed molar gas ratio and a true system was considered (Model 2, Condition E, Table 6), to 42.3% when Model 3 was used and no glucose was considered utilized in the production of acids or cells (Condition B, Table 7). The minimum hydrogen to butanol ratio (0.5 mole H_2/mole Butanol) was obtained when the model resulted in the highest value of the TNVP yield (Table 7). The maximum hydrogen to butanol molar ratio was 3.22 which occurred at the lowest value of the calculated TNVP yield (Model 2, Condition E, Table 6).

Tables 4–7 present the results of the computations related to the actual experimental observations of the process. The ratios between butanol, acetone, and ethanol could be different in the fermentations depending on the culture, fermentation medium and environmental conditions such as pH, temperature and redox potential. Another set of computations was completed whereby the ratio of 60 : 30 : 10 for butanol : acetone : ethanol was used. Table 8 presents the three sets of basic equations and Tables 9–12 present the outcome of the corresponding computations. Under the fixed-solvent-ratio assumption, the calculated TNVP yield ranged from 32.4% to 42.8% with corresponding values of the H_2/butanol ratio from 0.55 mole H_2/mole butanol to 3.38 mole H_2/mole butanol.

The results of the plotting of the TNVP yield versus the weight percents of butanol and ethanol are presented in Figs. 1 and 2. Figure 1 reflects the continuous variations in this parameter when no glucose carbon was incorporated into the acids or biomass (Condition B). Figure 2 has been developed by considering Condition D. These plots indicate that

Table 4. Stoichiometric coefficients, approach I, butanol: acetone: ethanol = 64: 30: 6. $C_6H_{12}O_6 + xNH_3 \rightarrow bC_4H_9OH + cCH_3 - CO - CH_3 + dC_2H_5OH + eCH_3 - COOH + fC_3H_9 - COOH + gCO_2 + hH_2 + x$ biomass $+ zH_2O$

Condition	Coefficient									TNVP yield %	mole H_2 / mole butanol
	b	c	d	e	f	g	h	x	z		
A	0.62	0.37	0	0	0	2.37	1.50	0	0.25	37.5	2.42
B	0.59	0.36	0.1	0	0	2.36	1.43	0	0.24	38.5	2.42
C	0.55	0.33	0.09	0.15	0.008	2.32	1.62	0	0.07	35.6	2.93

Table 5. Stoichiometric coefficients, Approach II, Model 1, butanol: acetone: ethanol = 64: 30: 6. $C_6H_{12}O_6 + xNH_3 \rightarrow bC_4H_9OH + cCH_3 - CO - CH_3 + dC_2H_5OH + eCH_3 - COOH + fC_3H_9 - COOH + gCO_2 + hH_2 + x$ biomass $+ zH_2O$

Condition	Coefficient									TNVP yield %	mole H_2 / mole butanol
	b	c	d	e	f	g	h	x	z		
B	0.60	0.36	0.09	0	0	2.33	1.36	0	0.28	38.6	2.27
C	0.57	0.34	0.09	0.15	0.009	2.2	1.27	0	0.28	36.5	2.33
D	0.54	0.33	0.08	0	0	2.12	1.17	0.14	0.53	35.0	2.15
E	0.52	0.31	0.08	0.14	0.008	2.01	1.09	0.13	0.52	33.5	2.1

Table 6. Stoichiometric coefficients, Approach II, Model 2, butanol: acetone: ethanol = 64: 30: 6. $C_6H_{12}O_6 + xNH_3 \rightarrow bC_4H_9OH + cCH_3 - CO - CH_3 + dC_2H_5OH + eCH_3 - COOH + fC_3H_7 - COOH + gCO_2 + hH_2 + x$ biomass $+ zH_2O$

Condition	Coefficient									TNVP yield %	mole H_2 / mole butanol
	b	c	d	e	f	g	h	x	z		
B	0.57	0.35	0.09	0	0	2.48	1.79	0	0.02	37.1	3.14
C	0.54	0.33	0.08	0.14	0.008	2.35	1.69	0	0.03	34.9	3.13
D	0.52	0.31	0.08	0	0	2.30	1.66	0.13	0.23	33.5	3.19
E	0.49	0.30	0.08	0.13	0.008	2.19	1.58	0.12	0.23	31.9	3.22

Table 7. Stoichiometric coefficients, Approach II, Model 3, butanol: acetone: ethanol = 64: 30: 6. $C_6H_{12}O_6 + xNH_3 \rightarrow bC_4H_9OH + cCH_3 - CO - CH_3 + dC_2H_5OH + eCH_3 - COOH + fC_3H_7COOH + gCO_2 + hH_2 + x$ biomass $+ zH_2O$

Condition	Coefficient									TNVP yield %	mole H_2 / mole butanol
	b	c	d	e	f	g	h	x	z		
B	0.66	0.39	0.10	0	0	1.98	0.33	0	0.88	42.3	0.5
C	0.6	0.36	0.09	0.16	0.009	1.98	0.64	0	0.65	38.6	1.07
D	0.56	0.34	0.09	0	0	1.98	0.77	0.14	0.76	36.3	1.37
E	0.52	0.31	0.08	0.14	0.008	1.98	1.0	0.13	0.57	33.5	1.92

Table 8. Equations representing models 1, 2, and 3 for the solution or Eq (8); butanol : acetone : ethanol = 60 : 30 : 10

Eq. no.	Model 1	Model 2	Model 3
9	Σ carbon = 0	Σ carbon = 0	Σ carbon = 0
10	Σ H = 0	Σ H = 0	Σ H = 0
11	Σ O = 0	Σ O = 0	Σ O = 0
12	b = 1.56c	b = 1.56c	b = 1.56c
13	b = 3.7d	b = 3.7d	b = 3.7d
14	b = 3.76e	b = 3.76e	b = 3.76e
15	b = 65.45f	b = 65.45f	b = 65.45f
16	b = 4x	b = 4x	b = 4x
17	b = 0.26g	g = 1.39h	g = 1.98

increase of the theoretical yield was always accompanied by an unfavourable decrease in the percentage of butanol and a simultaneous increase in the percentage of ethanol in the system. The maximum yield established from the plots occurred at the mutual ratio of solvents 45 : 35 : 20 (B : A : E).

Discussion

An economic evaluation of the butanol-acetone fermentation reveals that most of the production cost in an industrial process is related to the cost of the

Table 9. Stoichiometric coefficients, Approach I, butanol : acetone : ethanol = 60 : 30 : 10. $C_6H_{12}O_6 + xNH_3 \rightarrow bC_4H_9OH + cCH_3 - CO - CH_3 + dC_2H_5OH + eCH_3 - COOH + fC_3H_7 - COOH + gCO_2 + hH_2$ + x biomass + zH_2O

Condition	Coefficient									TNVP yield %	mole H_2 / mole butanol
	b	c	d	e	f	g	h	x	z		
A	0.61	0.39	0	0	0	2.39	1.56	0	0.22	37.7	2.56
B	0.56	0.36	0.15	0	0	2.37	1.45	0	0.2	38.5	2.56
C	0.52	0.33	0.14	0.14	0.008	2.33	1.63	0	0.05	35.6	3.13

Table 10. Stoichiometric coefficients, Approach II, Model 1, butanol : acetone : ethanol = 60 : 30 : 10. $C_6H_{12}O_6 + xNH_3 \rightarrow bC_4H_9OH + cCH_3 - CO - CH_3 + dC_2H_5OH + eCH_3 - COOH + fC_3H_7 - COOH + gCO_2 + hH_2$ + x biomass + zH_2O

Condition	Coefficient									TNVP yield %	mole H_2 / mole butanol
	b	c	d	e	f	g	h	x	z		
B	0.58	0.37	0.16	0	0	2.25	1.13	0	0.38	39.9	1.95
C	0.55	0.35	0.15	0.14	0.008	2.13	1.06	0	0.38	37.8	1.93
D	0.53	0.34	0.14	0	0	2.05	0.96	0.14	0.62	36.4	1.81
E	0.50	0.32	0.13	0.13	0.008	1.95	0.9	0.13	0.60	34.2	1.8

Table 11. Stoichiometric coefficients, Approach II, Model 2, butanol : acetone : ethanol = 60 : 30 : 10. $C_6H_{12}O_6 + xNH_3 \rightarrow bC_4H_9OH + cCH_3 - CO - CH_3 + dC_2H_5OH + eCH_3 - COOH + fC_3H_7 - COOH + gCO_2 + hH_2$ + x biomass + zH_2O

Condition	Coefficient									TNVP yield %	mole H_2 / mole butanol
	b	c	d	e	f	g	h	x	z		
B	0.54	0.35	0.15	0	0	2.48	1.79	0	0	37.4	3.31
C	0.52	0.33	0.14	0.14	0.008	2.36	1.70	0	0.005	35.6	3.27
D	0.49	0.32	0.13	0	0	2.30	1.66	0.13	0.20	33.8	3.38
E	0.47	0.30	0.13	0.12	0.007	2.20	1.59	0.12	0.20	32.4	3.36

Table 12. Stoichiometric coefficients, Approach II, Model 3, butanol: acetone: ethanol = 60: 30: 10. $C_6H_{12}O_6 + xNH_3 \rightarrow bC_4H_9OH + cCH_3 - CO - CH_3 + dC_2H_5OH + eCH_3 - COOH + fC_3H_9 - COOH + gCO_2 + hH_2 + x$ biomass $+ zH_2O$

Condition	Coefficient									TNVP yield %	mole H_2 / mole butanol
	b	c	d	e	f	g	h	x	z		
B	0.62	0.40	0.17	0	0	1.98	0.34	0	0.85	42.8	0.55
C	0.60	0.36	0.15	0.15	0.009	1.98	0.63	0	0.63	40.2	1.05
D	0.54	0.34	0.15	0	0	1.98	0.76	0.13	0.74	37.0	1.41
E	0.50	0.32	0.13	0.13	0.008	1.98	0.98	0.12	0.56	34.2	1.96

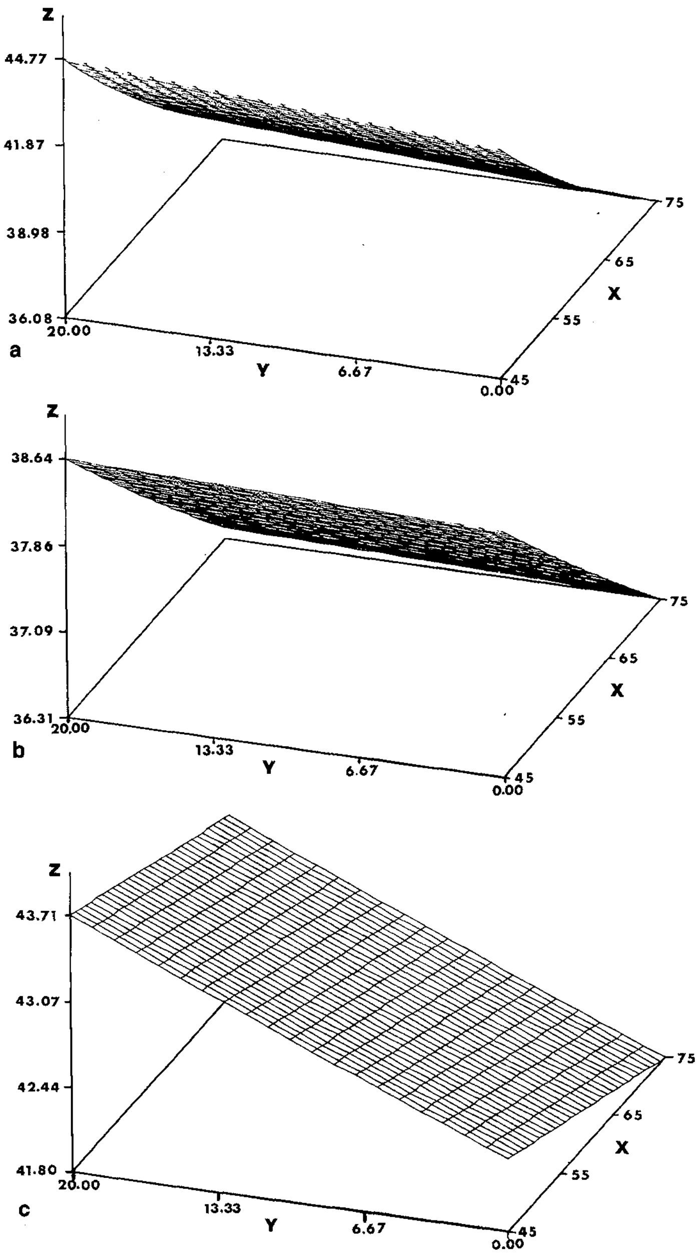

Fig. 1a–c. Continuous variations of the total solvent yield (z) with the weight fractions of butanol (x) and ethanol (y) in cultivation of *C. acetobutylicum* on glucose. **a** Model 1, Condition B; **b** Model 2, Condition B; **c** Model 3, Condition B

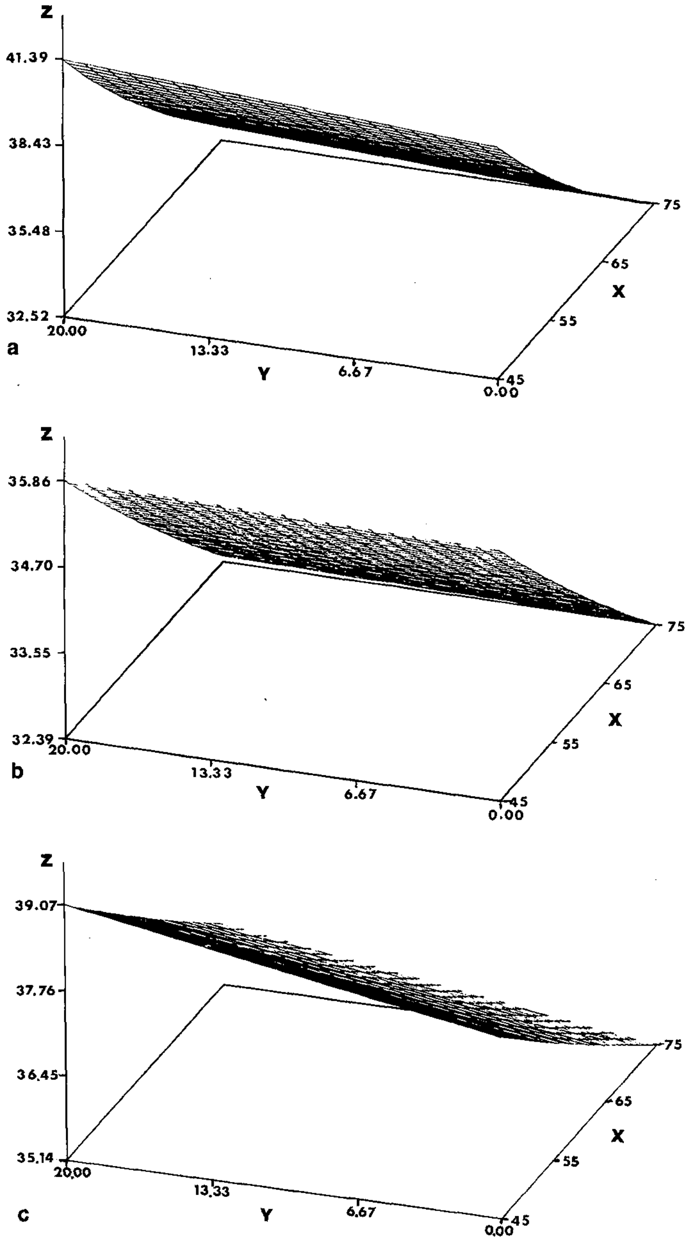

Fig. 2a–c. Continuous variations of the total solvent yield (z) with the weight fractions of butanol (x) and ethanol (y) in cultivation of *C. acetobutylicum* on glucose. **a** Model 1, Condition D; **b** Model 2, Condition D; **c** Model 3, Condition D

carbon and energy source. The total production costs for a molasses-based process were found to be slightly higher than the total project annual income even when all the fermentation by-products were taken into account (Lenz and Moreira 1980). This was mainly due to the cost of the molasses feedstock. It is obvious that the process conversion yield of the products from the carbon and energy source is crucially important for successful exploitation of the fermentation process.

In this paper, experimentally established values of the key fermentation parameters were used in developing models for the estimation of the TNVP yield reflecting the process efficiency of substrate conversion to the solvents. Two different approaches based on simple stoichiometric relationships between the substrate and the products and 30 different combinations of the variables found in Eq. (8) were considered. The values of the TNVP yield obtained in this study represent the conversion efficiency of glucose to the solvents considering that all the glucose carbon and energy were consumed in the production of the desired compounds and no other product was present in the system.

The actual TNVP yield obtained from the experiments referred to in this paper was ~33%. Considering the same ratios for butanol : acetone : ethanol, the models resulted in a maximum stoichiometric calculated TNVP yield of 42.3%. This value was obtained when using the Approach II, Model 3, and Condition B. The maximum stoichiometric calculated TNVP yield was 38.5% when Approach I was used, 38.6% when using Approach II and Model 1, and 37.11% when Approach II and Model 2 were used. Based on these stoichiometric calculated yield values, the efficiencies of 78%–89% can be calculated for the solvent production in the actual experimental biological system. The range of the calculated yield values of 11% is due to the differences in the glucose carbon, energy, and electrons divided among the solvents in the three models. It implies that the assumptions made in calculating the theoretical TNVP yield must be defined whenever this parameter is mentioned or used in process "conversion efficiency" calculations. The effect of the ratios between the solvents on the value of the TNVP yield is better presented in the three dimensional plots reflecting the interdependent variations in those parameters. The six plots presented in Figs. 1 and 2 are based on the models considered in this paper and can establish a method for predicting the variations in this key fermentation parameter.

Using the figures obtained in the stoichiometric yield computations, a stoichiometric relationship was developed between the glucose consumed, the solvents and acids produced and the gases evolved. The coefficients of these stoichiometric equations are tabulated in Tables 4–12. These relationships do not express the complicated mechanism involved in the production of the intermediates and the solvents. However, they are very important since they give a somewhat simplified picture of the whole process. The molar ratios between the products of the system in gas and liquid phases could also be calculated from these equations. Those equations which describe a real situation whereby all the components are present in the system (Tables 5–7 and 9–12, Condition E) are of particular practical importance.

Pondering the mechanisms involved in the production of butanol and hydrogen gas, one can see that formation of both of these compounds are convenient ways of disposing of the electrons brought into the bio-system in the substrate. Therefore, there may be a correlation between the production of these two compounds. A ratio of 2.8 mole of H_2/mole of butanol resulted from accumulative figures for total hydrogen and butanol respectively produced in the system during the batch experiments with *C. acetobutylicum.* This ratio ranged from 0.5 mole of H_2/mole of butanol to 3.2 mole of H_2/mole of butanol in the theoretical computations. The rather large fluctuations of this ratio in the computations were due to the freedom of the movements of the hydrogen atoms between H_2 and H_2O wherever there was no restriction on the molar value of the hydrogen gas in the models. Johnson et al. (1931) applied Eq. (2)–(6) in calculating the volumes of carbon dioxide and hydrogen which should evolve during the biosynthesis of the solvents and acids. Their considerations are not quite realistic since they do not account for the amount of carbon incorporated into the cellular structure of the biomass. As a result, higher volumes of the gases produced are obtained from those calculations. Freiberg (1925) presented another set of equations for the formation of acids by considering the cell build-up in the system. Calculating the gases evolved during the fermentation from his equations gives gas volumes closer to the experimental results. However, his equations are unbalanced and mainly hypothetical.

In seeking an answer to the question of the "theoretical yield" in the acetone-butanol fermentation, one should first examine the definition of this parameter and the obstacles in reaching the theoretical value during the actual experimental conditions. The acids (acetic and butyric) have been established to be the precursors of butanol and acetone in the metabolism of glucose by *C. acetobutylicum.* A complete conversion of these acids would then be necessary for the production of theoretical quantities of the solvents. The carbon, hydrogen and oxygen atoms incorporated in the cell structure should also be minimized. These assumptions will lead to Condition B whereby butanol, acetone and ethanol are assumed to be the only final products of the liquid phase. It is worth noticing that, due to the possible variation in the ratio between the solvents, it is not possible to establish a certain single value for the maximum theoretical yield which could be considered universal and applicable at any condition. The ratio of the solvents is dependent on the culture and culture conditions. Analysis of the metabolic pathways involved in the production of the solvents also reveals that the ratio between the solvents cannot be a constant fixed value. The energetic metabolism of *C. acetobutylicum* leading to the production of the solvents is branched. The efficiency of each branch is regulated in such a way that the overall efficiency of the system in the transformation of energy is optimized. This regulation effects the final ratio between the products of the system.

Computations of the TNVP in this work have been based on an experimentally determined ratio of 64 : 30 : 6 and on an often literature-quoted ratio of 60 : 30 : 10 between butanol, acetone, and ethanol. Comparison of the computed TNVP values shows that Approach II, Condition B, and Models 1 and 3 give the values of 39.9% and 42.8% respectively, for the ratio of 60 : 30 : 10 and 38.6% and 42.3% respectively for the ratio of 64 : 30 : 6 which are the highest values of the solvent yield on glucose in the computations. The plots (Figs. 1 and 2) indicated an ultimate maximum yield of 44.77% (Model 1, Condition B). However, this value was obtained at a ratio of 45 : 35 : 20 for butanol-acetone-ethanol which is rather undesirable. Model 3 also usually resulted in low values of hydrogen gas production and appears unrealistic. Therefore, a TNVP yield of 38.6%–39.9% resulting from Approach II, Model 1, and Condition B with a butanol : acetone : ethanol ratio of 64 : 30 : 6 to 60 : 30 : 10 has been considered in this work as the maximum theoretical yield under "acceptable" conditions. If one neglects the ethanol production in the system, a lower maximum TNVP yield of 38.5% (Approach I, Condition B) will result.

In 1981, Leung and Wang reported a theoretical stoichiometric equation by considering butanol and acetone as the only products of the system in the liquid phase. Their results gave a maximum theoretical solvent yield of 38%. A ratio of 2 mole butanol/mole acetone which resulted from their equation is higher than the normally observed ratio for the two compounds. The model reported by Phaff (1981) for the production of butanol and acetone also differs from reality leading to a ratio of 1 mole butanol/mole acetone.

The approach presented in this paper provides a simple method for expressing the stoichiometric relationships between the carbohydrate reactant and the products enabling the calculation of the theoretical TNVP yield without the need for any unrealistic assumptions. In using this approach, however, it should be borne in mind that, despite the validity of all the models considered here, and the perfect balance of the carbon and electrons in all of the equations, the value of the theoretical TNVP yield and the ratios between the gases and the solvents are dependent on the particular model utilized in the computations and on the ratio of the solvents produced by the microbial culture.

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